UNIVERSITY OF PISA

School of Graduate Studies
"Scienza del Farmaco e delle Sostanze Bioattive"

PhD THESIS
XXIII cycle (2008-2010)

"New agents against hypoxic tumours counteracting invasiveness and metabolism"

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CHIM/08

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AIMS OF THE THESIS

Tumour cells have abnormal growth rates, which arise from malfunctions in the regulatory mechanisms that oversee the cells' growth and development. This uncontrolled growth leads to a dramatic change in the physiology of the tumour itself. In particular, the development of hypoxic zones occurs as a result of a discrepancy between oxygen consumption and oxygen supply of the rapidly growing tumour. Hypoxia, in its turn, deeply modifies the metabolism and the behaviour of the tumour cells. Warburg effect, which is a shift from ATP generation through oxidative phosphorylation to ATP generation through glycolysis, even under normal oxygen concentrations, is the best characterized metabolic switch observed in hypoxic tumours. Hypoxia is also strongly associated with tumor progression and metastatic disease and this is likely because low oxygen tension is able to increase cell invasiveness. These pathological features make tumour hypoxia a poor prognostic factor in several kinds of malignancies.

The goal of this PhD Thesis was to interfere with these two main peculiar features of hypoxic tumours, glycolytic metabolism and invasiveness, by focusing our attention on two key enzyme: lactate dehydrogenase A (LDH-A), which is essential for energy production in hypoxic conditions and lysyl oxidase (LOX), which is strictly involved in the metastasis formation deriving from hypoxic tumours. Specifically, this Thesis aimed at designing and synthesizing small organic molecules able to inhibit LDH-A, so to be developed as starvation agents against hypoxic tumours. The other aim of this Thesis was to develop hypoxia-activated prodrugs as LOX inhibitors, in order to selectively block this enzyme in hypoxic cancer cells and, consequently, reduce or even completely stop the invasiveness of hypoxic tumours.

PREFACE

The first Chapter gives a broad overview of tumour hypoxia and the main pathological aspects of this condition, explaining why hypoxia is considered a poor prognostic factor in many cancers and introducing LDH-A and LOX in this context. Furthermore, the general strategies for attacking hypoxic tumours by inhibition of LDH-A and LOX are presented in Chapter 1.

Chapter 2 is focused on LOX, starting with the detailed description of this enzyme and previous research in this area, which support our choice to develop hypoxia-activated prodrugs as LOX inhibitors. Synthesis and biological results for the synthesized molecules are reported.

Chapter 3 deals with the main subject of this PhD Thesis, that is, LDH-A inhibition. Background of enzyme LDH-A is reported, including kinetic and structural features of human and *Plasmodium falciparum* enzyme isoforms, as well as inhibitors reported in the literature. A small overview of the most representative glycolytic inhibitors is also presented. The central part of this Chapter comprises the design and the synthesis of LDH-A inhibitors developed in my PhD project, which have been subdivided into structural classes and, for each group, enzymatic assays and molecular modeling studies are discussed. The last part of this Chapter is about cellular assays performed during the period spent at the University of Illinois at Urbana-Champaign (USA).

Detailed experimental procedures for the synthesis and characterization of LOX and LDH-A inhibitors are described in Chapter 4.

CHAPTER 1

“Introduction”

1.1. TUMOUR HYPOXIA

1.1.1. The main pathological aspects of hypoxic tumours

A distinctive feature of many solid tumours is the presence of hypoxic regions, which originate from an imbalance between blood supply and consumption of oxygen. This clinical consideration was made for the first time by Thomlinson and Gray about 50 years ago and was then confirmed thanks to the subsequent introduction in the 1990s of an oxygen electrode, called the "Eppendorf" electrode, that made it possible to accurately measure the oxygen pressure in human tumours [1, 2]. In order to explain this pathological aspect, we have to take into account the fact that cancer cells possess an elevated rate of growth, so their metabolic requirements surpass the vasculature ability to provide oxygen and nutrients. This pathological condition results in areas characterized by low levels of oxygen pressure (pO_2 < 5 mmHg corresponding to < 1% in the gas phase), and sometimes in necrotic regions, which have been detected in a wide range of cancers, such as brain, head and neck, cervix, breast tumours and soft tissues sarcomas. The development of this condition may be due to: *a*) temporary obstruction of microvessels or variable blood flow caused by structural and functional abnormalities of intratumoural vasculature (*perfusion-limited* or *acute hypoxia*); *b*) increased mean diffusion distances, about 100 μm or more, or adverse diffusion geometry caused by the irregular and fast tumour growth (*diffusion-limited* or *chronic hypoxia*) (Fig. **1.1**) [3, 4].

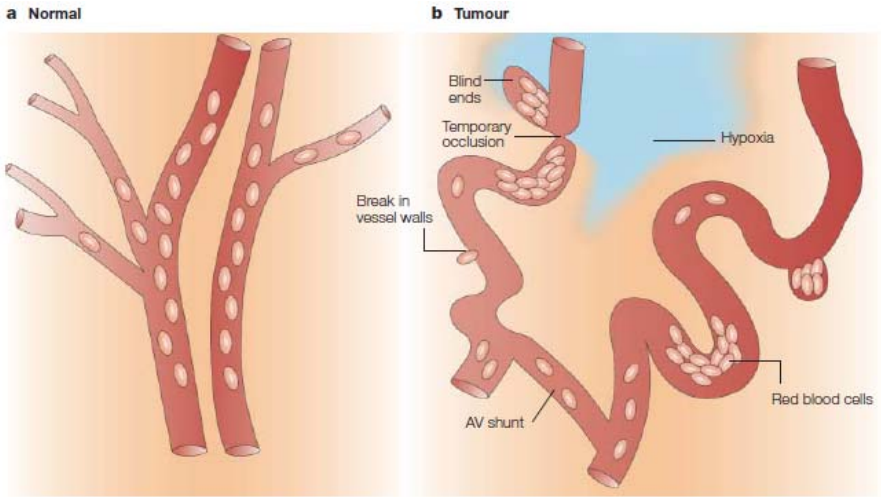

Figure 1.1. The vascular network of normal tissues (on the left) versus tumour tissues (on the right) [4].

Hypoxia, in turn, acts as a promoter of tumour progression, because it provokes an overexpression of a great number of genes implicated in the cell survival, leading to many changes in tumour cellular behaviour and metabolism. In particular, the transcription factor HIF-1α (Hypoxia-Inducible Factor-1α), that is overexpressed in hypoxic tumours, starts a transcriptional program that provides many solutions to hypoxia by up-regulating a series of downstream target genes, such as Lactate Dehydrogenase A (LDH-A), Lysyl Oxidase (LOX), together with Glucose Transporter-1 (GLUT-1), Carbonic Anhydrase IX (CA IX) and Vascular Endothelial Growth Factor (VEGF). In particular, the overexpression of two selected key enzymes, LDH-A and LOX at different oxygen concentrations and pH values in two tumour cells lines is shown in the graphs below (Fig. **1.2**) [5, 6]. The x axis reports the oxygen concentration and the relative expression of these enzymes is displayed on the y axis: it is evident that, depending on the pH, the relative expression of the two enzymes is increased in both cell lines at 1% of oxygen compared with normoxia condition that is 21% of oxygen.

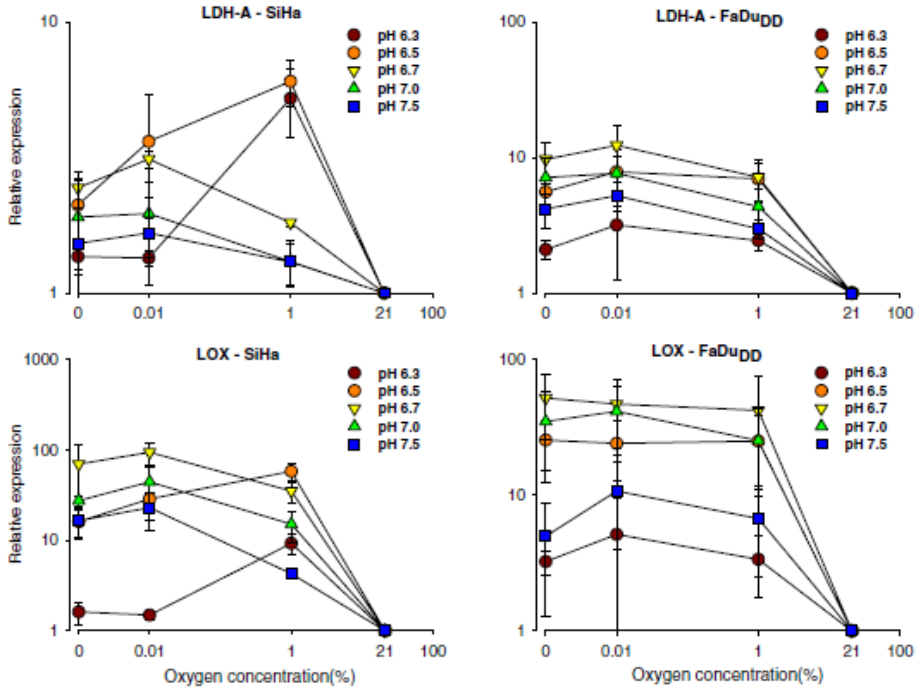

Figure 1.2. mRNA expression of LDH-A (top) and LOX (bottom) in SiHa (uterine cervix squamous cell carcinoma) and $FaDu_{DD}$ cells (pharyngeal squamous cell carcinoma) under different oxygen concentrations at various grades of acidosis. Cells were exposed to various oxygen levels at the different pH values for 24 h, then mRNA levels were determined, compared to untreated cells and plotted [5]. Copyright©2007 Elsevier B. V.

The main function of these proteins, together with other factors, is to protect cells from the hostile hypoxic environment and to help them to proliferate and to survive in adverse conditions, constituting adaptive responses to hypoxia. Consequently, tumour cells become able to overcome the nutritive deprivation by increasing their vascularization through angiogenesis, enhancing the glucose uptake, and optimizing its sugar metabolism. Moreover, tumour cells acquire the ability of invading surrounding healthy tissues in order to escape from the adverse environment (Fig. **1.3**). The overexpressed proteins mentioned above are considered among the main responsible factors contributing to the typical pathological alterations associated to tumours, although other molecular pathways that may be implied, such as oncogene activation and tumour suppressor loss, actively contribute to complete this process. Indeed, it is

recognized that the tumour microenvironment, involving several factors having both anti- and pro-tumour effects, makes the understanding of the tumour development and progression a very complex picture [7]. All these hypoxic tumour hallmarks result in the development of a more malignant and aggressive phenotype and provide a selective growth advantage over other competing cell populations. They also confer a higher survival ability to hypoxic tumour cells. As a consequence, a direct relationship between intratumoural hypoxia and poor patient prognosis has been observed [3, 8].

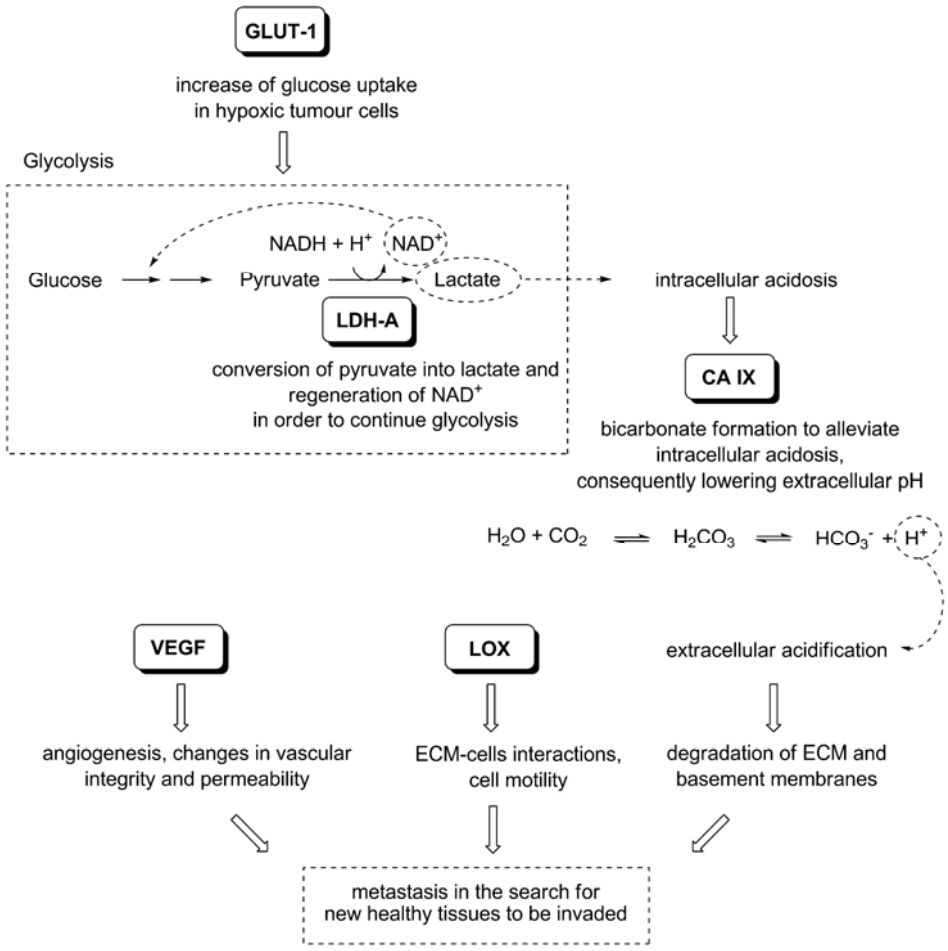

Figure 1.3. The biological relationships between the proteins up-regulated by HIF-1α in hypoxic conditions. ECM = extracellular matrix [9]. Copyright©2010 Bentham Science Publishers

One of the most important adaptive responses to hypoxia is the metabolic switch from oxidative to glycolytic pathway. Normally, cells produce most of the ATP from glucose through the oxidative phosphorylation (OXPHOS), but many cancer cells in hypoxic conditions obtain ATP by glycolysis and the final conversion of glucose to lactate, showing low levels of OXPHOS activity. This metabolic strategy is mainly permitted by the overexpression of LDH-A, a glycolytic enzyme which is a direct target of HIF-1α [10], although other enzymes belonging to the glycolytic pathway are also up-regulated. In fact, HIF-1α was proven to induce overexpression of glucose transporters GLUT-1 and GLUT-3, as well as of glycolytic enzymes hexokinase I and II (HK-I, HK-II), the liver isoform phosphofructokinase (PFK-L), aldolase A and C (ALD-A, ALD-C), phosphoglycerate kinase 1 (PGK-1), enolase α (ENO-α), pyruvate kinase M2 (PYK-M2), 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3 (PFKFB-3), and, of course, LDH-A [11]. As mentioned before, LDH-A converts pyruvate, the final product of glycolysis, to lactate, instead of undergoing the Krebs cycle in the mitochondria, due to the lack of oxygen that is the final electron acceptor in the electron transport chain. This reaction regenerates the cofactor NAD^+ and, therefore, permits the continuation of glycolysis (Fig. **1.4**). This kind of metabolism is maintained even when cells derived from hypoxic tumours are placed under normoxic conditions, confirming that the conversion of pyruvate into lactate, in order to obtain energy in conditions of low levels of oxygen, represents not only a simple adaptive mechanism to hypoxia, but above all a hallmark of hypoxic cancers involving epigenetic transformations that deeply affect some cellular pathways. A high rate of conversion of glucose into lactate with an elevated glucose uptake are the typical traits of the glycolytic phenotype and ensure adequate ATP levels and constant supply of intermediates essential for cell growth and division [12]. ATP generation via glycolysis is a less efficient energy production process (2 ATP molecules per glucose) than oxidative phosphorylation (36 ATP molecules per glucose). Hence, cancer cells consume more glucose than normal cells for maintaining an adequate ATP supply to proliferate. The fundamental role of this glycolytic phenotype is emphasized by studies demonstrating that increased glucose uptake coincides with the transition from pre-

malignant lesions to invasive cancer, since the cancer cells' dependence on glycolysis increases as malignant transformation increases [13, 14].

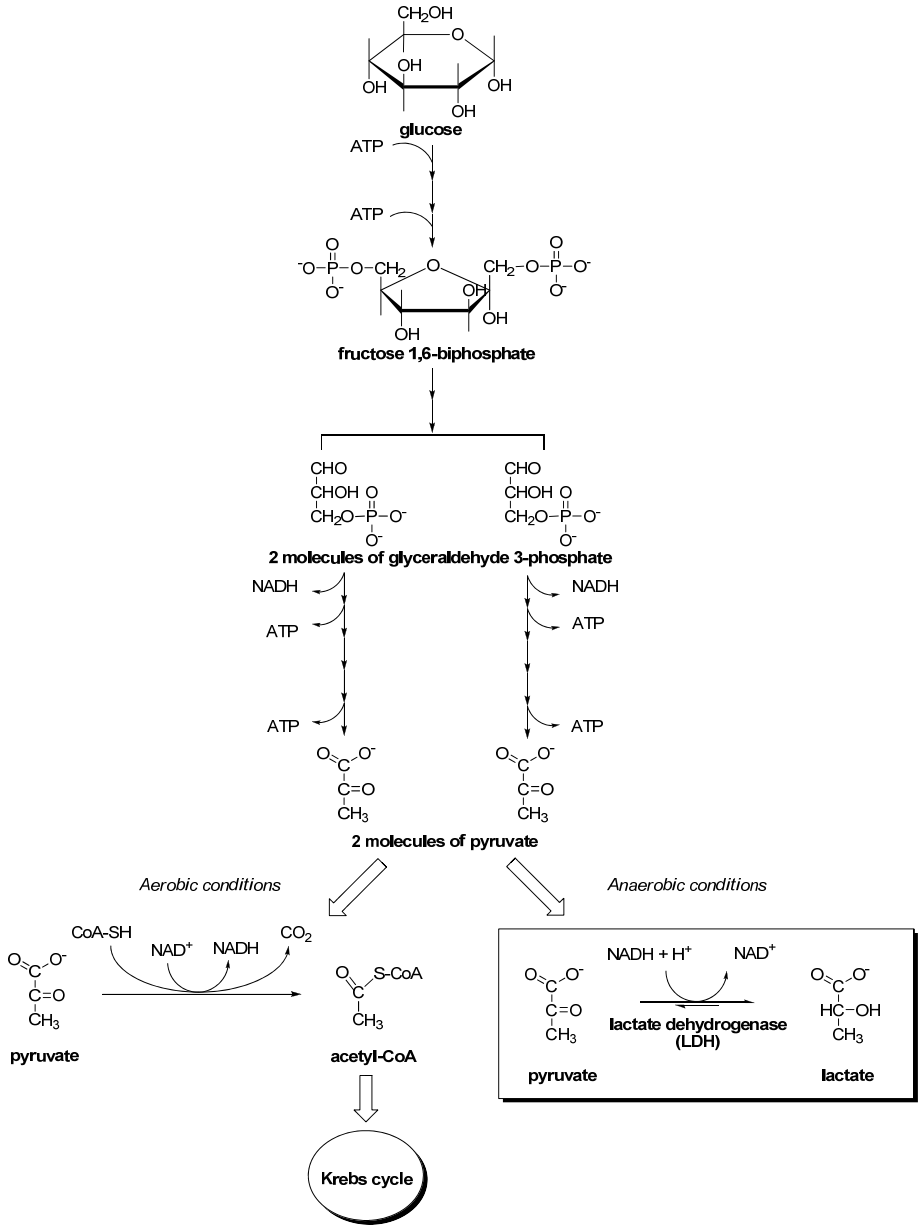

Figure 1.4. The glycolytic process and the two main metabolic pathways in which pyruvate is involved, depending on the presence of oxygen. The reaction catalyzed by LDH is highlighted (bottom-right) [9].

The increased production of lactate by malignant cells even under aerobic conditions is called aerobic glycolysis or "Warburg effect" and it has been observed only in tumour tissues. This phenomenon was first reported by Otto Warburg in the 1920s, hypothesizing that this altered glucose metabolism derives from impaired mitochondrial activity [15]. Nowadays, the "Warburg hypothesis" has proven incorrect, but an increased dependence on glycolytic activity for ATP generation in tumours even in the presence of oxygen has often been confirmed. The metabolic switch from the energetically efficient Krebs cycle to the tumour glucose utilization in glycolysis is regulated by a very wide network of pathways involving both oncogenes and tumour suppressor genes, thus playing a central role in the altered catabolic and anabolic processes of several tumours [16].

The invasive and aggressive behaviour is another typical pathological feature found in hypoxic tumours. Since metastasis is considered the main cause of cancer death, the understanding of this very complex process is an extremely challenging goal for the treatment of cancer. Metastasis is a multistep process starting with the detachment of cells form the original tumour. In order to metastasize, a malignant cell must dissociate from the primary tumour and intravasate into the vascular or lymphatic systems by degrading and migrating through the basement membrane and extracellular matrix. Once in the lymph or in the blood streams, this cell must evade the host immune system, migrate to its target site, and finally extravasate out of the circulation to implant in a healthy tissue where proliferation gives rise to cancer metastases [7, 17, 18]. A complex network of proteins of the extracellular matrix were found to be involved in metastasis formation, such as Matrix Metalloproteinases (MMPs), integrins, Connective Tissue Growth Factor (CTGF), which favour dissemination of malignant cells from a primary tumour site to a secondary site by different mechanisms. Moreover, the tumour itself becomes able to secrete a large number of growth factors and proteases, which permit to modify the local microenvironment making it more permissive for cell motility and adhesion. Even the extracellular acidosis, caused by the increased lactate production operated by LDH-A, is a factor promoting the entry of tumour cells into the blood vessels, so leading to metastasis formation. In this picture, a key role is played by another direct target of HIF-1, that is LOX, an amine oxidase of the extracellular matrix,

whose catalytic function contributes to the formation and the reinforcement of the ECM. This enzyme was found to be directly involved in the metastatic process. Numerous studies demonstrate that its expression is directly correlated to the invasiveness of a large number of tumours, causing disease progression and poor overall survival [19].

1.1.2. Hypoxia as an obstacle to medical treatment of cancer

Tissues possessing low levels of oxygen show resistance to radiotherapy and this is one of the principal clinical problems that compromises hypoxic cancer treatment documented as early as since 1953 [20]. The mechanism linking hypoxia to tumour radioresistance is generally known as the "oxygen enhancement effect" [21]. This phenomenon can be explained considering that DNA damage is usually provoked by direct ionization from ionizing radiations or is induced by oxygen radicals, such as hydroxyl radical, superoxide anion etc. In the presence of oxygen, DNA double-strand breaks cannot be repaired because O_2 reacts with the broken DNA forming stable organic peroxides which are hardly repaired by the cell, leading to fatal chromosome aberrations. Basically, oxygen fixes the DNA damage, making it permanent. On the other hand, in the absence of oxygen, the damage is more easily repaired: the broken DNA can be restored to its original form, thanks to reparative processes based upon reductions by –SH containing intracellular components. This mechanism explains why tumours with low levels of oxygen show a reduced effect on response to radiotherapy, compared with normally oxygenated tissues [4, 21, 22].

Hypoxic cells are also resistant to conventional anticancer therapies and this is mainly due to their distance from blood vessels, so they are not sufficiently reached by most anticancer drugs. We can also mention other reasons that are behind chemotherapy resistance. For example, anticancer chemotherapy is usually more efficient against cells in rapid proliferation due to the mechanisms of actions commonly associated to most of the currently available treatment regimens, so it causes lower effects to hypoxic cells, which are characterized by slower rates of proliferation compared to normally oxygenated cancer cells. Furthermore, hypoxia selects cells that up-regulate genes encoding for proteins involved in drug resistance. It follows that, at present, there are not adequate anticancer drugs able to efficiently kill hypoxic cells [4, 23].

In addition to radiotherapy and chemotherapy resistance, the higher malignant phenotype, typical of hypoxic cancer cells, is associated with a more metastatic and aggressive behaviour, consequently predisposing to the formation of metastases, that compromise curability of tumours by surgery [24].

Since hypoxic cells are resistant to standard therapies, it is clear that hypoxia has a negative impact on cancer treatment and prognosis. Nevertheless, the unique features of hypoxic tumours that do not occur in healthy tissues, such as the metabolic switch associated to the "Warburg effect", could be exploited in cancer-selective therapy.

1.2. LOX AND LDH-A: TWO KEY ENZYMES IN HYPOXIC INVASIVE TUMOURS

Tumour cells become able to survive in conditions of low oxygen concentration, that is restrictive to normal cells, thanks to many pathological alterations in their metabolism and behaviour. In particular, the glucose uptake is increased and then the internalized sugar is processed through aerobic glycolysis in order to generate energy for continuously growing cells, even in the absence of oxygen. Moreover, hypoxia represents a stimulus for invasion and metastasis, because tumour cells, living in areas of nutrient and oxygen deprivation, need to invade new healthy tissues more permissive for their growth (Fig. **1.5**). As a consequence, aerobic glycolysis and metastasis represents essential hallmarks of hypoxic cancer. In particular, in this PhD Thesis we focused our attention on two key enzymes, LOX and LDH-A, which play important roles in the metabolism and behaviour of tumour cells. LOX is deeply implicated in the progression of the tumour and in its spread in the surrounding tissues by metastasis formation; on the other hand, LDH-A supports the growth and the survival of hypoxic tumour cells, permitting the obtainment of ATP by glycolysis. Our interest is also justified by the almost total absence of suitable and selective inhibitors of these enzymes, so LOX and LDH-A can be considered innovative targets in the fight against invasive hypoxic tumours.

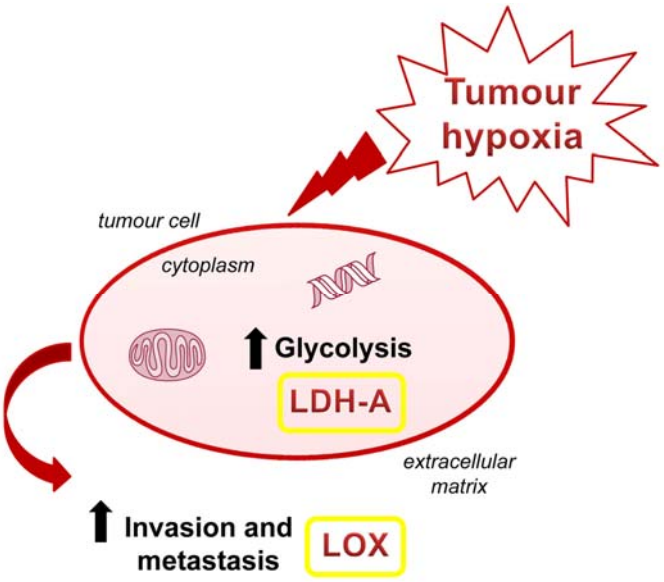

Figure 1.5. Tumour hypoxia-induced changes inside and outside the cancer cell. Increased glycolysis (in the cytoplasm) and increased invasive ability of tumour cells (in the extracellular environment) are schematically represented, together with the two key enzymes responsible for this altered behaviour, LDH-A and LOX.

1.2.1. Targeting LOX for the treatment of invasive hypoxic tumours

Metastasis constitutes an intricate process involving many tumours and stromal proteins necessary for the remodelling of the extracellular matrix and, of course, LOX represents one of the key ECM components involved in metastatic disease. The LOX involvement in metastatic disease is proved by numerous studies linking LOX to tumour development and metastasis formation [17]. LOX was found to be implicated in the promotion of the metastatic process *in vivo* and *in vitro*, being up-regulated by hypoxia-inducible factor-1 (HIF-1) in hypoxic tumour tissues [5, 6, 19]. An overexpression of LOX mRNA was identified principally in MDA-MB-231 cancer cell line, which is a highly invasive and metastatic breast cancer cell line, SiHa cervical cancer cells, and in head and neck cancers, but the same association was not found in the poorly invasive and non-metastatic human breast cancer phenotype MCF-7, demonstrating that this enzyme is required for the increased invasion in tumours and its expression seems to occur mainly in distant metastatic cancer tissues compared with primary tumours. Recently, LOX expression has been correlated to disease progression,

metastasis and poor overall survival also in oral cancers [25, 26]. LOX stimulates cell invasion through a mechanism mediated by hydrogen peroxide, a well-established LOX by-product [27-29].

In vitro and *in vivo* invasion assays demonstrated that only catalytically active LOX, secreted in the extracellular matrix, is responsible for the increased invasion ability in hypoxic tumours. In fact, since LOX is a copper-dependent enzyme, the use of a copper chelator that cannot enter the cell, such as bathocuprione disulphonate (BCS), results in a reduction of invasiveness, thus confirming that extracellular LOX involvement is the responsible for such invasion ability. Furthermore, when LOX catalytic activity was compromised by using LOX short hairpin RNA cells (shRNA), treatment with LOX antibody or administration of a LOX irreversible inhibitor such as β-aminoproprionitrile (BAPN), the invasion ability of tumour cells was significantly decreased and the formation of metastases was considerably reduced or completely blocked. This confirms that LOX is actively involved in the invasion ability of hypoxic tumour cells. This behaviour is also confirmed by the cellular shape: in air, wild-type tumour cells show a branching morphogenesis typical of invasive human cancer cells grown on collagen, which was further enhanced by hypoxia. On the other hand, the LOX shRNA cells grew in a markedly different manner, remaining more rounded in spheroid-like cell clusters, showing little branching in air or hypoxia. These results definitively indicate a role for LOX in the development of an invasive phenotype of hypoxic human tumours.

A recent study performed on a mouse model of mammary cancer proved the correlation among LOX activity, increased ECM stiffness due to collagen cross-linking, and progression to invasive disease. Pre-conditioning of mouse mammary fat pads with LOX-expressing fibroblasts promoted the growth and invasion of mammary cells and LOX inhibition with BAPN or LOX-specific antibody tempered tissues fibrosis, increased tumour latency, and decreased tumour incidence, suggesting that LOX-mediated cross-linkages likely regulate tumour progression by modifying the tumour microenvironment [7, 30].

Furthermore, LOX seems to play a critical role in premetastatic niche formation. Upon modification of the extracellular matrix and recruitment of CD11b+ myeloid cells to premetastatic sites, LOX prepares the target organs for the subsequent arrival of

metastatic tumour cells and enhances the establishment and growth of metastases. It appears that LOX alters tumour microenvironment and is involved in tumour invasion at different stages of this process. These findings together support the high potentiality of this perspective therapeutic target [31].

From a mechanistic point of view, the hypoxia-induced metastatic process starts with a series of adhesion and detachment interactions with the extracellular matrix by means of a cell pseudopode protrusion driven by actin polymerization. In particular, remarkable amounts of LOX are localized in proximity of the protrusion leading edge and on the many hair-like fibres sticking out from it. These events lead to invasion into the bloodstream and migration of the primary tumour cells, leaving behind a route of remodelled extracellular matrix that constitutes a sort of "highway" for the other cells, that can quite easily follow the first ones (Fig. **1.6** and **1.7**). As a consequence, it is confirmed to LOX the role of an ECM remodelling enzyme during invasion that allows cell motility, but also during extravasation and colonization processes. According to these findings, LOX could be considered not only a potential marker for identifying highly invasive/metastatic and hypoxic tumours, but its inhibition could be exploited for the prevention and also the treatment of aggressive and metastatic tumours, so revealing a new good therapeutic target for several kinds of metastatic cancers [19].

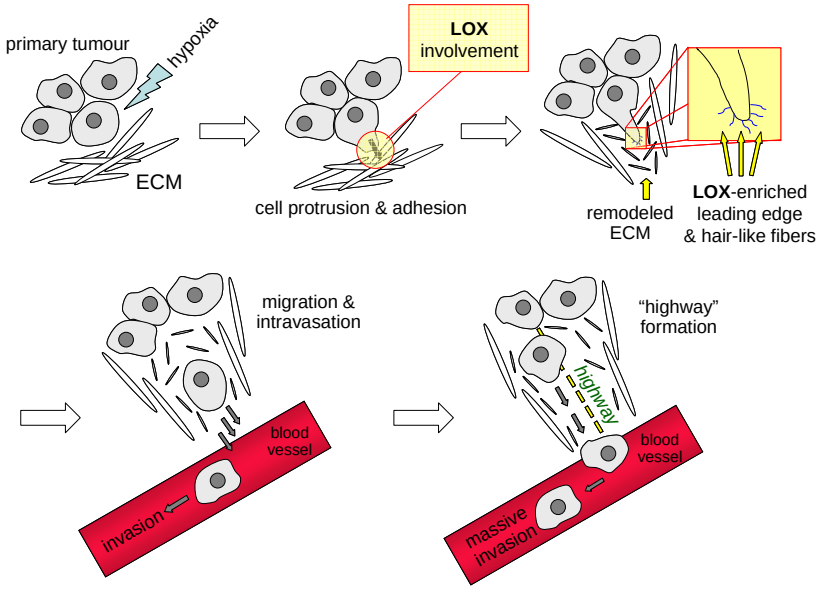

Figure 1.6. Metastasis early stage: ECM-remodeling and migration.

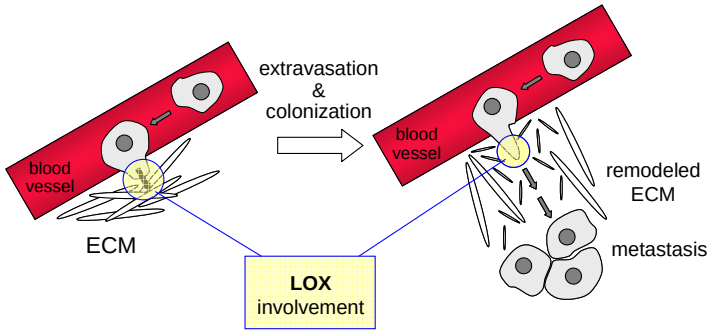

Figure 1.7. Metastasis later stage: extravasation and colonization of new tissues.

The lack of suitable LOX inhibitors to be used in clinic makes lysyl oxidase inhibition a very challenging goal in the field of anti-tumour research and there are wide margins of research work to do because LOX inhibitors have been poorly studied so far. LOX involvement in metastasis opens a new research way that is potentially selective, LOX being a hypoxia-induced and extracellular target, and also innovative, due to very few treatment options for patients with metastases. For these reasons, we have developed molecules able to selectively inhibit LOX in hypoxic environments, and the results are reported in Chapter 2 of this Thesis.

1.2.2. Exploiting tumour hypoxia: LDH-A inhibition

The much lower levels of oxygen in tumour cells compared to those in normal tissues constitute an useful opportunity to develop a selective anticancer therapy based on this peculiarity. At present, the most commonly and used strategies that have been attempted in this field are represented by hypoxia-activated pro-drugs [32] and by gene therapies based on the inhibition of HIF-1 in hypoxic conditions [33, 34].

Another way of taking advantage from the distinctive features of tumour cells is to consider their altered carbohydrate metabolism, that is the conversion of glucose to lactate even in the presence of normal oxygen pressure. The dependency of tumour cells on glucose metabolism constitutes the weak point of hypoxic cancers and it could be therapeutically exploited by interfering with LDH-A activity. Several attempts to inhibit the glycolytic enzymes, such as hexokinase (HK), up-regulated in hypoxic tumours and specially involved in malignancy progression have been realized. However, among the enzymatic checkpoints of the glycolytic pathway, LDH-A still remains one of the most attractive targets in this context [22, 35]. LDH-A is overexpressed in hypoxic tumour environment, whereas the other main isoform LDH-B is predominant in all living cells, both normal and malignant. The data reported in literature confirm that up-regulation of LDH-A ensures an efficient aerobic glycolysis in tumour cells, whereas this enzyme is not so necessary to healthy cells in normal conditions that generally use the aerobic oxidation pathway [5, 36-38].

It was discovered that when LDH-A levels are knocked down by means of shRNAs (short hairpin RNA) in tumour cell lines that show high glucose dependency and high LDH-A expression under both normal and hypoxic conditions, LDH-A activity is severely compromised and the ability of these cell populations to proliferate shows a remarkable reduction [39]. In particular, when the oxygen pressure is limited the effect is even more pronounced, because the proliferation was completely blocked and ATP levels were much lower. This suppression of aerobic production of lactate had also a great impact on OXPHOS activity and oxygen consumption, which were both significantly increased. Consistent with these observations, cancer cells with low levels of LDH-A possess a reduced ability to grow under hypoxic conditions and their tumorigenic potential is severely compromised. In order to determine if the growth

decrease is due to LDH-A knockdown and, as a consequence, to the inability of tumour cells to metabolize glucose to lactate, cDNA corresponding to human LDH-A was introduced into LDH-A-silenced cells. Ectopic expression of this enzyme restores the proliferation and reestablishes the glycolytic phenotype, thus confirming the specificity of LDH-A knockdown approach. Altogether, these data suggest that the A isoform of lactate dehydrogenase plays a fundamental role in tumour maintenance, because cancer cells rely on LDH-A activity for energy obtainment even when oxygen is not a limiting factor [39].

As regards possible side effects caused by this approach, humans suffering from a complete hereditary deficiency of LDH-A due to the lack of A subunit production have been studied. These individuals show myoglobinuria, that is, the presence of myoglobin in the urine usually associated with muscle damage, only after intense anaerobic exercise (exertional myoglobinuria), without displaying any symptoms under ordinary circumstances [40, 41].

All this evidence constitutes a good target validation for LDH-A. In fact, not only does the inhibition of LDH-A prevent the Warburg effect, by forcing hypoxic tumour cells to revert to OXPHOS metabolism from the glycolytic pathway, but it also attenuates cell proliferation, supporting the growing evidence that the glycolytic phenotype is responsible of tumorigenicity of hypoxic cancer cells. Up to now, unlike other glycolytic targets for which some suitable molecules as drug candidates have been found, only shRNAs have been used to selectively inhibit LDH-A in tumours, and there are not many small molecules able to selectively block LDH-A activity (Fig. **1.8**). As a consequence, antagonizing LDH-A could be considered an interesting, innovative, selective and also potentially non-toxic strategy of interfering with hypoxic cancer growth. Therefore, we focused our efforts in an attempt to develop small molecules able to selectively inhibit LDH-A, and our results are reported in Chapter 3 of this Thesis.

Table 2. Potential Metabolic Targets for the Treatment of Cancer

Target	Desired Effects	Examples of Compounds	Reference
Glycolysis			
Glucose uptake	Inhibition of glucose transport or of the initial steps of glycolysis	2-deoxyglucose has radiosensitizing and chemosensitizing effects	Simons et al. (2007)
Hexokinase (HK1 and HK2)	Inhibition of enzymatic activity and dissociation from mitochondria	3-bromopyruvate has potent antitumor effects in vitro and in vivo	Kim et al. (2007b); Pedersen (2007)
Pyruvate dehydrogenase kinase 1 (PDK1)	Inhibition of PDK1 for deinhibition of pyruvate dehydrogenase	Dichloroacetate (DCA)	Bonnet et al. (2007)
Lactate dehydrogenase A (LDHA)	Inhibition	siRNA	Fantin et al. (2006)
Pyruvate kinase (PK) isoenzyme PKM2	Translocation of PKM2 to the nucleus for induction of apoptosis	Somatostatin and its derivative TT-232 (in vitro)	Stetak et al. (2007)

Figure 1.8. Potential metabolic targets for the treatment of cancer with relative desired effects and compounds. For LDH-A inhibition (entry 4), only genetic techniques have been used [12].

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CHAPTER 2[‡]

"Inhibitors of lysyl oxidase"

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2.1. THE ENZYME LYSYL OXIDASE (LOX)

2.1.1. Biological functions

In the context of hypoxic tumours, the enzyme lysyl oxidase (LOX) plays a very important role, proving to be strictly involved in the tumour ability of invasion and metastasis, as already mentioned in Chapter 1.

Lysyl oxidase is an extracellular copper-dependent amine oxidase, which is secreted in the ECM as a *N*-glycosylated pro-enzyme of 50 kDa (proLOX) and then the inactive pro-enzyme is cleaved by extracellular proteases, so releasing the active mature form of LOX (32 kDa). LOX, which was discovered in 1968 [1], belongs to a larger family consisting of five members, because there are four LOX-like enzymes (LOXLs) with various degrees of similarity to LOX, thus forming a isozyme family comprising LOX, LOXL1, LOXL2, LOXL3, LOXL4, nevertheless their exact distinct roles have not been clarified yet [2, 3]. In addition to being found extracellularly, mature LOX has also been localized into the cellular nucleus, suggesting a nuclear activity, in fact it interacts with basic nuclear proteins like histone H1 [4], so intranuclear LOX is involved in modifying gene expression, although the exact intracellular role of LOX still needs a more exhaustive characterization.

The main biochemical function of LOX is to catalyze the covalent cross-linking of collagen and elastin molecules within the extracellular matrix (ECM), so stabilizing the fibrous deposits of these proteins in the ECM. These cross-links formed by the enzyme increase tensile strength and structural integrity necessary for normal connective tissue function, embryonic development, tissue remodeling and repair of diseased and aging connective tissues. In particular, it catalyzes the oxidation of the ε-amino group of peptidyl lysine to peptidyl aldehyde (α-aminoadipic-δ-semialdehyde, AAS) by consuming oxygen and water and producing ammonia and hydrogen peroxide (Fig. **2.1**). Then, the aldehyde group of AAS starts the formation of covalent cross-linkages, condensing either with a neighbouring ε-amino group of an unmodified lysine residue to form anhydrolysinonorleucine (deLNL) or with another peptidyl AAS to obtain an aldol condensation product (ACP) [5, 6].

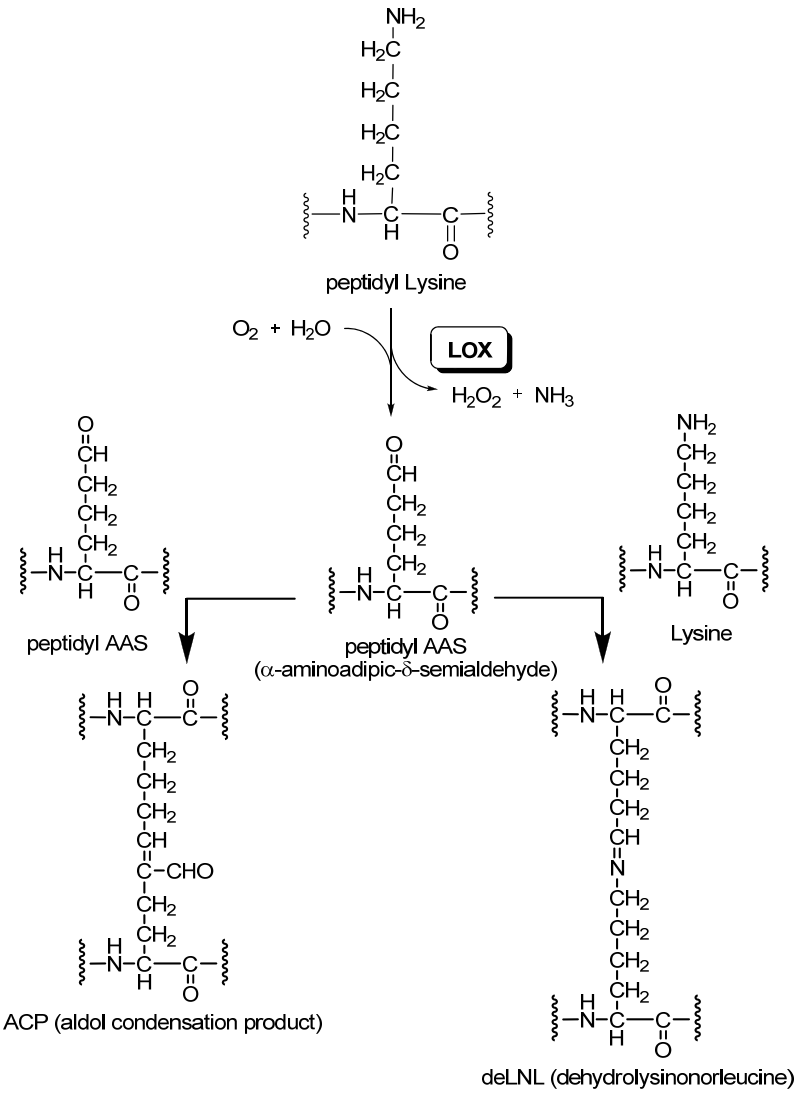


Figure 2.1. LOX-catalyzed reaction and spontaneous formation of crosslinkages from peptidyl AAS.

Although the X-ray structure of human LOX has never been obtained yet, it has been possible to determine the presence of a copper (II) ion in the LOX catalytically active site, which is bound to three histidine residues (not been exactly identified as of yet), and also of a covalently bound organic cofactor lysine tyrosylquinone (LTQ, Fig.

2.2), which is peculiar since other known amine oxidases are associated to cofactor trihydroxyphenylalanine quinine (TPQ).

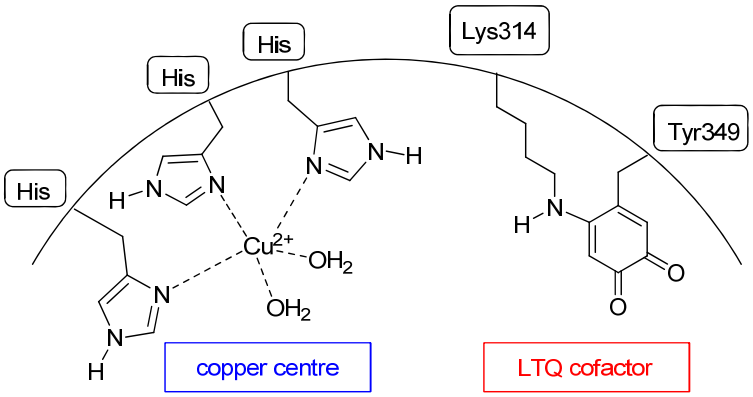


Figure 2.2. Active site of LOX. Copper atom bound to histidine residues is highlighted in blue, LTQ cofactor in red.

Probably, the cofactor LTQ is formed within the nascent enzyme, by an autocatalytical reaction between tyrosine 349 and lysine 314, in which Cu^{2+} catalyzes the oxidation of Tyr to quinone, followed by the covalent attack of the ε-amino group of lysine residue to the quinone ring, hence starting the formation of LTQ (Fig. **2.3**) [7].

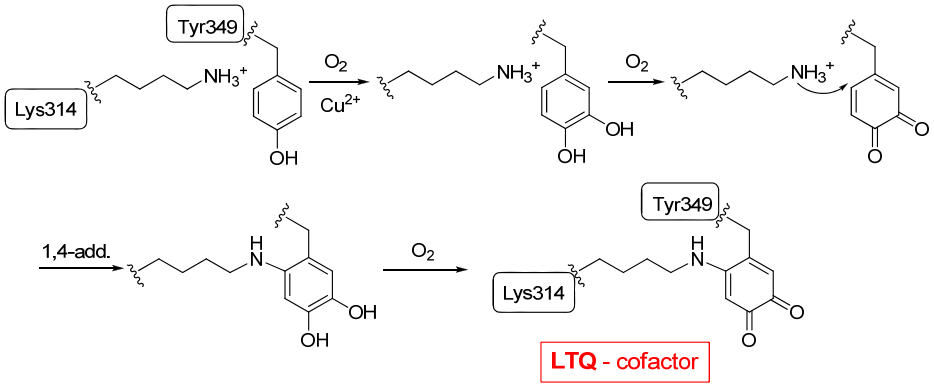


Figure 2.3. Autocatalytic formation of LTQ cofactor within LOX.

The catalytic cycle (Fig. **2.4**) performed by LOX starts with the condensation of its cofactor with the amino group of the substrate peptidyl lysine, then subsequent rearrangements of the imino double bond, hydrolysis and reoxidation complete the cycle affording the aldehyde product and regenerating the initial form of LTQ. It is supposed that the copper is involved in the reoxidation of the cofactor, due also to the fact that deprivation of this element compromises the catalytic activity of the enzyme.

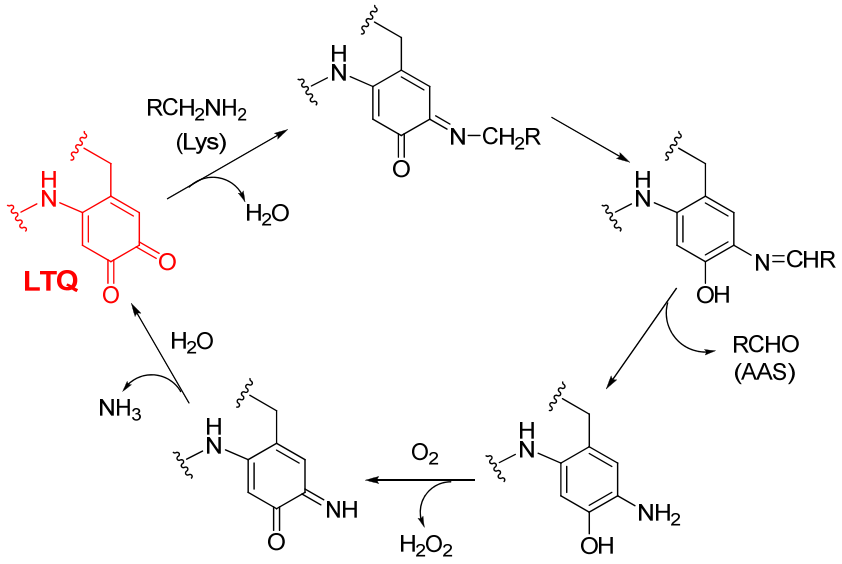


Figure 2.4. LTQ role in the LOX catalytic cycle.

The soluble precursors of collagen and elastin are not the only substrates of LOX, because it has been demonstrated that several basic proteins are oxidized *in vitro* by LOX. The selective preference for proteins with positive charges at physiological pH can be explained by the copious presence of anionic residues such as Asp and Glu in the catalytic active site (Fig. **2.5**), furthermore this abundance of negatively charged sites could be exploited in the development of LOX inhibitors.

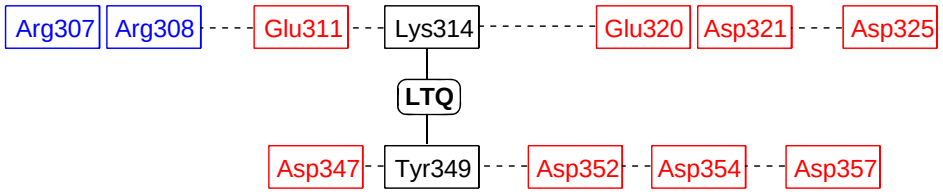


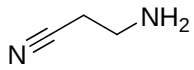
Figure 2.5. Charged aminoacid residues adjacent the LTQ cofactor (red: anionic; blue: cationic).

Beyond the deaminative oxidation of structural proteins of the ECM, LOX possesses other biological roles influencing cell behaviour, for example it has a potent chemoattractant activity for human peripheral blood monocytes and vascular smooth muscle cells, enhancing their migration with a mechanism mediated by hydrogen peroxide, a product of LOX catalytic activity [8, 9].

2.1.2. LOX inhibitors

BAPN (**2.1**, Fig. **2.6**) has been extensively used in assays devoted to demonstrate the role of LOX in metastasis formation, because it represents one of the very few known inhibitors of lysyl oxidase and so it constitutes the reference LOX inhibitor (IC_{50} = 10 μM). In particular, BAPN irreversibly inhibits LOX by forming covalent bonds within the catalytically active site, probably by a condensation between BAPN pharmacophoric primary amino-group and the quinone group of LOX covalently bound cofactor lysine tyrosylquinone (LTQ) [10, 11]. Recently, the reduction of the metastatic colonization potential on MDA-MB-231 cells due to LOX inhibition by BAPN was proved *in vivo* by bioluminescence imaging and microcomputed tomography [12]. Unfortunately, BAPN itself cannot be developed as a drug because of its many unwanted side effects. In fact, the small and "simple" molecular structure of BAPN is able to cross many physiological barriers and interact with a variety of biological targets, thus causing many unwanted side effects. Besides, the toxicity of BAPN is ascribed also to its LOX inhibitory property, because it is considered the main responsible for a syndrome called *Lathyrism* [13], a pathological condition involving several body districts, observed in people and animals after excessive ingestion of sweet pea (*Lathyrus odoratus*), which contains remarkable amounts of BAPN. Usually, this syndrome manifests in intoxicated

children or weanling animals, for which a mature connective tissues has not yet been developed.



2.1

Figure 2.6. Structure of BAPN (**2.1**).

In literature, there is a small number of LOX inhibitors, mainly because only few useful therapeutic uses had been envisaged so far. Most of them possesses primary amino group like BAPN itself, such as taurine **2.5** and compounds **2.6-2.14** [14-23] (Fig. **2.7**) or they are hydrazine-derivatives, such as isoniazide **2.2**, semicarbazide **2.3** and thiosemicarbazide **2.4** [24], so they should interact with LTQ cofactor similarly to BAPN, whereas only a few most recent inhibitors show heterocyclic scaffolds (compounds **2.15-2.16**). Besides, the presence of basic centers confirms the preference of LOX for cationic substrates. Unfortunately, most of these molecules not only are small and unspecific compounds, but they also have quite reactive structures and/or toxic functional groups, so they do not possess the proper "drug-like" requirements to be used as drugs, consequently they cannot be developed as anti-metastasis agents. Only the most recent LOX inhibitors such as benzazole **2.15** and pyridazinone **2.16**, possessing different structures, have been patented for treating diseases as scleroderma and fibrosis, respectively.

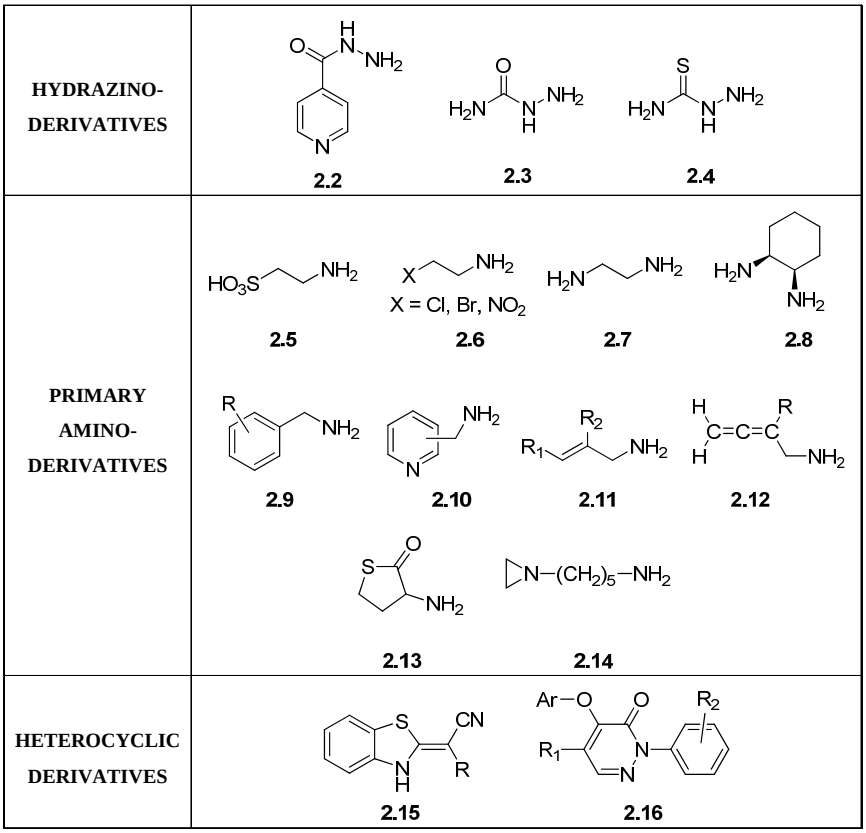


Figure 2.7. Structures of known LOX inhibitors (**2.2-2.16**), divided in three different structural classes (hydrazino-, primary amino- and heterocyclic derivatives).

At present, there are not small organic molecules able to selectively inhibit lysyl oxidase without provoking significant side effects in order to be used as anti-metastatic drugs, whereas there is a patent covering antibodies, polynucleotides, polypeptides and nucleic acids designed with the aim of blocking LOX activity in tumour tissues [25].

2.2. BIOREDUCTIVELY ACTIVATED LOX INHIBITORS

2.2.1. Hypoxia-activated cytotoxins: the nitroaromatic prodrugs

The lack of selectivity towards tumour cells is the responsible of dose-limiting side effects and toxicity associated with conventional chemotherapeutic agents. One approach to improve the therapeutic index and decrease side effects is to deliver selectively the drugs to tumour sites. The design of targeted prodrugs for tumour site-specific activation exploits the unique features associated with cancers, and in particular one of the well-known strategies exploits the low oxygen tension present in many solid tumours with the aim of developing prodrugs activated by hypoxia (hypoxia selective agents, HSA) [26].

In a general sense, this hypoxia-selective mechanism is based on a reductive process, by which an inactive prodrug is activated to a cytotoxic drug upon reduction in a hypoxia-dependant manner, but the same reductive reaction could occur also in oxygenated tissues, because reducing enzymes may be also present in aerobic regions. Nevertheless, in this case the electron of the prodrug radical is rapidly transferred back to molecular oxygen, forming superoxide, which is scavenged by the enzyme superoxide dismutase to less toxic species (but it can contribute to some unwanted toxic side effects), and regenerating the initial prodrug (Fig. **2.8**). This redox cycles is clearly advantageous, it ensures the cytotoxic action of the prodrug only in hypoxic areas, providing a selective treatment and minimizing the toxic side effects due to the activation of the drug in healthy oxygenated tissues. An ideal prodrug candidate has to meet some requirements to be developed as hypoxia-activated cytotoxin: it must be non toxic and stable and it must be able to diffuse efficiently through hypoxic tumour tissues but above all it must possess a reducible function, able to be converted to a potent cytotoxin when selectively activated by a specific enzyme or metabolic pathway present exclusively or predominantly in the targeted cancer cells, so exhibiting significantly greater toxicity to hypoxic compared to aerobic cells [27].

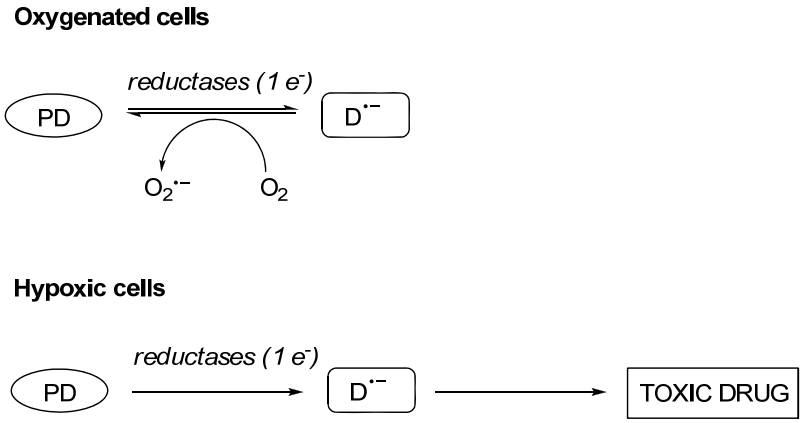
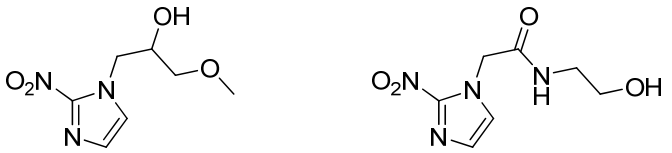


Figure 2.8. Mechanism of action of hypoxia selective agents in oxygenated and hypoxic cells. PD = prodrug, $D^{\bullet -}$ = prodrug radical.

Hypoxia-activated prodrugs can be divided into four main classes on the basis of the structure of the group that undergoes the initial reductive process (usually called the "trigger group"): quinone, nitroaromatics, *N*-oxides and metal complexes. Nitroaromatic class comprises a very wide group of efficient prodrugs whose biotransformation leads to drug activation in hypoxic tumours, but albeit the interest in their utilization is great, no clinically useful compounds have been obtained yet. Nitroreductive activation is also of current interest, due to the recent possibility of introducing specific exogenous nitroreductases into tumours [28]. Hypoxia-targeted prodrugs using nitroaromatic moieties were initially developed from the nitroimidazole-based radiosensitizers Misonidazole **2.17** and etanidazole **2.18** (Fig. **2.9**). They acts as mimetics of oxygen, causing fixation of radiation-induced damage to DNA or other biologically relevant biomolecules, when used in combination with radiation therapy, thus increasing the radiation sensitivity of hypoxic cells and, consequently, enhancing radiotherapeutic effect *in vivo*. Unluckily, they do not possess significant cytotoxicity or marked hypoxia selectivity. Moreover, they provoke neurotoxicity. Extensive studies and important progresses were made in the development of this class, starting from these first structures.



2.17 **2.18**

Figure 2.9. Structure of the radiosensitizers Misonidazole (**2.17**) and etanidazole (**2.18**).

Nitroreductase enzymes catalyze the reduction of aromatic nitro groups and usually the major metabolic products of nitroreductase-catalyzed reductions are the four-electron product hydroxylamine and the six-electron product amine, both of them passing by the first electron adduct of nitro group, the nitro radical anion. This first intermediate can be oxidized back by O_2 to the parent nitro group, so limiting the activation reaction to hypoxic areas, where the nitro radical anion is further reduced stepwise by irreversible reactions to hydroxylamine or amine (Fig. **2.10**). The reduction processes catalyzed by nitroreductases are particularly facilitated in cells characterized by total or partial absence of oxygen because they are rich in reduced cofactors.

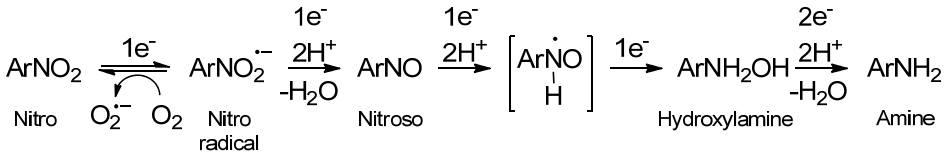


Figure 2.10. Reduction reactions of nitroaromatics and back-oxidation of nitro radical anion by oxygen.

The electronic reduction represents a very significant electronic density change in the structure of a nitroaromatic compound, thus it can be exploited as an efficient "electronic switch" to release or generate the latent active drug [29]. Nitroaromatics have been extensively used as pro-moieties to release a large number of anticancer agents and various linking groups have been employed to attach the cytotoxic portion to the masking nitroaromatic group. However, the most investigated mechanism of activation and release of drug involves the bioreduction of the nitro group of a nitrobenzyl portion to a hydroxylamine intermediate, followed by double bond

rearrangements and consequent spontaneous 1,6-elimination to release the cytotoxic drug (Fig. **2.11**).

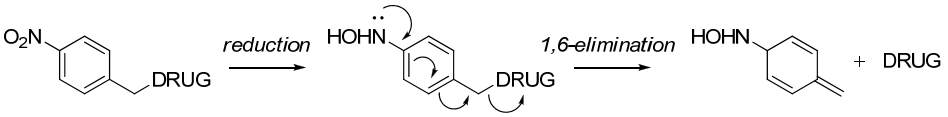


Figure 2.11. Activation and release of active drug from nitro-benzyl prodrug triggered by bioreduction and 1,6-elimination.

2.2.2. Pro-BAPNs as hypoxia-activated LOX inhibitors [30]

During my PhD project, in order to selectively deliver the reference LOX inhibitor BAPN to hypoxic tumours and block LOX-induced metastasis, we used the well-known hypoxia-sensitive nitroaromatic group to mask the pharmacophoric primary amino-group of BAPN. We developed a series of inactive nitroaryl pro-drugs of BAPN, that could be selectively activated to BAPN itself by bioreductive processes in the hypoxic environment of invasive tumours. Under hypoxic conditions, the masking nitro group is readily reduced to hydroxylamine or amine. Consequently, this transformation weakens the bond between the masking moiety and BAPN portion, promoting the spontaneous removal of the masking group and allowing the release of active BAPN selectively in hypoxic tumour cells (Fig. **2.12**).

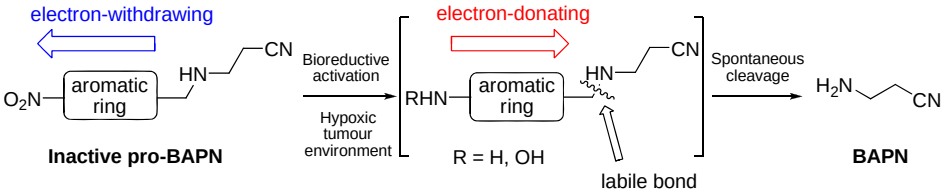


Figure 2.12. Activation mechanism of pro-BAPNs.

Exploiting this strategy, we designed and synthesized new pro-LOX inhibitors, which are nitrobenzyl- (**2.19** and **2.20**), nitrobenzylcarbonyl- (**2.21**) [31] and nitrofuranmethyl- (**2.22**) [32] derivatives of BAPN (Fig. **2.13**). In these preliminary series of prodrugs, the nitrogen of the pharmacophoric primary amino group of BAPN

was linked to nitroaromatic moieties by a methyl (**2.19**, **2.20** and **2.22**) or a methoxycarbonyl (**2.21**) unit.

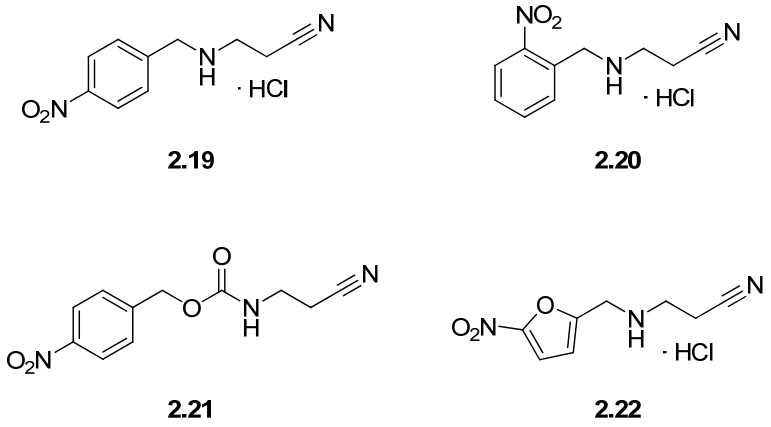
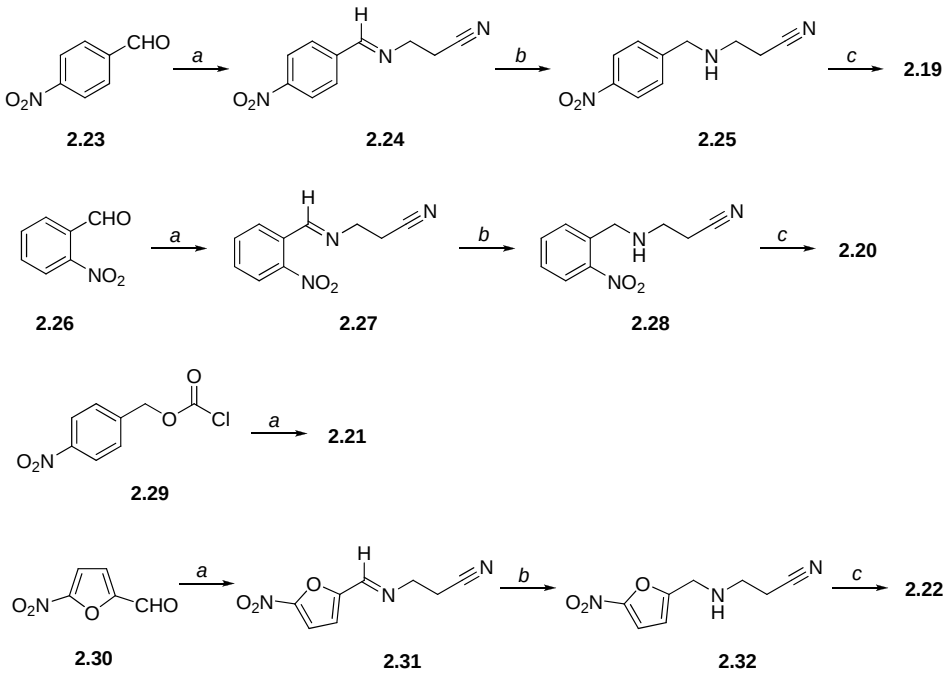


Figure 2.13. Structures of pro-BAPNs (**2.19**-**2.22**).

Synthesis of compounds **2.19**-**2.22** was accomplished as illustrated in Scheme **2.1**. Compounds **2.19**, **2.20** and **2.22** were prepared starting from the correspondent commercially available aldehyde precursors (**2.23**, **2.26** and **2.30**), whose condensation with an excess of β-aminopropionitrile in anhydrous dichloromethane afforded imine derivatives **2.24**, **2.27** and **2.31**, respectively. Then, reduction of the imine double bond with sodium borohydride at 0 °C afforded compounds **2.25**, **2.28** and **2.32**, and final treatment with $Et_2O{\cdot}HCl$ converted them into their corresponding water-soluble hydrochloride salts **2.19**, **2.20** and **2.22** [33]. Carbamate derivative **2.21** was simply obtained by a single step involving *p*-nitrobenzylchloroformate **2.29** and BAPN in DCM. Nitrofurane analogue **2.22** required a very slow addition of $NaBH_4$ to the intermediate **2.31** and strict control of the temperature to avoid the possible undesired reduction of the highly electron-deficient furan ring.

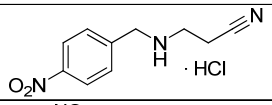
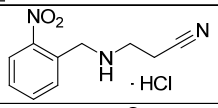
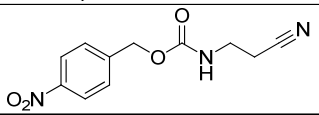
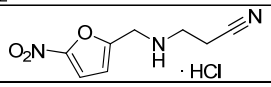


Scheme 2.1. a) β-aminopropionitrile, dry DCM, RT; b) $NaBH_4$, dry MeOH, 0 °C.; c) $Et_2O{\cdot}HCl$, dry MeOH, RT.

It has often been observed a direct association between the ease of activation of bioreductive prodrugs to their electrochemical reduction potential values [34, 35]: usually, a higher reductive potential value corresponds to a ready activation and, consequently, to a greater cytotoxicity in hypoxic cells. For this series of pro-BAPNs, cyclic voltammograms were recorded in iso-osmotic saline phosphate buffer solutions at pH 7.2, so measurements of their reduction potentials were obtained in order to evaluate their predicted ability to be reduced in the hypoxic environment (Table **2.1** and Fig. **2.14**). All the derivatives produced a single reduction peak when the potential was scanned from 0.0 to -1.8 V, which is the result of the reduction of their nitro group to the hydroxylamine product. Moreover, the voltammetry graph shows the irreversibility of the reductive process, missing a correspondent oxidation peak in the lower part of the curve. This indicates the irreversible transformation of the molecules (loss of the BAPN portion) following the NO_2/NHOH conversion. Among these compounds, analogues

2.19-2.21 displayed similar reduction potentials, with E° values ranging from -0.75 V of the two *para*-nitro derivatives **2.19** and **2.21** to -0.77 V for the *ortho*-nitro analogue **2.20**; while it is evident that nitrofuranmethyl-derivative **2.22** showed the less negative reduction potential (E° = -0.61 V), as shown by the blue line in the voltammogram. This means that **2.22** is much more inclined to reduction and, as a consequence, it should be more readily activated in hypoxic tumours releasing BAPN.

Table 2.1. Reduction potential values of compounds **2.19-2.22**. Measurements were recorded, using $5 \cdot 10^{-4}$ M solutions, at glassy carbon electrode in phosphate buffer (pH 7.2, 0.15 M NaCl), with a sweep rate of 0.1 V s^{-1}.

Compound	Structure	Reduction Potential (E°)
2.19		–0.75 V
2.20		–0.77 V
2.21		–0.75 V
2.22		–0.61 V

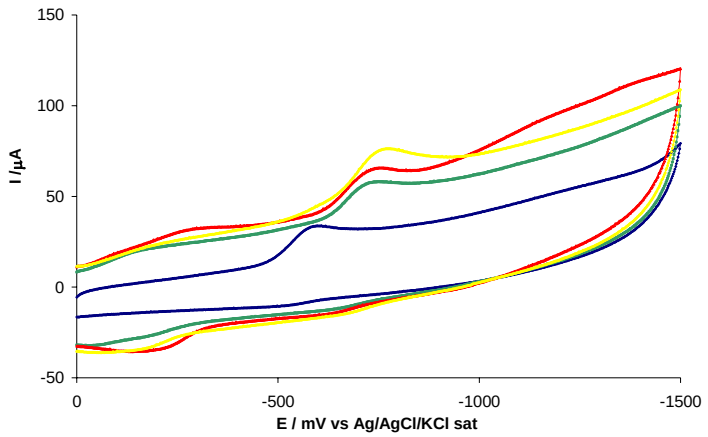


Figure 2.14. Cyclic voltammograms of compounds **2.19-2.22**. Measurements were recorded, using $5 \cdot 10^{-4}$ M solutions, at glassy carbon electrode in phosphate buffer (pH 7.2, 0.15 M NaCl), with a sweep rate of 0.1 V s^{-1}. Compound **2.22** in blue; **2.21** in red; **2.20** in yellow; **2.19** in green, in the graph.

2.3. BIOLOGICAL EVALUATION OF LOX INHIBITORS[30]

Compounds **2.19-2.22**, synthesized as pro-BAPNs molecules, were biologically assayed by the research group of Prof. Amato J. Giaccia at the University of Stanford (California, USA). Initially, their ability to inhibit LOX enzyme was tested, then they were submitted to *in vitro* invasion assays to measure their anti-invasive ability in tumour cells.

The inhibition potency of this series of inhibitors was assayed by using a conditioned medium (CM) from the MDA-MB-231 breast tumour cell line, which was incubated with the reference inhibitor BAPN and with 200 μM LOX inhibitors **2.19-2.22**, both in hypoxic and normoxic conditions (Fig. **2.15**). The LOX inhibition is related to the fluorescence development by a method previously described [36, 37]. This method consists in the reduction of the LOX by-product hydrogen peroxide by *N*-acetyl-3,7-dihydroxyphenoxazine (Amplex Red), catalyzed by the enzyme horseradish peroxidase, and this redox reaction produces as product fluorescent resorufin. This fluorometric measurement of hydrogen peroxide has been extensively used for measuring LOX activity, correlating a diminished fluorescence with a great LOX inhibition. It is clear from the bar graph that compounds **2.19**, **2.21** and **2.22** are much more active under hypoxia than in normoxia, consequently confirming their reduction and activation in conditions of low oxygen concentrations. On the other hand, the reference inhibitor BAPN does not show any significant difference in its activity between air and hypoxia, since it does not need any activation. In particular, compounds **2.21** and **2.22** provided the best results, thanks to their much greater inhibitory activities under hypoxic conditions, whereas their activities in the presence of air are almost the same of control and DMSO, thus showing a very selective behaviour.

ortho-Nitrobenzyl derivative **2.20** showed a very high fluorescence, if compared to the other derivatives and to control. This unpredictable result is likely generated by an interference with the fluorescence-based assay, possibly due to its high photo-instability, so, currently, optimization of the assay conditions for this specific derivative is in progress.

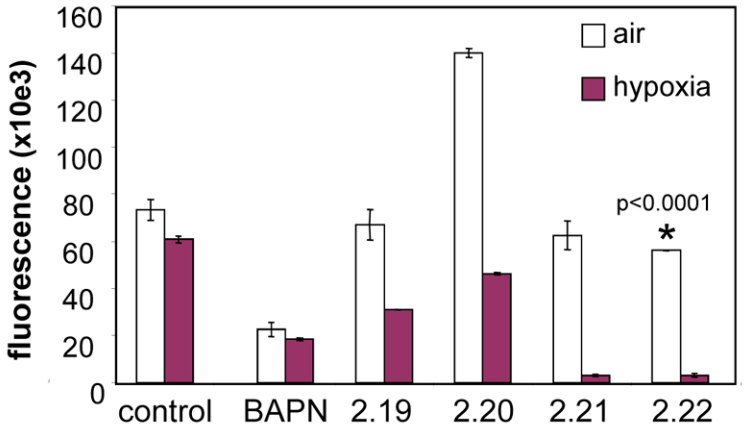


Figure 2.15. LOX inhibition bar graph: fluorescence assay for LOX activity on CM from MDA-MB-231 cells incubated in air (white bars) or hypoxia (2% O_2, purple bars) with 200 μM LOX inhibitors **2.19**-**2.22**.

Subsequently, the anti-invasion abilities of this series of compounds were determined. Cell invasion assays were performed *in vitro* on MDA-MB-231 breast cancer cells, following a method that has already been described [38]. Briefly, tumour cells were serum deprived and after 24 h seeded both on Matrigel-coated and uncoated inserts, then they were moved to chambers containing FBS as a chemo-attractant and incubated under normoxic or hypoxic conditions. The incubation with control or with the compounds was started 24 h before serum deprivation and continued for all the duration of the experiment. This tumour cell line is highly invasive and become even more aggressive when it is placed under oxygen deprivation, as shown by the control data in the graph (Fig. **2.16**), where the percentage of invasion under hypoxia is about two-fold higher than that in air. It is interesting to note that, even in this case, the most promising compounds are **2.21** and **2.22**. In fact, they possess not only enhanced activities in hypoxic conditions, but they also provoke a six-fold reduction of cell invasion under hypoxia, if compared to control experiments. In particular, the nitrofuran derivative **2.22** manifests the highest hypoxia-selectivity, since it maintains the same invasion level as control in normoxic conditions (30%), but it markedly reduces the level under air deprivation. The non-selective behaviour of the carbamate inhibitor **2.21** suggests that it may produce anti-invasive effects by means of a non-LOX-mediated

mechanism, showing an anti-invasive action even under normoxic conditions, although further studies need to be done to explain this phenomenon.

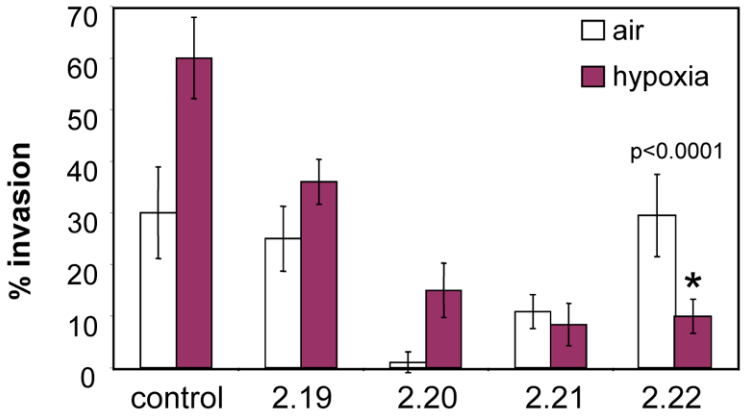


Figure 2.16. Cell invasion assay bar graph: transwell invasion assay to test ability of the synthesized compounds to inhibit *in vitro* invasion of MDA-MB-231 cells incubated in air (white bars) or hypoxia (2% O_2, purple bars) with 200 μM LOX inhibitors **2.19**-**2.22**.

Observing the data form both LOX inhibition and anti-invasion assays, we can conclude that pro-drug **2.22** proved to be the most active and hypoxia-selective compound and this result nicely correlates with its higher reduction potential (E° = –0.61 V) when compared to the other active members of the series of pro-BAPNs (**2.19** and **2.20**, E° = –0.75 V). Indeed, its least negative potential is associated with an easier bioreduction and activation of the nitrofuran masking portion, if compared to the other nitroaryl derivative **2.19**-**2.21**, and it is evident that this mechanism leads to more pronounced effects in hypoxia conditions in the performed biological assays.

2.4. CONCLUSIONS

To the best of our knowledge, the use of nitroaromatic pro-drugs to selectively inhibit the recently discovered target LOX is a completely new method to reduce or block the invasiveness of hypoxic tumours. Hypoxia-activated LOX inhibitors represent a new and effective therapeutic approach against metastatic tumours and it could be useful not only for the prevention of future metastasis, but also for the treatment of established metastatic conditions.

Pro-drug **2.22** showed a notable LOX inhibition activity and a marked anti-invasion ability in hypoxic conditions by selectively delivering LOX inhibitor BAPN to tumour tissues. Although the compound database is too small to demonstrate a definitive correlation, the hypoxia-enhanced activities of these prodrugs of BAPN appear to be strictly related to their electrochemical potential value, suggesting that the promising biological activities found with **2.22** are associated to the easier activation of its nitrofuran masking portion in hypoxic conditions, when compared to that of the other derivatives.

The direct involvement of the masking portions in the biological activity of the nitrofuranmethyl and *p*-nitrobenzyl derivatives **2.22** and **2.21**, which proved to be the most active compounds, are not to be excluded. In fact, the nitroaromatic groups, after detaching from BAPN under hypoxic conditions, might interact with LOX in a synergistic fashion with the active BAPN, even if attempts to demonstrate the involvement of chemical species derived from the masking portions have so far failed.

This research line can be further developed and expanded by using different masking moieties to be linked to BAPN or even by introducing completely new LOX inhibitors in the place of BAPN itself. This last goal could be achieved, for example, with derivatives able to bind LTQ cofactor, such as primary amino-derivatives, or even with compounds containing chelating groups, which can coordinate to the copper ion present in the LOX active site. Moreover, since LOX is an extracellular target, it can be accessible even to drugs that do not efficiently cross the cell membranes and this constitutes a further advantage, because higher concentrations of drugs in the tumour stroma are more likely to be reached than inside the cancer cell.

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CHAPTER 3‡

"Inhibitors of lactate dehydrogenase A"

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(a) Granchi, C.; Bertini, S.; Macchia, M.; Minutolo, F. Inhibitors of lactate dehydrogenase isoforms and their therapeutic potentials. *Curr. Med. Chem.*, **2010**, *17*, 672-697;
(b) Granchi, C.; Roy, S.; Giacomelli, C.; Macchia, M.; Tuccinardi, T.; Martinelli, A.; Lanza, M.; Betti, L.; Giannaccini, G.; Lucacchini, A.; Funel, N.; León, L.G.; Giovannetti, E.; Peters, G.J.; Palchaudhuri, R.; Calvaresi, E.C.; Hergenrother, P.J.; Minutolo, F. Discovery of *N*-hydroxyindole-based inhibitors of human lactate dehydrogenase isoform A (LDH-A) as starvation agents against cancer cells. *J. Med. Chem.*, **2011** (accepted for publication on Jan. 6, 2011) [jm-2010-01007q];

or included in patent applications:
(c) Minutolo, F.; Macchia, M.; Granchi, C.; Giannaccini, G.; Lucacchini, A. Composto inibitore dell'enzima lattato deidrogenasi (LDH) e composizione farmaceutica che comprende tale composto. IT/ PI2009A000140;
(d) Minutolo, F.; Macchia, M.; Granchi, C.; Roy, S.; Giannaccini, G.; Lucacchini, A. Compounds inhibitors of enzyme lactate dehydrogenase (LDH) and pharmaceutical compositions containing these compounds. PCT/EP2010/006740.

3.1. GLYCOLYTIC ENZYMES: POTENTIAL TARGETS AGAINST HYPOXIC TUMOURS

The peculiar tumour metabolism of hypoxic solid tumours has inspired the search for new therapeutic approaches able to selectively interfere with the Warburg effect. LDH inhibition is likely one of the most innovative way of attacking cancer of the past few years, but other drug candidates targeting glycolysis at different stages contribute to further demonstrate the central role of this metabolic pathway and the potential therapeutic selectivity of this kind of approach. In particular, glycolysis, the cytoplasmatic catabolic process responsible for ATP production in hypoxic cancers, starts with the internalization of glucose by means of specific transporters overexpressed in hypoxic tumours and comprises ten reactions, each catalyzed by specific enzymes, thus offering several possibilities for blocking the energy production in tumour cells (Fig. **3.1**) [1].

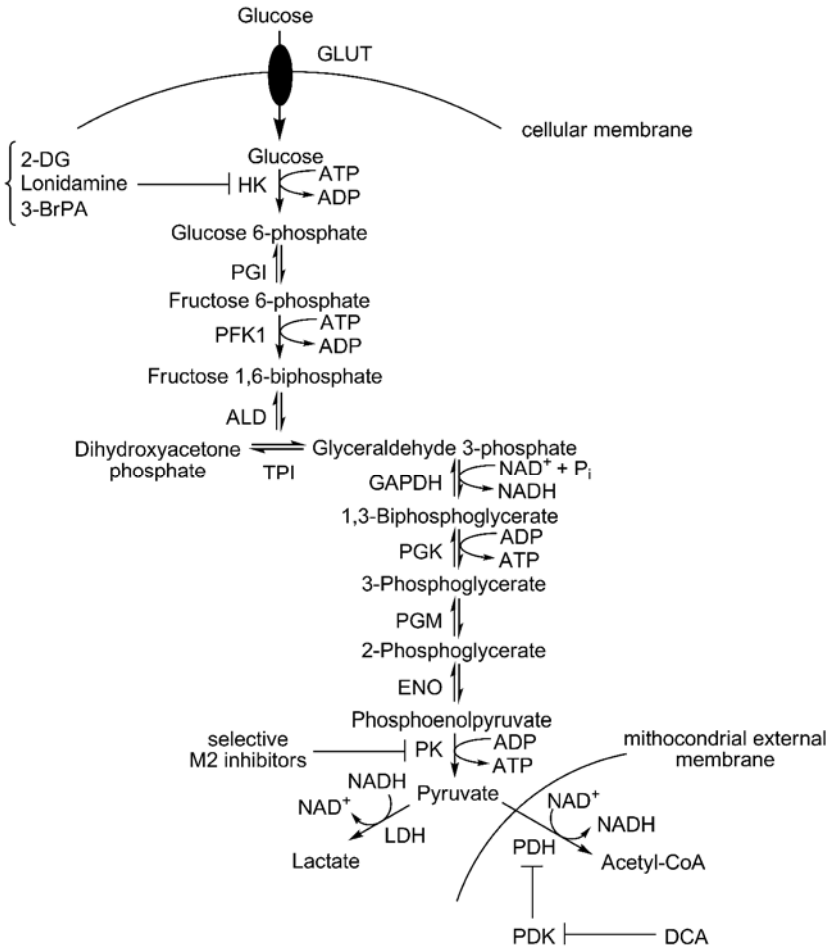
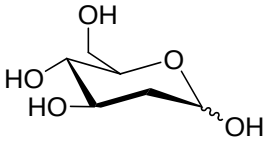


Figure 3.1. The glycolytic pathway: all the enzymes participating to glycolysis are indicated. Some of the most important glycolytic enzyme inhibitors are reported. HK = hexokinase, PGI = glucose 6-phosphate isomerase, PFK = phosphofructokinase, ALD = aldolase, TPI = triosephosphate isomerase, GAPDH = glyceraldehyde 3-phosphate dehydrogenase, PGK = phosphoglycerate kinase, PGM = phosphoglycerate mutase, ENO = enolase, PK = pyruvate kinase, PDH = pyruvate dehydrogenase, PDK = pyruvate kinase.

Hexokinase (HK), which exists in four different isozymes (HK I, II, III and IV), represents the first enzyme of glycolysis, catalyzing the irreversible phosphorylation of glucose to glucose-6-phosphate. This reaction constitutes a very important control site of glycolysis, transforming glucose into an anionic molecule, so trapping it into the cell, where it is ready to be utilized.

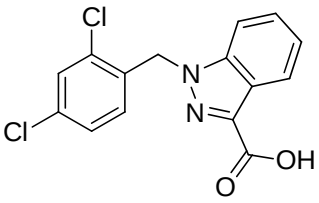
The glucose analogue 2-deoxyglucose (2-DG) (**3.1**, Fig. **3.2**) is one of the most important HK inhibitor, developed by Threshold Pharmaceuticals and now in phase I clinical trial [2, 3]. Phosphorylation of 2-DG by the enzyme HK leads to the accumulation of this molecule within the cell without being metabolized further, so blocking the next steps of glycolysis. However, the not entirely satisfactory action of this agent on hypoxic tumours and its toxic effects when used as a primary therapy limit the use of this molecule [4].



3.1

Figure 3.2. Structure of 2-deoxyglucose (**3.1**).

Lonidamine (**3.2**, Fig. **3.3**), a derivative of indazole-3-carboxylic acid developed by Threshold Pharmaceuticals, is an orally administered anticancer and antispermatogenic drug. Its mechanism of action still needs to be completely understood, but the interference in the glycolytic pathway by HK inhibition is well established [5]. Now it has completed a phase 3 trial; however, pancreatic and hepatic toxicity have been detected [6].

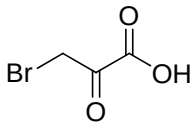


3.2

Figure 3.3. Structure of Lonidamine (**3.2**).

3-Bromopyruvate (3-BrPA), another well-known HK inhibitor, is a synthetic derivative of pyruvic acid (**3.3**, Fig. **3.4**). The supposed mechanism of inhibition is the

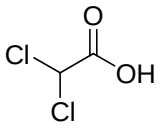
selective alkylation of sulfhydryl groups necessary for enzymatic function but not involved in glucose or ATP binding [7]. Preclincal studies on this compound showed promising results, but no clinical trials are yet available, probably due to its unspecific mode of action and also the low economical potential of this non-patentable structure [8].



3.3

Figure 3.4. Structure of 3-bromopyruvate (**3.3**).

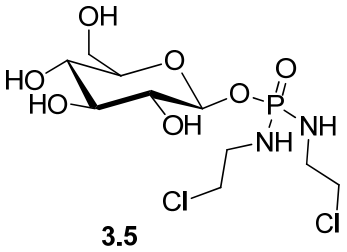
Pyruvate dehydrogenase (PDH) possesses a central role in glucose metabolism, favouring the switch from glycolysis to oxidative phosphorylation (OXPHOS), that could be beneficial to hypoxic tumour cells. This enzyme converts pyruvate to acetyl-CoA by pyruvate decarboxylation, so it directs the end product of glycolysis toward the Krebs cycle, promoting the restoration of normal OXPHOS to the detriment of aerobic glycolysis. PDH is inhibited by pyruvate dehydrogenase kinase (PDK) by means of a phosphorylation reaction requiring ATP as the phosphate source. Therefore, inhibition of PDK leads to reactivation of PDH and, indirectly, to the physiological demolition of pyruvate [9]. Dichloroacetate (**3.4**, Fig. **3.5**), a molecule already known for the treatment of lactic acidosis and inherited mitochondrial diseases, is a PDK inhibitor under phase I clinical study thanks to its marked anticancer action. Some side effects such as peripheral nerve toxicity, anxiolytic and sedative effects have been reported and recent evidences outline the fact that PDK-knockout does not block proliferation of hypoxic tumour cells [10]. Besides, being a non-patentable compound, DCA has received a limited interest by pharmaceuticals companies.



3.4

Figure 3.5. Structure of dichloroacetate (**3.4**).

The increased glucose uptake detected in hypoxic tumours was exploited by prodrugs like glufosfamide (**3.5**, Fig. **3.6**), composed by an alkylating moiety conjugated to β-D-glucose [11]. This prodrug, developed by Threshold Pharmaceuticals, which has already been evaluated in clinical trials on several kind of cancers [12], takes advantage of the glucose transporters overexpressed in tumours to be internalized within the cells and then the linkage between the sugar moiety and the cytotoxic portion is cleaved *in vivo*, thus releasing the active drug that is the alkylating phosphoramide mustard.

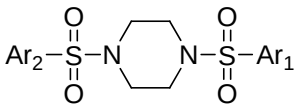


3.5

Figure 3.6. Structure of glufosfamide (**3.5**).

Pyruvate kinase (PK) is the last glycolytic enzyme catalyzing the irreversible transformation of phosphoenolpyruvate to pyruvate, producing ATP. PK is under complex control, allowing the cell to sense the levels of anabolic precursors as well as its energy status. It is noteworthy that the human fetal isoform of pyruvate kinase (PK-M2), a phosphotyrosine-binding protein, is overexpressed in hypoxic tumours. It switches from a tetrameric form with high affinity to its substrate phosphoenolpyruvate to a low affinity form and the balance between these two conformational states contributes to the control of glycolysis. Indeed, PK-M2 proved to be necessary for the

tumourigenicity, by promoting Warburg effect, even if its inhibition may lead also to controversial effects on cancer growth [13, 14]. For this reason, the search for PK-M2-selective inhibitors, either peptidic or small organic molecules, for disrupting tumour glycolysis [15, 16] is joined by the recent development of *N,N'*-diarylsulfonamides as PK-M2 activators (**3.6**, Fig. **3.7**), although these opposite effects on tumour cells still need to be verified [17].



3.6

Figure 3.7. General structure of *N,N'*-diarylsulfonamide derivatives (**3.6**).

3.2.THE ENZYME LACTATE DEHYDROGENASE (LDH)

3.2.1. Introduction

Although the roles of lactate dehydrogenases (LDHs) in biology have been well-established for many years, this enzyme is not commonly considered as a potential therapeutic target. However, its inhibition might be desirable in many pathologies, so different as cancer and malaria, where the modulation of the glucose metabolism may lead to clinically relevant effects.

Lactate dehydrogenases (LDHs) are a family of 2-hydroxyacid oxidoreductases that are found in almost all animal tissues, in microorganisms, in yeasts and in plants. They catalyze the reversible transformation of pyruvate to lactate with the simultaneous interconversion of NADH to NAD^+. LDH is one of the best characterized enzymes in the scientific literature because it is known since the early period of enzymology, with a well-studied kinetic mechanism and many crystal structures solved. As a matter of fact, LDH structures have been reported for dogfish [18], pig [19], *Bacillus steareothermophilus* [20], *Bifidobacterium longum* [21], *Plasmodium Falciparum* [22] and more recently also for the human enzymes M and H (isoforms typical of muscle and heart, respectively) [23].

The great attention directed to LDH is due to the central role that this enzyme plays in several metabolic pathways. It exerts its action principally during energy production in anaerobic conditions, that occur when there is a sudden demand for energy and, at the same time, oxygen is absent or in short supply, like in working human skeletal muscle. In this situation LDH converts pyruvate, that is the end product of the glycolytic process, to lactate. As a consequence, LDH permits to overcome a momentary oxygen debt in the form of accumulated lactate to be later discharged by the reoxidation of lactate to pyruvate when oxygen becomes available. In fact, under aerobic conditions, pyruvate can be further oxidized to CO_2 and H_2O in mitochondria through the Krebs (citric acid) cycle, generating ATP and NAD^+. However, when the Krebs cycle cannot start because of the lack of oxygen, the only source of ATP is glycolysis, so NAD^+ needs to be regenerated, being an electron acceptor essential for glycolysis to continue (Fig. **3.8**). The reduction of pyruvate to lactate, known as anaerobic homolactic

fermentation, represents an important mean of regenerating NAD^+, thus enabling the achievement of energy even in anaerobic conditions. The product of this reaction is the L-isomer of lactate in most of the cases, but certain lower animals and some microorganisms catalyze the formation of D-lactate isomer from pyruvate [24].

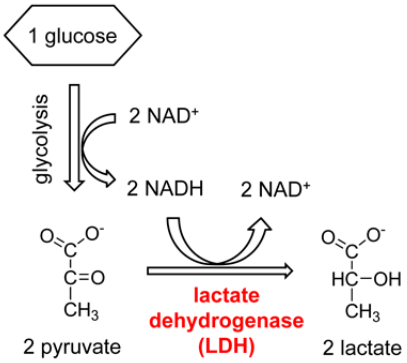


Figure 3.8. Reaction catalyzed by LDH and regeneration of the cofactor NAD^+, needed for the glycolytic process.

LDH performs also the reverse reaction in gluconeogenesis during the Cori cycle, a metabolic pathway that takes place mainly in the liver, in order to produce glucose, and subsequently ATP, from non saccharidic precursors during intense muscle activity, when the level of blood glucose decreases. In this physiological situation, lactate produced in anaerobic conditions is converted to pyruvate in the cytosol by LDH and subsequently internalized in mitochondria, where it undergoes two consecutive metabolic transformations in order to be used in the gluconeogenetic pathway. In this way gluconeogenesis also prevents lactic acidosis, that is the production of significant amounts of lactate during muscle activity by LDH, which causes muscle pain and cramps because of ionization of lactic acid in the blood [25].

The catalytic activity of LDH was first discovered in cell-free muscle extracts [26] and the enzyme was purified in a crystalline form for the first time by Straub in 1940 [27]. The accepted catalytic mechanism of LDH is rather simple and starts with the initial binding of NADH to the enzyme followed by binding of the pyruvate. Then, the LDH-NADH-pyruvate ternary complex undergoes a rate-limiting conformational

change, in which a substrate specificity loop closes to form a desolvated ternary complex, principally in order to bring the catalytical residue Arg109 into the active site where it polarizes the ketone functionality of pyruvate, thus promoting hydride and proton transfer to the substrate. Apart from the catalytic residues Arg109, Asp168, and His195, which are highly conserved in all LDHs, other amino acids are also involved in substrate discrimination and recognition, such as Gln102, Arg171, and Thr246, together with Arg109. For example, Gln102, Arg109 and Thr246 are implicated in pyruvate recognition by enclosing the methyl side chain of the native substrate, which is in fact oriented towards these residues (Fig. **3.9**). The catalytic mechanism involves a direct and stereospecific transfer of a hydride ion (H^-) from the C_4 carbon of the dihydronicotinamide ring of NADH to the ketone group of the pyruvate, in conjunction with proton donation (H^+) to the carbonyl oxygen of pyruvate from the catalytic diad Asp168/His195, finally producing lactate. The imidazole ring of His195 has the function of proton donor/acceptor in this reaction and it also orients the substrate in the proper position for its interaction with the C_4 of NADH. The other aminoacid of the catalytic diad, Asp168, interacts with His195 by stabilizing the protonated/cationic form of its imidazole ring through an H-bond between the side chain carboxylic group of Asp168 and imidazole ring of His195. The function of the last catalytic residue Arg171 is to fix the substrate through a strong two-point interaction between its side chain and the carboxylate of pyruvate. It is important to note that Ile250 with its hydrophobic side chain provides an environment suitable for the nicotinamide ring of NADH.

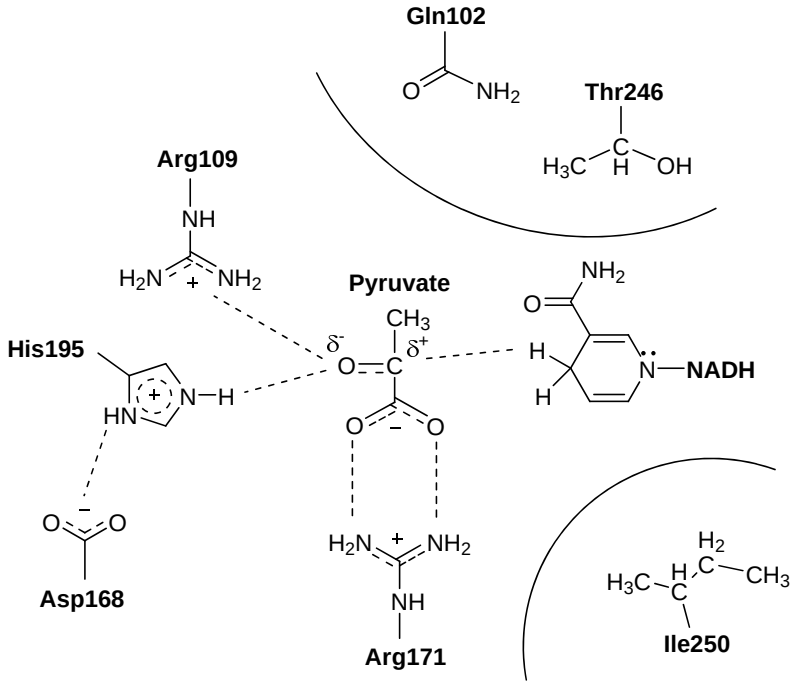


Figure 3.9. Schematic representation of the LDH catalysis mechanism. LDH active site bound to both substrate pyruvate and cofactor NADH; residues directly involved in catalytic interactions and substrate/cofactor recognition are reported.

The particular interest for this enzyme also rises from its importance as a diagnostic marker. A sudden increase in LDH activity in serum is associated with acute diseases, which cause a rapid release of this enzyme into the blood flow. In particular, the highest levels of LDH were found in patients with megaloblastic anaemia and malignancy, but the determination of total serum LDH is also widely used for the diagnosis of myocardial infarct, liver, hematological and skeletal muscle diseases and other secondary diseases. The pathological presence of LDH into the circulation could be explained by considering that LDH release from cells is caused by a defect in the plasma membrane; hence, LDH is considered as a hallmark of general cell damage for several tissues and it is often exploited as a diagnostic tool in human diseases [28].

LDH exists in several isozymic forms, for example in mammals the association of two principal types of subunits, which are genetically distinct, give rises to five

tetrameric isozymes. The two homotetramers and three heterotetramers are also present in all vertebrates with a characteristic tissue-specific expression relatively similar to the human case. Each species diverges in their physical-chemical properties, biological functions and developmental regulations [29].

Recent discoveries have made LDH a valid target for very different diseases, such as malaria and cancer. Isoforms expressed by the *Plasmodium* parasites, in particular *Plasmodium* LDHs (*p*LDHs) from *Plasmodium falciparum* (*pf*LDH), *vivax* (*pv*LDH), *ovale* (*po*LDH) and *malariae* (*pm*LDH), display high levels of similarity among them. The development of inhibitors of the parasite LDH isoforms has reached a potentially great therapeutic value. In fact, it is well established that lactate dehydrogenase from *Plasmodium falciparum* (*pf*LDH) is a key enzyme for the survival of the malarial parasite and, as a consequence, several small organic molecules have been designed and synthesized with the aim of attacking this antimalarial target [30-34].

On the other hand, the human isoform LDH-M, also referred to as LDH-A, is a relatively innovative anticancer target. The idea that the inhibition of this isoform could be useful to reduce tumour growth and invasiveness is very recent and nowadays LDH-A has been blocked only by genetic techniques [35], although many *pf*LDH inhibitors are not very selective and act also on human isoforms, including LDH-A.

3.2.2. *h*LDH isoforms

Human LDHs (*h*LDHs) are a family of tetrameric isozymes [36], that is to say, enzymes coded by different genes but translated into proteins with a high degree of similarity in sequence and activities. Each isozyme shows distinct kinetic parameters or regulatory properties. In mammals, each tetramer is composed by two different kinds of subunits, the M- and the H-type. As a consequence, there are five possible combinations of these polypeptide chains, corresponding to five forms of LDH. This composition gives rise to two homotetramers LDH-1 (H_4) and LDH-5 (M_4), which are also simply called LDH-H (or LDH-B) and LDH-M (or LDH-A), respectively, and three hybrid tetramers, LDH-2 (M_1H_3), LDH-3 (M_2H_2) and LDH-4 (M_3H_1) (Fig. **3.10**).

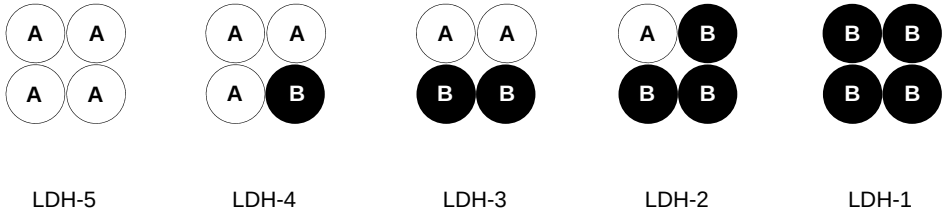


Figure 3.10. The two homotetramers (LDH-1 and LDH-5) and the three heterotetramers (LDH-2, LDH-3 and LDH-4) are schematically represented. A (or M) and B (or H) are the kinds of the subunits.

The numbers were assigned according to the different electrophoretic mobility of the isozymes. For example LDH-5 shows the lowest mobility, whereas LDH-1 is the fastest moving enzyme in this family. Each subunit is coded by a different gene, that are *ldh-a* and *ldh-b* for M and H subunit, respectively. Therefore, the five isozymes are also named LDH-B_4 or simply LDH-B (H_4), LDH-A_1B_3 (M_1H_3), LDH-A_2B_2 (M_2H_2), LDH-A_3B_1 (M_3H_1) and LDH-A_4 or LDH-A (M_4) (Fig. **3.10**). A third less common isoform, LDH-X or LDH-C_4, has been identified and corresponds to human *ldh-c* gene [37-39]. It should be noted that, although human LDH-C isoform seems to play a key role in male fertility, no evidences that this enzyme may be targeted by male contraceptive agents have been reported as of yet.

Each subunit has a molecular weight of about 35 kDa in humans, differing in aminoacid compositions, but possessing an high level of homology not only among different types of human subunits but also among subunits of the same type in different species. The names of the two types of subunits LDH-H and -M derive from the tissues in which they are mainly expressed. In fact in humans there is a specific distribution pattern: LDH-5 (M_4) and also LDH-4 (M_3H_1) are found especially in anaerobic tissues such as skeletal muscle (M stands for muscle), liver and neoplastic tissues; LDH-1 (H_4) and also LDH-2 (M_1H_3) strongly predominate in tissues with a principal aerobic metabolism such as cardiac muscle (H stands for heart), spleen, kidney, brain and erythrocytes. The intermediate LDH, isoform LDH-3, which possesses two subunits of each type, is mainly present in lymphatic tissues, platelets and many malignant tissues. LDH-X (C_4), the less known isoform of human LDHs and the most specialized of them all, was found only in testes and sperm, so its gene appears totally inactive in females.

Thus, the relative abundance of the five isoforms in each cell is completely dependent upon the numbers of subunits of each type available to combine. At the same time, the numbers of available subunits reflect the relative levels of activation of the corresponding genes.

As for the intracellular distribution of the isozymes, isoform LDH-X has a wide cellular distribution in sperm; in fact, it has been found in the cytosol, in the mitochondrial matrix between the inner and the outer mitochondrial membranes and in the plasma membrane. In contrast, the other five isozymes are mainly localized in the cytosol of somatic cells, but they have also been observed in mitochondria. Recently, it has been hypothesized that LDH has a fundamental role in an "intracellular lactate shuttle", a model that seems to be widely used in mammalian muscle and liver. In the intracellular lactate shuttle mechanism, cytosolic lactate from glycolysis is transported into the mitochondrial intermembrane space, oxidized to pyruvate by mitochondrial LDH and finally pyruvate is oxidized via the Krebs cycle that takes place exactly in mitochondria [40]. LDH isozyme distribution displays not only tissue specificity, but also differs between mitochondria and the surrounding cytosol in each tissue. For example, in the heart LDH-1 is mostly concentrated in the mitochondrial matrix, whereas LDH-5 in the skeletal muscle is equally present in cytosol and in mitochondria, but the same isoform in liver is more abundant in mitochondria.

Although all these isoforms are able to catalyze the transformation of pyruvate into lactate, when the number of H over M chains increases, isozymes become more efficient in catalyzing the reaction in the opposite direction, that is oxidation of lactate to pyruvate. In other words, the muscle-specific form LDH-5, and to a lower extent LDH-4, favour lactate formation from pyruvate, whereas the heart-specific forms, LDH-1 and LDH-2, promote the conversion of lactate to pyruvate, thus favouring its entrance into the Krebs cycle. This consideration is supported by the fact that LDH-1 shows a higher degree of affinity for lactate than LDH-5. In fact, LDH isoforms present in aerobic tissues (mainly LDH-1 and LDH-2) act as scavengers for lactate produced during glycolysis, either for resynthesis of glucose in the liver or for further production of pyruvate which is then degradated in the Krebs cycle in order to produce ATP [41]. This hypothesis follows the widely accepted theory by Kaplan and coworkers

concerning the differential physiological functions of the LDH isozymes, the so called "aerobic-anaerobic theory", which hypothesizes that LDH-1 is suited to aerobic metabolism, whereas LDH-5 is well-suited to tissues frequently exposed to oxygen limitations [24].

However, this theory does not take into due consideration some exceptions to the classical distribution pattern, such as human erythrocytes, which perform exclusively glycolysis, but synthesize predominantly the B subunit. Moreover, there are also differences in isoform distribution between organs of the same type in different species, which may not be easily rationalized by the Kaplan's theory [24].

3.2.3. Structural and kinetic features of *h*LDHs

LDH-M and LDH-H enzymes possess about 75% amino acid sequence identity, differing in 81 out of 331/332 amino acid positions. In particular, the residues that form the cofactor/substrate binding pockets are well conserved between the two isoforms and the differences generate only slight changes in the overall structures. The homotetramers have essentially the same tertiary structure, possessing identical folding of the main chain and the same α-helices and β-pleated sheets. Both proteins show high degrees of similarity with isoforms from other species such as those of pig and mouse. Moreover, the hybrid tetramers LDH-M_1H_3, LDH-M_2H_2 and LDH-M_3H_1 display macroscopic structures very similar to LDH-M_4 and H_4. Human tetramers are composed of four 330-residue subunits. Each monomer has two domains: *a)* a larger domain that assumes a typical "Rossmann fold" formed by residues 20-162 and 248-266; the classic "Rossmann fold" is a structural motif shared with many dinucleotide binding proteins, which was discovered by Rossmann et al. in 1975 [42] and then refined thanks to further studies by Bellamicina in 1996 [43]; *b)* a smaller accompanying domain, which is a "mixed α/β substrate binding" domain, comprising residues 163-247 and 267-331.

Despite the negligible structural diversity between LDH-M and -H, there are small but very significant dissimilarities in the kinetic properties of each form, that become even more pronounced when comparing them with LDH-X. The overall turnover rate is two-fold higher for LDH-M than -H, that is, LDH-M has higher V_{max}, whereas the LDH-H isoform shows about a three-fold increase in the ability to bind pyruvate compared

with the -M one, corresponding to a lower K_m value for pyruvate. Moreover, LDH-H is more sensitive to inhibition by high pyruvate concentrations. It has been supposed that the different kinetic properties probably arise from the way of substrate binding accompanied by the closure of the active-site loop. LDH-X has kinetic properties very different from the five isoforms deriving from the association of M and H subunits and possesses also a broader substrate specificity than the other isozymes [23].

3.3. PLASMODIUM LDH (*pf*LDH) AS AN ANTIMALARIAL TARGET

3.3.1. *pf*LDH role in the fight against malaria

Malaria is one of the major diseases affecting mankind, which is widely reemerging in many tropical and subtropical areas of the world, constituting a huge public health problem. It provokes more than 300 million new infections annually, resulting in at least one million deaths among which many children. The causative agents are protozoan parasites from the genus *Plasmodia*. In particular the most virulent *Plasmodium falciparum* is the main responsible for the infection in humans. Development of strains resistant against almost all the existing anti-malarial drugs, especially against chloroquine, is rapidly growing and also the spread of vectors resistant to insecticides is rising. Moreover, useful vaccines are not yet available. These facts cause an increasing need to find new drug candidates, which are effective towards the causative parasite *Plasmodium falciparum* and, in particular, which hit different targets from those considered by currently used anti-malarial therapies.

Plasmodial metabolic pathways that are either absent in humans, or at least very different, constitute good targets for selective anti-malarial therapies with a reduced risk of side effects. One of these approaches consists in exploiting the unusual dependence of *Plasmodium* on the glycolytic pathway, which is normally absent in human hosts. As a matter of fact, during its asexual erythrocytic cycle, that is, the portion of the parasite life cycle found in infected humans responsible for malarial symptoms, the parasite lacks a functional Krebs cycle. In this phase, its main source of ATP is the anaerobic

fermentation of glucose to lactate, consequently exhibiting levels of glucose consumption about 30-50-fold higher than that occurring in host human cells, producing high quantities of L-lactate [44, 45]. Hence, its lactate dehydrogenase isoform, (*pf*LDH) [46], which plays the essential role of regenerating NAD^+ needed for the continuity of the glycolytic cycle, is considered as the key enzyme in the parasite energy supply, constituting a potential drug target. Inhibition of *pf*LDH is expected to block ATP production and, consequently, to cause parasite death. However, due to the almost complete dependence for ATP production from glucose metabolism, many enzymes of the entire glycolytic pathway are expressed at high levels in the parasite compared with the host cells, and could be then considered as additional potential drug targets. This approach against the *Plasmodium* provides a potentially innovative strategy in the field of anti-malarial drugs and several authors have already considered *pf*LDH inhibition as a new valuable way of causing mortality of the parasite.

3.3.2. Unique features of *pf*LDH

One of the well-known difficulties in the search for an ideal drug candidate able to block energy production in the *Plasmodium* is the selectivity for *pf*LDH compared with human LDHs (*h*LDHs). Fortunately, *pf*LDH has several structural features that distinguish it from the human homologues, so that such peculiarities may be used to selectively block the parasite cell cycle without damaging the host. *pf*LDH is a 316 amino acid protein coded by a single gene on chromosome 13. Beyond the several features that the parasite and the human enzymes have in common, there are some significant differences between them. As regards the similarity, *pf*LDH has the same overall “Rossmann fold” topology observed in other mammalian LDHs. It also displays high similarity in the primary structure, and the essential catalytic residues Arg109, Asp168, Arg171 and His195, implicated in substrate and coenzyme binding, are well conserved [47]. However, overlapping the crystal structures of both *pf*LDH and *h*LDHs, two key differences were observed, involving both the cofactor and substrate sites. The positioning of the NADH cofactor is slightly different between these isoforms, because of changes in the amino acid sequence in the cofactor binding pocket. A superimposition of the two catalytic sites shows the displacement of the cofactor

nicotinamide ring by about 1.2 Å in *pf*LDH compared to human LDHs and other mammalian isoforms [22]. This shift can be explained by considering that human Ser163, a widely conserved residue among *h*LDH isoforms, is replaced by a leucine in *pf*LDH. Consequently, hydrogen bonding between the OH of serine and the amide group of nicotinamide is no longer possible in the parasite, and the more hydrophobic side chain of leucine in *pf*LDH is buried into the core of the protein, thus altering the conformation of this region.

It was also observed that a change occurs both in the sequence and in the secondary structure of a mobile loop region, called the substrate specificity loop, which closes down on the active site during catalysis, helping to define the catalytic site. This loop includes residues from 98 to 110 in human H and M isoforms and possesses a quite highly conserved sequence in all other known LDHs, with the exception of *pf*LDH, where not only is the sequence different, but there is also a five residue insertion immediately in front of the conserved catalytic residue Arg109, from residues 104 to 108 (^{104}DKEWN^{108}), which forms an extended specificity loop that bulges away from the protein surface, thus forming a distinctive cavity adjacent to the catalytic site possessing a cleft-shape (Fig. **3.11**). These changes result in a larger volume of the active site in *pf*LDH relative to its human counterparts, which may constitute a further advantage when designing selective inhibitors for the parasite isoform.

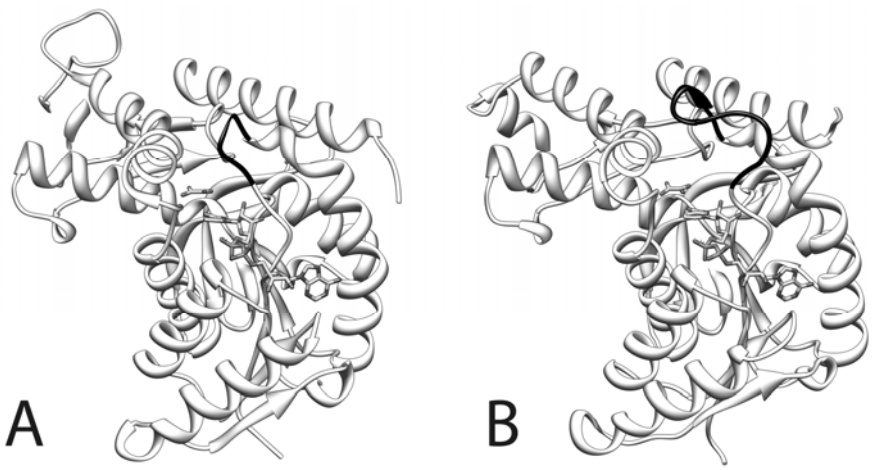


Figure 3.11. Comparison between LDH-A (**A**, pdb code 1I10) and *pf*LDH (**B**, pdb code 1LDG). The substrate specificity loop (residues 98-110) is highlighted in black in both structures. Oxamate and NADH are reported as reference structures.

Other differences are found in: *a)* the *N*-terminus, which turns out to be more extended in human LDH, and wraps around adjacent subunits; *b)* in the structure of the antigenic loop (residues 205-221). Furthermore, the most significant modifications of residues that are present in the binding site of all known LDHs, but are not conserved in *pf*LDH are: *1)* a neutral Gln102, which contributes to define the active site, is replaced by a positively charged lysine; *2)* Ile250, with its hydrophobic side chain stacking against the nicotinamide ring, is substituted by a proline; *3)* Thr246, adjacent to both the nicotinamide group and the substrate site, is replaced again by a proline; *4)* finally, an asparagine is present in the place of the conserved anionic Asp197 in the active site [22].

*pf*LDH possesses also unique kinetic properties when compared to other isoforms. For example, mammalian LDH is inhibited by the substrate pyruvate and by the tight complex formed by pyruvate and NAD^+, whereas *pf*LDH is only weakly sensible to inhibition by the substrate and the pyruvate-NAD^+ complex.

Cofactor selectivity also reflects the changes in the *pf*LDH active site. For example, it is well established that a variety of NAD^+ analogs with chemical modifications at the 3-position of the pyridine ring can be used as cofactors by LDHs, with no relevant

differences between *pf*LDH and human isoforms in discriminating these cofactors. Nevertheless, the NADH analogue APADH (3-acetylpyridine adenine dinucleotide) displays some relevant differences between the parasite and the mammalian isoforms. In fact, when $APAD^+$ replaces NAD^+, *pf*LDH increases its K_{cat} and its catalytic efficiency is several hundred fold higher. Since loop closure is the rate-limiting step, this loop movement for *pf*LDH is faster when $APAD^+$ is bound to the enzyme than when NAD^+ is present. On the other hand, $APAD^+$ proved to be a poor cofactor for the two human isoforms LDH-A and LDH-B. This may be due to the fact that the replacement of the carboxamide moiety of NADH with an acetyl group in APADH disrupts the hydrogen-bonding network normally occurring with Ser163 present in human LDHs (Fig. **3.12**). On the contrary, in the parasite this H bond is not present, and the remainder of the cofactor binding site adapts to favour binding of both NADH and APADH [48].

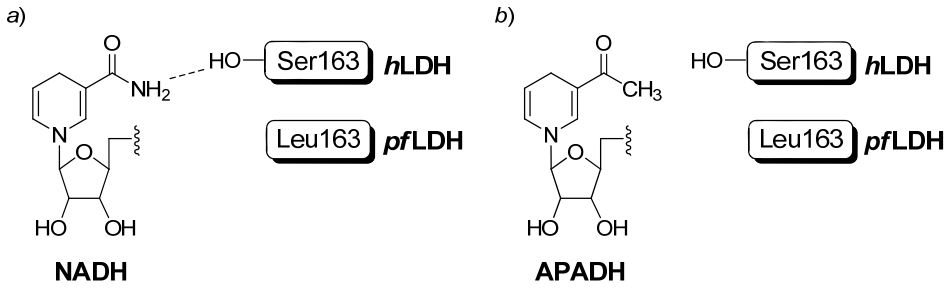


Figure 3.12. Differences in the interactions between human (*h*LDH) or plasmodial (*pf*LDH) LDH and the two cofactors NADH and APADH are schematically represented. *a*) the carboxamide group of NADH interacts only with *h*LDH through a hydrogen bond with the hydroxyl of Ser163; *b*) the methyl group of APADH is unable to establish this interaction.

These structural diversities, as well as the kinetic differences, observed between the human and malarial LDH constitute the basis for a possible development of selective *pf*LDH inhibitors. In addition, the observation that LDHs from four *Plasmodium* species (*pf*LDH, *pv*LDH, *po*LDH, *pm*LDH) possess a high degree of structural similarity supports the idea that inhibitors which are effective against multiple parasite species is a real possibility [31, 49, 50].

3.4. LDH INHIBITORS - BACKGROUND

3.4.1. Perspective therapeutic applications [51]

The roles of human LDH-A in cancer progression and that of *pf*LDH in *Plasmodium* survival discussed above confer a validation to these two enzymes as promising therapeutic targets in the treatment of hypoxic tumours and malaria, respectively. Nowadays, more progresses has been made on *pf*LDH as anti-malaria target, whereas LDH-A is a more innovative tumor target, since research efforts in this direction are still at an early stage. In fact, many small organic molecules have been synthesized in attempts to inhibit *pf*LDH and, consequently, causing *Plasmodium Falciparum* death [30-33]. On the contrary, until the discovery of the relationship between LDH-A activity and the "Warburg effect" by Leder *et al.* in 2006 [35], LDH-A had not been seen as a possible target in the treatment of cancer. At the moment, genetic inhibition of LDH-A through shRNA remains the most selective way of interfering with LDH-A catalytic activity and still no reports about small organic molecules able to reverse the "Warburg effect" by inhibiting LDH-A are present in the literature, to the best of our knowledge.

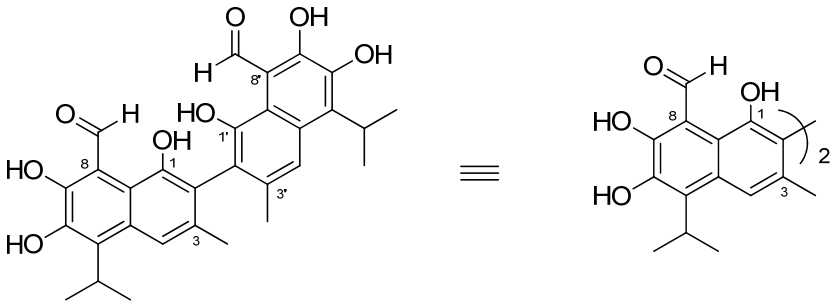
A restricted number of examples of unspecific inhibitors of human isoforms, showed good levels of inhibition of LDH-X and, therefore, may be considered for the development of male contraceptive drugs [37].

In this chapter, we provide an overview of the most important class of inhibitors of *pf*LDH and LDH-A in an attempt to delineate the most important structural features needed to interact with this class of isozymes, and to find whether there are selective lead structures to be subsequently functionalized and developed. All the molecules herein reported were initially designed and synthesized in the search for antimalarial drugs, but also tested on other mammalian isoforms in order to detect their degree of selectivity.

3.4.2. Gossypol and its derivatives

Gossypol ("Gossyp" from its *Gossypium* origin, the cotton plant, and "ol" from its phenolic nature) is a polyphenolic binaphthyl disesquiterpene compound. It exists as two optically active forms generated by atropisomerism that originates from the reduced

rotation ability about the C2-C2' binaphtalenic bond (**3.7**, Fig. **3.13**). It is usually extracted from the cotton seeds belonging to the *Gossypium* species and the tropical tree *Thespesia populnea*, both of which are members of the *Malvaceae* family [52]. It was isolated for the first time in 1886 during cottonseed oil refinement as a mixture containing other molecules, but the chemical structure was unequivocally established only in 1938 as a consequence of its physical and chemical properties [53]. In the cotton plan, its biosynthesis occurs via dimerization of hemigossypol and plays the role of rendering the plant resistant to various kinds of pathogens and insects, acting as a natural insecticide together with other sesquiterpenoids present inside the plant [54].



3.7

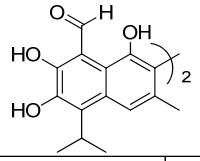
Figure 3.13. Structure of gossypol (**3.7**).

Gossypol has initially received a great deal of attention for its potential therapeutic application as a male antifertility agent, thanks to its spermicidal action [55, 56]. Later, further promising biological properties of gossypol were found, such as antitumour, antioxidant, antiviral, and antiparasitic activities [57]. In particular, among its numerous biological activities, the antiparasitic effect against the malarial parasite *Plasmodium Falciparum* is considered as one of the most interesting activities. The main problem that has stopped any further development is the gossypol general toxicity, leading to cardiac arrhythmias, hypokalemia, renal failure, muscle weakness, fatigue and, in some cases, even paralysis [58-60]. One of the structural reasons for the unspecific toxicity of gossypol may lie in the presence of two functional aldehyde groups. These groups can easily bind primary amino groups belonging to lysine residues of biological important

proteins, leading to the formation of stable Schiff's bases and, consequently, affecting the activity of many enzymes [61].

Gossypol preferentially acts on redox reactions catalyzed by NAD^+/NADH-based enzymes such as many dehydrogenases, which possess a common structural homology showing the typical dinucleotide "Rossmann fold". This evidence is also confirmed by the fact that most of the biological activities of gossypol may result from inhibition of several of these dehydrogenases. For example, its antifertility action has been mainly attributed to inhibition of LDH-C_4 [37, 62], its antitumour activity in some cell lines seems due to the action on LDH-A [63] and especially the antimalarial activity is provoked via the inhibition of *pf*LDH, which are all NAD^+/NADH-linked enzymes. As a consequence, gossypol is generally considered a non-selective competitive inhibitor of LDH relative to NADH (Table **3.1**) [61].

Table 3.1. Activity profile of gossypol (**3.7**).



Cpd	K_i (μM) *pf*LDH	K_i (μM) LDH-A	K_i (μM) LDH-B	K_i (μM) LDH-C
3.7	0.7[a]	1.9[a]	1.4[a]	4.2[b]

[a] Data from ref. [48]; [b] data from ref. [37].

Unluckily, the above-mentioned toxicity of gossypol precludes its direct use as a therapeutic agent. For this reason, many derivatives or analogs of gossypol that do not retain the aldehyde functional groups were prepared in order to verify if the resulting compounds were able to maintain the beneficial biological activities of gossypol, with a reduction of its toxic side effects. However, the possibility of obtaining derivatives by direct modifications of gossypol aldehyde groups has been limited because of the presence of the phenolic hydroxyl groups in the 1 and 1' (*peri*) positions, that complicate the chemistry of the CHO groups at the 8 and 8' positions. As a consequence, in many derivatives obtained by synthetic transformations of gossypol the

1,1'-hydroxyl groups are absent [31, 37], acylated [30] or incorporated into the functional groups replacing the aldehydes [48].

Initially, some gossypol analogs were prepared and tested for other purposes, such as inhibition of aldose reductase [64] or anti-HIV activity [65, 66]. More recently, in most cases, gossypol derivatives were designed as new potential antimalarial drugs (Fig. **3.14**). However, the *pf*LDH inhibition is often accompanied by a comparable inhibitory action on other LDH isoforms, especially the human ones, because of their low selectivity [30, 31].

In the series of peri-acylated gossylic nitriles (compounds **3.8-3.11**, Fig. **3.14**, Table **3.2**), the aldehyde groups are converted into nitriles and also the *peri* (1 and 1') hydroxyl groups are derivatized through acylation (R_1 = acyl group) in order to stabilize the nitrile derivatives; free *ortho* and *meta* phenolic hydroxyls groups are instead present [30]. Compounds **3.8-3.11** were evaluated in enzymatic inhibition assays against *pf*LDH (Table **3.2**). They were also evaluated *in vitro* for antimalarial activities against both chloroquine-sensitive and chloroquine-resistant strains of *Plasmodium Falciparum* (data not shown in the table). All the inhibition values show a particular pattern according to the length of the acyl-substituent: the activity substantially increases with the length of the *peri*-acyl group, with the propionylated derivative **3.11** displaying the lowest K_i (0.3 μM). All the experimental data are in agreement with the assumption that peri-acylated gossylic nitriles are competitive inhibitors of NADH binding to *pf*LDH. However, inhibition values do not show any significant improvements over gossypol (Table **3.1**).

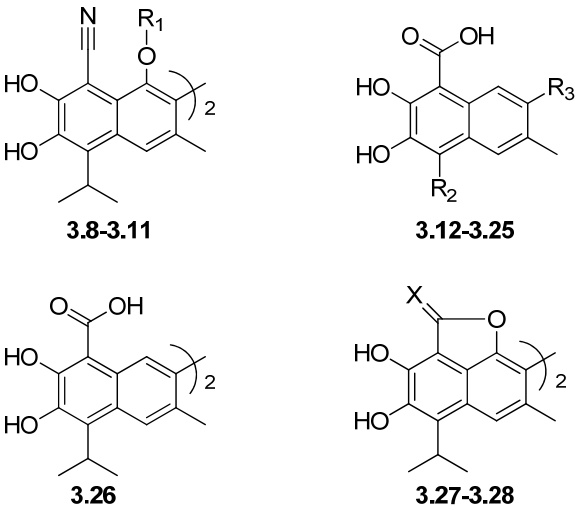
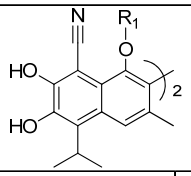
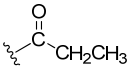
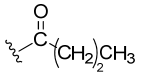
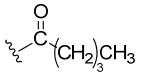


Figure 3.14. Structures of gossypol derivatives and analogs: peri-acylated gossylic nitriles (compounds **3.8-3.11**), derivatives of 8-deoxyhemigossylic acid (**3.12-3.25**), 1,1'-dideoxygossylic acid (**3.26**), gossylic lactones (**3.27-3.28**).

Table 3.2. Peri-acylated gossylic nitriles (**3.8-3.11**).[a]



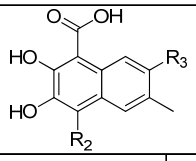
Cpd	R_1	K_i (μM) *pf*LDH
3.8		0.8
3.9		1.1
3.10		0.6
3.11		0.3

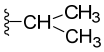
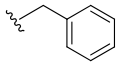
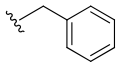
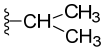
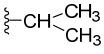
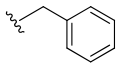
[a] Data from ref. [30].

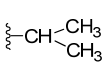
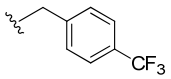
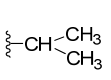
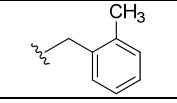
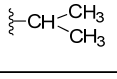
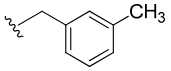
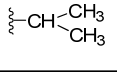
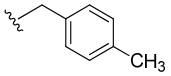
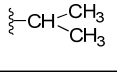
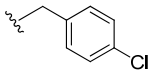
Another group of gossypol analogs (compounds **3.13-3.25**, Fig. **3.14**, Table **3.3**) is structurally related to the sesquiterpene 8-deoxyhemigossylic acid (2,3-dihydroxy-6-methyl-4-(1-methylethyl)-1-naphtoic acid) **3.12** (Table **3.3**) [31, 37]. The aim of the design of these substituted 2,3-dihydroxy-1-naphtoic acids was to test the role of the alkyl group in the 4-position (R_2) of the 8-deoxyhemigossylic scaffold, replacing the isopropyl group with a methyl or a *n*-propyl substituent (**3.13-3.18**) and contemporarily to analyze the role of the substituent in the 7-position (R_3), which is the coupling position in the formation of disesquiterpenes like gossypol itself (**3.19-3.25**). All these molecules compete with NADH cofactor for binding both to *pf*LDH and human LDHs. The reference compound 8-deoxyhemigossylic acid **3.12** is an equally potent inhibitor of *pf*LDH and LDH-A (K_i of 2 and 3 μM, respectively), but shows a 30-fold higher K_i value for LDH-B (91 μM), whereas its dimer 1,1'-dideoxygossylic acid **3.26** (Table **3.4**) is non-selective in the inhibition of all these three isoforms with very similar K_i values in the low micromolar range. Thus, if the dimerization of **3.12** increases activity only on LDH-B and **3.12** and its dimer **3.26** show similar inhibitory abilities against LDH-A and *pf*LDH, it seems that only half of the gossypol backbone is necessary for the inhibition of LDH-A and *pf*LDH. For this reason, it was investigated if addition of groups at the 7-position, the coupling position in the gossypol derivatives, has any effect on the inhibitory properties. It is interesting to note that the introduction of a benzyl group in the 7-position (compound **3.20**) determined a strong inhibition of human isoforms A and B, but not of malarial enzyme, compared with the reference structure, whereas the introduction of a substituted benzyl group completely changed this pattern. For example, the *p*-(trifluoromethyl)benzyl derivative **3.21** is more active against *pf*LDH than human LDHs, showing selectivity for the malarial enzyme. As regards modification in the 4-position (R_2), compounds **3.13-3.15** possessing a methyl group in this position are selective for *pf*LDH and LDH-A, with improvements in their inhibitory properties for all the three LDHs when a large group such as a benzyl is present in the 7-position (compound **3.15**). Among the 4-isopropyl-substituted derivatives **3.19-3.25**, compound **3.24**, substituted in the 7-position with a *p*-tolylbenzyl group, is the most active, showing a high activity and selectivity for LDH-A (K_i = 30 nM). In summary, substituents at both the 4- and the 7-position are important in determining potency and

selectivity for this class of inhibitors. The *p*-(trifluoromethyl)benzyl derivative **3.21** shows that the development of selective *pf*LDH inhibitors is possible. Moreover, even a certain degree of selectivity for LDH-A over LDH-B is showed by many compounds belonging to the 2,3-dihydroxy-1-naphtoic acid family, such as derivatives **3.22-3.25**, in spite of the high similarities of the two human isoforms.

Table 3.3. 2,3-Dihydroxy-1-naphtoic acids (**3.12-3.25**).

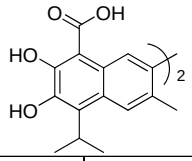


Cpd	R_2	R_3	K_i (μM) *pf*LDH	K_i (μM) LDH-A	K_i (μM) LDH-B
3.12[a]		H	2	3	91
3.13[a]	—CH_3	H	22	34	>250
3.14[a]	—CH_3	—CH_3	13	4	190
3.15[a]	—CH_3		8	0.5	39
3.16[a]	—$CH_2CH_2CH_3$	H	6	1	49
3.17[a]	—$CH_2CH_2CH_3$	—CH_3	0.1	0.1	19
3.18[a]	—$CH_2CH_2CH_3$		0.3	0.05	1
3.19[a]		—CH_3	1	2	78
3.20[a]			0.7	0.2	7

3.21[a]			0.2	13	81
3.22[b]			n.t.[c]	3	>125
3.23[b]			n.t.	0.2	34
3.24[b]			n.t.	0.03	8
3.25[b]			n.t.	1	8

[a] Data from ref. [31]; [b] data from ref. [37]; [c] n.t. = not tested.

Table 3.4. Activity profile of 1,1'-dideoxygossylic acid (**3.26**).[a]



Cpd	K_i (μM) *pf*LDH	K_i (μM) LDH-A	K_i (μM) LDH-B
3.26	1.2	1.3	0.7

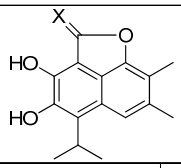
[a] Data from ref. [48].

Subsequently, compound **3.18** (called FX11 by *Le et al.*) was further selected as a candidate small organic molecule LDH-A inhibitor of the dihydroxynaphtoic acid group, due to its ability to inhibit preferentially LDH-A as opposed to the malarial and B isoform (Table **3.3**) [67]. It was retested on the purified human liver LDH-A showing a K_i values of 8 μM. Furthermore, it was demonstrated that FX11 affects cellular energy supply significantly, diminishing cellular production of lactate, by targeting LDH-A. FX11 proved to be the first compound up to now able to reduce tumour growth in tumour cells that are dependant on glycolysis and in xenograft models, supporting the essential role of LDH-A in tumorigenesis and tumour maintenance. Although these

encouraging results, the highly reactive cathecolic structure of FX11 could contribute to its biological activity by means of off-target effects, making this compound unsuitable as drug.

Other two compounds that can be included in the gossypol-like series are lactone **3.27** and iminolactone **3.28** (Fig. **3.14**, Table **3.5**). They were considered as antimalarial agents thanks to their good abilities to inhibit *pf*LDH [48, 68], although they were initially studied as anti-HIV agents [65, 66] or aldose reductase inhibitors [64]. Unfortunately, gossylic lactone **3.27** is completely non selective, whereas gossylic iminolactone **3.28**, that differs from gossylic lactone **3.27** only for a nitrogen/oxygen atom exchange, is generally less active than **3.27** against *pf*LDH (40-fold reduction) and against LDH-B (230-fold reduction); it keeps a good activity on LDH-A with a K_i of 2.5 μM and is more selective for LDH-A than its lactone analogue (Table **3.5**).

Table 3.5. Gossylic lactone and iminolactone (**3.27-3.28**).[a]



Cpd	X	K_i (μM) *pf*LDH	K_i (μM) LDH-A	K_i (μM) LDH-B
3.27	O	0.4	0.6	0.4
3.28	NH	16	2.5	92

[a] Data from ref. [48].

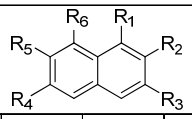
The low selectivity of many gossypol derivatives and analogs in the inhibition of a particular LDH isoform is in agreement with the ability of these classes of compounds of inhibiting various kinds of dehydrogenases, that may contribute to a wide range of side effects. Nevertheless, in spite of the high homology between enzymes belonging to the dehydrogenase family, some of these dihydroxynaphtoic acids proved to be able to discriminate LDH-A, LDH-B and *pf*LDH. This observation enhances the chances of developing potent and selective LDH inhibitors structurally related to gossypol.

3.4.3. Naphthoic acids

A further noteworthy class of LDH inhibitors is the naphthoic acid family, characterized by a simple naphthalene scaffold substituted by carboxylic acid or sulphonic acid groups [69]. As previously demonstrated, the pharmacophore core of gossypol is a naphthalene bicycle substituted in various positions with different functional groups (like hydroxyls, carboxyls). Consequently, starting from this consideration, some commercially available compounds containing the naphthalene moiety as central scaffold were selected (compounds **3.29-3.34**, Table **3.6**) in order to determine the inhibitory ability of molecules possessing this kind of scaffold on both *pf*LDH and human LDHs. Unfortunately, these compounds are very modest LDH inhibitors, showing IC_{50} values in the millimolar range. These biological data raise the question as to whether strong simplifications of gossypol structure can be detrimental for anti-LDH activity. However, some of these compounds inhibit preferentially *pf*LDH (compounds **3.33-3.34**), whereas others (**3.29-3.30**) show some preference for *h*LDHs. The crystal structures of three enzyme-inhibitor complexes highlight two different binding modes for this naphtoic acids in the *pf*LDH active site. Compounds **3.30** (2,6-naphtalene disulphonic acid, simply called NDSA) and **3.32** (3,7-dihydroxy naphthalene-2-carboxylic acid, DHNCA) lie vertically in the active site, in a manner similar to pyruvate: in this position, the sulphonate of **3.30** and the carboxylate of **3.32** are able to establish charged hydrogen bonds with Arg171 and, possibly, a weak hydrogen bond with His195, while the two hydroxyls of **3.32** and the second sulphonate group of **3.30** interact with the backbone carbonyl group of Pro246, via a water-mediated hydrogen bond. This positioning is reinforced through van der Waals contacts that the naphthalene moiety establishes in the hydrophobic groove that it occupies. The second type of binding mode is showed by the horizontal position assumed by compound **3.29** (2,6-naphtalenedicarboxylic acid, NDCA) in the active site, bridging the cofactor and the substrate binding sites. One carboxylate takes part in a charged hydrogen bond with His195 and two water-mediated contacts with Arg171, while the other carboxylate interacts with the backbone nitrogen of Ile31 via a hydrogen bond. In addition, Ser245 which is present only in *pf*LDH, permits the formation of several water-mediated interactions. The naphthalene ring is located in a hydrophobic pocket

that seems like a cleft, mainly delimited by residues unique to *pf*LDH, such as Pro246, Tyr247 and Pro250 (Thr, Ser and Ile respectively, in human).

Table 3.6. Naphtoic acids (**3.29-3.34**).[a]

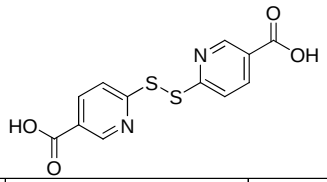


Cpd	R_1	R_2	R_3	R_4	R_5	R_6	IC_{50} (mM) *pf*LDH	IC_{50} (mM) *h*LDH[b]
3.29	H	H	COOH	H	COOH	H	5.1	1.4
3.30	H	H	SO_3H	H	SO_3H	H	21	9.8
3.31		H	H	H	H	SO_3H	0.52	1.1
3.32	H	H	OH	COOH	OH	H	2.4	5.0
3.33	H	OH	COOH	H	H	OH	1.7	150
3.34	H	H	Br	COOH	OH	Br	0.31	5.9

[a] Data from ref. [68]; [b] unspecified human LDH isoform used.

In this section it is worth mentioning 6,6'-dithiodinicotinic acid **3.35** (Table **3.7**) [69], which is somewhat different from the naphtoic acid derivatives for two structural characteristics: a disulphur linker that separates the two aromatic rings and two pyridine rings in the place of a pair of fused phenyl rings. Compound **3.35** is non-selective in its inhibition of *pf*LDH and *h*LDH, showing comparably poor IC_{50} values in the millimolar range.

Table 3.7. Activity profile of 6,6'-dithiodinicotinic acid (**3.35**).[a]

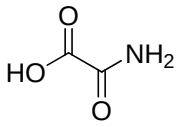


Cpd	IC_{50} (mM) *pf*LDH	IC_{50} (mM) *h*LDH[a]
3.35	6.6	4.6

[a] Data from ref. [68]; [a] unspecified human LDH isoform used.

3.4.4. *N*-Substituted oxamic acids

Oxamic acid (**3.36**, Fig. **3.15**, Table **3.8**), a structural isoster of pyruvic acid, represents a well-known substrate-like inhibitor of LDHs and, consequently, it is competitive with the pyruvate substrate binding. Both human LDH structures (LDH-A and LDH-B) [23] and *pf*LDH were crystallized as ternary complexes in the presence of the NADH cofactor and **3.36** [22]. Oxamate has attracted a great deal of attention thanks to its better inhibition properties against *pf*LDH than human LDHs, raising the question whether the development of selective inhibitors related to the oxamate moiety was possible.



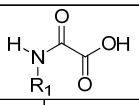
3.36

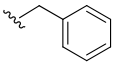
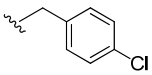
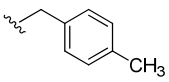
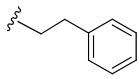
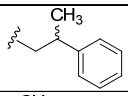
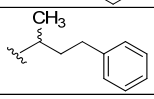
Figure 3.15. Structure of oxamic acid (**3.36**).

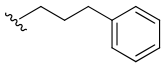
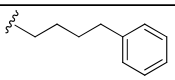
A first series of *N*-substituted oxamic acids **3.37**-**3.45** (Table **3.8**), which show a competitive inhibition with the pyruvate binding site, was synthesized and tested against human LDH-A, -B and -C [37]. The intent was to determine if structural modifications of the oxamic acid scaffold, such as the introduction of rather simple aryl and alkyl groups on the nitrogen of the oxamate amide group, allowed the development of

selective inhibitors. Unluckily, this class did not prove to be very promising on any of the human isoforms LDH-A/C since the K_i values of these compounds are in the millimolar range and are not even very selective (Table **3.8**). On the other hand, excellent inhibition levels of *pf*LDH were found with some of these oxamate analogs. In particular, *N*-benzyl derivatives **3.38**-**3.40**, and their higher homologues **3.41** and **3.44** possessing a slightly longer aliphatic spacer between the nitrogen atom and the phenyl ring, all displayed IC_{50} values in the sub-micromolar range, thus resulting as highly selective inhibitors for the parasite isoform over the human subtypes.

Table 3.8. Oxamic acid (**3.36**) and its derivatives (**3.37-3.45**) assayed on human, parasite and bovine LDH isoforms.



Cpd	R_1	K_i (mM) LDH-A	K_i (mM) LDH-B	K_i (mM) LDH-C	IC_{50} (μM) *pf*LDH	IC_{50} (μM) *m*LDH[d]
3.36[a]	H	-	-	-	94.4	116
3.37[b]		>10	6	>10	-	-
3.38[b]		0.4	0.4	2	0.08[c]	-
3.39[b]		1	0.5	0.3	0.07[c]	-
3.40[b]		2	0.5	0.6	0.09[c]	-
3.41[b]		7	3	5	0.10[c]	-
3.42[b]		>10	2	>10	-	-
3.43[b]		0.8	9	>10	-	-

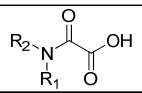
3.44[b]		0.9	0.7	0.7	0.035[c]	-
3.45[b]		2	3	0.6	41[a]	88[a]

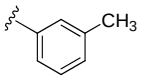
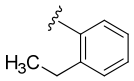
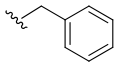
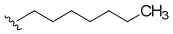
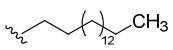
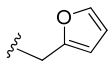
[a] Data from ref. [34]; [b] data from ref. [37]; [c] data from ref. [69]; [d] *m*LDH = mammalian LDH extracted from bovine heart.

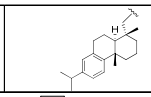
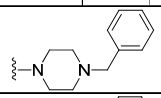
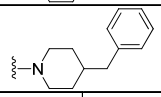
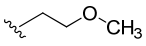
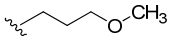
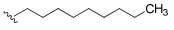
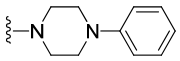
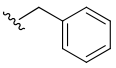
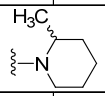
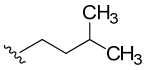
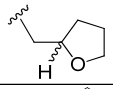
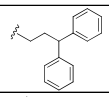
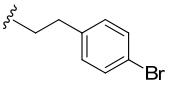
Choi and co-workers reported another series of mono- and di- *N*-substituted oxamic acids obtained by an automated synthetic strategy directed to find potential antimalarial drugs acting against *pf*LDH [34]. This library of molecules (compounds **3.46**-**3.66**, Table **3.9**) was also tested on mammalian LDH (*m*LDH), extracted from bovine heart, in order to see if these oxamic-based structures can be selective *pf*LDH inhibitors. Most oxamic acids show *pf*LDH selectivities and, in particular, compound **3.55** is the most active against *pf*LDH with an IC_{50} value of 14 μM, although the highest degree of selectivity for the plasmodial enzyme, about 5-fold, is obtained by compound **3.51**. Some compounds inhibit only *m*LDH, for example compounds **3.59** and **3.61**, showing a high selectivity for the mammalian isoform. Compound **3.52** is the most active inhibitor of mammalian dehydrogenase of this series with an IC_{50} value of 20 μM, although it also inhibit *pf*LDH (IC_{50} = 59 μM). Observing the inhibition values within this class, we can assert that oxamic acids that are disubstituted on the nitrogen atom with bulky groups (for example **3.47**) are generally less active on both enzymes than the other compounds, maybe due to the steric hindrance that makes the entrance of these molecules in the active site difficult. This consideration is confirmed by the fact that the most active compounds **3.55** and **3.57** on both dehydrogenases have small groups on the oxamate nitrogen atom (R_1 = H and R_2 = small alkyl ethers). The binding mode of this class of compounds was supported by molecular docking studies on *pf*LDH showing that oxamic acid and its derivatives interact similarly with catalytical residues of the enzyme, with some differences caused by the presence of additional groups on the nitrogen atom. These substituents occupy part of the NADH binding site by establishing new interactions, that could explain why, for example, compound **3.55** is more active

than **3.57**. In fact compound **3.55**, which differs from **3.57** only by a methylene group less in the ether chain, reaches and establishes a hydrogen bond with Asn140, which is near the hydroxyl of NADH ribosyl moiety, whereas the longer ether side chain of compound **3.57** cannot interact with this residue in the same way. Compound **3.64** positions its two phenyl rings near both the nicotinamide and ribosyl rings of the cofactor and Leu163, which is a residue unique to *pf*LDH (in human it is replaced by a serine). Usually, Leu163 establishes a H-bond between its amide oxygen atom and the amide group of the NADH nicotinamide moiety. As a consequence, the perspective introduction of a hydrogen bond donor on one of the two phenyl groups of compound **3.64** is expected to improve *pf*LDH selectivity [34], supporting the idea that the exploitation of structural features exclusive of *pf*LDH is the best strategy to obtain selective inhibitors as anti-malarial drugs.

Table 3.9. Oxamic acid derivatives (**3.46-3.66**) assayed on parasite and bovine LDH isoforms.[a]



Cpd	R_1	R_2	IC_{50} (μM) *pf*LDH	IC_{50} (μM) *m*LDH[b]
3.46			97.9	107
3.47			>200	158
3.48	H		160	>200
3.49	H		>200	150
3.50	—CH_3		>200	186
3.51	H		43	>200

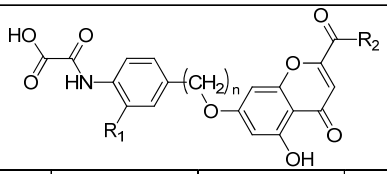
3.52	H		59	20
3.53			169	46
3.54			101	59
3.55	H		14	25
3.56	H		47	133
3.57	H		43	31
3.58	H		88	>200
3.59			>200	32
3.60	—CH_3		>200	168
3.61			>200	43
3.62	H		157	>200
3.63	H		188	>200
3.64	H		35	90
3.65	H		146	>200
3.66	H		51	>200

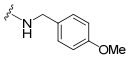
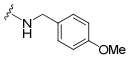
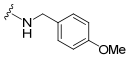
[a] Data from ref. [34] [b] *m*LDH = mammalian LDH extracted from bovine heart.

A series of larger oxamic acid derivatives containing a chromene portion (**3.67**-**3.71**, Table **3.10**) was designed with the principal aim of inhibiting *pf*LDH, by means of compounds that mimic the shape of the bioactive conformation of the NADH cofactor, in an attempt to fill both the substrate and the cofactor binding sites and, consequently, to improve the inhibitory activities in comparison with the previous class of substituted oxamic acids [32]. The chromene group was considered as a suitable replacement of the diphosphate moiety of NADH, possessing a greater rigidity and hydrophobicity than the diphosphate group. This group was also chosen in view of its easy synthetic accessibility. The chromene moiety, connected covalently to the nitrogen of the oxamate portion through an aryl-alkyl linker, generally showed a binding mode similar to that of the NADH phosphate group, possessing hydrogen bonding acceptor portions (OH and C=O) comparable to oxygen atoms of the phosphate group. Moreover, the *N*-aryl group directly linked to the oxamic acid is a good mimic of the nicotinamide ring of the cofactor. This observation suggests that the substituted oxamate moiety and the chromene group together may constitute a scaffold that can bind to almost the entire LDH active site. These compounds were tested against *pf*LDH, and also, for comparison, against mammalian LDH (*m*LDH) extracted from bovine heart. In addition, it was observed a certain degree of inhibition even on malate dehydrogenase (MDH) (data not shown in the table), a very important enzyme in glucose metabolism of *Plasmodium Falciparum*, which may substitute *pf*LDH when this enzyme is blocked. This dual enzyme inhibition might increase the ability of oxamic derivatives to block ATP production in the parasite. Similarly to simple oxamic acid **3.36**, some of these derivatives show good levels of *pf*LDH selectivity over *m*LDH (Table **3.10**). It is evident that the presence of a second COOH group on the chromene terminal moiety (**3.67**, **3.68**) is detrimental for the inhibitory potency, whereas amide analogs (**3.69**-**3.71**) generally show very good inhibitory properties against *pf*LDH. Compound **3.71** is the most potent and selective, as well as its structurally related analogs **3.69** and **3.70**. The *ortho*-substituent to the *N*-oxamic acid (R_1) has a marked influence on the inhibition. In fact, the introduction of a methoxy group in that position causes an improvement of the inhibitory activity against *pf*LDH (compare **3.71** to **3.69**), whereas a cyano group causes a slight decrease in inhibitory potency (**3.70**). This result can be explained by the

replacement of human Ser163 with plasmodial Leu163 and the consequence that this substitution provokes. The hydrogen bond between the hydroxyl of Ser163 present in human LDHs and the amide of the nicotinamide ring is impossible in *pf*LDH, where no H-bond takes place on Leu163. *Ortho*-substituents (R_1) are positioned near to *pf*Leu163, in close proximity to the amide group of the cofactor nicotinamide ring. As a consequence, the introduction of groups able to act as H-bond acceptors, such as a methoxy, in the *ortho* position (**3.71**) improves the activity and the selectivity of this class of compounds for *pf*LDH. Molecular docking studies confirmed that compounds **3.69** and **3.71** bind in the same way in both the cofactor and the substrate binding sites, establishing hydrogen bonds with catalytical residues and with amino acids delineating the cofactor binding site.

Table 3.10. Chromene-based oxamic acids (**3.67**-**3.71**).[a]



Cpd	R_1	R_2	n	IC_{50} (μM) *pf*LDH	IC_{50} (μM) *m*LDH[b]
3.67	OMe	OH	1	87.3	>232
3.68	OMe	OH	2	191	>225
3.69	H		2	3.13	>187
3.70	CN		2	8.25	>179
3.71	OMe		2	1.75	11.4

[a] Data from ref. [32]; [b] *m*LDH = mammalian LDH extracted from bovine heart.

3.4.5. Azole-based compounds

Another important class in the field of anti-LDH drugs is constituted by the azole-based inhibitors (Fig. **3.16**) [33]. They were identified in a high throughput enzymatic screening with the aim of finding antimalarial drugs, but, as it often happened for other classes of LDH inhibitors, they showed inhibitory properties against both *pf*LDH and *h*LDHs. However, some of them possess good levels of *pf*LDH selectivity, by inhibiting preferentially this isoform at low micromolar concentrations. The active compounds are mixed competitive inhibitors towards both NADH and pyruvate and are competitive against lactate. These compounds were also tested *in vitro* against drug-sensitive and drug-resistant strains of *Plasmodium Falciparum*, showing IC_{50} values in agreement with those obtained in enzymatic assays. In details, four parent kinds of azole-based compounds were identified with a common pharmacophoric portion: a hydroxyl and a carboxyl moieties in adjacent positions, that are 3 and 4 positions of the azole ring (Fig. **3.16**).

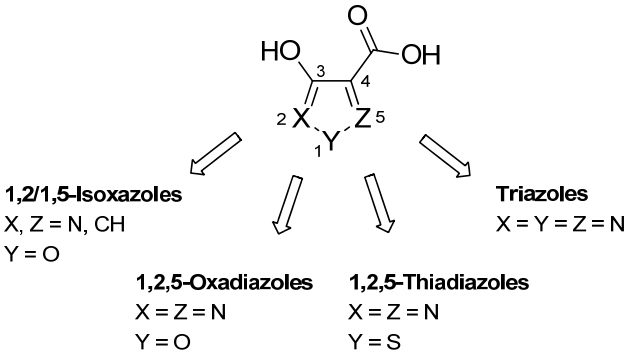
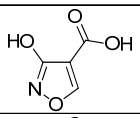
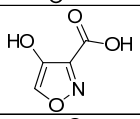
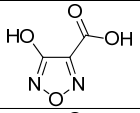
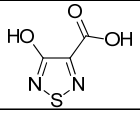


Figure 3.16. General chemical structures of 1,2 or 1,5-isoxazole, 1,2,5-oxadiazole, 1,2,5-thiadiazole and triazole classes [33].

For each class, derivatives with various substituents in the pentacyclic ring were synthesized in order to explore structure-activity relationships of the azole class (Table **3.11**). From the IC_{50} values reported against both *pf*LDH and *h*LDH (human heart isoform LDH-B), it is evident that C3-hydroxyl and C4-carboxyl are essential for activity. Both 1,2- and 1,5-isoxazole scaffolds are active against the parasite isoform,

provided they maintain the "OH/COOH" pharmacophoric portion, as in compound **3.72** and **3.73**, respectively, although compound **3.72** showed higher levels of inhibition on both *pf*LDH and *h*LDH-B than its 1,5-regioisomer **3.73**. The data confirmed that vicinal OH and COOH groups are fundamental for the inhibitory activity within this class. In fact, both methoxy-substituted derivative of **3.72** and its *N*-CH_3 tautomer are inactive, as well as its ester analog; the introduction of a small methyl group in position 5 also causes a loss of activity on both isoforms (Fig. **3.17**). A further confirmation of the strict requirement to keep the "OH/COOH" pharmacophoric portion is given by the 1,2,5-oxadiazole class, since only compound **3.74** displayed inhibitory properties for both *pf*LDH (IC_{50} = 0.65 μM) and, to a much lesser extent, *h*LDH-B (IC_{50} = 72.05 μM).

Table 3.11. Azole-based compounds **3.72-3.75**.[a]

Cpd	Structure	IC_{50} (μM) *pf*LDH	IC_{50} (μM) *h*LDH-B
3.72		1.1	54
3.73		16	>100
3.74		0.65	72.05
3.75		0.14	10.27

[a] Data from ref. [33].

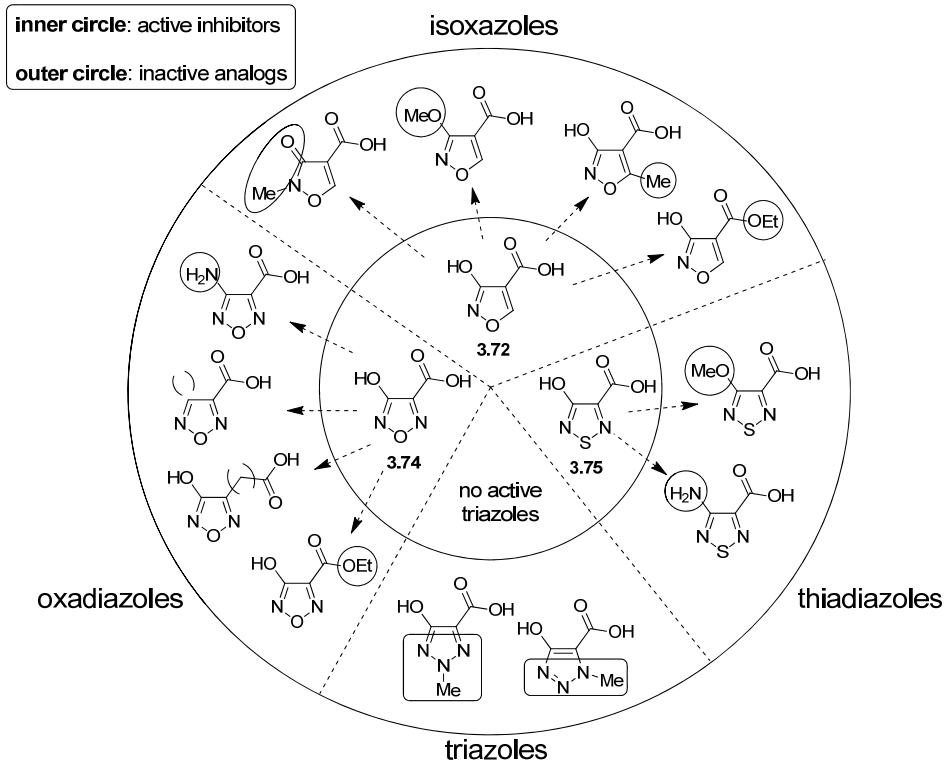


Figure 3.17. Structural modifications of active azoles (inner circle) leading to inactive inhibitors (outer circle).

Similar results were obtained with 1,2,5-thiadiazole derivatives, where the 3-hydroxy-4-carboxy-substituted derivative **3.75** exhibited an excellent inhibitory potency against *pf*LDH (IC_{50} = 0.14 μM) with a >70-fold selectivity over the human heart isoform. However, also in this case, the replacement of the 3-OH group, with either an amino or a methoxy group caused a complete loss of activity (Fig. **3.17**).

These data obtained within the azole classes show that changes of heteroatoms are sometimes useful; in fact replacement of the oxygen in position 1 (Y, Fig. **3.16**) with a sulfur atom gives more potent, but also less selective, *pf*LDH-inhibitors (compare **3.75** with **3.74** and **3.72**), whereas the replacement of the same oxygen with a nitrogen atom, leading to triazoles, is detrimental for activity. In fact, the few examples of OH/COOH-substituted triazoles reported (Fig. **3.17**) did not show any relevant inhibition of *pf*LDH

and *h*LDH-B, in spite of the fact that the pharmacophoric portion is present in these compounds.

These biological results can be explained by observing the complexes of *pf*LDH with the most active azole derivatives **3.72**, **3.74** and **3.75** obtained by X-ray studies: in all the cases, the carboxylate group interacts with Arg109 and Arg171 in the active site through a bifurcated salt bridge like the substrate pyruvate and, at the same time, the hydroxy group establishes a hydrogen bond network with the side chains of Arg109, His195 and with the backbone of Leu140 (Fig. **3.18**).

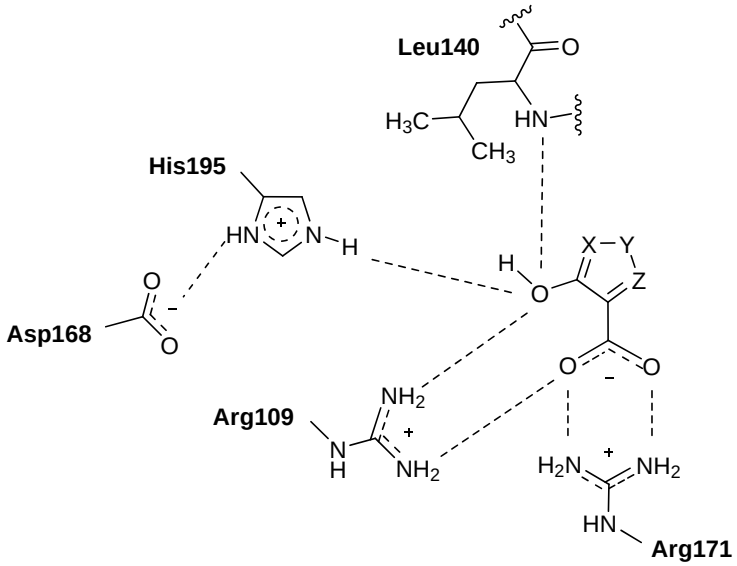


Figure 3.18. Schematic illustration of the interactions between the "OH/COOH"-substituted azole scaffold and the *pf*LDH active site. Residues directly involved in the interactions are represented.

Moreover, the crystal structures of the enzyme-inhibitor complexes explain the *pf*LDH-selectivity showed by the azole-based compounds. As a matter of fact, the azole rings bind in the active site of both LDHs, stacking parallel to the nicotinamide ring of the cofactor NADH. In the *pf*LDH structure complexed to **3.72**, **3.74** and **3.75**, the portion of the azole ring bearing the carboxylic moiety is in close proximity to Ser245 and Pro246 and the hydroxyl group of Ser245 side chain is hydrogen bonded to the oxygen or the sulfur atom at position 1 of the azole ring of the inhibitor via a water

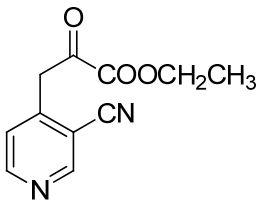
molecule. Differently, in the human isoform the oxygen or the sulfur of the azole ring of **3.72**, **3.74** or **3.75** is only bound to a water molecule, and they do not interact with the enzyme, because in this case the equivalent residue is a tyrosine (Tyr248) that projects away from the inhibitor molecule. The analysis of these complexes suggests that the H-bond between Ser245 and the heterocyclic atom at position 1 is responsible for the selectivity for *pf*LDH and this hypothesis was also confirmed by site-directed mutagenesis studies, performed to change Ser245 to alanine. As a consequence, the disposition of the inhibitors in the LDH active site can also explain why even a simple methyl group introduced at 5 position of the ring (Fig. **3.17**) causes a loss of activity; in fact, Pro246 (threonine in human LDH) is located next to the carboxylate function leaving very little space for the introduction of a group at position 5 of the ring.

Initial toxicity and pharmacokinetic studies of these compounds highlight their low cytotoxicity and favorable drug-like properties, making them possible candidates as antimalarial drugs. However, because of the moderate potency levels displayed by these inhibitors, an improvement in their activity is necessary for considering them as possible candidates for clinical trials. Furthermore, their relatively small sizes preclude their use as selective therapeutic agents, as they may unspecifically interact with several biological targets.

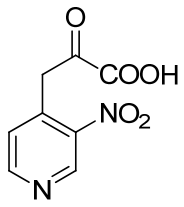
3.4.6. Other LDH inhibitors

In addition to the LDH inhibitor classes reported in the previous sections, other examples of compounds endowed with a certain ability of LDH inhibition were reported.

A series of pyridylpyruvates were designed as mimics of the substrate pyruvate. As a matter of fact, they contain a pyruvate-like moiety linked to the 4-position of a pyridine ring, resembling in part the transition state of the conversion of pyruvate into lactate with the involvement of NADH [70]. Among them, compounds **3.76** and **3.77** resulted active against the muscle isoform of rabbit LDH, showing IC_{50} values in the range of 70-100 μM (Fig. **3.19**).



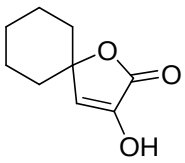
3.76



3.77

Figure 3.19. Structures of ethyl 3-(3-cyano-4-pyridyl)pyruvate (**3.76**) and 3-(3-nitro-4-pyridyl)pyruvic acid (**3.77**).

Spirolactone **3.78** (Fig. **3.20**) proved to be a LDH inhibitor non-competitive with respect to pyruvate (K_i = 7.5 mM), but competitive with NADH (K_i = 0.3 mM, K_i values originated from enzymatic assays on the muscle isoform of rabbit LDH) [71]. This molecule was originally synthesized for the therapeutic treatment of lactacidosis, together with other aromatic β,γ-unsatured α-ketoacids. The spiro structure is completely new in the field of LDH inhibitors. However, this compound possesses a latent carboxylate function (lactone), confirming the fundamental role that this functional group has in the interaction process with the target enzyme.

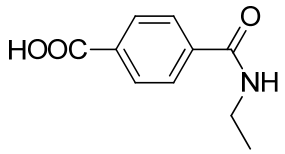


3.78

Figure 3.20. Structure of 3-hydroxy-2-oxo-1-oxaspiro[4.5]-dec-3-ene (**3.78**).

Over the past few years, several metal complexes have been evaluated for their anti-cancer properties. In particular, ruthenium complexes have already been investigated as cytotoxic agents [72]. However, their pharmacological mechanism of action remains unclear. Trigun et al. reported an example of a ruthenium (II) complex (**3.79**, Fig. **3.21**) containing 4-carboxy-*N*-ethylbenzamide (CNEB) moieties as an inhibitor of *h*LDH-A and, in particular, the cytotoxicity of this complex was demonstrated in Dalton's

lymphoma [73]. Complex **3.79** displays a non-competitive inhibition of LDH activity with a K_i value of 32 μM. It was suggested an allosteric inhibition for this complex, constituting the first example of a new alternative mechanism of LDH inhibition, never reported before for other compounds.

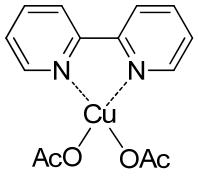


= CNEB

$Ru(CNEB)_2(bpy)_2 \cdot 2PF_6$ = Ru(II)-CNEB complex (**3.79**)

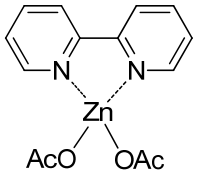
Figure 3.21. Structure of CNEB and the empirical composition of Ru(II)-CNEB complex (**3.79**).

The same authors also developed Cu and Zn-bipyridyl complexes (**3.80** and **3.81** respectively, Fig. **3.22**) [74], exploiting the good biocompatibility of copper (Cu^{2+}) and zinc (Zn^{2+}), since these two metal ions are present in several proteins and take part to many physiological reactions. The evaluation of their interactions with all the five LDH isoforms in mouse tissues afforded average binding constants of 1 mM for Cu-bpy complex **3.80** and 7 μM for Zn-bpy **3.81**. Although Cu-bpy **3.80** proved to be a weaker LDH inhibitor than its Zn-based analog **3.81**, its inhibition of LDH activity was detected in all mouse tissues, unlike the Zn-analog which showed inhibition only in kidney, heart and liver.



$Cu(bpy)(AcO)_2 \cdot H_2O$ = Cu-bpy complex

3.80



$Zn(bpy)(AcO)_2 \cdot H_2O$ = Zn-bpy complex

3.81

Figure 3.22. Structure of Cu-bpy (**3.80**) and Zn-bpy (**3.81**) complexes. bpy = 2,2' bipyridine.

3.4.7. Features and perspectives of LDH inhibitors

The fundamental biological role of LDH in sugar metabolism has been extensively studied so far in humans. However, this enzyme has attracted a great deal of attention also thanks to its possible involvement as an innovative anti-malarial target. Moreover, quite recently, the possibility of a LDH involvement in hypoxic tumour maintenance has been confirmed, transforming human isoform-5 (LDH-A) in an even more attractive target in the oncology field. These two very interesting roles of *h*LDH-A and *pf*LDH have been differently exploited as of yet. As a matter of fact, the strategy of inhibiting LDH of *Plasmodium falciparum* in the search for an ideal antimalarial therapy, able to overtake the emerging resistance typical of several *Plasmodium* strains, has been followed and, nowadays, interest in *pf*LDH inhibitors in the field of antimalarial research is continuously growing. The risk/benefit ratio in this case depends on the selectivity that the inhibitors have for the parasite isoform of the enzyme, so that they do not interfere with any of the metabolic pathways of the patient. The synthesis of several small organic molecules in order to find a lead candidate against *pf*LDH shows that, unluckily, it is not an easy task to obtain molecules that selectively interact with the parasite isoform. Nevertheless, this low selectivity reveals the possibility of developing inhibitors of the human A isoform, opening a challenging research line for the discovery of new anticancer drugs. In fact, the activity of some compounds on the human A isoform was initially discovered as a side effect of *pf*LDH inhibition. To the best of our knowledge, the only application of LDH inhibitors in the anti-cancer treatment is represented by a recent patent [75], where well known natural products, such as flavonoids and other polyphenols, are reported to inhibit LDH-A. As a therapeutic perspective, the selective inhibition of the muscle isoform (LDH-A) overexpressed in tumors would guarantee a low toxicity to the patient. In fact, the blockage of this enzyme might lead to toxicity only if combined with an intense physical activity, otherwise it does not seem to cause any particular side effect in humans [76, 77].

The structural similarity shared by most of LDH inhibitors could be the starting point from which development of new inhibitors can begin. The features that the existing LDH inhibitors have in common are principally the presence in adjacent positions of an acidic group, such as carboxylate, lactone or sulphonate, and a hydroxyl

or carbonyl moiety. This observation is in agreement with the substrate structures, such as those of pyruvate and lactate that are α-ketoacid and α-kydroxyacid, respectively (Fig. **3.23**), and finds its explanation in the structure of the active site, which is characterized by the presence of many polar and basic residues able to establish attractive interactions with these molecular portions. On this basis, LDH-inhibitors may be classified as *lactate-like*, if they present an hydroxy-acid motif, which resembles the polar portion of lactic acid, or as *pyruvate-like*, if they have a C=O group directly linked to the carboxylate, similarly to what happens in pyruvic acid (Fig. **3.23**).

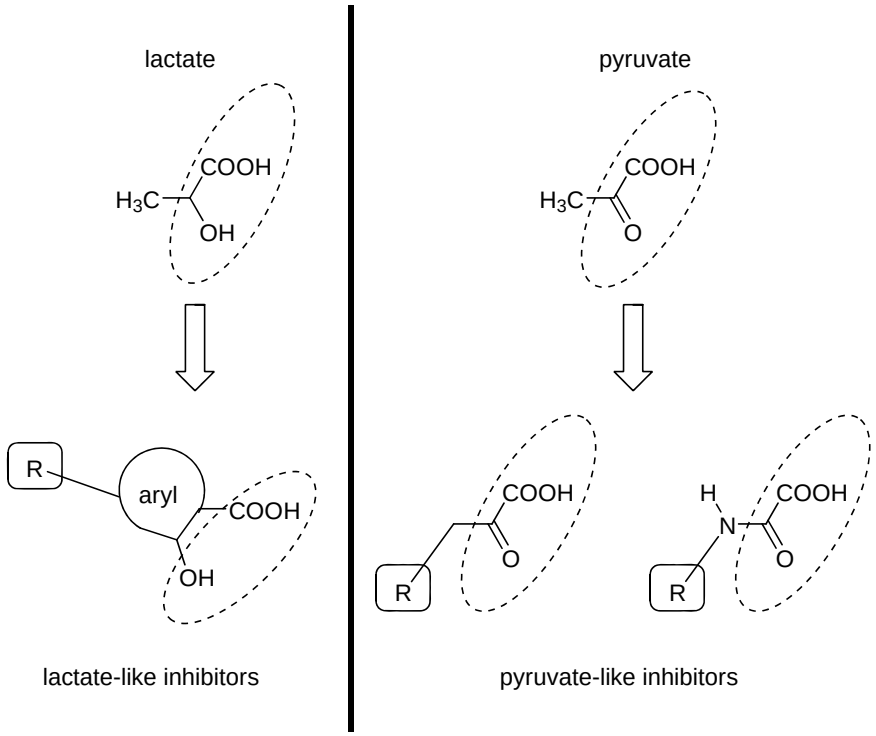


Figure 3.23. Generic structural features for the two main classes (lactate-like and pyruvate-like) of LDH inhibitors.

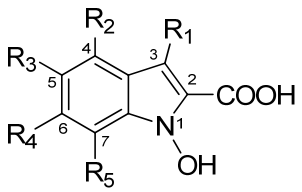
Moreover, most inhibitors reported so far possess a hydrophobic aromatic scaffold, mimicking the NADH cofactor. Hence, a valid strategy for obtaining more potent inhibitors could consist in combining substrate-like moieties, possessing a hydroxyl-

carboxylic pattern, with various kinds of aromatic cycles, which resemble part of the cofactor structure. In fact, the most potent inhibitors so far developed occupy both the substrate and the cofactor binding sites. The isozyme selectivity may also be improved by exploiting the known structural characteristics that distinguish each LDH isoforms, although the data as of yet available are not sufficient to provide a valid rationale to support this purpose.

3.5. *N*-HYDROXYINDOLES AS LDH-A INHIBITORS

3.5.1. The *N*-hydroxyindole nucleus in Medicinal Chemistry

As highlighted in the previous section, all the existing LDH inhibitors have a very high similarity in their molecular structure, sharing a carboxylate-hydroxyl/carbonyl moiety supported on an aromatic scaffold, so partially mimicking the structures of the LDH substrates, lactate or pyruvate. The identification of these pharmacophoric groups was the starting point of our search for new LDH-A inhibitors, hence some series of OH-COOH substituted aromatic scaffolds were developed during my PhD research work. Most of the synthesized compounds possess a *N*-hydroxyindolic structure (NHI) with a carboxylic group in position 2 of the heterocycle (structure **NHI**, Fig. **3.24**) [78]. This group of molecules retains the OH-COOH motif observed in the other LDH inhibitors, with the difference of the *N*-OH group that is slightly less acidic than the phenol group [79].



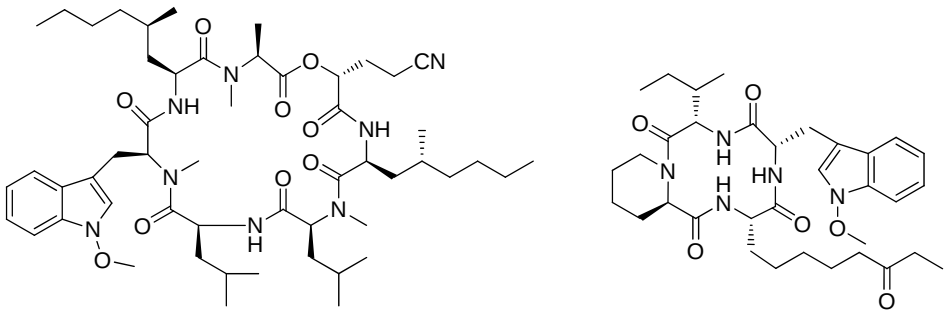
NHI

Figure 3.24. Structure of *N*-hydroxyindole-2-carboxylic acid derivatives (generally abbreviated **NHI**).

N-hydroxyindoles are not only scarcely represented chemical entities in nature, but also a relatively new and unexplored class of heterocyclic compounds in the field of Medicinal Chemistry.

Until 1996, there were not examples of *N*-hydroxyindoles isolated as natural products, although it was hypothesized the existence of 1-hydroxytryptophan derivatives in living organism, undergoing nucleophilic substitution reactions with 1-OH as a leaving group for explaining the occurrence of biologically relevant indole-based molecules such as serotonin, melatonin, trypthophan derivatives etc. (1-

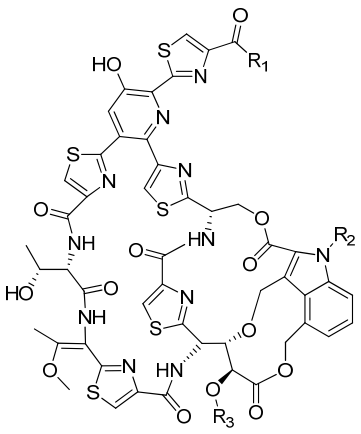
Hydroxindole Hypotheses) [80]. At last, in 1996, two peptides bearing a 1-methoxytrypthophan residue in their structures were isolated as natural products: HUN-7293 **3.82**, a cyclic heptadepsipeptide from fungal broth which is a potent inhibitor of cell adhesion molecule expression [81] and Apicidin **3.83**, a cyclic tetrapeptide isolated from *Fusarium pallidoroseum* with antiprotozoal activity against Apicomplexan parasites [82, 83], demonstrating the existence in nature of NHI-like structures (Fig. **3.25**).



3.82 **3.83**

Figure 3.25. Structures of HUN-7293 (**3.82**) and Apicidin (**3.83**).

More recently, other natural products containing the NHI unit were discovered such as Nocathiacins I, III and IV (**3.84-3.86**, Fig. **3.26**) isolated from bacteria species *Nocardia* and the fungus *Amicolaptosis* [84-86], and Thiazomycin and Thiazomycin A (**3.87-3.88**, Fig. **3.26**) isolated from *Amycolatopsis fastidiosa* [87], possessing a highly substituted NHI moiety within their structure. They all belong to the thiazolyl peptide class and they are among the most potent *in vivo* antibacterial agents known at the moment, although their clinical use has been limited by their poor physicochemical properties, in particular, by their low aqueous solubility. These discoveries in nature increased the interest toward the NHI motif, in particular for the potential application of this challenging heterocycle in Medicinal Chemistry.



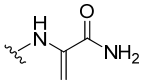
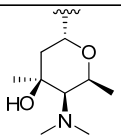
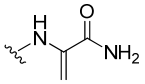
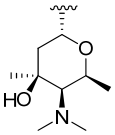
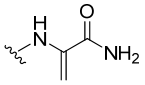
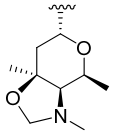
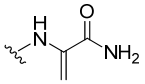
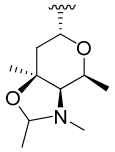
Name	R_1	R_2	R_3
3.84		OH	
3.85		OH	H
3.86	H	OH	
3.87		OH	
3.88		OH	

Figure 3.26. Structures of Nocathiacins I, III and IV (**3.84-3.86**), Thiazomycin (**3.87**) and Thiazomycin A (**3.88**).

The almost total absence of NHI structure in Medicinal Chemistry is mainly due to the lack of suitable methods for the assembly this scaffold, but also the presumed

instability of this organic framework might have contributed to neglect its use in the design of bio-relevant molecules. Somei [80, 88] and Acheson [89] were the first who showed interest in the synthesis of NHI molecules, succeeding in creating quite simple and efficient procedures for their synthesis. Briefly, the best methods discovered by Somei are: 1) conversion of 2-nitrotoluene **I** to enamine **II** by heating it with *N*,*N*-dimethylformamide dimethylacetal (DMF/DMA) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), followed by reductive cyclization of the intermediate nitroenamine **II** with titanium chloride or zinc and ammonium chloride to give *N*-hydroxyindole **III**; 2) oxidation of 2,3-dihydroindoles **IV** in methanol/water with H_2O_2 in the presence of a catalytic amount of sodium tungstate (Fig. **3.27**).

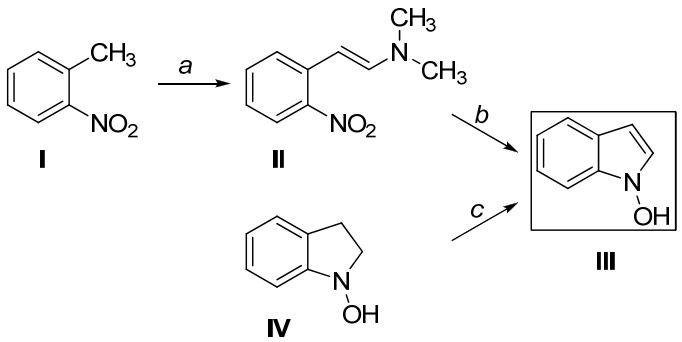


Figure 3.27. a) DMF/DMA, DBU; b) $TiCl_3$ or Zn, NH_4Cl, H_2O/Et_2O; c) H_2O_2 30%, $Na_2WO_4 2H_2O$, H_2O/MeOH.

Nevertheless, the instability of the so obtained 1-hydroxyindoles still continued to be the main obstacle to their isolation. 1-Hydroxyindole is so reactive, that it has only been obtained in solution, polimeryzating to a green powder on attempted isolation. Substitutions of the indole nucleus with electron withdrawing or resonance stabilizing groups, methylation of the 1-OH group, insertion of bulky groups in position 3 or substituents in position 2 able to form hydrogen-bonds with the *N*-hydroxyl group, are all factors that contribute to the increase in stability of the molecule. For this reason, *N*-hydroxyindole-2-carboxylic acids (Fig. **3.24**) can be readily obtained and isolated, being relatively stable thanks to the hydrogen bond between the hydrogen atom of the 1-OH group as H-bond donor and the oxygen atom of the 2-carbonyl group as H-bond

acceptor or, in the case of its tautomer *3H*-indole 1-oxide, which is less representative than its non-charged counterpart, between the H atom of the carboxylic group and the oxygen of N-O group (Fig. **3.28**).

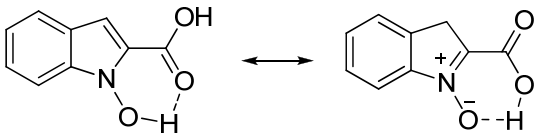


Figure 3.28. Intramolecular hydrogen bonds in *N*-hydroxyindole-2-carboxylic acids.

During the last decade, K.C. Nicolaou has directed his attention to the synthesis of substituted *N*-hydroxyindole-2-carboxylic acids, reporting a new synthetic procedure for their construction that was applied to the total synthesis of Nocathiacin I, whose structure contains a NHI domain [90-92]. The synthetic scheme adopted by Nicolaou is showed in Fig. **3.29**. Commercially available nitrotoluene **I** is alkylated with sodium hydride and dimethyl oxalate. Treatment of ketoester **II** with Eschenmoser's salt (*N*,*N*-dimethyleneiminium chloride) in the presence of NaH lead to α,β-unsaturated nitroester **III**, which is cyclized using $SnCl_2$ and, then, the so-obtained reactive nitrone **IV** is immediately subjected to 1,5-addition by a nucleophile to afford *N*-hydroxyindole **V** substituted at the 3-position.

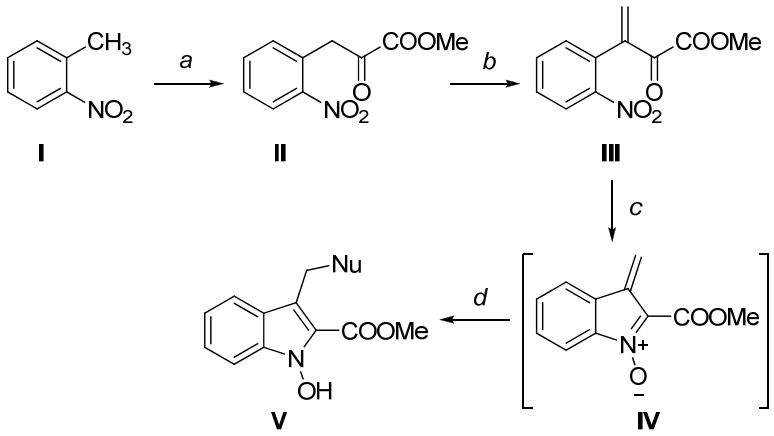


Figure 3.29. a) NaH, $(COOMe)_2$, DMF; b) NaH, $CH_2{=}NMe_2^+Cl^-$, THF; c, d) $SnCl_2\,2H_2O$, MS 4Å, NuH, DME. MS = molecular sieves; NuH = nucleophile; DME = 1,2-dimethoxyethane.

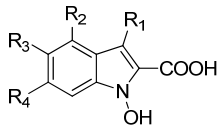
A recent paper by Penoni *et al.* [93] reports a completely new method for NHI construction, via a cylcoaddition of nitrosoarenes and alkynes. Anyway, at the present, the Nicolaou's way of synthesis represents the most suitable method to generate *N*-hydroxyindoles with a carboxylic group in position 2 of the nucleus. Consequently, we followed the Nicolaou's procedure for the synthesis of our new NHI derivatives, making only minor adjustments when necessary.

3.6. *N*-HYDROXYINDOLES SUBSTITUTED WITH SMALL GROUPS

3.6.1. Synthesis

We started to synthesize compounds belonging to NHI class, selecting a series of exploratory substituents in order to test if it was possible to obtain satisfactory levels of LDH-A inhibition using this scaffold. We chose several small substituents such as halogens, methyl, ethyl, cyano, trifluoromethyl and carboxy group and also small heterocycles such as tetrazole and oxadiazolone group, that are carboxylic acid isosters.

Compounds **3.89a-p** (Fig. **3.30**) were synthesized according to the general synthetic procedure showed in Scheme **3.1**. Some starting materials were commercially available (opportunely substituted *o*-nitrotoluenes **3.90a-l** or 1-ethyl-2-nitrobenzene **3.90m** and 1-nitro-2-propylbenzene **3.90n**) and others were appropriately synthesized (**3.90o-p**, see Scheme **3.2**). These *ortho*-alkyl-nitroaryl precursors were alkylated with an excess of dimethyl oxalate and sodium hydride in dry DMF at –15 °C to room temperature [91], then the so obtained ketoesters **3.91a-n** were subjected to a reductive cyclization process with stannous chloride dihydrate in the presence of 4Å molecular sieves in anhydrous DME to afford methyl ester *N*-hydroxyindoles **3.92a-n** [92], which were finally hydrolyzed in the dark with a 2N aqueous solution of litium hydroxide in a MeOH/THF mixture, until the disappearance of the precursors on TLC, to give final products **3.89a-n** [94].



3.89a-p

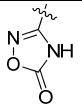
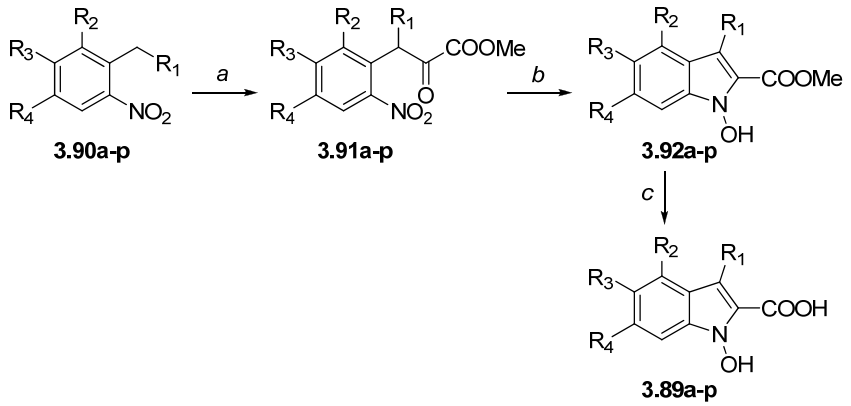
Cpd	R_1	R_2	R_3	R_4
3.89a[a]	H	H	H	H
3.89b[a]	H	Br	H	H
3.89c[a]	H	Cl	H	H
3.89d[a]	H	H	H	Br
3.89e[a]	H	CH_3	H	H
3.89f	H	H	F	H
3.89g	H	H	COOH	H
3.89h	H	H	H	F
3.89i	H	H	CN	H
3.89j	H	H	H	CN
3.89k[a]	H	F	H	H
3.89l[a]	H	CF_3	H	H
3.89m[a]	CH_3	H	H	H
3.89n[a]	CH_2CH_3	H	H	H
3.89o	H	H	H	
3.89p	H	H	H	

Figure 3.30. First exploratory *N*-hydroxyindoles **3.89a-p**; [a] compounds **3.89a-e** and **3.89k-n** were previously synthesized during experimental graduation thesis in the same research laboratory.



Scheme 3.1. a) $(COOMe)_2$, NaH 60%, dry DMF, –15 °C to RT; b) for **3.91a-n**: $SnCl_2 \cdot 2H_2O$, dry DME, 4Å MS, 0 °C to RT; for **3.91o-p**: $H_2PO_2Na \cdot H_2O$, Pd-C 10%, H_2O/THF (1:1), RT; c) aqueous 2N LiOH, THF/MeOH (1:1), RT.

Synthesized precursors **3.90o** and **3.90p** were submitted to the same synthetic steps as compounds **3.90a-n**, with the exception of the reductive cyclization reaction (step *b* in Scheme **3.1**). In these two cases, the chosen conditions ($SnCl_2$ and DME) did not promote only the proper cyclization to build the NHI scaffold, but they also gave a mixture of *N*-hydroxyindole and the over-reduced side product indole. In fact, the over-reduction of the nitro group to amine (*path b*, Fig. **3.31**) leads to the formation of indole and this side reaction is contemporaneous with the reduction to hydroxylamine that produces NHI (*path a*, Fig. **3.31**). These mixtures lowered the yields of the cyclization step and they were of difficult separation either by crystallization or column chromatography, due to the very similar R_f values of the formed products. Anyway, NHI and the corresponding indole can be easily detected on TLC, by the typical fluorescence of the indole stain visible at the wavelength of 254 nm and by staining the TLC with a solution of copper sulfate in a MeOH/water mixture: Cu^{2+} complexes with the N-OH group, resulting in a violet spot on TLC plate which clearly indicates the presence of the *N*-hydroxy compound. For these two substrates we decided to change conditions by using sodium hypophosphite monohydrate and catalytic palladium over charcoal in a mixture H_2O/tetrahydrofuran: this reducing system had already been successfully utilized in the past for the selective reduction of nitro-groups to hydroxylamines, but it was not used for the preparation of *N*-hydroxyindole systems

like ours. Exploiting this reducing system, we successful got the desired NHI **3.92o-p** and, in other cases, this method proved to be effective in reducing the amounts of the over-reduced side products, when compared to other conditions (see following schemes) [95, 96]. Although in most cases the separation of the desired NHI compound from the indole analogue, or the use of alternative reaction conditions were successful in obtaining the pure product, some compounds, such as for example compounds **3.89g** and **3.89i**, were obtained in mixture with a certain (low) percentage of the correspondent indole analogue. Besides, for the ketoester **3.91o-p** formation, we doubled the equivalents of both sodium hydride and dimethyl oxalate (8 eq of NaH and 10 eq of $(COOMe)_2$), due to the presence of the carboxylic acid bioisosters present on the molecules which can consume NaH in addition to that needed for the proper functionalization of the toluenic methyl groups.

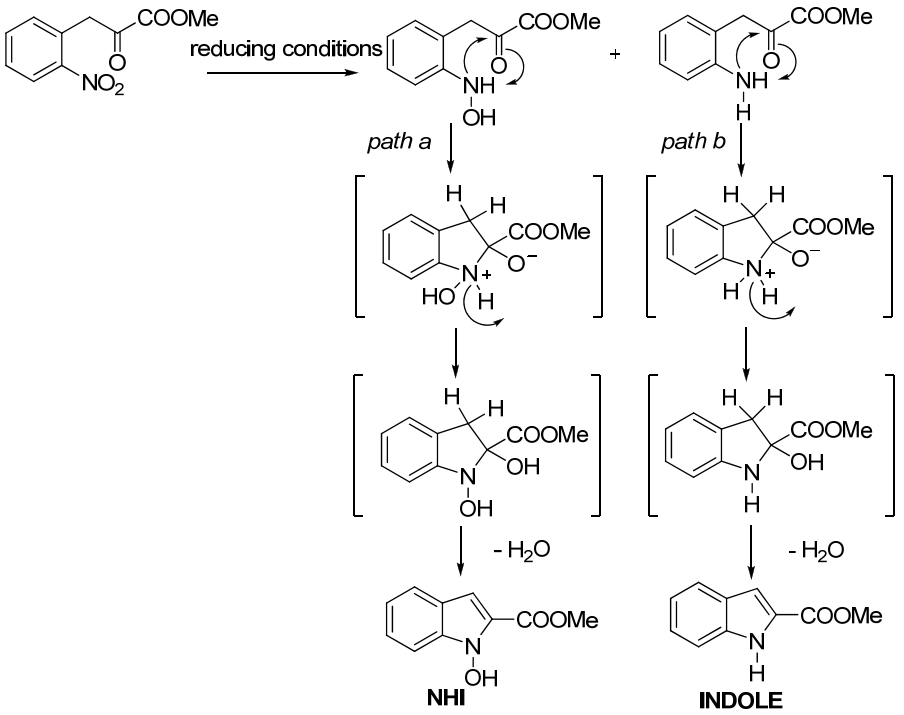
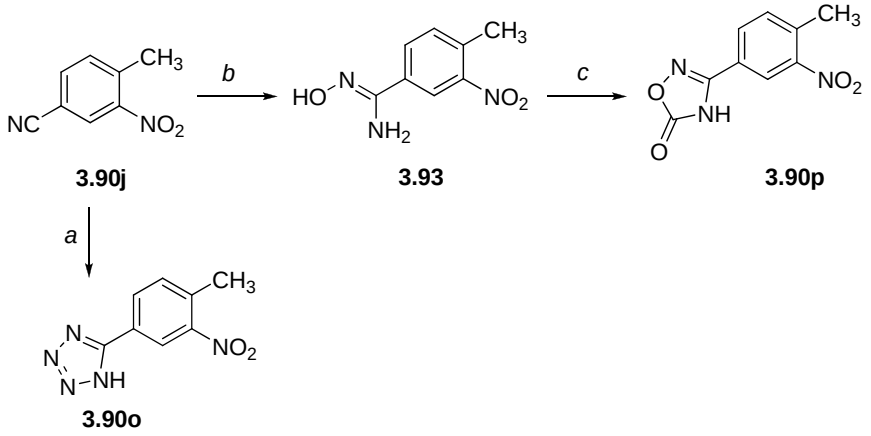


Figure 3.31. Mechanism of reductive cyclization (*path a*) and formation of the indolic by-product (*path b*).

As for compound **3.89o-p**, precursors **3.90o** and **3.90p** were respectively prepared by a zinc-promoted addition of sodium azide to benzonitrile **3.90j** in water [97] and by formation of amidoxime **3.93** from benzonitrile **3.90j** with hydroxylamine hydrochloride and diisopropylethylamine (DIEA) and its subsequent cyclization with DBU and 1,1-carbonildiimidazolo (CDI) (Scheme **3.2**) [98].While tetrazole is a common carboxylic acid bioisostere [99], 1,2,4-oxadiazol-5-one is a less commonly used acidic heterocycle, with physico-chemical properties that are slightly different from those of the carboxylic group, which may lead to an improved bioavailability of compounds bearing this moiety [100]. In spite of these small synthetic problems requiring optimizations, the introduction on the NHI nucleus of an additional carboxylic group (**3.89g**) as well as carboxylic acid bioisosteres (**3.89o-p**) was encouraged by the observation of the X-ray structure of the LDH-A active site (see docking sections below), which normally hosts the substrate pyruvate or lactate and the cofactor NADH, so this cavity is rich in cationic residues such as arginines, which may give rise to favourable attractive interactions with deprotonatable groups of perspective inhibitors.



Scheme 3.2. a) NaN_3, $ZnBr_2$, H_2O, reflux; b) DIEA, $NH_2OH \cdot HCl$, abs. EtOH, reflux; c) DBU, CDI, 1,4-dioxane, reflux.

In the ketoester formation (step *a*, Scheme **3.1**), the colour of the reaction mixture is indicative of the progress of the reaction. After a certain time, depending on the substrate, it is possible to observe the development of an intense colour, varying from

cherry red to violet-blue, due to the deprotonated enolic form of the ketoester **III** (Fig. **3.32**) that is present in the basic medium of the reaction, and to the possibility of delocalizing the negative charge on the substituted phenyl ring. Hence, the development of that colour suggests that the formation of the desired product is taking place.

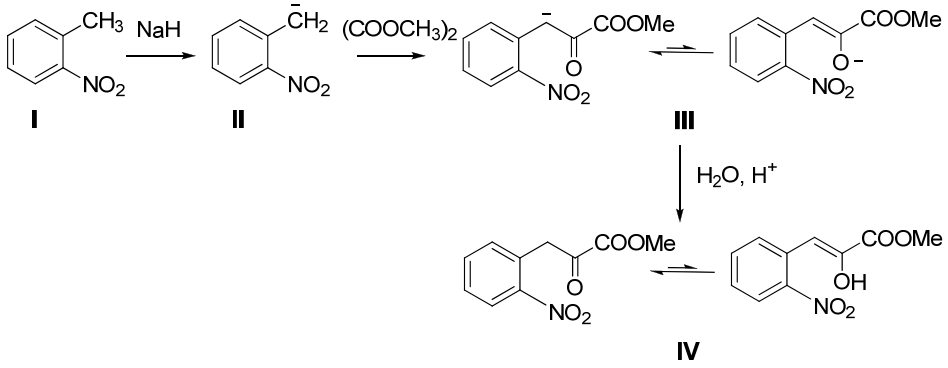


Figure 3.32. Mechanism of ketoester formation.

3.6.2. Enzymatic assays

Methods. All the synthesized compounds were assayed on human LDH-A (as LDH-5, or LDH-A_4) and LDH-B (as LDH-1, or LDH-B_4) purified isoforms, by the research group of Prof. G. Giannaccini at the University of Pisa.

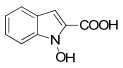
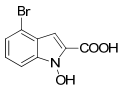
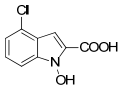
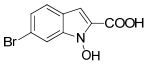
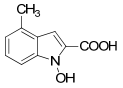
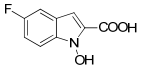
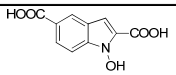
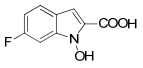
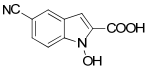
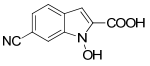
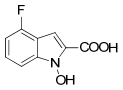
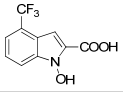
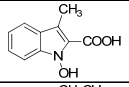
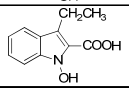
A spectrophotometric assay was used to monitor the rate of the decrease of the absorbance of NADH to NAD^+ at 340 nm, in the absence of interfering reactions, running the reaction in the forward direction (pyruvate $\rightarrow$ lactate). Assays were performed at 37 °C, with all reagents (the enzyme, the cofactor NADH, the substrate pyruvate and the candidate inhibitor) dissolved in 0.1 M sodium phosphate buffer solution (NaH_2PO_4 and Na_2HPO_4), at pH 7.4. Compounds, tested in concentration range = 25-100 μM, were initially dissolved in stock solutions of DMSO (concentrations of DMSO during the initial rate measurements did not exceed 0.5%).

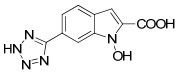
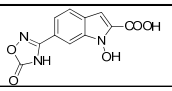
In the initial screening, using 25 μM NADH and 2 mM sodium pyruvate, we evaluated the percentual inhibition of the compounds, then additional kinetic studies were carried out only on compounds that showed a $\geq$ 50% inhibition. The LDH inhibitory activities of the compounds that resulted promising from the first "single

point" analysis were measured by standard enzyme kinetic experiments. Kinetic parameters for pyruvate and NADH were obtained, adding 0.005 units of LDH-A in 100 mM sodium phosphate buffer, performing the assays in pyruvate-saturating conditions (2 mM sodium pyruvate and 6.25 μM to 150 μM NADH), in order to verify the competition with NADH, or in NADH-saturating conditions (200 μM NADH and 25 μM to 2 mM sodium pyruvate), to check the ability of the compounds to compete with the natural enzyme substrate. Hence, K_i values for each single inhibitor were determined using double-reciprocal plots (Lineweaver-Burk plots). LDH-B enzyme activity was measured for compounds displaying significant inhibition properties on the desired A isoform, only in pyruvate-saturating conditions.

Results and discussion. Enzymatic results derived from the simplest NHI derivatives substituted with a series of exploratory groups **3.89a-p** (Section 3.6.1) are shown in Table **3.12**. The smallest member of this group, the unsubstituted derivative **3.89a**, showed a very modest but encouraging inhibition on both isoforms (16% on LDH-5 at 250 μM, and 22% on LDH-1 at 125 μM), due to the wide possibility of further modifying and developing this scaffold. The introduction of halogens in various positions gave poor results (**3.89c**, **d**, **h**, **k**) or provoked the total loss of activity (**3.89f**), with the exception of the 4-bromosubstituted compound **3.89b**, which almost reaches a 50% inhibition, but the shift of the bromine atom to position 6 (**3.89d**) deeply decreases the inhibition value. Cyano, methyl, trifluoromethyl substitutions, as well as carboxylic group and COOH-mimicking heteroaryl portion did not significantly improve the inhibition on the A isoform, when compared to the unsubstituted analogue **3.89a**. On the other hand, observing the inhibition percentages of the 3-susbtituted compounds **3.89m** and **3.89n**, we can suppose that bulky groups in this position of the heterocycle are not well tolerated, due to the reduction in activity when the methyl group (**3.89m**) is replaced by the ethyl (**3.89n**), although small groups may be tolerated. As for the B isoform, compounds **3.89c**, **h** and **i** showed a trend similar to compound **3.89a**, displaying a modest activity also on this isoform, but most of the substituted derivatives completely lost the activity on LDH-1. The inhibition level (K_i = 275 μM) reached by 3-CH_3-substituted derivative **3.89m**, is noteworthy, since it represents the first of our compounds to be slightly selective for LDH-1.

Table 3.12. Inhibition data on LDH-5 (LDH-A_4) and LDH-1 (LDH-B_4) for compounds **3.89a-p**.

Cpd	Structure	Inhibition values	
		LDH-5[a]	LDH-1[b]
3.89a[d]		16%	22%
3.89b[d]		48%	n.a.
3.89c[d]		17%	14%
3.89d[d]		22%	n.a.
3.89e[d]		26%	n.a.
3.89f		n.a.	n.a.
3.89g	 10% indole[e]	6%	n.a.
3.89h		13%	20%
3.89i	 30% indole[e]	8%	18%
3.89j		n.a.	n.a.
3.89k[d]		5%	n.a.
3.89l[d]		10%	n.a.
3.89m[d]		18%	K_i = 275 μM (NADH)
3.89n[d]		13%	n.a.

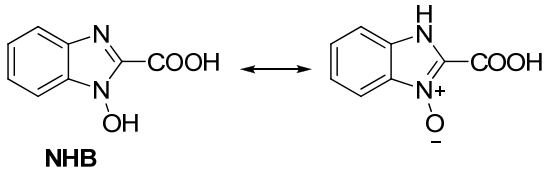
3.89o		15%	n.a.
3.89p		13%[c]	n.t.

[a] compounds tested at 250 μM, [b] compounds tested at 125 μM, [c] compounds tested at 100 μM, [d] compounds **3.89a-e** and **3.89k-n** were previously synthesized by undergraduate students during experimental thesis in the same research laboratory, [e] compounds tested as a mixture with the reported percentage of indole. n.a. = not active, n.t. = not tested.

3.7. N_1-HYDROXYBENZIMIDAZOLES AND N_1-HYDROXYBENZIMIDAZOL-N_3-OXIDES AS LDH-A INHIBITORS

3.7.1. Synthesis

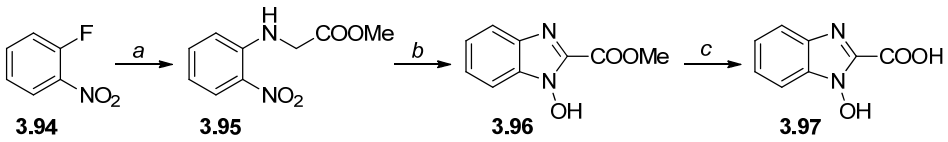
After the synthesis of this first series of *N*-hydroxyindoles, we decided to explore different heterocycles with an high degree of similarity with NHI cycle. In particular, we decided to introduce a nitrogen atom in position 3 of the NHI heterocycle, so creating N_1-hydroxybenzimidazoles (**NHB**) as new target molecules (Fig. **3.33**). This kind of structure maintains the pharmacophoric carboxylic group in position 2 and the hydroxyl moiety linked to the heterocyclic nitrogen in adjacent position, as for the NHI class, but the introduction of a second nitrogen atom in the cycle opens up the way for new possible interactions, in particular hydrogen bonds, with residues present in the enzyme active site.



NHB

Figure 3.33. N_1-hydroxybenzimidazole structure (generally abbreviated **NHB**, on the left). Its 1*H*-benzimidazole 3-oxide tautomer is displayed (on the right).

Such kind of structure had been previously reported for purposes that are completely different from our intents [101]. In my PhD Thesis, we synthesized the non substituted NHB **3.97** following a straightforward procedure previously reported for other amino-substituted N_1-hydroxybenzimidazoles (Scheme **3.3**) [102]. Commercially available 1-fluoro-2-nitrobenzene **3.94** was heated to reflux with glycine methyl ester hydrochloride in methanol in the presence of sodium bicarbonate to afford the *N*-arylglycinic derivative **3.95**. The choice of using methanol instead of ethanol as the solvent derived from the results obtained in a previous attempt, when the same reaction performed in ethanol caused the formation of the ethyl ester product, derived from a transesterification side reaction. Intermediate **3.95** was treated with a freshly prepared solution of sodium methoxide in methanol to cyclize it to benzimidazole **3.96** [103]. Final hydrolysis of the methyl ester with an aqueous solution of lithium hydroxide yielded the desired product **3.97**.

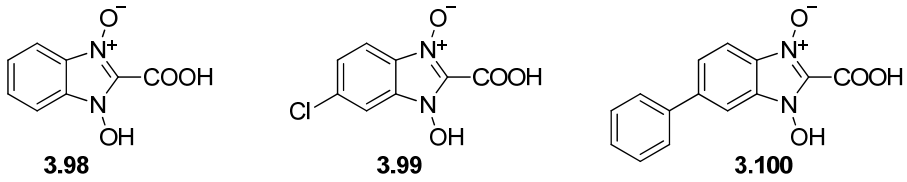


3.94 **3.95** **3.96** **3.97**

Scheme 3.3. a) $H_3N^+CH_2COOMeCl^-$, $NaHCO_3$, MeOH, reflux; b) NaOMe, MeOH, RT; c) aqueous 2N LiOH, THF/MeOH (1:1), RT.

N_1-hydroxybenzimidazol-N_3-oxide (**NHO**) derivatives resemble the precedent NHB class, but the *N*-oxide functionality introduces a new polar moiety in the structure, which can lead to new potential binding interactions with the enzyme. Thanks to the availability of the commercial substrates and the easy synthetic routes, the reference non

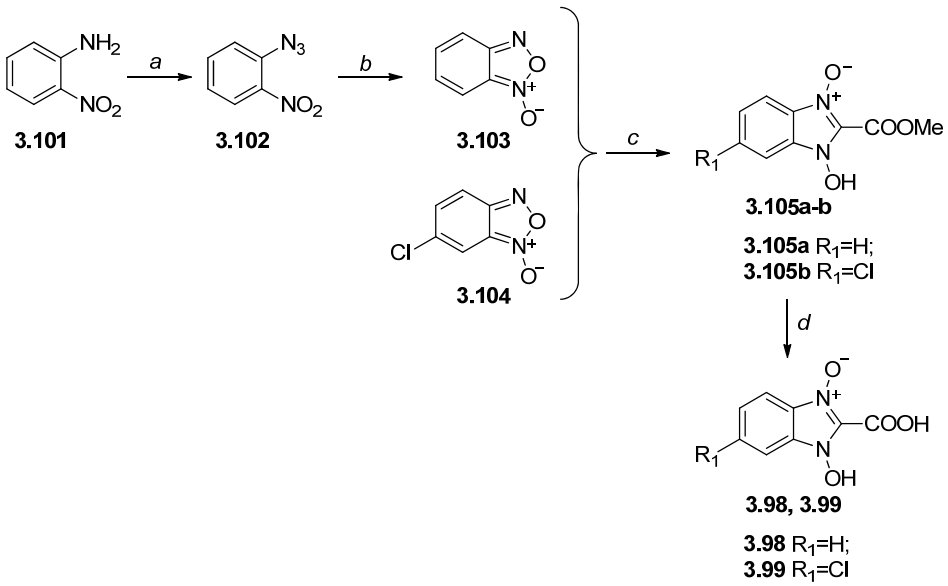
substituted N_1-hydroxybenzimidazol-N_3-oxide **3.98**, the 6-chloro and the 6-phenyl derivatives **3.99** and **3.100**, respectively, were synthesized with the aim to explore the effect of substituents of this nucleus on the enzyme inhibitory properties (Fig. **3.34**).



3.98 **3.99** **3.100**

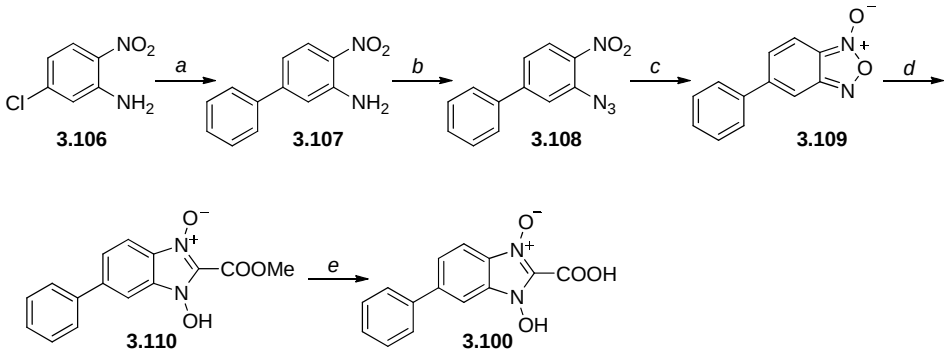
Figure 3.34. N_1-hydroxybenzimidazol-N_3-oxide (**NHO**) derivatives **3.98-3.100**.

Benzofuroxan derivative **3.103** was prepared by cyclocondensation in boiling toluene of nitrophenyl azide **3.102**, which could be easily obtained from 2-nitroaniline **3.101** [104], whereas the precursor 5-chlorobenzofuran-3-oxide **3.104** was commercially available (Scheme **3.4**). Benzofuroxans **3.103** and **3.104** were then transformed into the corresponding 2-methyl ester N_1-hydroxybenzimidazol-N_3-oxides by following two different procedures previously developed for similar compounds. Compound **3.103** was stirred at room temperature with methyl phenylsulfonlyacetate in a methanolic solution of potassium hydroxide to yield **3.105a** [105]. Unfortunately, the same attempt with this reactant for **3.104** was fruitless, even after heating, so in this latter case we preferred to change conditions using methyl nitroacetate with diethylamine, and these conditions successfully afforded compound **3.105b** [106]. Then, for both intermediates, alkaline hydrolysis of their ester groups produced final compounds **3.98** and **3.99**.



Scheme 3.4. a) $NaNO_2$, NaN_3, aqueous HCl, H_2O, -5/0 °C to RT.; b) toluene, reflux; c) for **3.103**: $PhSO_2CH_2COOMe$, methanolic 8% KOH, MeOH, RT; for **3.104**: $CH_3COOCH_2NO_2$, Et_2NH, THF, RT; d) aqueous 2N LiOH, THF/MeOH (1:1), RT.

The last compound of this structural class, phenyl-substituted derivative **3.100**, was prepared by reacting 5-chloro-2-nitroaniline **3.106** with benzeneboronic acid using the cross coupling conditions developed by Greg Fu [107], comprising tris(dibenzylideneacetone)dipalladium as the catalyst with tricyclohexylphosphine as the ligand, that are suitable for the less reactive aryl chlorides (Scheme **3.5**). From this point, the phenyl nitroaniline **3.107** followed the same synthetic scheme described for compound **3.99**, with the use of the opportunely substituted nitroalcane in diethylaniline for the formation of the cycle, although this final synthetic step was characteized by a considerably low efficiency. Previously, we had tried the insertion of the phenyl ring directly on the commercially available 5-chlorobenzofuran-3-oxide **3.104**, to decrease the number of reactions steps and speed up the synthesis of the desired compound, but the chlorobenzofuroxan did not react properly under these conditions, so we decided to insert the phenyl ring on the simpler precursor, as shown in Scheme **3.5**.



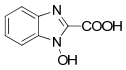
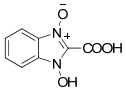
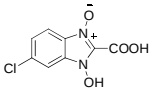
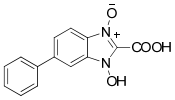
Scheme 3.5. a) $C_6H_5B(OH)_2$, Cs_2CO_3, $Pd_2(dba)_3$, Pcy_3, dry 1,4-dioxane, 80 °C; b) $NaNO_2$, NaN_3, aqueous HCl, H_2O, -5/0 °C to RT.; c) toluene, reflux; d) $CH_3COOCH_2NO_2$, Et_2NH, THF, RT; e) aqueous 2N LiOH, THF/MeOH (1:1), RT.

3.7.2. Enzymatic assays

The preliminary exploration of new similar *N*-hydroxy-based heterocycles has so far proved to be fruitless, as shown by the results reported in Table **3.13**. In particular, unsubstituted N_1-hydroxybenzimidazole **3.97** and N_1-hydroxybenzimidazol-N_3-oxide **3.98** were completely deprived of any inhibitory activity. Even the 6-phenyl N_1-hydroxybenzimidazol-N_3-oxide **3.100** showed no detectable inhibition of LDH-A. Compound **3.99**, bearing a chlorine atom in position 6, displayed an almost undetectable weak inhibition of the LDH-A isoform, which is barely above baseline (about 2% at 250 μM), whereas it showed a certain inhibition of the heart isoform (17% at 125 μM).

Overall, this preliminary screening did not show any promising results associated to the NHB and NHO derivatives herein reported.

Table 3.13. Inhibition data on LDH-5 (LDH-A_4) and LDH-1 (LDH-B_4) for compounds **3.97-3.100**.

Cpd	Structure	Inhibition values	
		LDH-5[a]	LDH-1
3.97		n.a.	-
3.98		n.a.	-
3.99		2%	17%[b]
3.100		n.a.	-

[a] compounds tested at 250 μM, [b] compounds tested at 125 μM. n.a. = not active

3.8. ARYL-SUBSTITUTED *N*-HYDROXYINDOLES

3.8.1. Synthesis

The preliminary biological results obtained from N_1-hydroxyindole, N_1-hydroxybenzimidazole and N_1-hydroxybenzimidazol-N_3-oxide classes (see Sections 3.6 and 3.7) demonstrated that *N*-hydroxyindole derivatives proved to be the more promising structures to be developed as LDH-A inhibitors, so we decided to focus our attention on this class. After the introduction of small groups on the NHI nucleus, we further developed this scaffold by inserting non-substituted phenyl rings in different positions, so obtaining the first aryl-substituted compounds (mono-aryl derivatives **3.111-3.113** and di-aryl derivative **3.114**, Fig. **3.35**). 3-Phenyl-substituted-*N*-hydroxyindole was not synthesized because of synthetic difficulties and of a presumable low affinity for the LDH-A active site since this portion of the inhibitor is hosted by the substrate pocket (see below) which is rather small and, therefore, is not able to fit large phenyl ring. As for position 7, our attempts to introduce a phenyl ring did not afford the

desired product, due to its high instability, in fact during the last step of hydrolysis of the methyl ester the compound polymerized and it was impossible to get the final product.

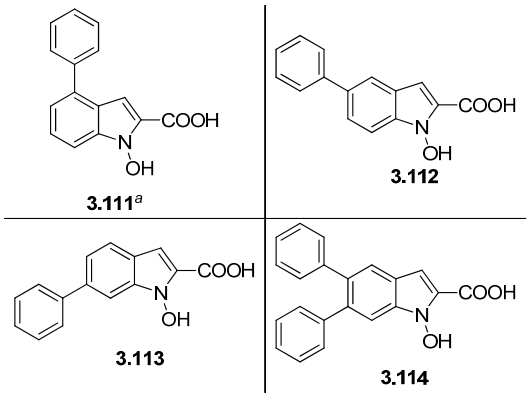
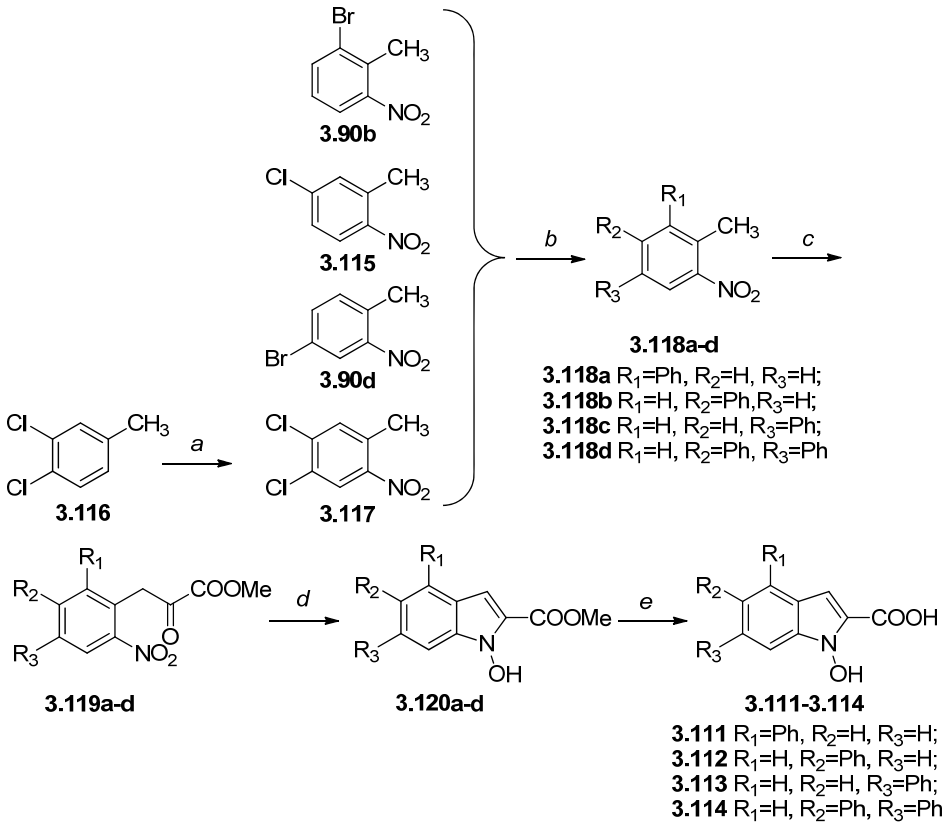


Figure 3.35. Aryl-substituted compounds **3.111-3.114**; [a] compound **3.111** was previously synthesized during experimental graduation thesis in the same research laboratory.

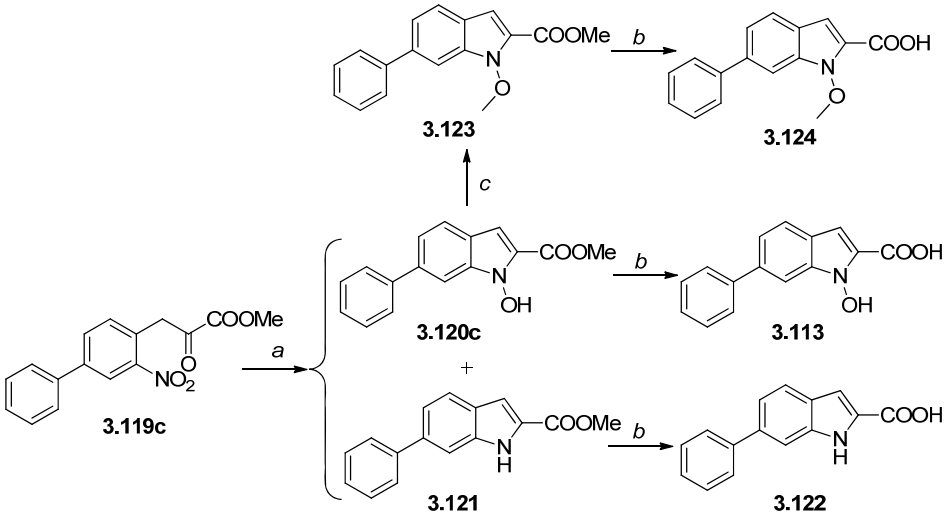
In the first step of the synthesis, halogenated precursors **3.90b**, **3.90d** and **3.115** were subjected to a cross-coupling reaction with phenylboronic acid using the classical thermal Suzuki conditions [108] to get phenyl-substituted nitrotoluenes **3.118a-c** (Scheme **3.6**). Compounds **3.117**, which was obtained by nitration with potassium nitrate in concentrated sulfuric acid from the commercially available 3,4-dichlorotoluene **3.116** [109], was subjected to slightly different cross-coupling conditions, using the phase transfer catalyst tetrabutylammonium bromide, potassium phosphate as the base, long reaction times (48 h) and higher temperatures (125 °C) [110], in order to obtain the double substitution, because previous attempts under milder reaction conditions (Suzuki) afforded a mixture of the inseparable mono- and di-phenyl substituted derivatives. For the reduction-promoted cyclization of compound **3.119c** and **3.119d**, we exploited synthetic procedures different from those previously described (ipophosphite with palladium and stannous chloride in DME), because we experienced that the choice of the best reaction conditions strongly depends on the structure of the substrate we were going to cyclize. In fact, several attempts were necessary to find out

the most suitable reducing agent in each case. In particular, for **3.119c** we used stannous chloride in the presence of tiophenol and triethylammine: PhSH and the basic conditions slightly decrease the reductive ability of bivalent tin, thus counteracting the exhaustive reduction of the nitro group to amine; this adjustment proved to reduce the indole formation and, therefore, properly promote the partial reduction/cyclization step which furnished the desired product [111]. In the same synthetic step run on **3.119d**, we found out that metallic lead in the presence of a triethylammonium formate (TEAF) buffer solution in methanol permitted an optimal reaction outcome [112].



Scheme 3.6. a) KNO_3, conc. H_2SO_4, 5 °C to RT; b) $C_6H_5B(OH)_2$, $Pd(OAc)_2$, PPh_3, aqueous 2M Na_2CO_3, distilled toluene, abs. EtOH, 100 °C, 24 h; for **3.117**: $Pd(OAc)_2$, TBAB, K_3PO_4, H_2O, 125 °C, 48 h; c) $(COOMe)_2$, NaH 60%, dry DMF, –15 °C to RT; d) for **3.119a**: $SnCl_2{\cdot}2H_2O$, dry DME, 4Å MS, 0 °C to RT; for **3.119b**: $H_2PO_2Na{\cdot}H_2O$, Pd-C 10%, H_2O/THF (1:1), RT; for **3.119c**: $SnCl_2{\cdot}2H_2O$, C_6H_5SH, Et_3N, CH_3CN, RT; for **3.119d**: Pb, TEAF, MeOH, 55 °C; e) aqueous 2N LiOH, THF/MeOH (1:1), RT.

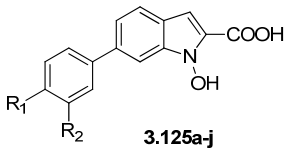
During precedent efforts for the cyclization of compound **3.119c**, using $SnCl_2$ and DME, predominant amounts of indoles were obtained (34:66 for **3.120c**/**3.121**, Scheme **3.7**). In that case, we separated NHI **3.120c** from its indole analogue **3.121** by column chromatography; they were separately hydrolyzed to get also the indole-carboxylic derivative **3.122** (Scheme **3.7**). The methyl ester **3.120c** and the indole derivative **3.122** were used in biological assays as valuable tools to establish the importance of the pharmacophoric portions, COOH and OH respectively, needed for LDH-A inhibition. With the same aim, an example of *O*-methylated NHI (**3.124**) was also synthesized [95], to verify the importance of the free OH group in the enzyme inhibition assays. To this purpose, NHI **3.120c** was treated with iodomethane and DBU in THF to give ester **3.123**, which was finally hydrolyzed to compound **3.124**.



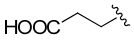
Scheme 3.7. a) $SnCl_2 \cdot 2H_2O$, dry DME, 4Å MS, 0 °C to RT; b) aqueous 2N LiOH, THF/MeOH (1:1), RT; c) CH_3I, DBU, THF, RT.

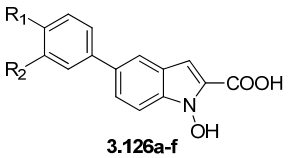
Afterwards, we were particularly attracted by the effects that could be generated by the insertion of variously substituted aryl rings. Hence, we began to introduce substituents on the phenyl rings, with a particular focus on 5- and 6-phenyl-substituted derivatives, encouraged by the promising biological results obtained with compounds

3.112-3.114 (see enzymatic assay Section below). We inserted various groups possessing different electronic and steric properties, such as halogens, methoxy, trifluoromethoxy, trifluoromethyl, carboxy (directly bound to the phenyl ring or with an ethylene chain linker) and methylsulfonyl groups, in *meta* and *para* positions of the phenyls, with the aim to increase the interactions of our inhibitors (**3.125a-j**, **3.126a-f**, Fig. **3.36**) with the LDH-A binding site.



3.125a-j

Cpd	R_1	R_2
3.125a	COOH	H
3.125b	H	COOH
3.125c		H
3.125d		H
3.125e	OCH_3	H
3.125f	H	OCF_3
3.125g	OCF_3	H
3.125h	CF_3	H
3.125i	F	H
3.125j	Cl	H



3.126a-f

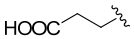
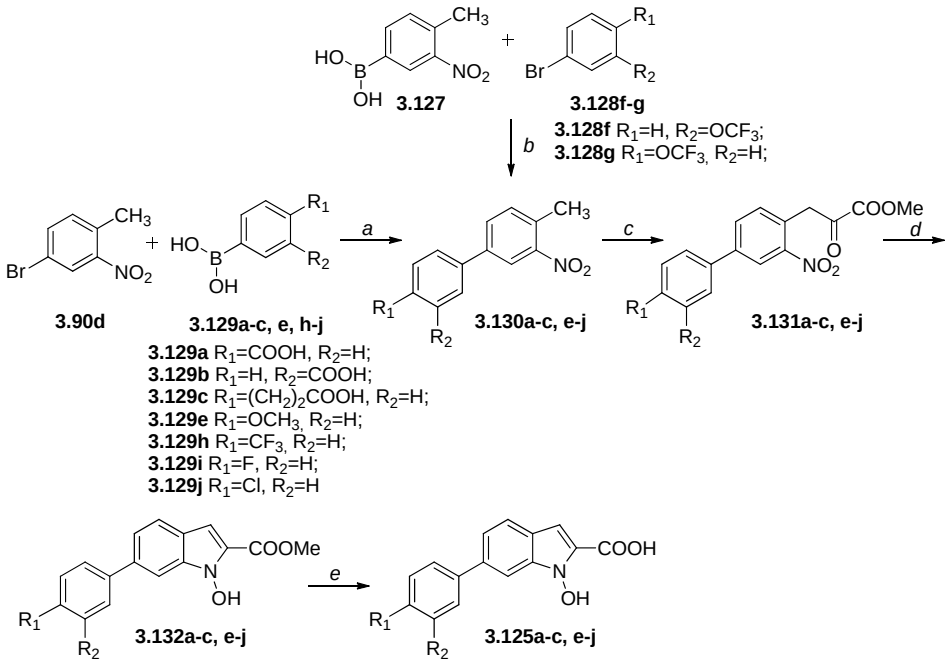
Cpd	R_1	R_2
3.126a	H	COOH
3.126b		H
3.126c	OCH_3	H
3.126d	CF_3	H
3.126e	F	H
3.126f	Cl	H

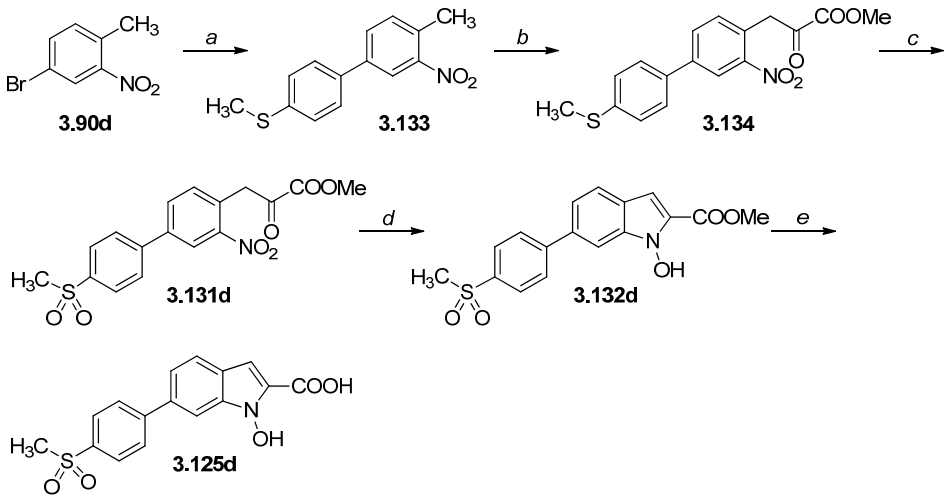
Figure 3.36. Structures of 6- and 5-phenyl-substituted compounds **3.125a-j** and **3.126a-f**, respectively.

The same synthetic scheme (ketoester formation, reduction-promoted cyclization and hydrolysis) was followed also in the synthesis of these two series of compounds, including 6-aryl-substituted compounds **3.125a-j** (Scheme **3.8-3.9**) and 5-aryl-substituted compounds **3.126a-f** (Scheme **3.10**). All the halogenated precursors **3.90d**, **3.115** and the nitrotoluenic boronic acid **3.127** ("umpolung" reagent) were subjected to

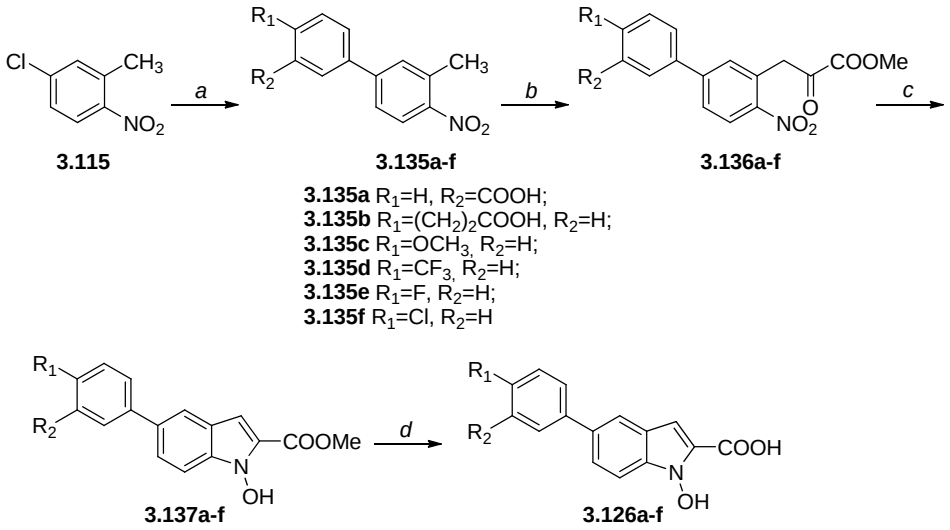
cross-coupling reactions using classical conditions or the more advantageous microwave-assisted Suzuki coupling for the significantly reduction of the reaction time (classical method: 24 h, microwave-assisted coupling: 5 to 20 minutes) [113, 114]. For the synthesis of **3.125d** (Scheme **3.9**), coupling reaction with 4-(methylthio)-benzeneboronic acid was followed by the ketoester **3.134** formation with NaH and $(COOMe)_2$ and, subsequently, the oxidation of methylthio- to methyl-sulphonic group was performed by using oxone as oxidant in dioxane [115]. This sequence of steps was optimized after the unsuccessful attempt of converting the methyltio moiety before the alkylation step: in that case we obtained not only the proper formation of the ketoester chain, but also the alkylation of the methyl-sulfonyl group. We supposed that the presence of two S=O groups linked to the methyl group increases the acidity of the methyl protons, so promoting the removal of a proton by sodium hydride and the subsequent attack by dimethyl oxalate.



Scheme 3.8. a) for **3.129a-b-e**: $Pd(OAc)_2$, PPh_3, aqueous 2M Na_2CO_3, distilled toluene, abs. EtOH, μW, 20 min.; for **3.129c**: $Pd(OAc)_2$, PPh_3, aqueous 2M Na_2CO_3, distilled toluene, abs.EtOH, 100 °C, 24 h; for **3.129h-i**: $Pd(OAc)_2$, TBAB, K_3PO_4, H_2O, 125 °C, 24 h; for **3.129j**: $Pd(OAc)_2$, TBAB, Na_2CO_3, H_2O, μW, 5 min.; b) $Pd(OAc)_2$, TBAB, Na_2CO_3, H_2O, μW, 5 min.; c) $(COOMe)_2$, NaH 60%, dry DMF, –15 °C to RT; d) for **3.131a-b-e**: $SnCl_2 \cdot 2H_2O$, dry DME, 4Å MS, 0 °C to RT; for **3.131c**: $H_2PO_2Na \cdot H_2O$, Pd-C 10%, H_2O/THF (1:1), RT; for **3.131f-g-h-i-j**: $SnCl_2 \cdot 2H_2O$, C_6H_5SH, Et_3N, CH_3CN, RT; e) aqueous 2N LiOH, THF/MeOH (1:1), RT.



Scheme 3.9. a) 4-(methyltio)-benzene boronic acid, $Pd(OAc)_2$, PPh_3, aqueous 2M Na_2CO_3, distilled toluene, abs. EtOH, 100 °C, 24 h; b) $(COOMe)_2$, NaH 60%, dry DMF, –15 °C to RT; c) Oxone® 50% w/w, 1,4-dioxane, 0 °C to RT; d) $SnCl_2 \cdot 2H_2O$, dry DME, 4Å MS, 0 °C to RT; e) aqueous 2N LiOH, THF/MeOH (1:1), RT.



Scheme 3.10. a) all the cross-coupling reactions were performed with appropriate substituted-phenylboronic acids; for: **3.135a-b**: $Pd(OAc)_2$, PPh_3, aqueous 2M Na_2CO_3, distilled toluene, abs. EtOH, 100 °C, 24 h; for **3.135c-d-e-f**: $Pd(OAc)_2$, TBAB, Na_2CO_3, H_2O, μW, 5 min; b) $(COOMe)_2$, NaH 60%, dry DMF, –15 °C to RT; c) for **3.136a-b**: $H_2PO_2Na \cdot H_2O$, Pd-C 10%, H_2O/THF (1:1), RT; for **3.136c**: $SnCl_2 \cdot 2H_2O$, dry DME, 4Å MS, 0 °C to RT; for **3.136d-e-f**: $SnCl_2 \cdot 2H_2O$, C_6H_5SH, Et_3N, CH_3CN, RT; d) aqueous 2N LiOH, THF/MeOH (1:1), RT.

Subsequently, we designed compounds bearing contemporarily halogenated groups (Cl, CF_3) and a phenyl on the NHI scaffold, with the aim of further stabilizing the structure and, possibly, improving the inhibitory activity of one of the most active compounds obtained up to now, that is, compound **3.113** (Fig. **3.37**), with electron-withdrawing groups on the NHI nucleus. Moreover, the choice of the target molecules was also dictated by the synthetic accessibility of the desired products. Hence, we initially synthesized derivatives **3.138** and **3.139** (Fig. **3.37**), both of them possessing a phenyl ring in position 6, together with either a trifluoromethyl in position 4, or a fluorine atom in position 5, respectively. Thanks to the very good results obtained in biological assays by compound **3.139** (see enzymatic assay Section below), we were attracted by the possibility to further develop this compound, by adding a phenyl in position 7, which was impossible to introduce on the unsubstituted NHI scaffold, due to the high instability of the structure itself, as previously explained, to afford compound **3.140** (Fig. **3.37**). Moreover, we wanted to combine in compound **3.141** (Fig. **3.37**) both the structural features of compound **3.139** and the *p*-chloro-substitution of the derivative **3.125j**, which proved to be very promising for its high LDH-A inhibition ability. We completed our design of this series of 6-phenyl-substituted NHIs with a final structural modification: the introduction in the 6-phenyl group of an additional chlorine atom in the *meta* position with respect to the already present Cl atom (compound **3.142**, Fig. **3.37**), in order to verify if the introduction of this atom could positively affect the affinity of these chloro-substituted molecules for the enzyme.

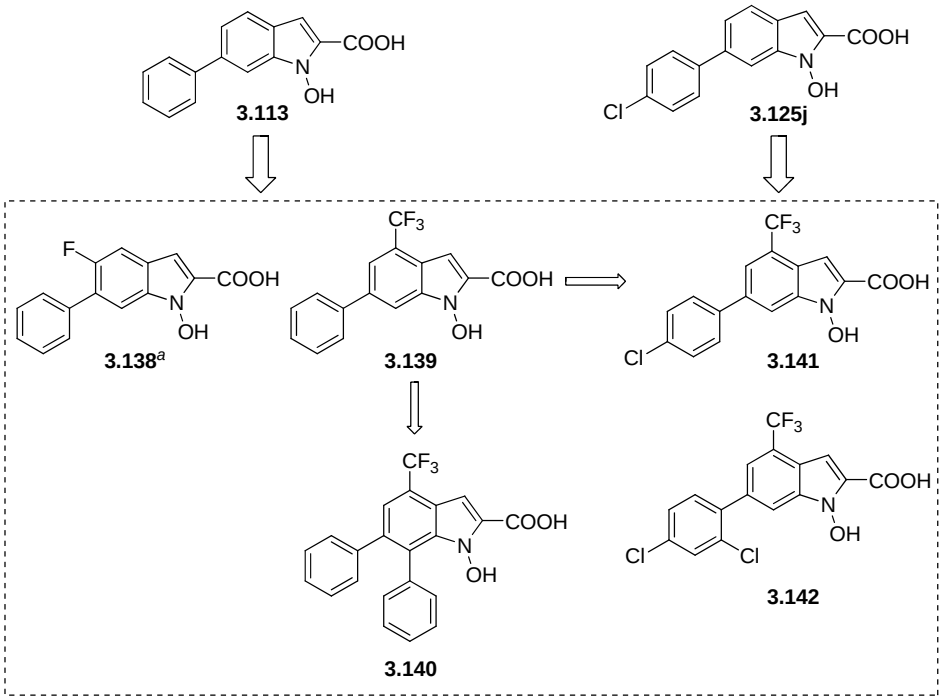
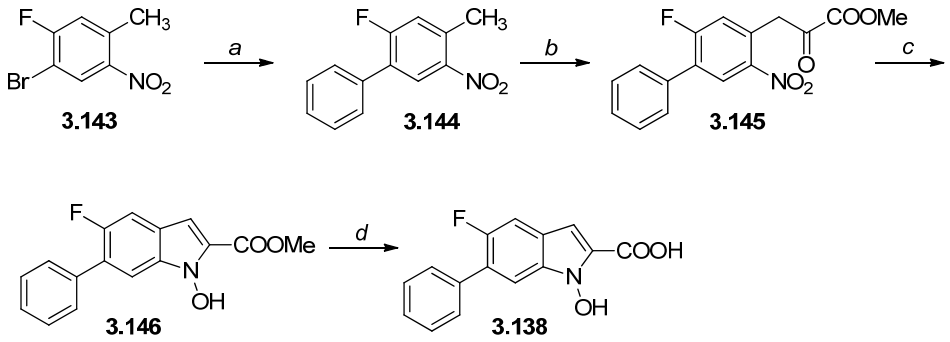


Figure 3.37. Compounds **3.138**-**3.142** deriving from structural development of compounds **3.113** and **3.125j**; [a] compound **3.138** was previously synthesized during experimental graduation thesis in the same research laboratory.

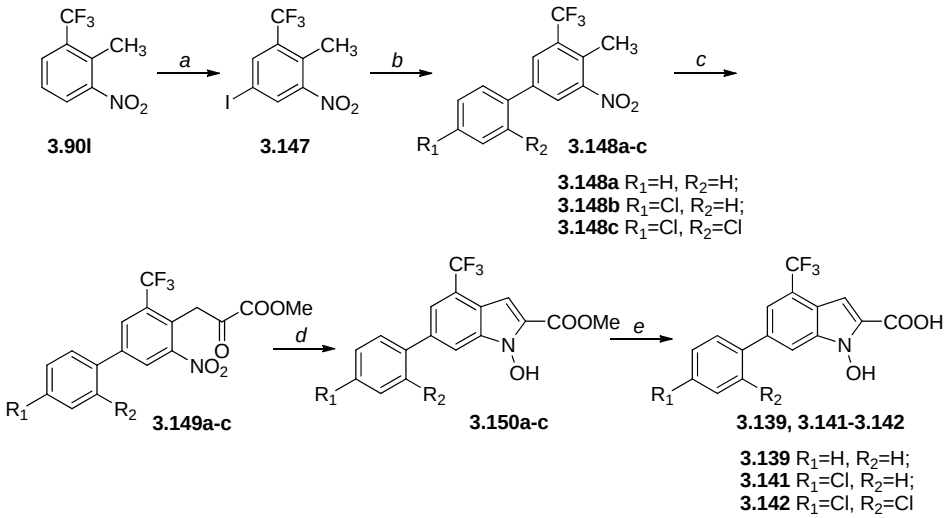
Compound **3.138** was synthesized by following the same synthetic scheme seen for the other phenyl-substituted derivatives (Scheme **3.11**). Commercially available 4-bromo-5-fluoro-2-nitrotoluene **3.143** was submitted to a cross-coupling reaction, where only the most reactive bromine could be replaced by the aryl group under classical Suzuki conditions using $Pd(PPh_3)_4$ as catalyst. Then the so obtain product **3.144** was alkylated with dimethyl oxalate, then cyclized with $SnCl_2$ in DME and finally hydrolyzed with lithium hydroxide to get the free acid **3.138**.



Scheme 3.11. a) $C_6H_5B(OH)_2$, $Pd(OAc)_2$, PPh_3, aqueous 2M Na_2CO_3, distilled toluene, abs. EtOH, 100 °C, 24 h; b) $(COOMe)_2$, NaH 60%, dry DMF, –15 °C to RT; d) $SnCl_2 \cdot 2H_2O$, dry DME, 4Å MS, 0 °C to RT; e) aqueous 2N LiOH, THF/MeOH (1:1), RT.

For the synthesis of CF_3-derivatives **3.139**, **3.141** and **3.142** (Scheme **3.12**), commercially available 2-methyl-3-nitrobenzotrifluoride **3.901** was iodinated with *N*-iodosuccinimide (NIS) in concentrated sulfuric acid to give iodo-aryl derivative **3.147** [116] in order to permit, in the next step, the exchange of the iodine with the appropriate aryl ring by a ligand-free cross-coupling in a microwave reactor, which afforded compounds **3.148a-b**. For the synthesis of compound **3.148c**, we preferred the milder thermal Suzuki reaction to the harsher ligand-free microwave conditions, in order to avoid the potential over-reaction of the chlorine atoms present in the aryl substituent.

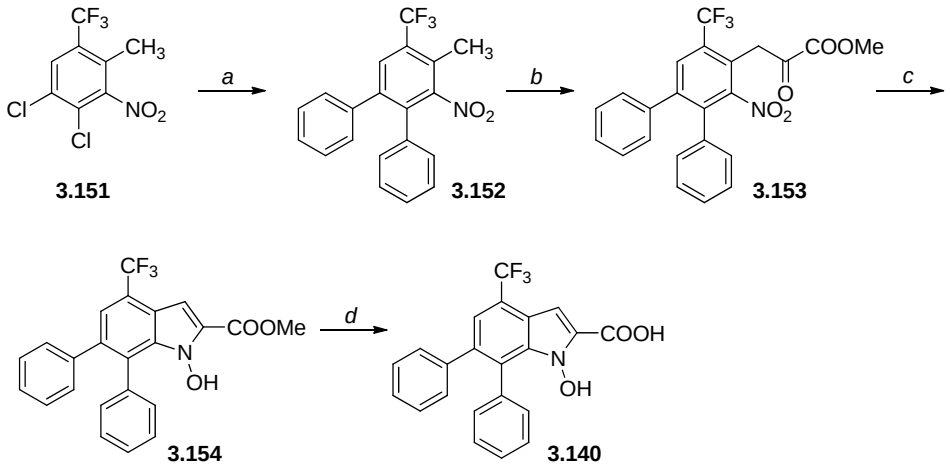
During the synthesis of the compounds bearing the 4-trifluoromethyl group, the previously described conditions in the ketoester formation (sodium hydride, DMF, dimethyl oxalate) gave very low yields of the desired intermediate, due to the formation of side products, whose interpretation by NMR signals was difficult. For such reason, we changed the base, using potassium *tert*-butoxide in the place of sodium hydride, in anhydrous diethyl ether and methanol, with dimethyl oxalate; under these conditions we obtained a reddish suspension where the potassium salt of the desired ketoester precipitates in the reaction mixture as soon as it forms [117]. The ketoseters **3.149a-c** so obtained were then cyclized with stannous chloride in DME and finally hydrolyzed to get final products **3.139**, **3.141** and **3.142**.



Scheme 3.12. a) NIS, conc. H_2SO_4, 5-10 °C to RT; b) for **3.148a-b**: appropriate substituted phenylboronic acids, $Pd(OAc)_2$, TBAB, Na_2CO_3, H_2O, μW, 5 min; for **3.148c**: 2,4-dichlorobenzeneboronic acid, $Pd(OAc)_2$, PPh_3, aqueous 2M Na_2CO_3, distilled toluene, abs. EtOH, 80 °C, 24 h; c) $(COOMe)_2$, *t*BuOK, dry Et_2O-MeOH (10:1), 0 °C to RT; d) $SnCl_2 \cdot 2H_2O$, dry DME, 4Å MS, 0 °C to RT; e) aqueous 2N LiOH, THF/MeOH (1:1), RT.

For the double phenyl insertion on 3,4-dichloro-2-nitro-6-trifluoromethyltoluene **3.151** (Scheme **3.13**), a microwave-assisted Suzuki coupling with an excess of benzeneboronic acid (3 equivalents) and an increased reaction time (10 minutes) compared with standard conditions, was used and biphenyl-substituted nitrotoluene **3.152** was successfully obtained. In this case the use of *tert*-butoxide gave almost no reaction, whereas sodium hydride afforded the proper ketoester **3.153**.

Several attempts were performed for the reduction-promoted cyclization of the ketoester, always obtaining mixtures of the NHI and the indole side product. The optimization of this step led us to finally use zinc dust, previously activated with iodine in tetrahydrofuran in refluxing conditions, in the presence of an aqueous solution of ammonium chloride, albeit also this method gave a certain percentage of indole, which was eliminated by chromatographic purification [92].



Scheme 3.13. a) $C_6H_5B(OH)_2$, $Pd(OAc)_2$, TBAB, Na_2CO_3, H_2O, μW, 10 min; b) $(COOMe)_2$, NaH 60%, dry DMF, –15 °C to RT; c) Zn dust, I_2, NH_4Cl, dry THF, H_2O, RT; d) aqueous 2N LiOH, THF/MeOH (1:1), RT.

All the 4-CF_3-6-aryl-substituted derivatives showed very good results in the LDH-A inhibition assays, so the next step was to further substitute the NHI scaffold to probe the chemical space available for a better inhibition of the enzyme. We planned, as an additional structural modification, the introduction of a small group in position 3 such as a methyl, in order to see if that introduction was tolerated by the enzyme binding site. Therefore, we synthesized compounds **3.155** and **3.156** (Fig. **3.38**).

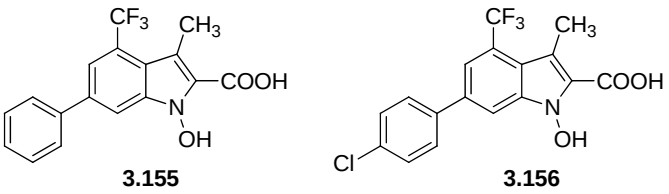


Figure 3.38. 3-Methyl-substituted compounds **3.155** and **3.156**.

We optimized the synthetic procedure for the introduction of the 3-methyl group. In Fig. **3.39**, the main synthetic strategies we attempted in the search for the best method are reported. In the first two strategies (path I and II), ketoester was treated with potassium or lithium *tert*-butoxide in the presence of methyl iodide [118] with the aim to deprotonate the methylene moiety and insert the methyl group, but in both cases a

mixture of *O*- and *C*-methylated derivatives was obtained, whose separation by purification techniques was quite challenging. Moreover, the recovery of starting material by hydrolysis of the formed *O*-methylated side-product proved to be quite difficult. In the last case (path III), where we tried to introduce the methyl group already in the initial nitro-toluene precursor, hydroxymethylation with formaldehyde and potassium hydroxide [119], followed by OH/Br exchange with tetrabromomethane, triphenylphosphine in DCM [120], and subsequent reduction with sodium borohydride as the reducing agent and anhydrous 1-methyl-2-pyrrolidinone (NMP) as the solvent [121] gave a mixture of the desired ethyl-substituted derivative together with the unsaturated styrene by-product derived from an undesired elimination reaction.

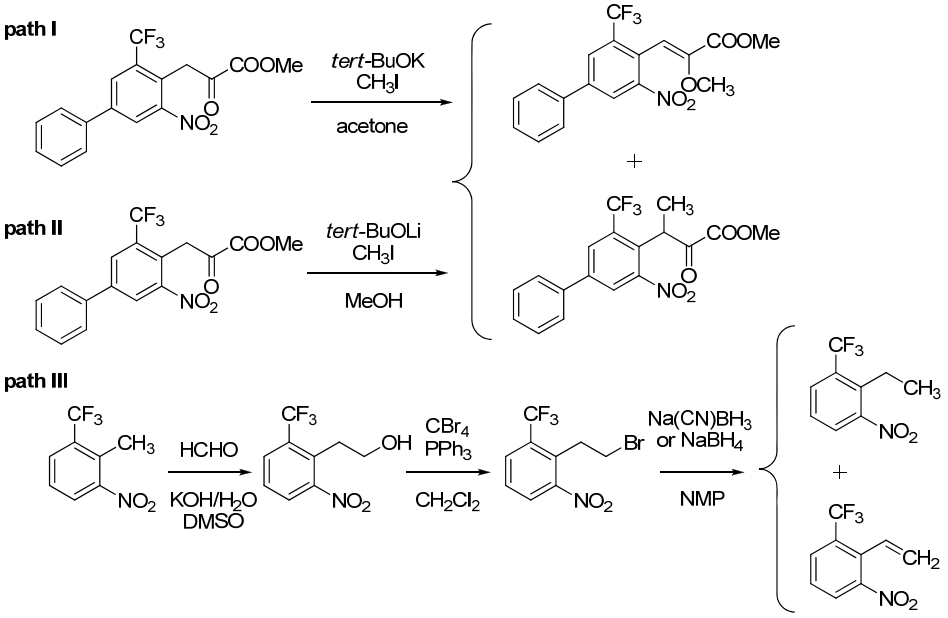
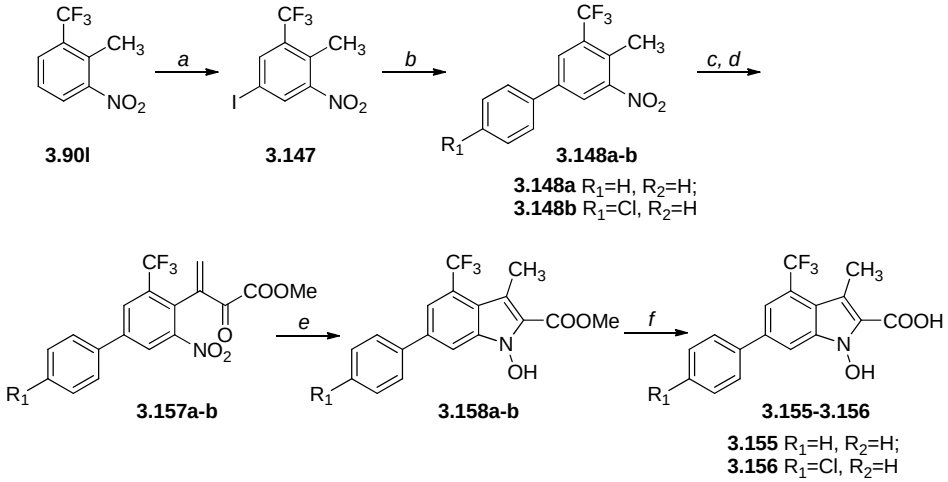


Figure 3.39. Synthetic strategies (**path I**, **II** and **III**) adopted for the insertion of the 3-methyl group are schematically represented. Reagents and conditions are briefly summarized on the arrows.

In the light of these insufficient results, we decided to follow the Nicolaou's method used for the insertion of several kinds of groups in position 3 of the NHI scaffold [92]. This approach consists in treating the potassium salts of ketoesters with Eschenmoser's salt (*N*,*N*-dimethylmethilideneammonium chloride) in THF to give the corresponding

α,β-unsaturated ketoesters **3.157a-b** and then carrying out the reductive cyclization in the presence of triethylsilane as the hydride donor to efficiently afford 3-methyl-*N*-hydroxyindoles **3.158a-b** (Scheme **3.14**).



Scheme 3.14. a) NIS, conc. H_2SO_4, 5-10 °C to RT; b) appropriate substituted phenylboronic acids, $Pd(OAc)_2$, TBAB, Na_2CO_3, H_2O, μW, 5 min; c) $(COOMe)_2$, *t*BuOK, dry Et_2O-MeOH (10:1), 0 °C to RT; d) $CH_2=N^+Me_2Cl^-$, dry THF, 0 °C to RT; e) $SnCl_2 \cdot 2H_2O$, trietyhlsilane, dry DME, 4Å MS, 0 °C to RT; f) aqueous 2N LiOH, THF/MeOH (1:1), RT.

For completing the series of the aryl-substituted NHI, we envisaged the possibility of introducing heterocycles or other different aromatic moieties on the NHI nucleus in the place of the aryl rings present in the previously synthesized products, in order to enlarge the molecular diversity of the series of aryl-substituted *N*-hydroxyindoles. In particular, we focused our synthesis on the introduction of biphenyl, naphthalene, furan, 1,3-benzodioxolane and 2,2-difluoro-1,3-benzodioxolane groups in position 6 (compounds **3.159a-f**, Fig. **3.40**) and *p*-chlorophenoxy portion in position 5 (compound **3.160**, Fig. **3.40**). The choice of these products was also dictated by the commercial availability of the precursors and on the synthetic accessibility of the resulting target molecules.

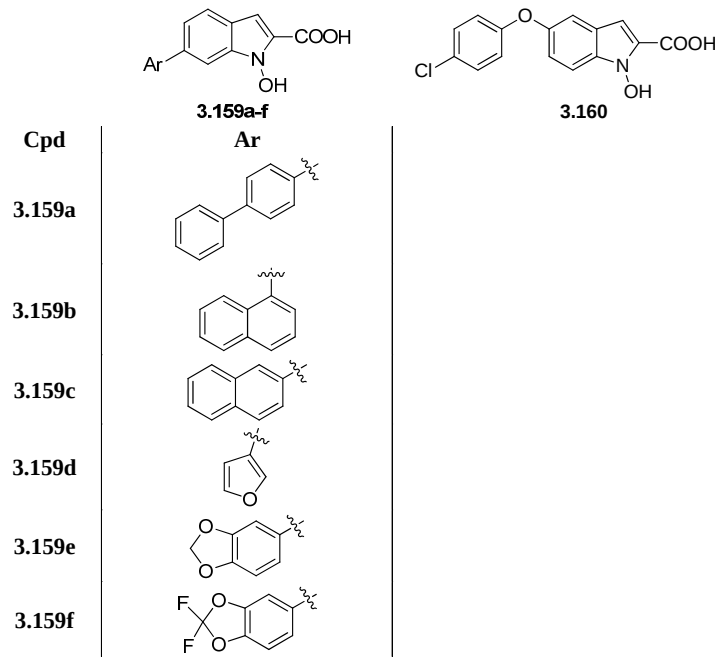
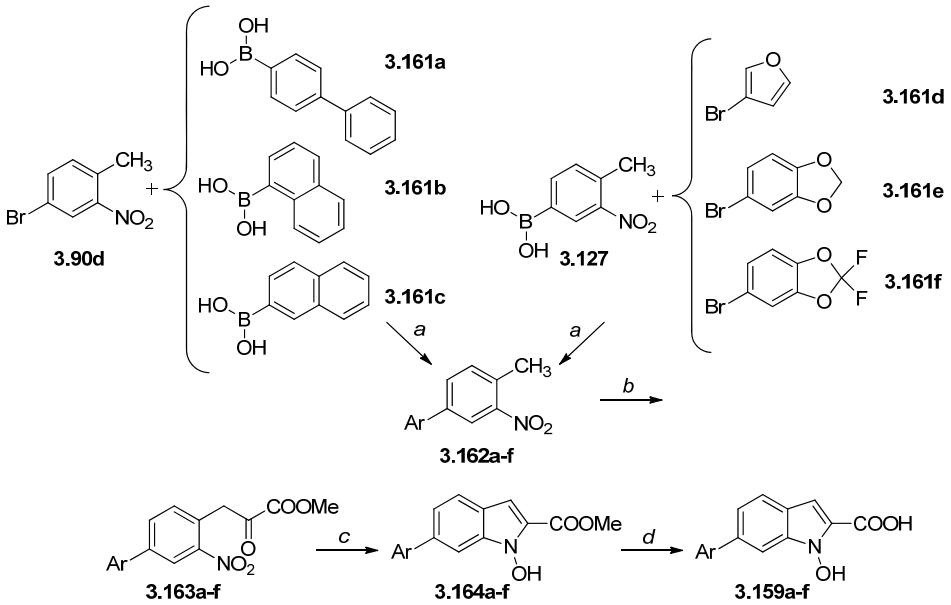


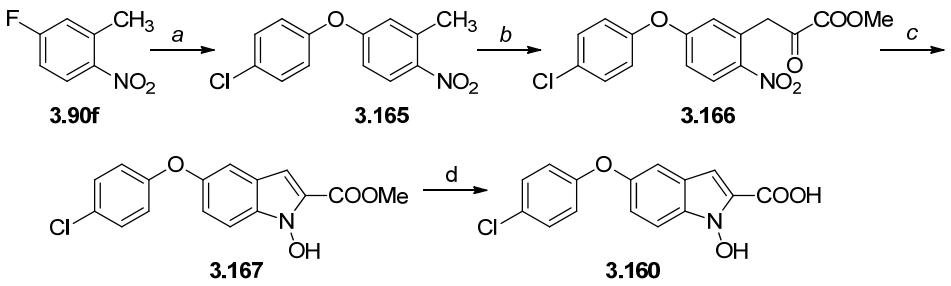
Figure 3.40. Structures of heterocyclic compounds **3.159a-f** and **3.160**.

Nitrotoluenic precursors **3.162a-f** were synthesized by a microwave-assisted Suzuki coupling starting from commercially available 4-bromo-2-nitrotoluene **3.90d**, 4-biphenyl or 1-/2-naphtalene-boronic acids (**3.161a-c**). Otherwise, the commercial availability of the "umpolung" reagent 4-methyl-3-nitrobenzeneboronic acid **3.127** allowed us to run the cross-coupling reactions with bromo-substituted derivatives **3.161d-f** (Scheme **3.15**). According to the same synthetic scheme previously described for the construction of the NHI scaffold, the so obtained compounds **3.162a-f** were reacted with NaH and dimethyl oxalate to give ketoesters **3.163a-f**, whose reductive cyclization was performed with $SnCl_2$ in the presence of thiophenol, and finally methyl esters of **3.164a-f** were hydrolyzed with lithium hydroxide. Similarly, the procedure adopted for the synthesis of compound **3.160** was accomplished as shown in Scheme **3.16**, where commercially available 5-fluoro-2-nitrotoluene **3.90f** was treated with *p*-chlorophenol in the presence of potassium carbonate in dimethylsulphoxide to yield

compound **3.165** [122], then the final steps were practically identical to those described in Scheme **3.15**.



Scheme 3.15. a) $Pd(OAc)_2$, TBAB, Na_2CO_3, H_2O, µW, 5 min; b) $(COOMe)_2$, NaH 60%, dry DMF, –15 °C to RT; c) $SnCl_2 \cdot 2H_2O$, C_6H_5SH, Et_3N, CH_3CN, RT; e) aqueous 2N LiOH, THF/MeOH (1:1), RT.



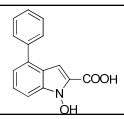
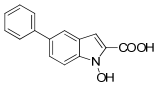
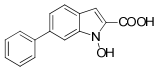
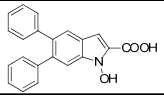
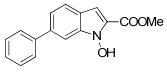
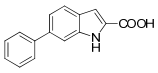
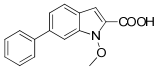
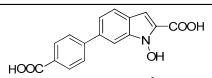
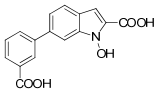
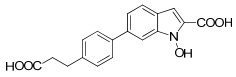
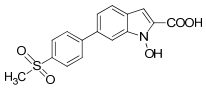
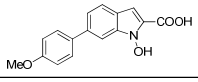
Scheme 3.16. a) 4-chlorophenol, K_2CO_3, DMSO, RT to 80 °C; b) $(COOMe)_2$, NaH 60%, dry DMF, –15 °C to RT; d) $SnCl_2 \cdot 2H_2O$, dry DME, 4Å MS, 0 °C to RT; e) aqueous 2N LiOH, THF/MeOH (1:1), RT.

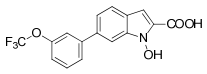
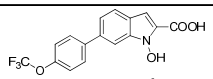
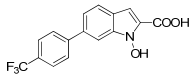
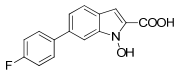
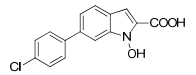
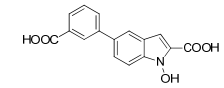
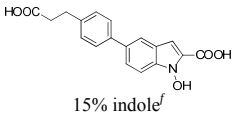
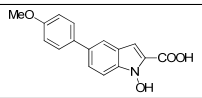
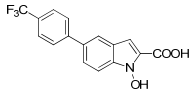
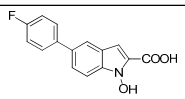
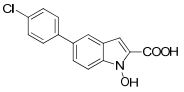
3.8.2. Enzymatic assays

Following the first series of exploratory compounds, aryl-substituted derivatives were tested. Here again, we checked the activity on the B isoform only for those compounds that displayed relevant inhibition levels of the A isoform (Table **3.14**). In spite of the poor results obtained with 4-phenyl-substituted compound **3.111**, compounds **3.112** and **3.113**, bearing a phenyl ring in positions 5 and 6, respectively, showed an evident increase in the inhibitory potency, with K_i values in a range of 10-20 μM in pyruvate-saturating conditions. Moreover, they proved to be competitive also in NADH-saturating conditions, showing K_i values of 15.7 and 35.5 μM for **3.112** and **3.113**, respectively. A synergistic effect, generated by joining the substitutions present in compounds **3.112** and **3.113**, was found in the di-phenyl derivative **3.114** which reached a K_i value of 5 μM. Moreover, compounds **3.112-3.114** did not show any appreciable activity on the other isoform, so demonstrating to be selective for LDH-A. The lack of activity of methyl ester **3.120c**, indole **3.122** and *O*-methylated derivative **3.124** (analogues of compound **3.113**) constituted the proof that the OH-COOH motif represents the pharmacophoric group needed for LDH-A inhibition. In fact, when COOH or *N*-OH are methylated or the *N*-OH is substituted with *N*-H the inhibition activity is completely loss (**3.120c** and **3.124**) or dramatically reduced (**3.122**). The indole derivative **3.122** proved to be a very modest non-selective LDH inhibitor, showing also a small inhibition percentage (8%) on the B isoform. An activity worsening was provoked by the introduction of methylsulfonic (**3.125d**) and methoxy groups (**3.125e** and **3.126c**) in position 4 of the phenyl substituent, when compared to their corresponding unsubstituted analogues **3.113** and **3.112**. The presence of a carboxylic group showed controversial effects: when the COOH moiety is directly linked to the phenyl ring, scarce results were obtained (compounds **3.125a** and **b**), with the exception of the *m*-COOH-5-phenylsusbstituted derivative **3.126a** (K_i = 19 μM for NADH). However, when the acid group is spaced by an ethylene chain linker (**3.125c** and **3.126b**) the activity sensibly increases. Trifluoromethoxy-substituted NHIs **3.125f** and **3.125g** are among the most potent and selective compounds assayed up to now, as well as *para*-chloro derivatives **3.125j** and **3.126f**, reaching K_i values in the low micro molar range. On the contrary, the presence of the trifluoromethyl group and the fluorine

atom in *para* of the phenyl rings in both positions (5 for compounds **3.126d-e** and 6 for compounds **3.125h-i**) resulted to be detrimental for the activity on the enzyme.

Table 3.14. Inhibition data on LDH-5 (LDH-A_4) and LDH-1 (LDH-B_4) for compounds **3.111-3.114**, **3.120c**, **3.122**, **3.124**, **3.125a-j**, **3.126a-f**.

Cpd	Structure	Inhibition values	
		LDH-5[a]	LDH-1
3.111[e]		n.a.	-
3.112		K_i = 10.4 μM (NADH) 15.7 μM (Pyr)	n.a.[a]
3.113		K_i = 19.8 μM (NADH) 35.5 μM (Pyr)	n.a.[a]
3.114		K_i = 5.0 μM (NADH) 7.5 μM (Pyr)	n.a.[b]
3.120c		n.a.	-
3.122		12%	8%[a]
3.124		n.a.	-
3.125a	 31% indole[f]	3%	-
3.125b		32%[c]	-
3.125c		K_i = 117.6 μM (NADH)	n.a.[b]
3.125d		17%	-
3.125e		18%	-

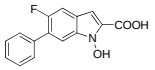
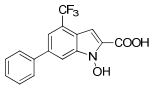
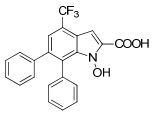
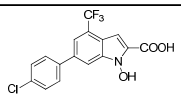
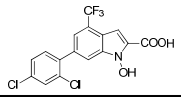
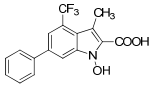
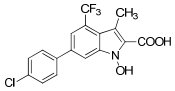
3.125f		K_i = 8.5 μM (NADH) 33 μM (Pyr)	n.a.[b]
3.125g	 10% indole[f]	K_i = 7.5 μM (NADH) 40 μM (Pyr)	n.a.[b]
3.125h		K_i = 42 μM (NADH) 25 μM (Pyr)	n.a.[b]
3.125i		K_i = 67 μM (NADH) 17 μM (Pyr)	n.a.[b]
3.125j		K_i = 5.0 μM (NADH) 56 μM (Pyr)	n.a.[b]
3.126a		K_i = 19.4 μM (NADH) 27.2 μM (Pyr)	n.a.[b]
3.126b	 15% indole[f]	55%[c]	27%[c]
3.126c		25%[c]	-
3.126d		30%[d]	-
3.126e		5%[d]	-
3.126f		K_i = 16 μM (NADH) 100 μM (Pyr)	n.a.[b]

[a] compounds tested at 125 μM, [b] compounds tested at 100 μM, [c] compounds tested at 250 μM, [d] compounds tested at 50 μM, [e] compound **3.111** was previously synthesized by undergraduate students during experimental thesis in the same research laboratory, [f] compounds tested as a mixture with the reported percentage of indole. n.a. = not active.

The co-presence of halogenated substituents and aryl rings on the NHI scaffold provided very good results (Table **3.15**). Initially, we found that 4-CF_3-6-phenyl-substituted compound **3.139** caused a 87% of inhibition of LDH-A at 125 μM, with a K_i

as low as 8.9 μM, together with a minimal activity (11%) on LDH-B. On the contrary, the insertion of a fluorine atom in position 5, as in compound **3.138**, led to a total loss of activity. The other CF_3-derivatives **3.140-3.142**, close analogues of lead compound **3.139**, generally maintained a very high potency on the desired isoform, without showing any relevant activity on LDH-B, so slightly improving their biological profiles. In details, the additional presence of the 7-phenyl ring in compound **3.140** lowered the activity if compared to **3.139** (K_i = 17 μM instead of 8.9 μM), whereas the activity turned out to be sensibly improved upon insertion of a chlorine atom in *para*-position of the phenyl ring (**3.141**) and, even more, when two chlorine atoms were present in *para*- and *ortho*-positions. In fact, dichlorophenyl-substituted compound **3.142** proved to be the most active LDH-A inhibitor among all the so far synthesized *N*-hydroxyindole derivatives, in both pyruvate- (K_i = 1.5 μM) and cofactor-saturating conditions (K_i = 1.6 μM), associated with a total selectivity for this isoform (0% inhibition on LDH-B at 100 μM). 3-Methyl-substituted derivatives **3.155** and **3.156** maintained approximately the same activity of their corresponding non-methylated analogues **3.139** and **3.141**, respectively, with the only peculiarity of a remarkable increase of the K_i value showed in NADH-saturating conditions for compound **3.155** (98 μM), when compared to that obtained by its analogue **3.139** (4.7 μM).

Table 3.15. Inhibition data on LDH-5 (LDH-A_4) and LDH-1 (LDH-B_4) for compounds **3.138-3.142**, **3.155-3.156**.

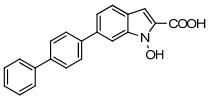
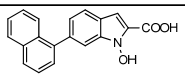
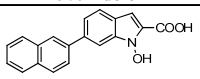
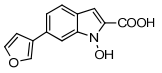
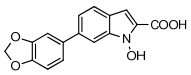
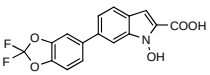
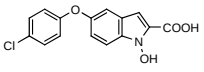
Cpd	Structure	Inhibition values	
		LDH-5	LDH-1[a]
3.138[c]		n.a.	-
3.139		K_i = 8.9 μM (NADH) 4.7 μM (Pyr)	11%[b]
3.140		K_i = 17 μM (NADH) 14.3 μM (Pyr)	n.a.
3.141		K_i = 2.6 μM (NADH) 16 μM (Pyr)	n.a.
3.142		K_i = 1.5 μM (NADH) 1.6 μM (Pyr)	n.a.
3.155		K_i = 10 μM (NADH) 98 μM (Pyr)	n.a.
3.156		K_i = 10 μM (NADH) 6 μM (Pyr)	n.a.

[a] compounds tested at 100 μM, [b] compounds tested at 125 μM, [c] compound **3.138** was previously synthesized by undergraduate students during experimental thesis in the same research laboratory. n.a. = not active.

In accordance with the previous results, most of the subsequently synthesized *N*-hydroxyindoles, substituted with aromatic portions in position 6 of the central ring, resulted to be active (Table **3.16**). Biphenyl derivative **3.159a** showed a very high activity on LDH-A (K_i = 4.5 μM in pyruvate-saturating conditions), so confirming that the insertion of hydrophobic moieties in this position of the nucleus is favourable for the inhibition abilities of our compounds, although this molecule showed also an undesired 20% inhibition on the B isoform. Naphthalene-substituted compounds **3.159b-c** maintained a good activity (K_i values ranging from 10 to 15 μM), and they proved to be completely LDH-A selective. The insertion of a furan ring as in compound **3.159d**

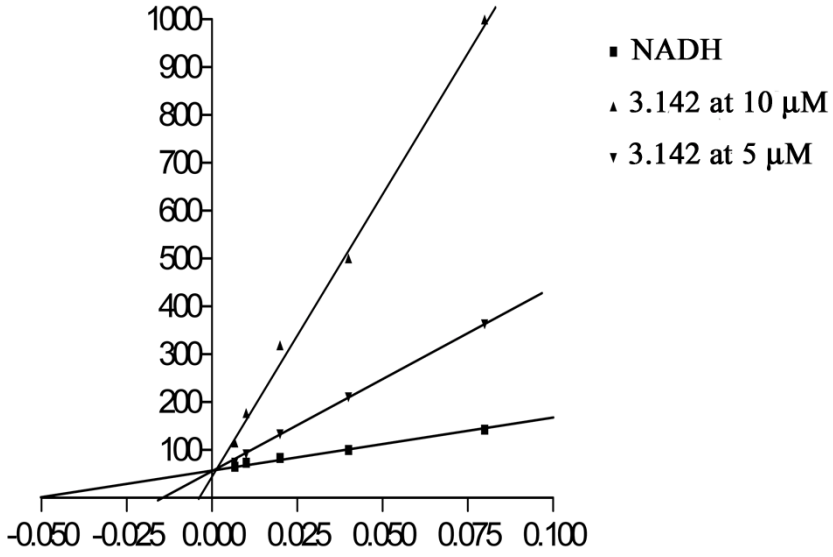
provoked a small decrease in the potency (K_i = 54 μM). The introduction of the benzodioxolane group (compound **3.159e**), which produced a dramatic drop in the inhibitory activity, reaching only a 9% inhibition at 125 μM. This situation is reverted if two fluorine atoms are inserted in the methylene bridge linking the two benzodioxolane oxygen atoms (**3.159f**), restoring an acceptable K_i value (52 μM *vs.* NADH; 17 μM *vs.* Pyr). Unluckily, compound **3.160**, possessing a *p*-chlorophenyl ring in position 5 spaced by an oxygen atom form the central nucleus, was not tested in enzymatic assays, due to its very low solubility even when relatively high concentrations of DMSO were used in the assay.

Table 3.16. Inhibition data on LDH-5 (LDH-A_4) and LDH-1 (LDH-B_4) for compounds **3.159a-f**, **3.160**.

Cpd	Structure	Inhibition values	
		LDH-5	**LDH-1**
3.159a[a]	 10% indole[e]	K_i = 4.5 μM (NADH) 13.5 μM (Pyr)	20%[b]
3.159b	 9% indole[e]	K_i = 10 μM (NADH) 7 μM (Pyr)	n.a.[b]
3.159c	 13% indole[e]	K_i = 15.3 μM (NADH) 13 μM (Pyr)	n.a.[b]
3.159d	 15% indole[e]	K_i = 54 μM (NADH) 20 μM (Pyr)	n.a.[c]
3.159e		9%[d]	-
3.159f		K_i = 52 μM (NADH) 17 μM (Pyr)	n.a.[c]
3.160		n.t	-

[a] compound **3.159a** was tested using a 2% DMSO, for solubility problems, [b] compounds tested at 50 μM, [c] compounds tested at 100 μM, [d] compounds tested at 125 μM, [e] compounds tested as a mixture with the reported percentage of indole. n.a. = not active. n.t. = compound **3.160** was not tested for solubility problems.

As a summary of these fruitful enzyme kinetic studies, we can say that all the compounds that proved to be active in inhibiting LDH-A, with K_i values in the low micromolar range, were found to behave as competitive inhibitors with respect to both NADH and pyruvate in the conversion of pyruvate to lactate catalyzed by this enzyme, whereby NADH is converted to NAD^+. This competitive behaviour is confirmed by the Lineweaver-Burk plots, where the lines obtained in the absence and presence of various concentrations of the inhibitor intersect on the ordinate. This would suggest that these compounds likely occupy both the cofactor and the substrate binding sites. A graphical representative example of the double-reciprocal plots obtained with compound **3.142**, which is the most potent and selective LDH-A inhibitor obtained so far, in competition with both the cofactor NADH (top, Fig. **3.41**) and the substrate pyruvate (bottom, Fig. **3.41**) is reported below.



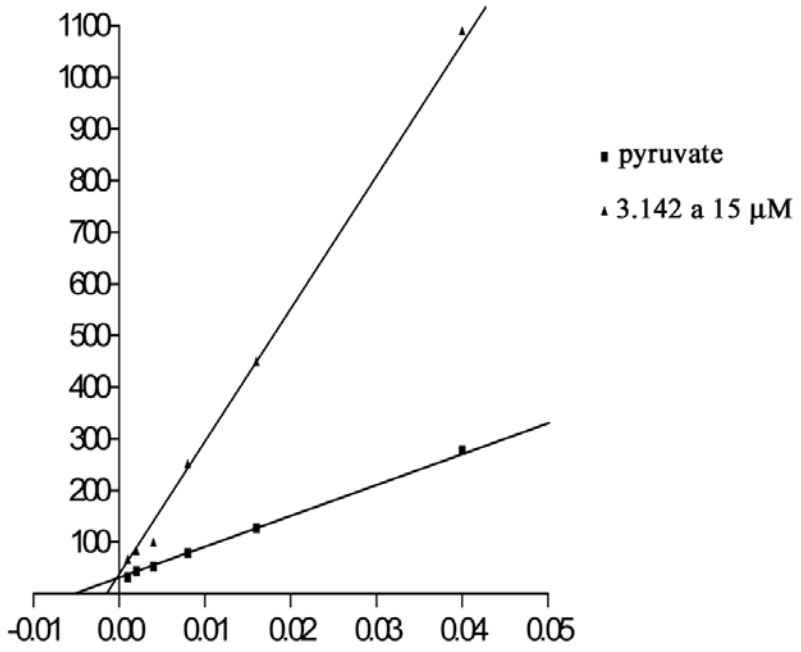


Figure 3.41. Lineweaver-Burk plots determined from enzyme kinetic experiments human LDH-5 (LDH-A_4) in the presence of inhibitor **3.142** at different concentrations in competition with NADH (top) and pyruvate (bottom).

3.8.3. Molecular modeling studies

Methods. In order to perform docking calculations, the X-ray structure of the human muscle L-LDH M chain complexed with NADH and Oxamate [23] (PDB code 1I10) was taken from the Protein Data Bank [123]. With the aim of obtaining an open loop conformation form of the protein, the loop structure from A96 to K118 was taken from the open conformation of the rabbit muscle L-LDH M chain (PDB code 3H3F) [124]. The structure was directly replaced since between the two loop sequences there is one hundred percent of identity. The so obtained structure was placed in a rectangular parallelepiped water box, an explicit solvent model for water, TIP3P, was used, and the complex was solvated with a 8 Å water cap. Chlorine ions were added as counterions to neutralize the system. Two steps of minimization were then carried out. In the first stage, we kept the complex fixed with a position restraint of 500 kcal/mol•$Å^2$ and we

solely minimized the positions of the water molecules. In the second stage, we minimized the entire system through 5000 steps of steepest descent followed by conjugate gradient (CG) until a convergence of 0.05 kcal/mol•Å^2 was attained. The ligands were built using Maestro 9.0 [125] and were minimized using the CG method until a convergence value of 0.05 kcal/mol•Å^2 was reached. The minimizations were carried out in a water environment model (generalized-Born/surface-area model) using the MMFFs force field and a distance-dependent dielectric constant of 1.0. Automated docking was carried out by means of the GOLD program, version 4.1.1 [126]. The region of interest used by GOLD was defined in order to contain the residues within 10 Å from the original position of NADH and oxamate in the X-ray structures. The "allow early termination" option was deactivated, the remaining GOLD default parameters were used, and the ligand was submitted to 30 genetic algorithm runs by applying the ChemScore fitness function. The best docked conformation was taken into account. The docking of the analysed compounds was carried out using the procedure described above and using as the target protein the structure extracted from the minimized average structure of the LDH-**3.139** complex obtained from the MD simulations.

All the molecular dynamics (MD) simulations were performed using AMBER 10 [127]. MD simulations were carried out using the parm03 force field at 300 K. The complex was placed in a rectangular parallelepiped water box, an explicit solvent model for water, TIP3P, was used, and the complex was solvated with a 10 Å water cap. Chlorine ions were added as counterions to neutralize the system. Prior to MD simulations, two steps of minimization were carried out using the same procedure described above. Particle mesh Ewald (PME) electrostatics and periodic boundary conditions were used in the simulation [128]. The MD trajectory was run using the minimized structure as the starting conformation. The time step of the simulations was 2.0 fs with a cutoff of 10 Å for the nonbonded interaction, and SHAKE was employed to keep all bonds involving hydrogen atoms rigid. Constant-volume periodic boundary MD was carried out for 400 ps, during which the temperature was raised from 0 to 300 K. Then 9.6 ns of constant pressure periodic boundary MD was carried out at 300 K using the Langevin thermostat to maintain constant the temperature of our system. In the first 1.2 ns, all the α carbons of the protein were blocked with a harmonic force

constant, which decreased during these 1.2 ns from 10 to 1 kcal/mol•Å^2, while in the last 7.8 ns, no constraints were applied. General Amber force field (GAFF) parameters were assigned to the ligand, while partial charges were calculated using the AM1-BCC method as implemented in the Antechamber suite of AMBER 10. The final structure of the complex was obtained as the average of the last 5.5 ns of MD minimized by the CG method until a convergence of 0.05 kcal/mol•Å. The average structure was obtained using the ptraj program implemented in AMBER 10.

Results and discussion. In order to understand and characterize the interaction of the synthesized compounds with LDH-A active site, one of the most promising ligands, compound **3.139** (Section 3.8.1), was analyzed by docking modeling studies into an open loop conformation of the enzyme. The compound was docked into LDH-A using the GOLD program [126] and the best pose was used as starting geometry for molecular dynamics (MD) calculations. Ten nanoseconds of MD simulation with explicit water were carried out, in the first 1.2 ns all the α carbons of the protein were blocked with a harmonic force constant, which decreased during these 1.2 ns from 10 to 1 kcal/mol•Å^2, while in the last 7.8 ns no constraints were applied. The system reached an apparent equilibrium after about 0.5 ns of MD, since the total energy for the residual nanoseconds remained constant (see panel A in Fig. **3.42**). Analyzing the root-mean-square deviation (RMSD) from the initial model of the α carbons of the proteins, we observed that, after an initial increase, the RMSD remained approximately constant around the value of 1.5 Å during the last 5.5 ns (see panel B in Fig. **3.42**).

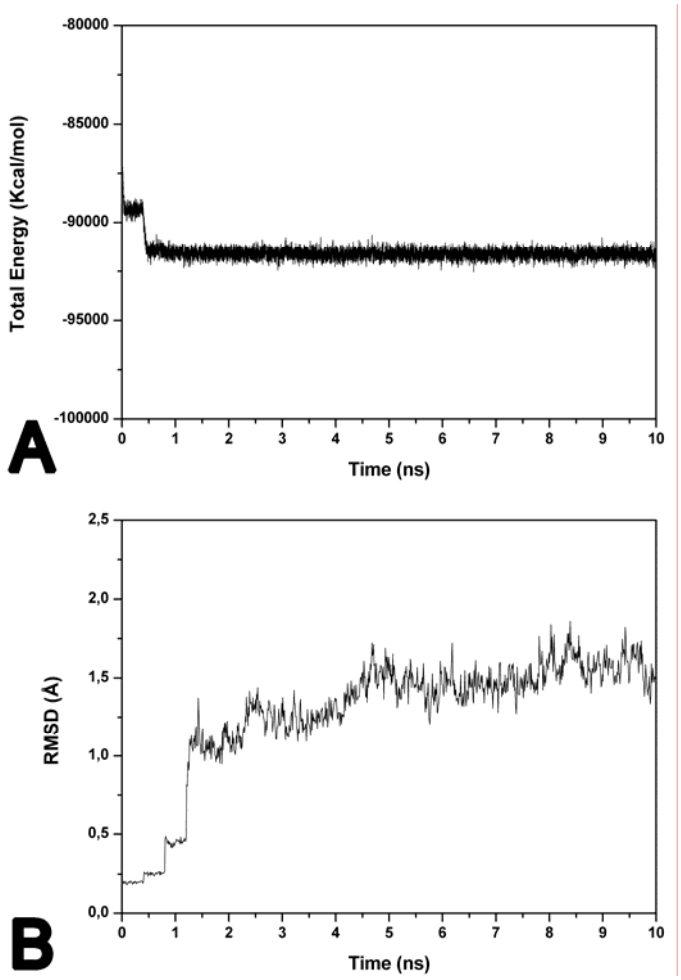


Figure 3.42. Analysis of the MD simulation of the LDH-**3.139** complex. Total energy (kcal/mol) of the system plotted *vs* time (A) and RMSD in angstrom of the α carbon of the protein from the starting model structure during the simulation *vs* time (B) are reported.

Fig. **3.43** shows the minimized structures of the average of the last 5.5 ns of the MD simulation. In the proposed model the carboxylic group of compound **3.139** shows a strong interaction with R169 and T248 (see panel A in Fig. **3.43**), whereas the *N*-hydroxy group shows an H-bond interaction with the nitrogen backbone of T248 and a water molecule that mediates the interaction of **3.139** with the catalytic H193 (see panels A and B in Fig. **3.43**).

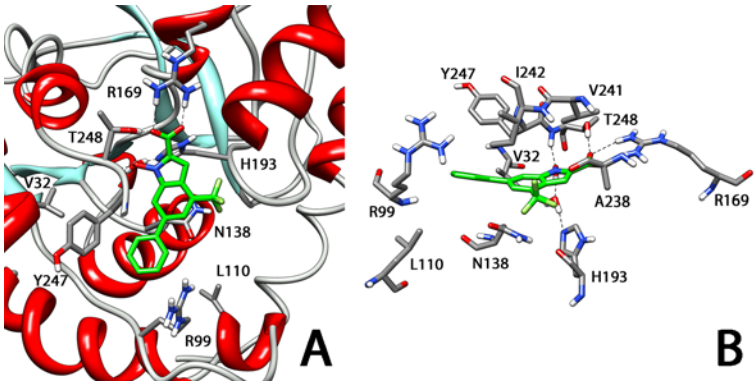


Figure 3.43. MD simulation results for the complex of LDH with **3.139**. Overall disposition of the ligand into LDH (A, residues 240-245 are hidden for a better visualization) and LDH-ligand interactions (B).

An important role of residues R169, T248 and H193 for the ligand interaction has been already highlighted by the analysis of the X-ray structure of the LDH complexed with the natural substrate pyruvate and NADH (Fig. **3.44**). In fact, this structure confirms the interactions between the pyruvate carboxylate group with the arginine and threonine residues of the enzyme, as well as that occurring between the carbonyl oxygen atom of the substrate and the above-mentioned histidine aminoacid (compare Figures **3.43** and **3.44**). With regards to the 6-phenyl substituent, it shows lipophilic interactions with V32 and Y247.

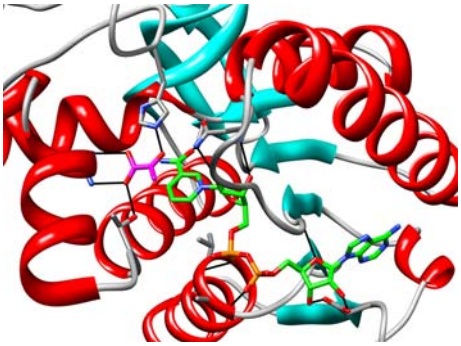
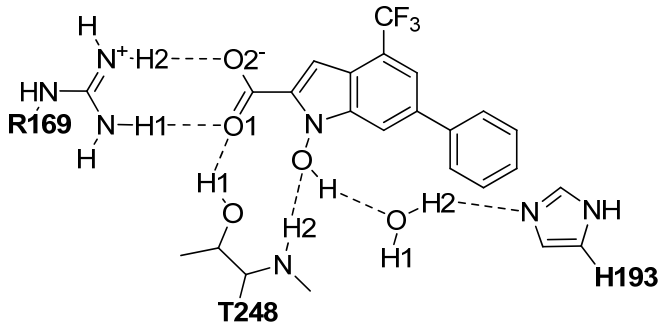


Figure 3.44. A representative example of a 1.80 Å resolution X-Ray structure of the complex of LDH with NADH (green) and pyruvate (purple): detailed view of the catalytically active site [PDB code: 3D4P].

The H-bond analysis of the last 5.5 ns of the MD simulation seems to confirm the interactions described above. In particular, as shown in Table **3.17**, it is interesting to note that the interaction between the water molecule (bound to H193) and the *N*-hydroxy group is highly conserved during MD, confirming the important role of this *N*-hydroxy substituent. Furthermore, the analysis of the X-ray structure of *Plasmodium falciparum* isoform of lactate dehydrogenase complexed with 3,7-dihydroxy-2-naphthoic acid [69], revealed the presence of a water molecule able to mediate a H-bond between an OH group of this ligand and the catalytic H193 of the enzyme, thus supporting the possible presence of this water molecule also in the human LDH-A form, as we have found with our calculations. As for the 4-(trifluoromethyl)indole central scaffold, it results to be placed in a cleft mainly delimited by H193, G194, A238, V241, I242, T248. Moreover, N138 is placed in close proximity to the trifluoromethyl substituent in our model. According to our calculations, the 6-phenyl group is directed towards the entrance of the binding site cavity and shows lipophilic interactions with I242 and Y247. Overall, this molecular portion seems to partially overlap with the region occupied by the cofactor NADH in the human LDH-A X-ray structure [23].

Table 3.17. Hydrogen Bonds Analysis of the LDH-**3.139** interactions during the last 5.5 ns of MD Simulation.



donor	acceptorh	acceptor	distance (Å)	% occupied
WAT@O	LIG@H	LIG@O3	2.6	99
LIG@O1	T248@H1	T248@OG1	2.7	93
LIG@O2	R169@H2	R169@NH2	2.8	88
LIG@O1	R169@H1	R169@NH1	2.8	70
LIG@O3	T248@H2	T248@N	2.9	51
H193@NE2	WAT@H2	WAT@O	2.8	41
H193@NE2	WAT@H1	WAT@O	2.8	37

In order to analyze the interactions of other synthesized and tested compounds, two representative compounds belonging to the aryl-substituted *N*-hydroxyindole class, compounds **3.112** and **3.114**, were docked in the minimized structure of the protein extracted from the complex with **3.139**.

Then the resulting protein-ligand complexes were energy minimized. Docking of compound **3.112** (Fig. **3.45**) shows a binding mode very similar to that found for **3.139**, with the carboxylic group of compound **3.112** showing a strong interaction with R169 and T248 and the *N*-hydroxy group displaying an H-bond interaction with the nitrogen backbone of T248 and a water molecule that mediates the interaction of **3.112** with the catalytic H193. The shift of the 6-phenyl group to position 5 determines the loss of the interaction with V32 and Y247 and the presence of a lipophilic interaction with L110. Therefore, in agreement with the good activity of both **3.139** and **3.112**, they show a

very similar binding disposition with the formation of the same hydrogen bonds and similar lipophilic interactions.

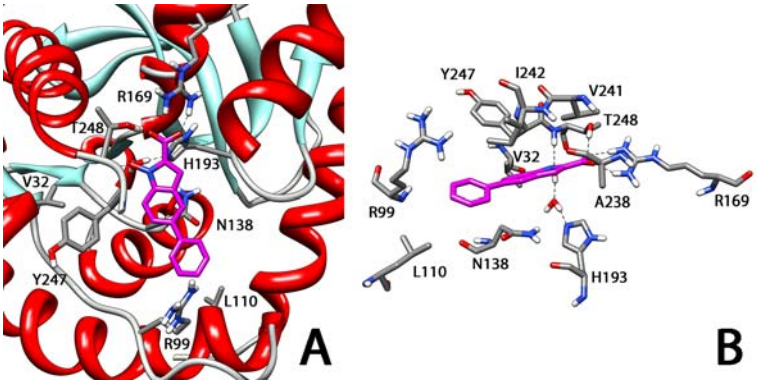


Figure 3.45. Docking analysis of compound **3.112**. Overall disposition of the ligand into LDH (A, residues 240-245 are hidden for a better visualization) and LDH-ligand interactions (B).

In agreement with the very high activity of compound **3.114**, the docking analysis of this compound highlights the same H-bonds showed by compound **3.139** and **3.112**, the lipophilic interactions of the 6-phenyl ring with V32 and Y247 and the interaction of the 5-phenyl ring with L110 (see Fig. **3.46**).

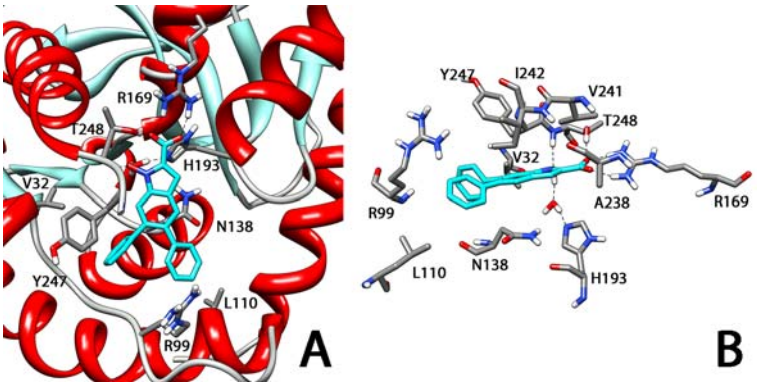


Fig. 3.46. Docking analysis of compound **3.114**. Overall disposition of the ligand into LDH (A, residues 240-245 are hidden for a better visualization) and LDH-ligand interactions (B).

3.9. TRIAZOLE-BASED DERIVATIVES

3.9.1. Synthesis

During my PhD work, we directed our attention to the design and the synthesis of triazole-based *N*-hydroxyindoles due to the synthetic accessibility of this kind of structure, obtained by Huisgen 1,3 dipolar cycloaddition, generally simply referred as *Click Chemistry.* Although this popular definition, the term *Click Chem*istry is used improperly for this reaction, because it was coined by K. Berry Sharpless referring to a more general concept of a chemistry able to generate substances quickly and safely, by joining small units together, giving high yields and inoffensive by-products, without the need of complex purification processes (such as chromatography, while distillation and crystallization are preferred) and without the use of toxic solvent, so usually preferring water-based reactions [129]. Certainly, one of the most famous reactions within the *Click Chemistry* philosophy is the Huisgen cycloaddition, consisting in a copper-catalyzed triazole formation by a reaction between azides and terminal alkynes at room temperature in a mixture of water and *tert*-butanol, with no efforts to exclude oxygen from the reaction medium. However, even this simple reaction may require high temperature and it may results in the production of a mixture of the 1,4 and 1,5 regioisomers, although the copper(I) catalyst strongly directs towards the 1,4-regioselectivity, so affording 1,4-disubstituted 1,2,3-triazoles (Fig. **3.47**). As for Cu(I) catalyst, the best sources proved to be Cu^{2+} salts, such as $CuSO_4 \cdot 5H_2O$, which are reduced *in situ* by the reductant sodium ascorbate.

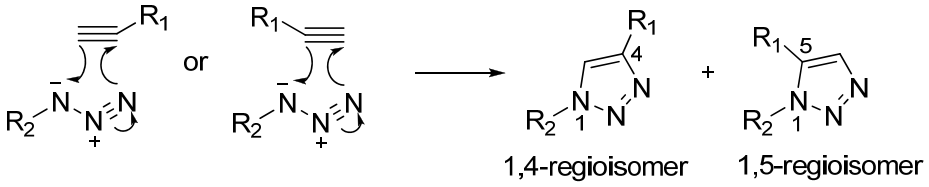


Figure 3.47. Possible formation of the 1,4- and 1,5-regiosiomers from the Huisgen cycloaddition.

The proposed mechanism for the catalytic cycle, which is still under study, is exemplified in Fig. **3.48**. It starts with the formation of Cu(I) acetylide (step **A**,

supported also by the lack of reactivity shown by internal alkynes), then azide attack on the copper atom of this intermediate (step **B**), which subsequently leads to the formation of the triazole (step **C**) where the copper is still linked on the just formed heterocycle. Finally, the cycle ends with the detachment and the regeneration of the copper catalyst and the formation of the triazole (step **D**).

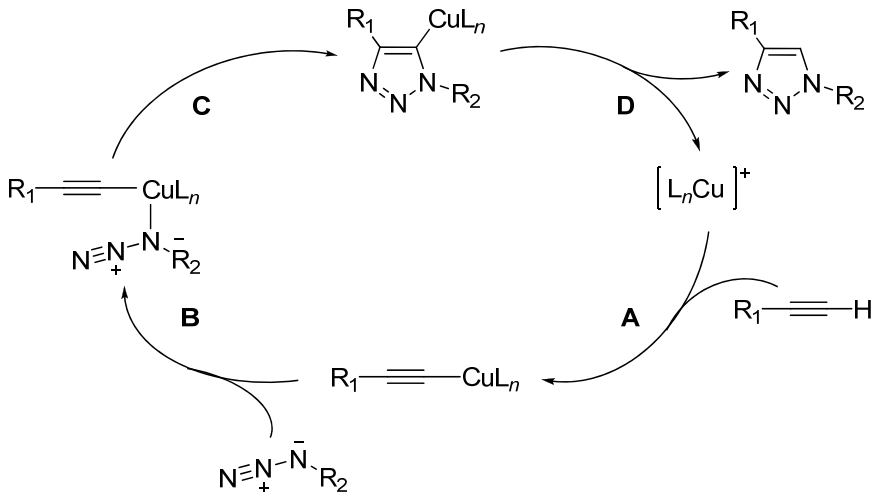


Figure 3.48. Probable catalytic cycle leading to triazole formation in the Huisgen cycloaddition.

Using this reliable and simple strategy, which shows a wide tolerance for both the alkyne and the azide substrates, we obtained a relatively numerous class of triazole-bearing NHI derivatives. We then investigated the effect on LDH-A inhibition caused by presence of variously substituted triazoles in position 4, 5 and 6 of the NHI scaffold, as shown by synthesized compounds **3.168a-c**, **3.169a-d** and **3.170a-f**, respectively (Fig. **3.49**). In details, we introduced a series of lipophilic and hydrophilic exploratory groups on the triazole ring, which acts as a linker between them and the NHI heterocycle. Possibly, these substituents may be placed in the entrance groove of the LDH active site, which normally hosts the cofactor NADH, so they may potentially gain new interactions with the enzyme, some of which may be similar to those established with NADH itself.

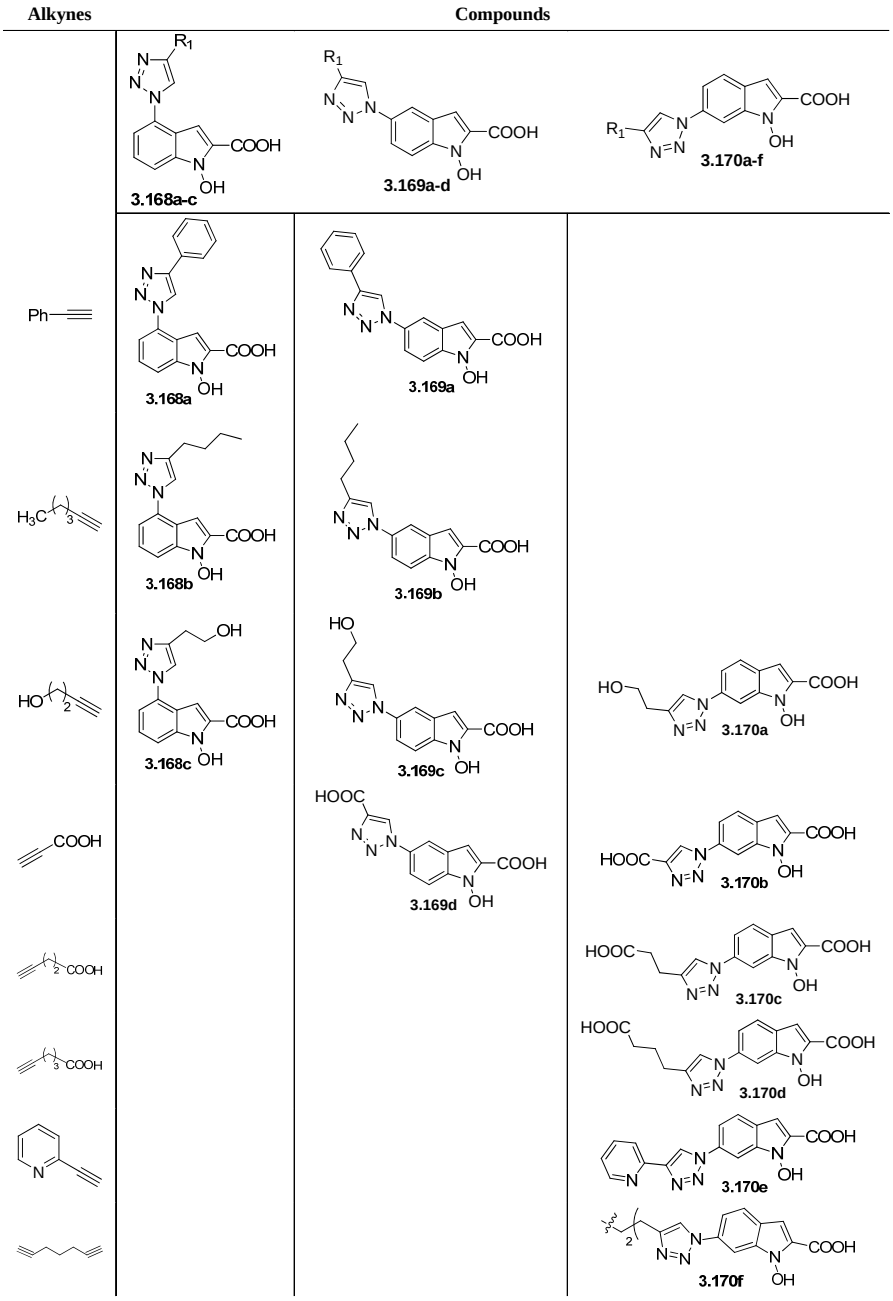
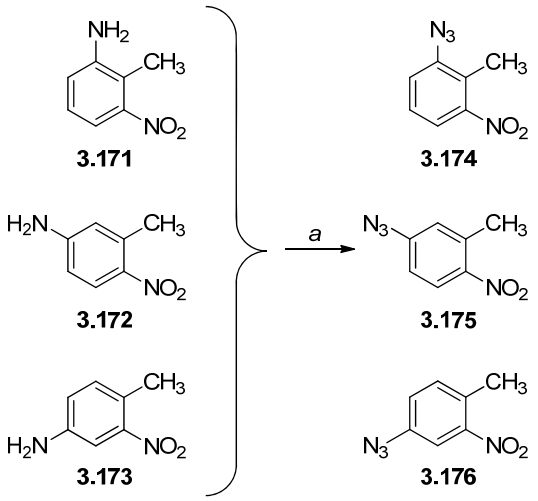


Figure 3.49. Structural class of triazole derivatives **3.168a-c**, **3.169a-d** and **3.170a-f**. Commercially available terminal alkynes, used in the cycloaddition reaction for triazole formation, are listed on the left column.

The azide precursors **3.174-3.176** were synthesized from the opportune commercially available methyl-nitro-substituted aniline **3.171-3.173**, dissolved in aqueous hydrochloridric acid, in the presence of sodium nitrite and sodium azide (Scheme **3.17**). Sodium nitrite is the source of the nitrosyl cation (NO^+), which undergoes a nucleophilic attack by the primary amminic nitrogen of the aniline, resulting in the formation of a diazonium cation, which eliminates molecular nitrogen and reacts with sodium azide by incorporating the N_3 moiety in the aromatic structure [130].

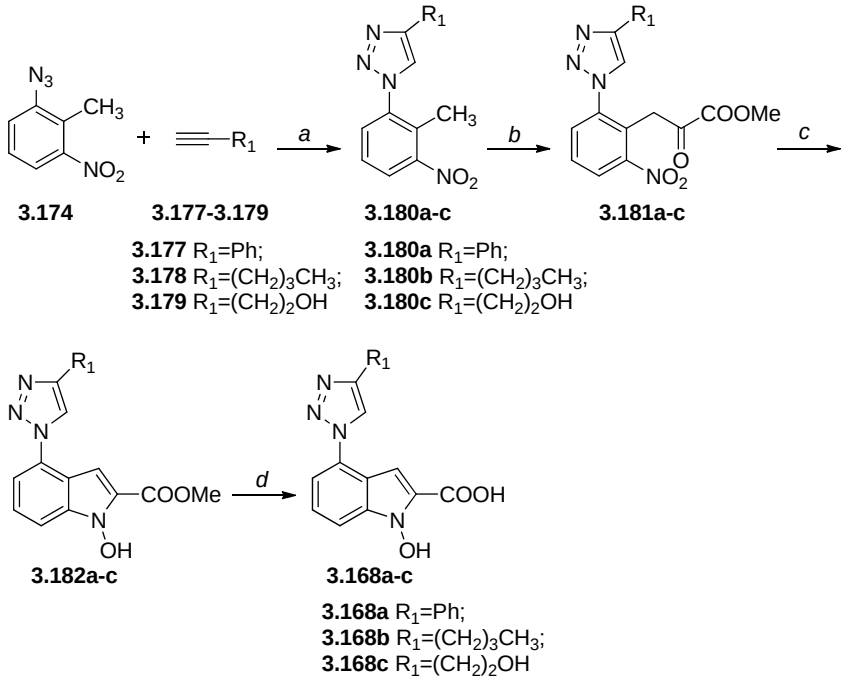


Scheme 3.17. a) $NaNO_2$, NaN_3, aqueous HCl, H_2O, -5/0 °C to RT.

Compounds **3.168a-c**, possessing a triazole moiety in position 4 of the NHI heterocycle, were synthesized from azide **3.174** by *"click"* reactions with different terminal alkynes, such as, phenylacetylene **3.177**, 1-hexyne **3.178** and 3-butyn-1-ol **3.179**, in the presence of catalytic amount of copper sulfate pentahydrate and sodium ascorbate in a mixture of water and *tert*-butanol, affording triazole derivatives **3.180a**, **3.180b** and **3.180c**, respectively (Scheme **3.18**). In particular, only condensation of azide **3.174** and phenylacetylene **3.177** gave good yield at room temperature, whereas in the other two cases longer reaction times and higher temperatures (80 °C) were required to obtain the precipitation from the reaction mixture of the desired products [131, 132].

The resultant triazoles were then subjected to the same synthetic pathway described before for the synthesis of other NHI derivatives, which comprises ketoester formation, cyclization with stannous chloride in DME and hydrolysis of the methyl esters.

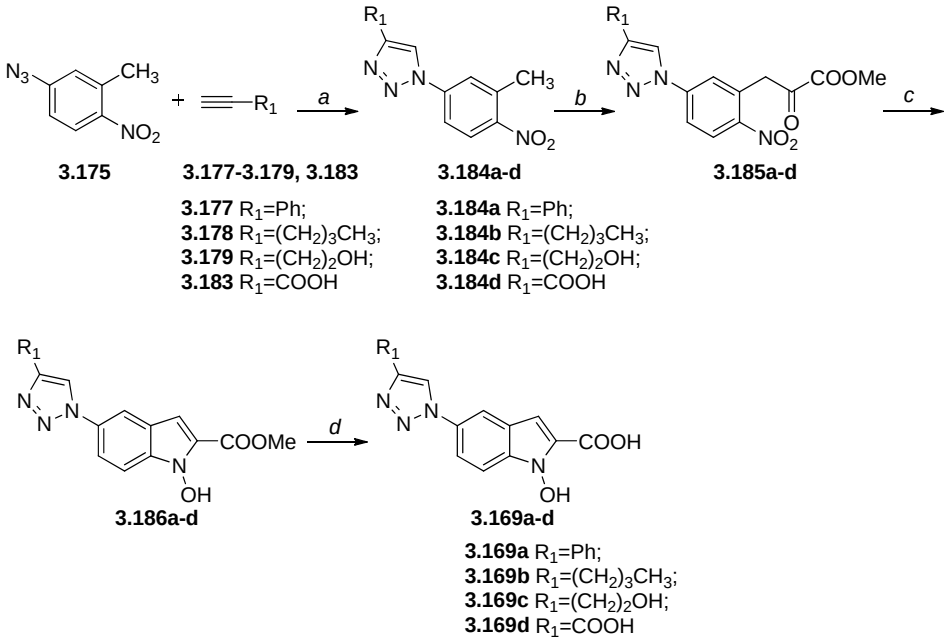
During previous attempts, we tried to treat the azide derivatives with sodium hydride and dimethyl oxalate to form the ketoester chain directly, in order to maintain the azide group up to the reductive cyclization step and then subject these common azide intermediates to the treatment with different alkynes, in an attempt to postpone as much as possible the diversification step. Unfortunately, azides are instable precursors and they polymerize under alkylating conditions, so we were forced to apply the synthetic strategy displayed in Scheme **3.18**.



Scheme 3.18. a) appropriate alkyne, $CuSO_4 \cdot 5H_2O$, sodium ascorbate, H_2O/*t*BuOH (1:1), RT; for **3.178** and **3.179**: 80 °C b) $(COOMe)_2$, NaH 60%, dry DMF, –15 °C to RT; c) $SnCl_2 \cdot 2H_2O$, dry DME, 4Å MS, 0 °C to RT; d) aqueous 2N LiOH, THF/MeOH (1:1), RT.

The same sequence of reactions shown in Scheme **3.18** was adopted for the preparation of compounds **3.169a-d** (Scheme **3.19**). During the synthesis of compound

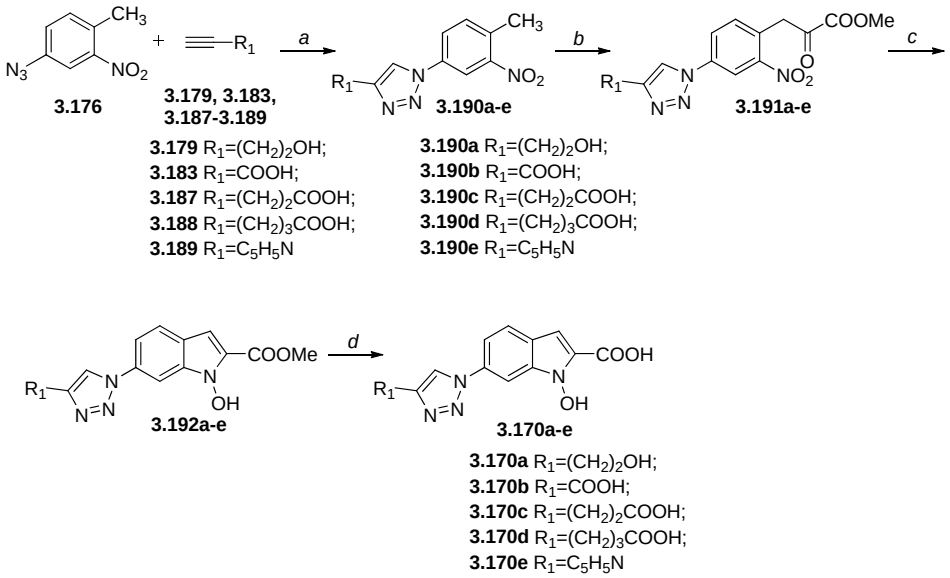
3.184a, we observed the simultaneous formation of its 1,5-regioisomer, however we succeeded in the isolation from the reaction mixture of the desired 1,4-regioisomer, which was unambiguously identified in the NMR spectrum by the presence of the typical singlet over 8 ppm relative to the triazole proton.



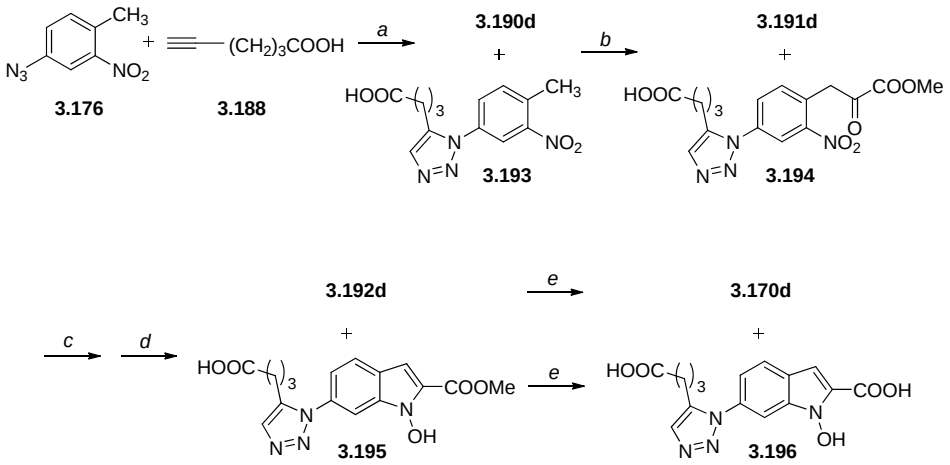
Scheme 3.19. a) appropriate alkyne, $CuSO_4 \cdot 5H_2O$, sodium ascorbate, H_2O/*t*BuOH (1:1), RT; for **3.179** and **3.183**: 80 °C b) $(COOMe)_2$, NaH 60%, dry DMF, –15 °C to RT; c) $SnCl_2 \cdot 2H_2O$, dry DME, 4Å MS, 0 °C to RT; d) aqueous 2N LiOH, THF/MeOH (1:1), RT.

Scheme **3.20** displays the synthesis of compounds **3.170a-e**, bearing the triazole substituents in position 6. Even in this series, some substrates required heating in the cycloaddition step (step *a*) for an efficient completion of the reaction. In particular the condensation of **3.176** with 4-pentynoic acid **3.187** was particularly slow, so we increased the temperature up to 100 °C in a closed vessel; even in this case isolated yield of intermediate **3.190c** remained rather low (39%). It is important to note that the same reaction with 5-hexynoic acid **3.188** led to the formation of a mixture of 1,4- and 1,5-regioisomer triazoles **3.190d** and **3.193** (Scheme **3.21**), which were not separable by

chromatographic methods, so we pursued all the following steps on that regioisomeric mixture, until the reductive cyclization step when it was possible to separate the two isomers **3.192d** and **3.195**. From this point, we separately hydrolyzed also the 1,5-isomer **3.195** to give the derivative **3.196**. As for this series of 6-triazole-substituted NHI derivatives, the synthesis of the phenyl-triazole derivative (formed by reaction with phenylacetylene) could not be completed due to the formation of excessively high amounts of indole side-product in the reductive cyclization step of the corresponding ketoester, as well as to the degradation of the very small amounts of the NHI methyl ester during the hydrolysis step.

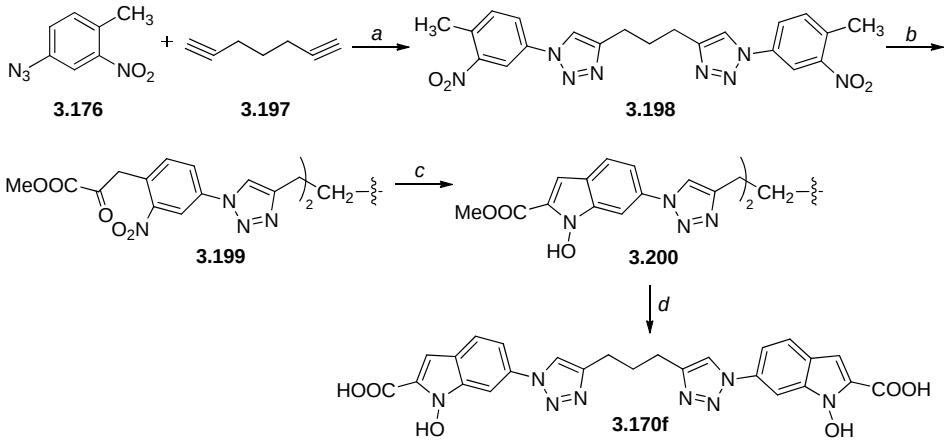


Scheme 3.20. a) appropriate alkyne, $CuSO_4 \cdot 5H_2O$, sodium ascorbate, H_2O/*t*BuOH (1:1), RT; for **3.179**: 80 °C; for **3.187**: 100 °C; for **3.189**: 45 °C; b) $(COOMe)_2$, NaH 60%, dry DMF, –15 °C to RT; c) $SnCl_2 \cdot 2H_2O$, dry DME, 4Å MS, 0 °C to RT; d) aqueous 2N LiOH, THF/MeOH (1:1), RT.



Scheme 3.21. a) $CuSO_4 \cdot 5H_2O$, sodium ascorbate, H_2O/*t*BuOH (1:1), RT; b) $(COOMe)_2$, NaH 60%, dry DMF, –15 °C to RT; c) $SnCl_2 \cdot 2H_2O$, dry DME, 4Å MS, 0 °C to RT; d) chromatographic separation; e) aqueous 2N LiOH, THF/MeOH (1:1), RT.

Later, we carried out the synthesis of "NHI-dimer" **3.170f**, in which 2 *N*-hydroxyindole portions are spaced by a 1,3-ditriazole-propylene chain. The synthesis was accomplished as illustrated in Scheme **3.22**, starting from the cycloaddition of 2 equivalent of azide **3.176** with 1 equivalent of 1,6-heptadiyne **3.197**.



Scheme 3.22. a) $CuSO_4 \cdot 5H_2O$, sodium ascorbate, H_2O/*t*BuOH (1:1), RT; b) $(COOMe)_2$, NaH 60%, dry DMF, –15 °C to RT; c) $H_2PO_2Na \cdot H_2O$, Pd-C 10%, H_2O/THF (1:1), 40 °C; d) aqueous 2N LiOH, THF/MeOH (1:1), RT.

During biological evaluation of our triazole-based inhibitors (see enzymatic assay Section below), the phenyl-derivative **3.168a** showed an appreciable inhibitory activity, and also COOH-containing triazole compounds, such as for example **3.169d**, displayed some levels of inhibitions. Consequently, we applied our efforts in the synthesis of more challenging triazole derivatives, trying to combine the phenyl substitution and the presence of a carboxylic group. In this research line, compounds **3.201-3.205** (Fig. **3.50**), bearing carboxy-substituted phenyl groups (*m*- or *p*-COOH) in position 4 of the triazole rings, were prepared, with the same aim of enriching of polar moieties our inhibitors and so acquiring new possible interactions in the enzyme active site.

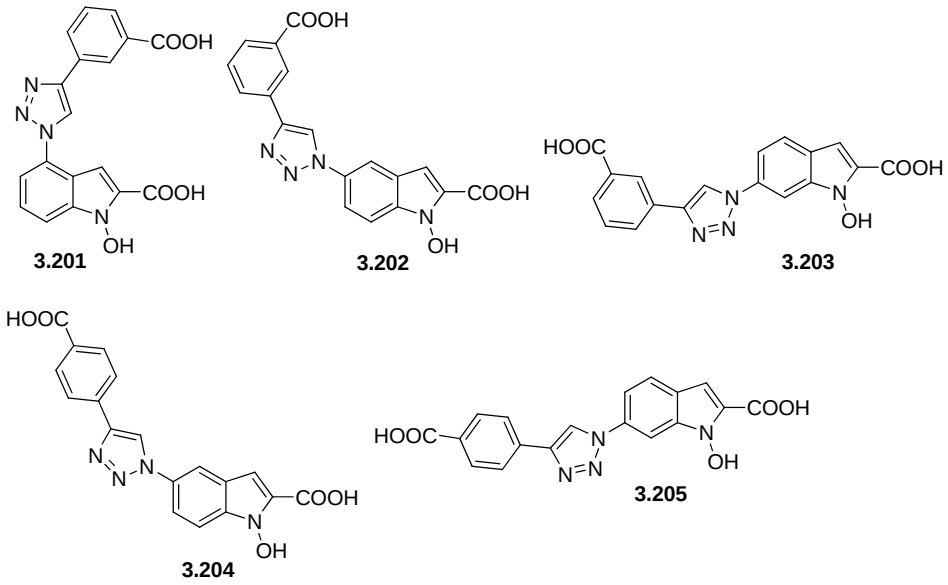
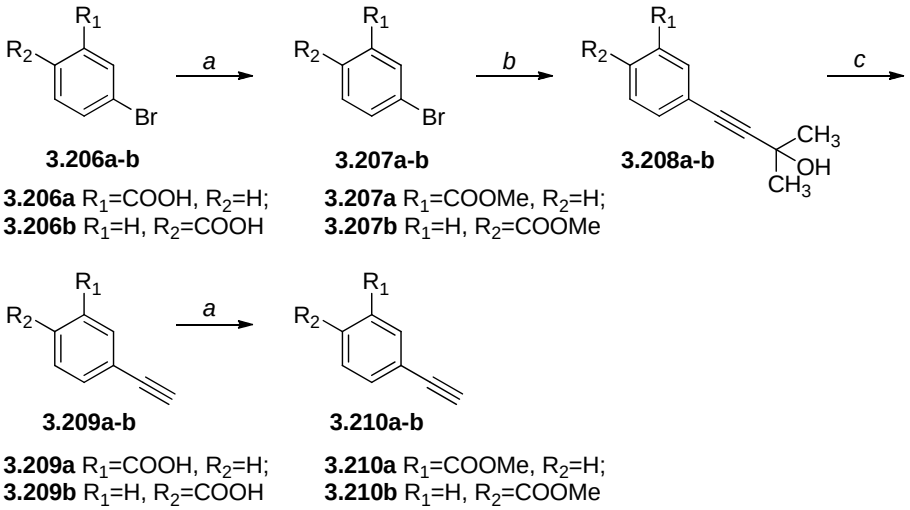


Figure 3.50. Triazole derivatives **3.201-3.205**.

For the synthesis of this series of compounds, the appropriate terminal alkynes **3.210a** and **b** were synthesized according to Scheme **3.23**, by exploiting a reliable and simple common procedure for the preparation of both *meta* and *para*-ethynyl benzoic acids. Commercially available 3- and 4-bromobenzoic acids **3.206a** and **b** were subjected to a Fischer esterification in methanol with a catalytic amount of sulfuric acid in refluxing conditions to give the corresponding methyl esters **3.207a** and **b**.

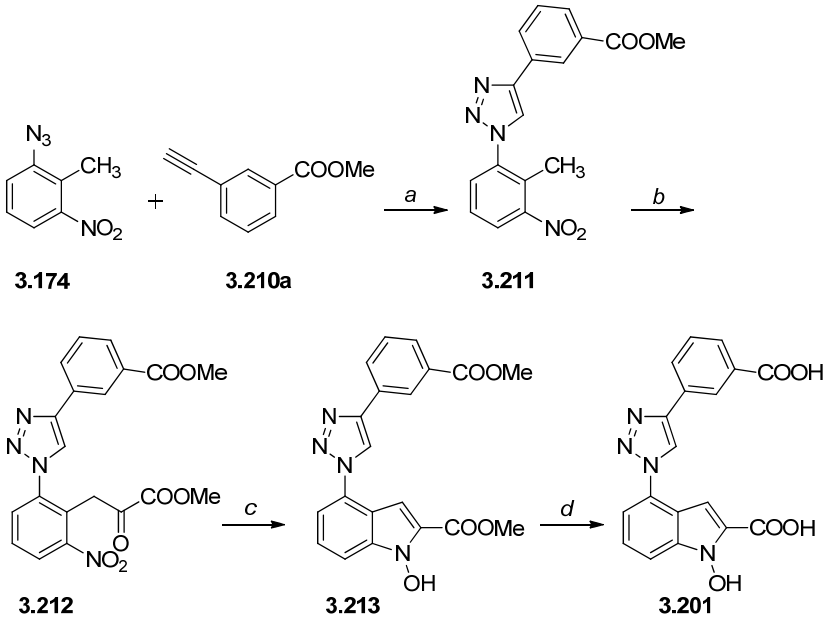
Subsequently, these aryl bromides were reacted with the alkyne 2-methyl-3-butyn-2-ol (often abbreviated as MEBYNOL) in a Sonogashira coupling which is carried out with tetrakis(triphenylphosphine)palladium as the catalyst and copper iodide as the co-catalyst, in the presence of triethylamine [133]. The supposed mechanism includes two catalytic cycles, one relative to the copper catalyst and another one relative to the palladium catalyst. Briefly, it starts with the formation of a π-complex between the alkyne and the copper atom, increasing the acidity of the terminal proton, so that a weak base as triethylamine can remove it. This intermediate then reacts with the palladium-aryl bromide complex, affording the desired product and regenerating the catalyst. The protecting groups of alkynes **3.208a** and **b** needed to be removed by treatment with sodium hydroxide and butanol in refluxing conditions [134], but the desired cleavage was accompanied by the simultaneous saponification of the methyl esters, so we decided to esterify again the benzoic acids **3.209a** and **b** in order to facilitate the next synthetic steps and purifications.



Scheme 3.23. a) MeOH, conc. H_2SO_4, reflux; b) 2-methyl-3-butyn-2-ol, $Pd(OAc)_2$, PPh_3, CuI, Et_3N, dry DMF, reflux; c) NaOH, *n*-BuOH, reflux.

For the synthesis of compound **3.201** (Scheme **3.24**), azide **3.174** and alkyne **3.210a** were subjected to a Huisgen cycloaddtion in a microwave reactor at 300 W of power,

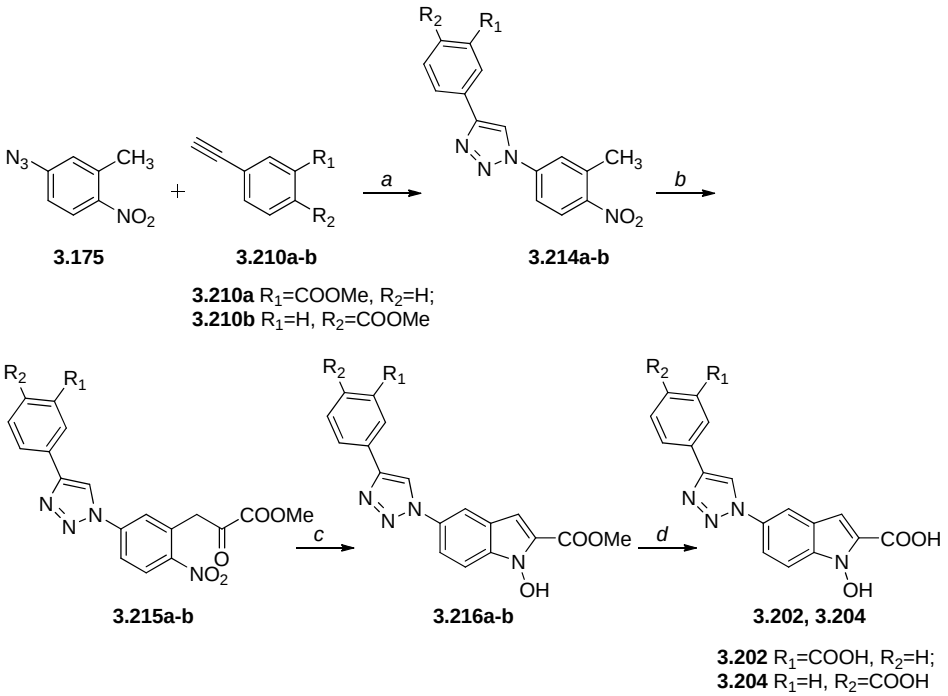
which permitted a favourable reduction of reaction times, albeit this harsh conditions favoured the simultaneous formation of the undesired 1,5-regioisomer together with the desired 1,4-adduct [135]. The chromatographic separation of the mixture of the two isomers afforded pure compound **3.211**, which was then alkylated with sodium hydride and dimethyl oxalate, cyclized using ipophosphite and palladium over charcoal and at last hydrolyzed in the presence of increased (double) amounts of lithium hydroxide in order to efficiently saponify both the methyl esters present in the structure **3.213** to give compound **3.201**. The corresponding product, bearing the carboxylic acid in position *para* of the phenyl ring, could not be synthesized, because of the unsuccessful formation of the ketoester intermediate, for reasons that we are not able to find as of yet.



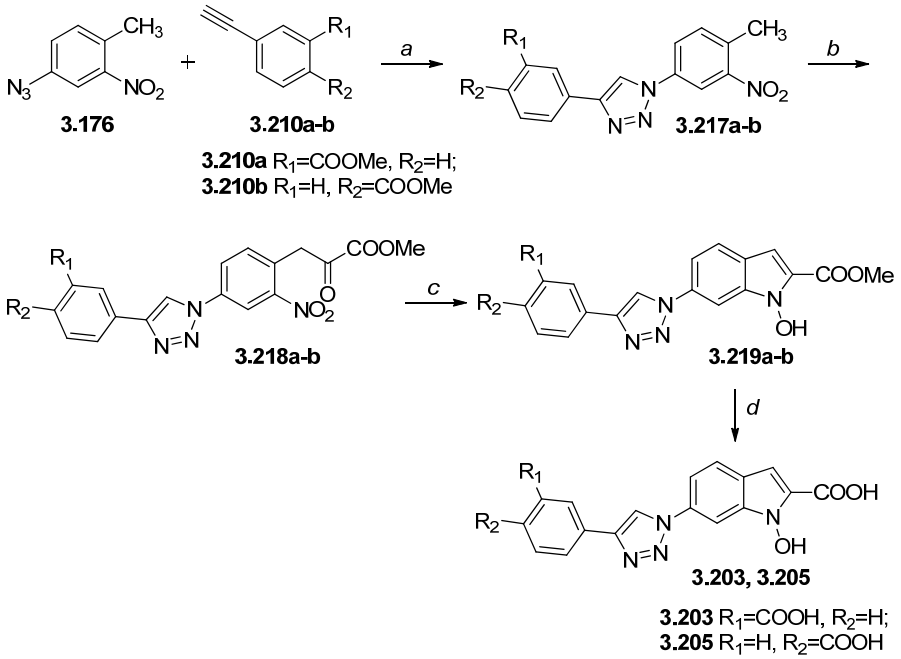
Scheme 3.24. a) $CuSO_4 \cdot 5H_2O$, sodium ascorbate, H_2O/*t*BuOH (1:1), μW, 15 min.; b) $(COOMe)_2$, NaH 60%, dry DMF, –15 °C to RT; c) $H_2PO_2Na \cdot H_2O$, Pd-C 10%, H_2O/THF (1:1), RT; d) aqueous 2N LiOH, THF/MeOH (1:1), RT.

The synthetic procedure used for compound **3.201** (Scheme **3.24**), was adopted also for the preparation of the similar derivatives **3.202**, **3.204** and **3.203**, **3.205** (Schemes **3.25-3.26**). In all the cases, microwave-assisted triazole formation was preferred to the

classical conditions for the abbreviation of the reaction time. The lower regioselectivity of this procedure was not an obstacle to the obtainment of the desired compounds, thanks to the successful chromatographic purifications.



Scheme 3.25. a) $CuSO_4 \cdot 5H_2O$, sodium ascorbate, H_2O/*t*BuOH (1:1), μW, 15 min.; b) $(COOMe)_2$, NaH 60%, dry DMF, –15 °C to RT; c) $SnCl_2 \cdot 2H_2O$, C_6H_5SH, Et_3N, CH_3CN, RT; d) aqueous 2N LiOH, THF/MeOH (1:1), RT.



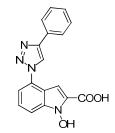
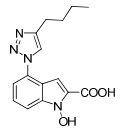
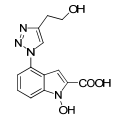
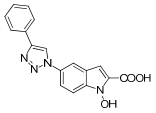
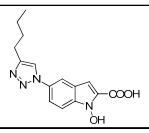
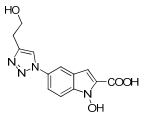
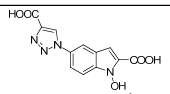
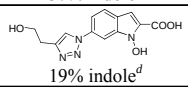
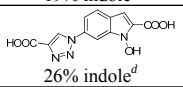
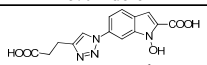
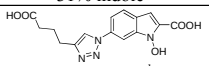
Scheme 3.26. a) $CuSO_4 \cdot 5H_2O$, sodium ascorbate, H_2O/*t*BuOH (1:1), μW, 15 min.; b) $(COOMe)_2$, NaH 60%, dry DMF, –15 °C to RT; c) for **3.218a**: Pb, TEAF, MeOH, 55 °C; for **3.218b**: $SnCl_2 \cdot 2H_2O$, dry DME, 4Å MS, 0 °C to RT; d) aqueous 2N LiOH, THF/MeOH (1:1), RT.

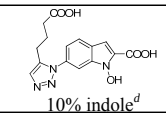
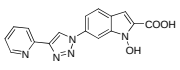
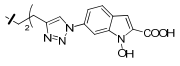
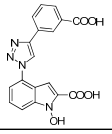
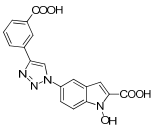
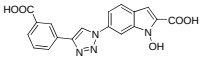
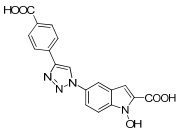
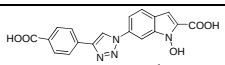
3.9.2. Enzymatic assays

The series of triazole-based compounds (Table **3.18**) generally revealed lower potencies on the enzyme, with only a few exceptions. The insertion of triazole group in position 4 of the central heterocycle led to the obtainment of active compounds only in the case of the phenyl-substituted derivative **3.168a**, while the use of alkyl (**3.168b**) or hydroxyl-alkyl chains (**3.168c**) as substituents of the triazole ring did not produce active inhibitors. Compounds **3.169a-d**, bearing the triazole motif in position 5, were mostly inactive (**3.169a** and **3.169c**), or they were not tested for solubility problems in the assay conditions (**3.169b**); among them, compound **3.169d**, possessing a carboxylic group directly linked to the triazole ring, showed a weak 33% of inhibition on the enzyme. A similar behaviour was observed in the series of derivatives bearing the triazole moiety in position 6 of the NHI scaffold. In this series, variable percentages of inhibition could only be found with those derivatives bearing a carboxylic group, either directly linked

to the triazole ring (**3.170b**), or spaced by an alkyl chain (**3.170c**, **3.170d** and its 1,5-regioisomer **3.196**). Among these compounds the percentages of inhibition range from 6-8% of the carboxy-propyl derivatives **3.170d** and **3.196** to 27% of the carboxy-ethyl analogue **3.170c**, whereas compound **3.170b** possesses an intermediate potency, which is, by the way, lower than that of its 5-substituted analogue **3.169d**. Among these 6-triazole-substituted NHIs, the hydroxyethyl chain of compound **3.170a** and the use of a pyridine cycle in compound **3.170e** did not get any positive results: **3.170a** was completely inactive and **3.170e** was too insoluble in the assay conditions. We were initially encouraged by the biological data obtained with the carboxylic-substituted triazole derivatives, as well as by the promising K_i value of phenyl-triazole derivative **3.168a**, we planned to combine their structural features (COOH group and phenyl ring bound to the triazole moiety) and, therefore, we synthesized compounds **3.201**-**3.205**. Unluckily, only the *meta*-carboxylic derivatives **3.201** (K_i = 57 μM *vs.* NADH and 86 μM *vs.* Pyr) and **3.203** (K_i = 50 μM *vs.* NADH and 45 μM *vs.* Pyr) maintained an acceptable potency, albeit lower than non-carboxylic analogue **3.168a**. Moreover, compound **3.201** showed a very similar inhibition potency also on the other isoform (K_i = 88 μM *vs.* NADH on LDH-B) and it represents the first compound of this class that does not show an isoform-selective inhibition. The other COOH-phenyl-substituted compounds of the triazole series (compounds **3.202**, **3.204** and **3.205**) displayed only modest activities (**3.202** and **3.205**) or they were totally inactive (**3.204**). Finally, the dimer **3.170f** showed a very good inhibitory activity on LDH-A, but, unfortunately, it proved to possess a low level of isoform-selectivity because it also caused a notable 32% inhibition on LDH-B.

Table 3.18. Inhibition data on LDH-5 (LDH-A_4) and LDH-1 (LDH-B_4) for compounds **3.168a-c**, **3.169a-d**, **3.170a-f**, **3.196**, **3.201-3.205**.

Cpd	Structure	Inhibition values	
		LDH-5[a]	LDH-1[b]
3.168a		K_i = 27.5 μM (NADH) 43.7 μM (Pyr)	n.a.
3.168b		n.a.	-
3.168c		5%	-
3.169a		n.a.	-
3.169b		n.t.	-
3.169c		n.a.	-
3.169d	 37% indole[d]	33%	-
3.170a	 19% indole[d]	n.a.	-
3.170b	 26% indole[d]	17%	-
3.170c	 31% indole[d]	27%	-
3.170d	 30% indole[d]	6%	-

3.196	 10% indole[d]	8%	-
3.170e		n.t.	-
3.170f		K_i = 20.7 μM (NADH) 37.4 μM (Pyr)	32.6%
3.201		K_i = 57 μM (NADH) 86 μM (Pyr)	K_i = 88.7 μM (NADH)
3.202		15%[c]	-
3.203		K_i = 50 μM (NADH) 45 μM (Pyr)	n.a.[c]
3.204		n.a.[b]	-
3.205	 26% indole[d]	27%[c]	-

[a] compounds tested at 250 μM, [b] compounds tested at 125 μM, [c] compounds tested at 100 μM, [d] compounds tested as a mixture with the reported percentage of indole. n.a. = not active. n.t. = compounds not tested for solubility problems.

3.9.3. Molecular modeling studies

Molecular modeling studies of one of the most active triazole derivatives, **3.168a**, demonstrated that the presence of a triazole-phenyl group in position 4 allows the interaction of the compound with a different zone of the binding site. As shown in Fig. **3.51**, docking of compound **3.168a** shows a binding mode of the central 1-hydroxy-*H*-indole-2-carboxylic scaffold very similar to that found for the other analyzed compounds. The carboxylic group of compound **3.168a** shows a strong interaction with R169 and T248 and the *N*-hydroxy group shows an H-bond interaction with the nitrogen

backbone of T248 and a water molecule that mediates the interaction of **3.168a** with the catalytic H193. As shown in Fig. **3.51**, the phenyl group linked to the triazole ring displays a favorable π- π interaction with Y239.

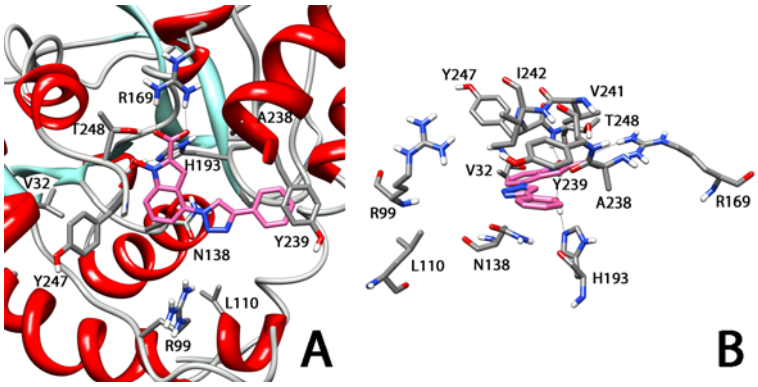


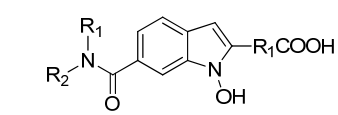
Fig. 3.51. Docking analysis of compound **3.168a**. Overall disposition of the ligand into LDH (A, residues 240-245 are hidden for a better visualization) and LDH-ligand interactions (B).

3.10. AMIDE AND SULFONAMIDE DERIVATIVES

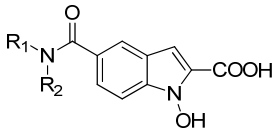
3.10.1. Synthesis

Part of my PhD work concerned the synthesis of amide- and sulfonamide-substituted NHI derivatives. We initially planned to introduce amide groups in positions 5 and 6 of the NHI structure to explore if the presence of these kinds of groups could be beneficial for LDH-A inhibition activity. The first synthesized amides **3.220a-d** and **3.221a-d** are summarized in Fig. **3.52**. The choice of the combinations of the various substituents on the amidic nitrogen was dictated by the availability of the precursors and also by the synthetic feasibility of both intermediates and target molecules. The use of the cyclopropane sulfonamide group, which is a versatile building block for many biologically active compounds, rises from our initial idea to introduce polar moieties on *N*-hydroxyindoles in order to gain new hydrophilic interactions in the LDH-A active site between the enzyme and our inhibitors. In particular, this group can be considered

as a bioisoster of a phosphate group, then it should be able to mimick the diphosphate portion present in the LDH-cofactor NADH.



3.220a-d



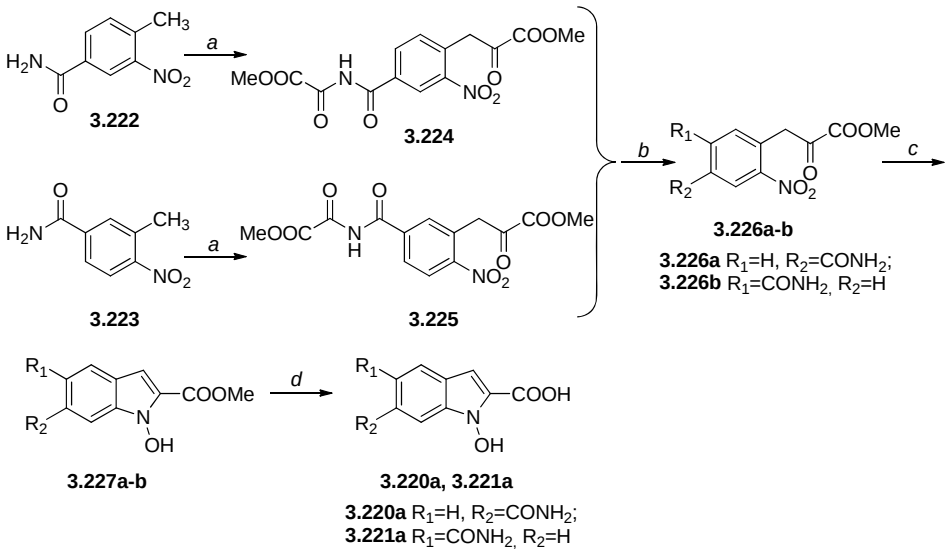
3.221a-d

Cpd	R_1	R_2
3.220a	H	H
3.220b	CH_3	
3.220c	CH_3	CH_3
3.220d	H	

Cpd	R_1	R_2
3.221a	H	H
3.221b	CH_3	
3.221c		
3.221d	H	

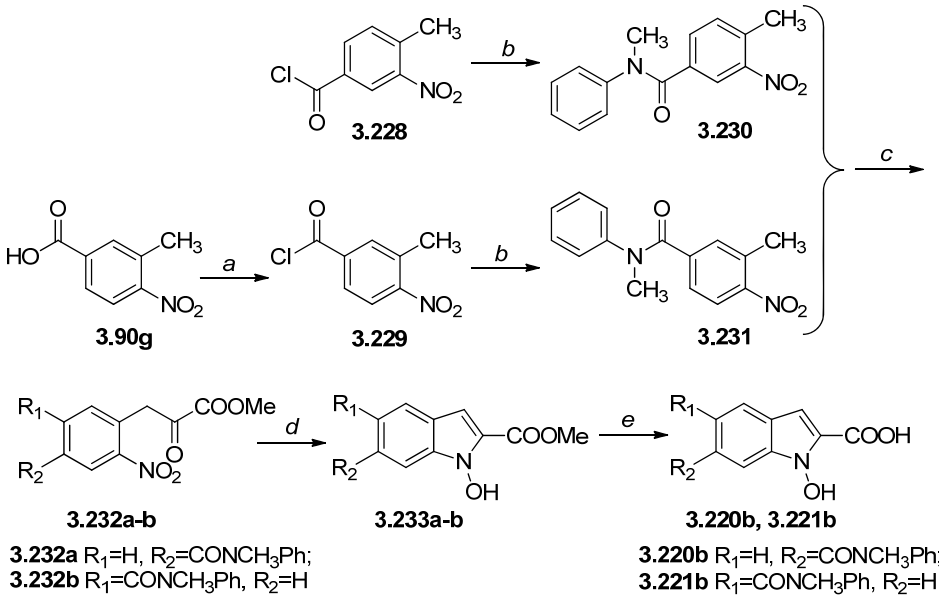
Figure 3.52. Structures of 6- and 5-amido-substituted compounds **3.220a-d** and **3.221a-d**, respectively.

The synthesis of *N*-unsubstituted amide derivatives **3.220a** and **3.221a** was accomplished as shown in Scheme **3.27**. Commercially available benzamides **3.222** and **3.223** were treated with sodium hydride and dimethyl oxalate for the synthesis of the respective ketoesters, but the proper formation of ketoester chains was accompanied by the alkylation of the primary amidic groups, thus producing compounds **3.224** and **3.225**. These labile immidic products were subsequently hydrolyzed by exploiting the slight acidity of silica gel in methanol to selectively remove the -COCOOMe moiety linked to the amidic nitrogen, whereas the more stable ketoester chain was not affected by these conditions. Then, desired ketoesters **3.226a-b** were cyclized with stannous chloride by carefully maintaining the temperature at 0 °C for all the reaction time to prevent the formation of the over-reduced indole side-product; however, even under those conditions, **3.227a** was not obtained as a pure compound, but it was isolated in a mixture containing a 50% of the corresponding indole. Finally hydrolysis yielded the desired products **3.220a** (together with a 50% of its indole analogue) and pure **3.221a**.

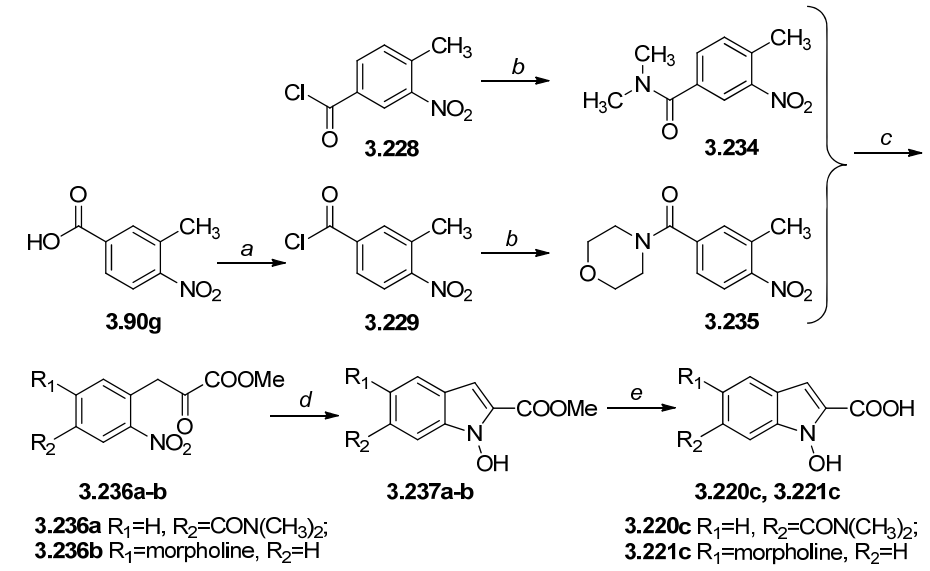


Scheme 3.27. a) $(COOMe)_2$, NaH 60%, dry DMF, –15 °C to RT; b) SiO_2, MeOH, RT; c) $SnCl_2 \cdot 2H_2O$, dry DME, 4Å MS, 0 °C; e) aqueous 2N LiOH, THF/MeOH (1:1), RT.

Amides **3.220b** and **3.221b** were prepared starting from the acid chlorides **3.228** and **3.229**: 4-methyl-3-nitrobenzoylchloride **3.228** is commercially available, while **3.229** was obtained by reaction of 3-methyl-4-nitrobenzoic acid **3.90g** with oxalyl chloride in dichloromethane (Scheme **3.28**). Then, acid chlorides **3.228** and **3.229** were reacted with an excess of *N*-methylaniline in DCM in a closed vessel, affording amides **3.230** and **3.231**, which, at this point, followed the same synthetic steps of alkylation, reductive cyclization and hydrolysis seen before for the synthesis of other NHI derivatives. It is interesting to note that only ketoester **3.232b** was obtained with a low yield, due to the contemporaneous formation of a methyl ester side product, generated by a nucleophile attack of MeO^-, formed in the reaction medium from dimethyl oxalate, to the amidic group, resulting in the formation of a di-ester side product, which could be easily separated from the desired intermediate by column chromatography. Compounds **3.220c** and **3.221c** were achieved by means of a synthetic path similar to the one exemplified in Scheme **3.28**; in this case we used dimethylamine and morpholine instead of *N*-methylaniline for the preparation of their amidic nitrotoluene intermediates **3.234** and **3.235** (Scheme **3.29**).

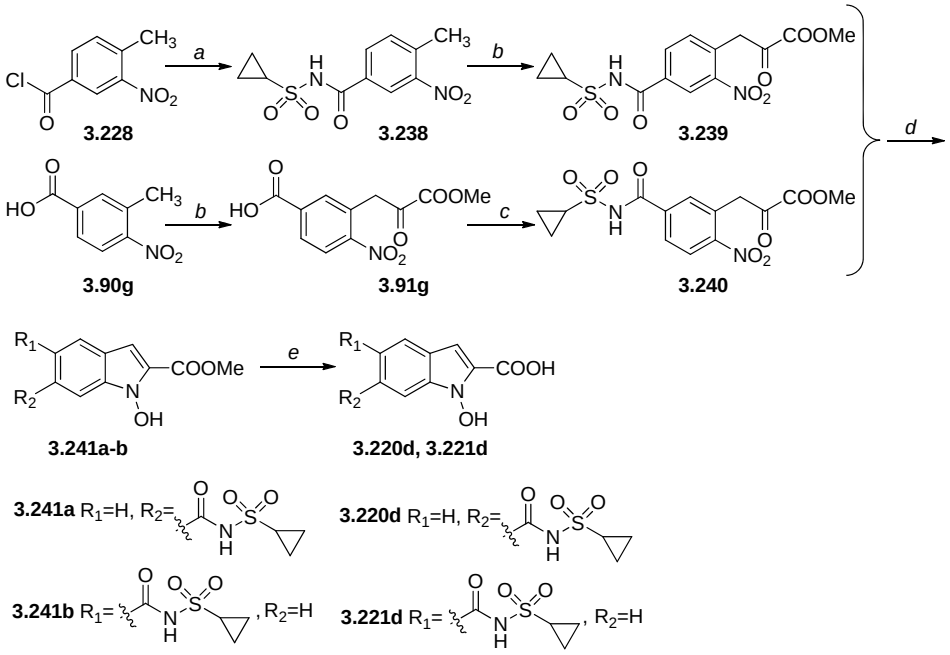


Scheme 3.28. a) $(COCl)_2$, DCM, RT; b) *N*-methylaniline, DCM, RT; c) $(COOMe)_2$, NaH 60%, dry DMF, –15 °C to RT; d) $SnCl_2 \cdot 2H_2O$, dry DME, 4Å MS , 0 °C; e) aqueous 2N LiOH, THF/MeOH (1:1), RT.



Scheme 3.29. a) $(COCl)_2$, DCM, RT; b) for **3.234**: dimethylamine, DCM, RT; for **3.235**: morpholine, DCM, RT c) $(COOMe)_2$, NaH 60%, dry DMF, –15 °C to RT; d) $SnCl_2 \cdot 2H_2O$, dry DME, 4Å MS, 0 °C; e) aqueous 2N LiOH, THF/MeOH (1:1), RT.

Cyclopropansulfonamide moiety was inserted in position 6 of the NHI nucleus by a reaction of commercially available cyclopropane sulfonamide (abbreviated CPS, in Scheme **3.30**) with 4-methyl-3-nitrobenzoylchloride **3.228** using triethylamine as the base [136] and in position 5 by condensation with the synthesized benzoic derivative **3.91g** with CDI as the condensing agent and DBU as the base [137]. In both cases, the reactions proceeded with good yields (70-80%), so both the carboxylic acid and acid chloride proved to be equally efficient condensation substrates for CPS, pending the use of respectively appropriate reaction conditions. Then ipophosphite and palladium promoted the reductive cyclization of ketoesters **3.239** and **3.240** to compounds **3.241a** and **3.241b**, respectively, which were hydrolyzed to get the desired products **3.220d** and **3.221d**.



Scheme 3.30. a) CPS, Et_3N, DCM, 0 °C to RT; b) $(COOMe)_2$, NaH 60%, dry DMF, –15 °C to RT; c) CPS, CDI, DBU, dry THF, RT; d) $H_2PO_2Na{\cdot}H_2O$, Pd-C 10%, H_2O/THF (1:1), RT; e) aqueous 2N LiOH, THF/MeOH (1:1), RT.

Amide derivatives proved to be very weak LDH-A inhibitors (see enzymatic assay Section below), therefore we did not complete all the possible structural combinations of these substituents in the various positions of the NHI scaffold. We rather shifted our interest to the design of sulfonamide-substituted *N*-hydroxyindoles. We started this series with the synthesis of three representative compounds, to find the best position for the introduction of this kind of moiety, as well as, the most appropriate substituents of the sulfonamidic nitrogen atom for the development of this class. These compounds are: **3.242**, which bears a *N*,*N*-dimethyl sulfonamide in position 6; **3.243**, possessing a *N*-methyl-*N*-phenyl sulfonamide in the same position 6; **3.244**, where the same *N*-methyl-*N*-phenyl sulfonamide is shifted to position 5 (Fig. **3.53**).

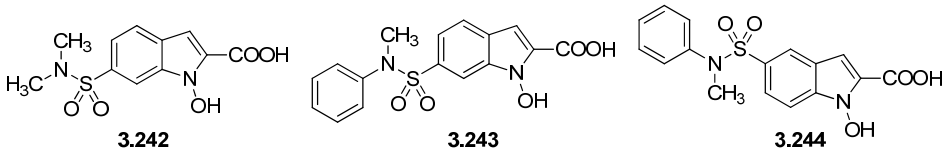
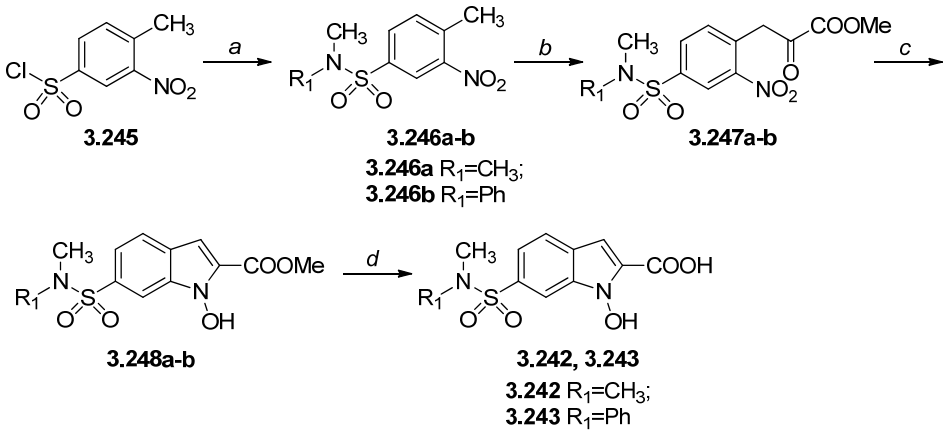


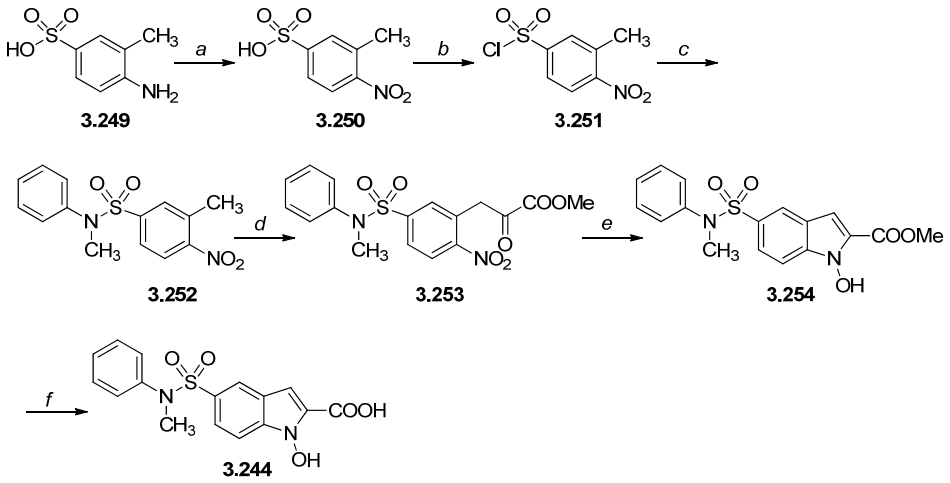
Figure 3.53. Sulfonamide compounds **3.242-3.244**.

The preparation of compounds **3.242** and **3.243** proceeded following a synthetic pathway that is similar to that already described for the amide derivatives (Scheme **3.31**). 4-Methyl-3-nitrobenzenesulfonylchloride **3.245** was reacted with an excess of the appropriate amine, dimethylamine for **3.246a** and *N*-methyalaniline for **3.246b**, to get the relative sulfonamide products. Pure cyclized methyl ester **3.248a** was obtained by simply treating **3.247a** with stannous chloride. On the contrary, the same reaction conditions applied to **3.247b** produced a relevant percentage of indole side product, whereas we were pleased to find that the use of sodium ipophosphite and catalytic palladium over charcoal gave the pure desired intermediate **3.248b**.



Scheme 3.31. a) for **3.246a**: dimethylamine, DCM, RT; for **3.246b**: *N*-methylaniline, DCM, RT; b) $(COOMe)_2$, NaH 60%, dry DMF, –15 °C to RT; c) for **3.247a**: $SnCl_2 \cdot 2H_2O$, dry DME, 4Å MS, 0 °C; for **3.247b**: $H_2PO_2Na \cdot H_2O$, Pd-C 10%, H_2O/THF (1:1), RT; d) aqueous 2N LiOH, THF/MeOH (1:1), RT.

For the synthesis of compound **3.244** (Scheme **3.32**), 3-methyl-4-nitrobenzensulfonylchloride **3.251** was not commercially available, so 4-amino-3-methylbenzenesulfonic acid **3.249** was first subjected to oxidation of the aniline NH_2 group with hydrogen peroxide in refluxing acetic acid [138]. The resulting nitro-substituted sulfonic acid derivative **3.250** was converted into sulfonyl chloride **3.251** by using thionyl chloride as the chlorinating agent in the presence of some drops of DMF, that acts as the catalyst, at 100 °C in a closed vessel [139]. Then, the synthesis followed the same steps we used for the preparation of compound **3.243**, with the exception of the $SnCl_2$-promoted reductive cyclization of ketoester **3.253**, which was carried out in the presence of thiophenol and triethylamine, because these additives proved to improve the efficiency of that step.



Scheme 3.32. a) H_2O_2 35%, CH_3COOH, 75 °C; b) $SOCl_2$, dry DMF, 100 °C; c) *N*-methylaniline, DCM, RT; d) $(COOMe)_2$, NaH 60%, dry DMF, –15 °C to RT; e) $SnCl_2 \cdot 2H_2O$, C_6H_5SH, Et_3N, CH_3CN, RT; f) aqueous 2N LiOH, THF/MeOH (1:1), RT.

Among these first sulfonamide derivatives, only compound **3.243** showed a notable inhibition activity on the enzyme, so we planned to make some structural modifications on this molecule that could provide an improvement in its activity on LDH-A, such as: 1) substitution of the *N*-methyl group with a bulkier and more lipophilic *n*-butyl group (compound **3.255**, Fig. **3.54**); 2) introduction of substituents in the *para* position of the sulfonamidic phenyl ring (compounds **3.256-3.259**, Fig. **3.54**); 3) introduction of a phenyl spacer between the NHI core and the sulfonamide groups (compounds **3.260-3.261**, Fig. **3.54**).

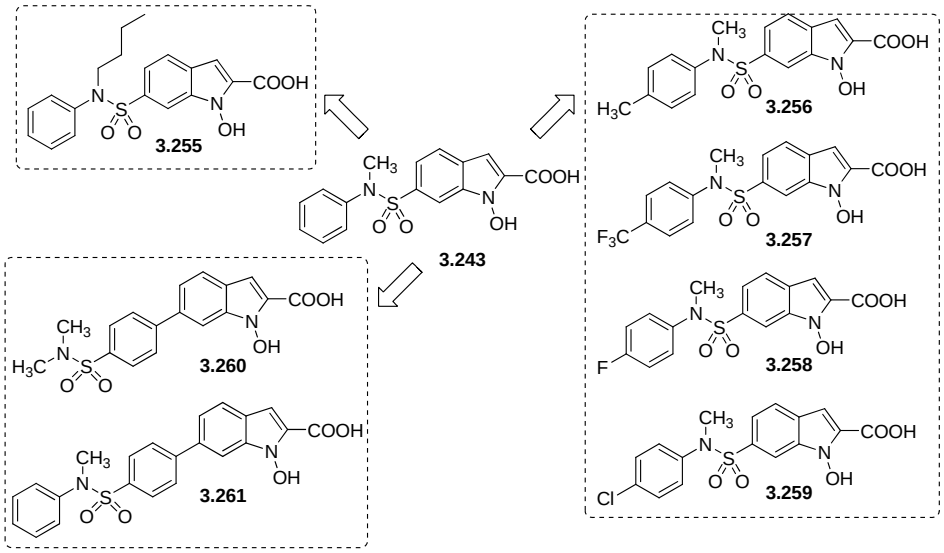
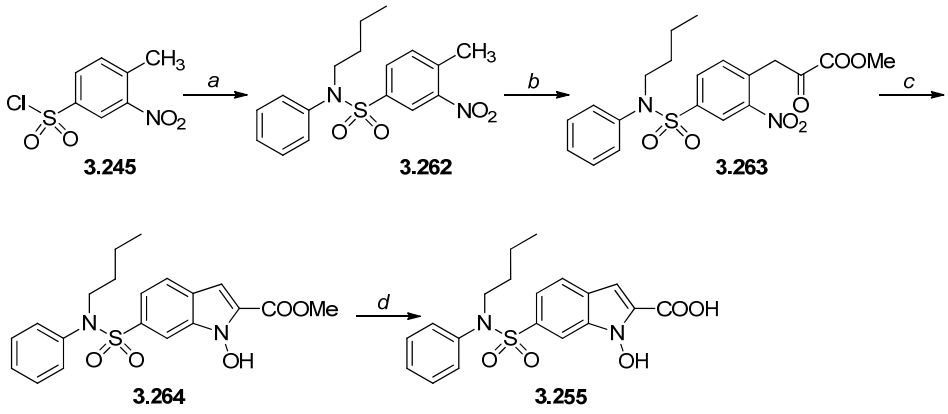


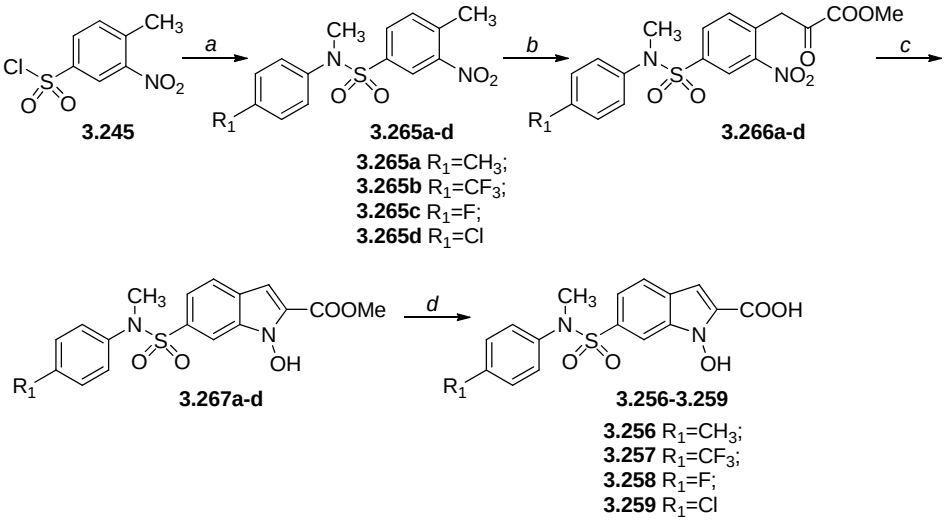
Figure 3.54. Compounds **3.255-3.261** deriving from structural developments of compound **3.243**.

Compound **3.255** (Scheme **3.33**) was obtained in the same manner as its *N*-methyl-substituted analogue **3.243**, with the only exceptions of the use of the appropriate *N*-butylaniline for the sulfonamide formation (step *a*, Scheme **3.33**) and of the reagents used for the cyclization step (stannous chloride with thiophenol/triethylamine, step *d*, Scheme **3.33**).



Scheme 3.33. a) *N*-buthylaniline, DCM, RT; b) $(COOMe)_2$, NaH 60%, dry DMF, –15 °C to RT; c) $SnCl_2 \cdot 2H_2O$, C_6H_5SH, Et_3N, CH_3CN, RT; d) aqueous 2N LiOH, THF/MeOH (1:1), RT.

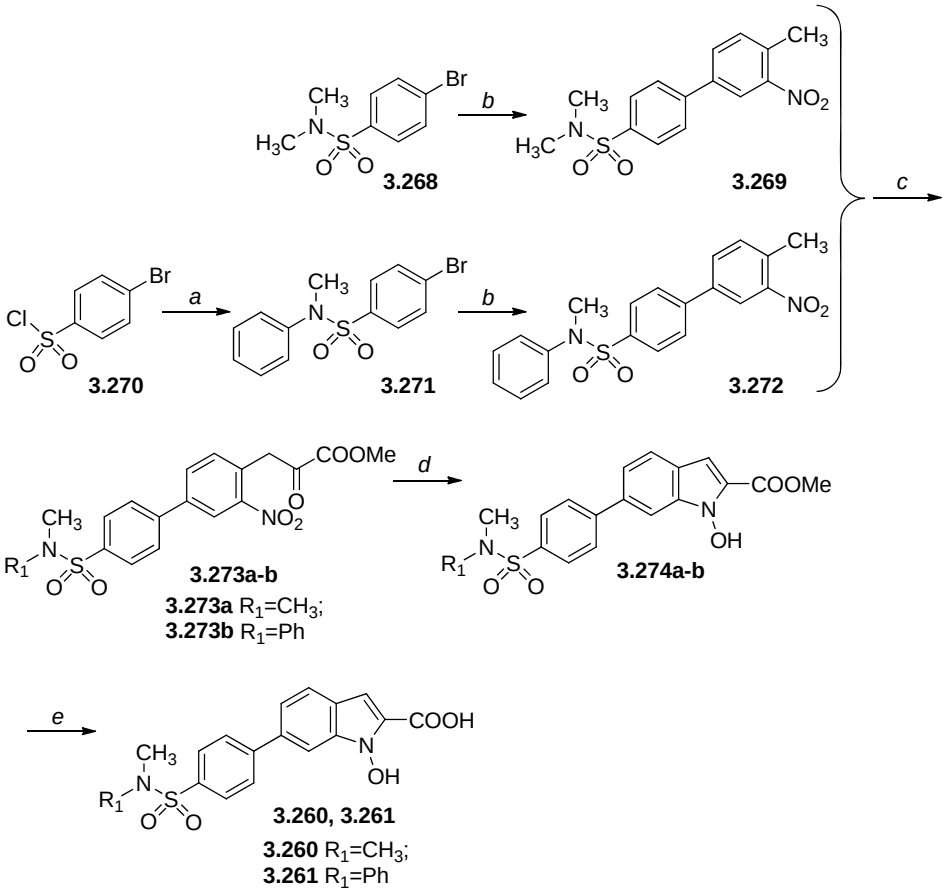
Inhibitors **3.256-3.259** were obtained as illustrated in Scheme **3.34**. For the sulfonamide formation step, only **3.265a** could be obtained in high yield by simple condensation of sulfonyl chloride **3.245** with an excess *N*-methyl-*p*-toluidine in dichloromethane, whereas in the other three cases (**3.265b-d**) the use of catalytic 4-dimethylaminopyridine (DMAP) in pyridine was preferred, because these latter conditions proved to considerably reduce reaction times and increase the isolated yields. In the cyclization step of all the sulfonamido-substituted ketoesters **3.266a-d**, the use of $SnCl_2$ and $PhSH/Et_3N$ always produced the cyclized methyl esters **3.267a-d** in complex product mixtures containing a certain amount of impurities. After an optimization study, we found out that the use of metallic lead in TEAF afforded purer cyclized products, similarly to what we experienced in the preparation of cyclized intermediate **3.120d** previously described (Scheme **3.6**). This method was, then, preferred for the reaction of these sulfonamido-substituted substrates producing intermediates **3.267a-d**.



Scheme 3.34. a) for **3.265a**: *N*-methyl-*p*-toluidine, DCM, RT; for **3.265b-c-d**: appropriate *p*-substituted *N*-methylaniline, pyridine, DMAP, dry DCM, 0 °C to RT; b) $(COOMe)_2$, NaH 60%, dry DMF, –15 °C to RT; c) Pb, TEAF, MeOH, 55 °C; d) aqueous 2N LiOH, THF/MeOH (1:1), RT.

The subsequent structural evolution concerned the introduction of a phenyl in position 6 as a linker between the *N*-hydroxyindole cycle and the sulfonamide group

(compounds **3.260-3.261**, Fig. **3.54**). These compounds were inspired by the combination of the structural characteristics contained in 6-phenyl-substituted NHI **3.113** and sulfonamides such as **3.243**, which both proved to be active LDH-A inhibitors. Initially, we started our synthetic studies (Scheme **3.35**) on the simpler *N*,*N*-dimethyl-sulfonamide derivative by reacting 4-bromo-*N*,*N*-dimethylbenzenesulfonamide **3.268** with 4-methyl-3-nitrobenezeneboronic acid **3.127** in a microwave-assisted Suzuki coupling. Then, the same procedure was applied to the synthesized bromo derivative **3.271**, but with poorer results (only 40% yield), so this substrate was subjected to a Suzuki coupling in the classical thermal conditions, this time obtaining compound **3.272** in 70% yield. Subsequently, the two nitrotoluenic substrates **3.269** and **3.272** were treated with sodium hydride and dimethyl oxalate to give ketoesters **3.273a-b**, then $SnCl_2$-induced cyclization in the presence of thiophenol/triethylamine and hydrolysis with lithium hydroxide afforded final products **3.260** and **3.261**.



Scheme 3.35. a) *N*-methylaniline, DCM, RT; b) for **3.268**: 4-methyl-3-nitrobenezeneboronic acid **3.127**, $Pd(OAc)_2$, TBAB, Na_2CO_3, H_2O, μW, 5 min; for **3.271**: 4-methyl-3-nitrobenezeneboronic acid **3.127**, $Pd(OAc)_2$, PPh_3, aqueous 2M Na_2CO_3, distilled toluene, abs. EtOH, 100 °C, 24 h; c) $(COOMe)_2$, NaH 60%, dry DMF, –15 °C to RT; d) $SnCl_2 \cdot 2H_2O$, C_6H_5SH, Et_3N, CH_3CN, RT; e) aqueous 2N LiOH, THF/MeOH (1:1), RT.

Compound **3.259**, possessing a chlorine atom in *para* position of the phenyl ring, proved to be the best LDH-A inhibitor (see enzymatic assay Section below) generated from the structural modifications of compound **3.243**. On this basis, we completed our SAR studies of this sulfonamide class, by changing the position of the chlorine atom. So we decided to shift it form the *para* to the *meta* and *ortho* positions of the phenyl substituent, thus producing compounds **3.275** and **3.276**, respectively (Fig. **3.55**).

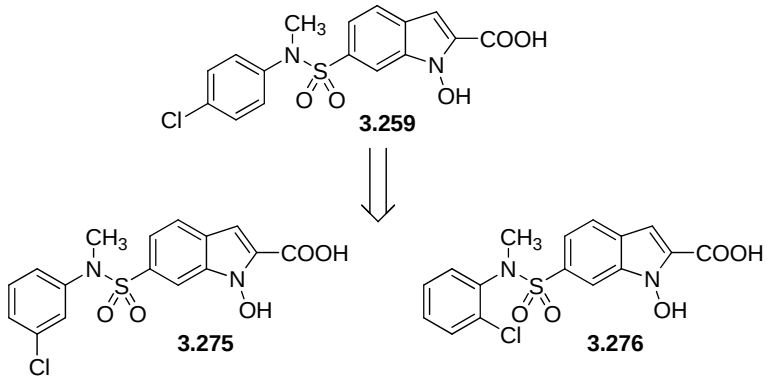
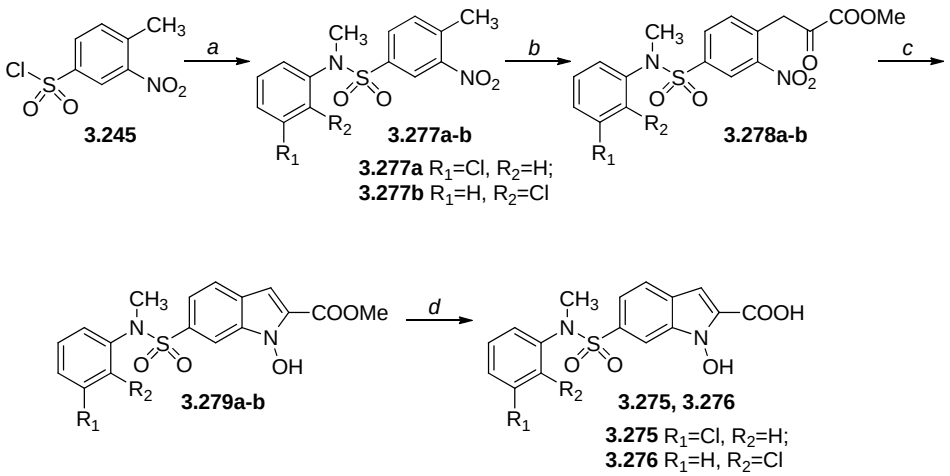


Figure 3.55. Chloro-substituted compounds **3.275** and **3.276** deriving from structural modifications of compound **3.259**.

Sulfonamides **3.277a** and **3.277b** were synthesized from commercially available 4-methyl-3-nitrobenzenesulfonylchloride **3.245** by respectively using different reaction conditions (Scheme **3.36**). Solvent-free condensation of **3.245** with 3-chloro-*N*-methylaniline in the presence of anhydrous sodium bicarbonate successfully afforded compound **3.277a** [140]. Unfortunately, the same reaction conditions produced sulfonamide **3.277b** in very low yields, so we switched back to the already used condensation conditions, comprising catalytic DMAP and pyridine, which demonstrated to be the most suitable for the synthesis of this compound. For the cyclization step, we initially tried the same Pb/TEAF conditions that were efficiently employed for *para*-substituted ketoesters **3.266a-d**: however, in this case these reagents did not afford the proper cyclized products **3.279a-b**, whereas they promoted the retro-Claisen reaction, forming the nitrotoluenic derivatives **3.277a-b**. On the contrary, we optimized the stannous chloride/thiophenol conditions for these substrates, by decreasing the equivalents of the tin salt (1 eq. instead of 1.5 eq) for compounds **3.278a-b**. With this way we were able to limit the formation of the over-reduced indole side products.



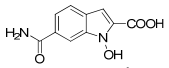
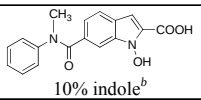
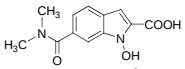
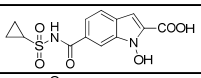
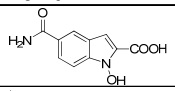
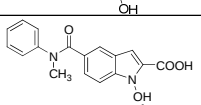
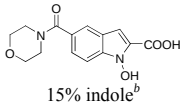
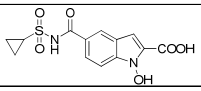
Scheme 3.36. a) for **3.277a**: 3-chloro-*N*-methylaniline, anhydrous $NaHCO_3$, RT; for **3.277b**: 2-chloro-*N*-methylaniline, pyridine, DMAP, dry DCM, 0 °C to RT; b) $(COOMe)_2$, NaH 60%, dry DMF, –15 °C to RT; c) $SnCl_2 \cdot 2H_2O$ (1eq.), C_6H_5SH, Et_3N, CH_3CN, RT; d) aqueous 2N LiOH, THF/MeOH (1:1), RT.

3.10.2. Enzymatic assays

Enzymatic results regarding amide derivatives are summarized in Table **3.19**. Generally, amides did not show encouraging results, all of them possessing very low inhibition percentages. The simple 6-amide-susbtituted derivative **3.220a** proved to be slightly more active on the enzyme when compared to its analogue substituted in position 5 **3.221a**, with a 8% inhibition on LDH-A. The insertion of two methyl groups on the amidic nitrogen, as in compound **3.220c**, nearly preserved the same very modest activity of **3.220a** (6%), whereas the replacement of one of the two *N*-methyl groups with a phenyl ring (**3.220b**) caused the complete loss of inhibition activity. On the contrary, the insertion of methyl and phenyl groups on the amidic nitrogen in position 5 in compound **3.221b** led to a slightly increased potency with respect to that of compound **3.221a**, so displaying an opposite trend relative to the 6-amide derivatives. When the nitrogen atom was included in a morpholine cycle (**3.221c**), no significant activity could be detected. Compounds **3.220d** and **3.221d**, bearing a cyclopropanesulfonamide group on the amidic nitrogen in positions 6 and 5, respectively, were both active, but with low inhibition percentage. Anyway **3.221d**, with a poor 11% of inhibition at 250 μM, proved to be the most active compound of this

unsuccessful amide series, together with the *N*-methyl-*N*-phenylamide derivative **3.221b**, confirming that insertion of amides in position 5 of the *N*-hydroxyindole scaffold is preferred to the same modification in position 6.

Table 3.19. Inhibition data on LDH-5 (LDH-A_4) and LDH-1 (LDH-B_4) for compounds **3.220a-d**, **3.221a-d**.

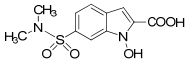
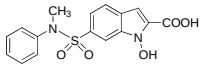
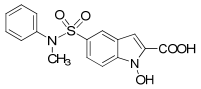
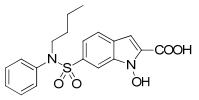
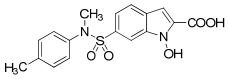
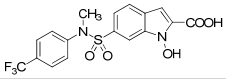
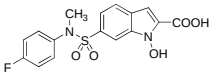
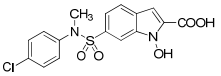
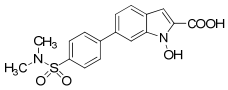
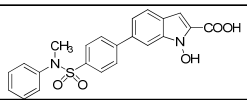
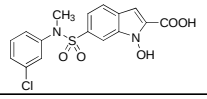
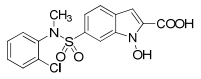
Cpd	Structure	Inhibition values	
		LDH-5[a]	LDH-1
3.220a	 50% indole[b]	8%	-
3.220b	 10% indole[b]	n.a.	-
3.220c	 40% indole[b]	6%	-
3.220d		6.5%	-
3.221a		n.a.	-
3.221b	 50% indole[b]	11%	-
3.221c	 15% indole[b]	n.a.	-
3.221d		11%	-

[a] compounds tested at 250 μM, [b] compounds tested as a mixture with the reported percentage of indole. n.a. = not active.

A different situation was found within the sulfonamide class of inhibitors, as reported in Table **3.20**. In this case, the low activity showed by 5-sulfonamido-substituted compound **3.244** shifted our main interest in the development of sulfonamide derivatives bearing this kind of substituents in position 6. In any case, the 30% inhibition at 50 μM of this compound, being much better than the data obtained

with its amide analogue **3.221b**, already confirmed that the sulfonamide class could be more promising than the previous amide series. From an analysis of the activity shown by the first 6-sulfonamide compounds obtained (**3.242** and **3.243**), it is evident that the presence of a phenyl ring on the sulfonamido-nitrogen atom is highly beneficial, contrarily to what had been previously observed for amide derivatives **3.220b** and **3.220c**. As a matter of fact, *N*,*N*-dimethyl-substituted compound **3.242** had basically no activity on LDH-A, whereas its *N*-methyl-*N*-phenyl-substituted analog **3.243** proved to be a very promising sulfonamide derivative, with a K_i value of 17.1 μM (NADH) and 9.3 μM (Pyr) on LDH-A, and no activity detected on LDH-B. Substituting the methyl with a bulkier alkyl chain (*N*-butyl analogue **3.255**), the activity was substantially unmodified versus the cofactor NADH, while it decreased versus the substrate pyruvate, but still maintaining the high selectivity against the desired isoform. The *para*-substituted derivatives **3.256-3.259** were selective LDH-A inhibitors (K_i values ranging from 125 to 6 μM), and in particular the *para*-chlorophenyl-substituted compound **3.259** gave excellent results (K_i = 6.6 μM *vs.* NADH and 5.6 μM *vs.* Pyr). When the chlorine atom was moved from the *para* position of the phenyl ring of **3.259** to the *ortho*-(**3.276**), or *meta*-positions (**3.275**), the activities of the resulting compounds suffered from a slightly decrease, but both compounds maintained acceptable inhibition values on LDH-A. When a phenyl ring was inserted as a "linker" between the sulfonamide group and the NHI nucleus, we obtained results comparable to those achieved in the absence of this spacer moiety. In fact, derivative **3.260** possessing two methyl groups on the nitrogen was completely inactive (see compound **3.242** for comparison). On the other hand, derivative **3.261**, where one of the two *N*-methyl groups is replaced by a phenyl ring, displayed a good potency (K_i = 16 μM *vs.* NADH and 22.5 μM *vs.* Pyr) on the enzyme (see compound **3.243** for comparison).

Table 3.20. Inhibition data on LDH-5 (LDH-A_4) and LDH-1 (LDH-B_4) for compounds **3.242-3.244**, **3.255-3.261**, **3.275-3.276**.

Cpd	Structure	Inhibition values	
		LDH-5[a]	**LDH-1**[b]
3.242	 5% indole[e]	5%	-
3.243		K_i = 17.1 μM (NADH) 9.3 μM (Pyr)	n.a.[c]
3.244		30%[d]	-
3.255		K_i = 17 μM (NADH) 100 μM (Pyr)	n.a.
3.256		K_i = 20 μM (NADH) 20 μM (Pyr)	n.a.
3.257		K_i = 26 μM (NADH) 6 μM (Pyr)	n.a.
3.258		K_i = 125 μM (NADH) 28 μM (Pyr)	n.a.
3.259		K_i = 6.6 μM (NADH) 5.6 μM (Pyr)	n.a.
3.260		n.a.	-
3.261		K_i = 16 μM (NADH) 22.5 μM (Pyr)	n.a.
3.275		K_i = 48 μM (NADH) 43 μM (Pyr)	n.a.
3.276		K_i = 14 μM (NADH) 25 μM (Pyr)	n.a.

[a] compounds tested at 250 μM. [b] compounds tested at 100 μM, [c] compounds tested at 125 μM, [d] compounds tested at 50 μM, [e] compounds tested as a mixture with the reported percentage of indole. n.a. = not active.

3.10.3. Molecular modelling studies

In order to analyze the interaction of a representative sulfonamide derivative, compound **3.243** was docked in the minimized structure of the protein extracted from the complex with compound **3.139**.

Molecular modelling studies demonstrated that the substitution of the phenyl ring in position 6 (**3.139**) with the *N*-methyl-*N*-phenylsufonamide group in compound **3.243** determines a different interaction of the aromatic ring. As showed in Fig. **3.56**, the docking of compound **3.243** highlights the same disposition of the 1-hydroxy-*H*-indole-2-carboxylic scaffold already showed by **3.139**, **3.112** and **3.114** (see Section 3.8.3), with the formation of the H-bonds with R169, T248 and the water molecule that mediates the interaction with the catalytic H193. Furthermore, as shown in Fig. **3.56**, the phenyl ring of **3.243** displays a favourable T-shaped, perpendicular arrangement with the aromatic ring of Y247 [141].

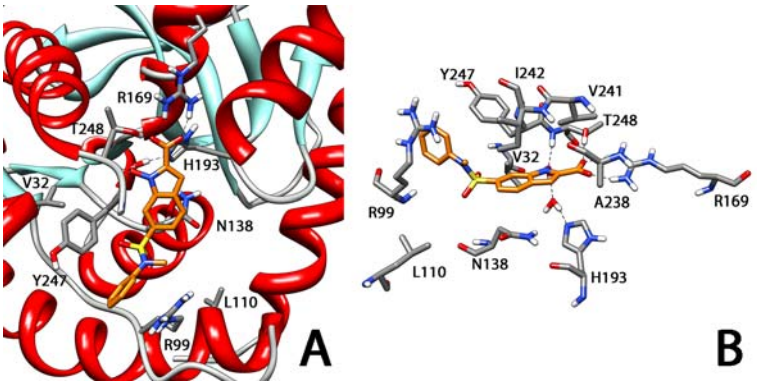


Figure 3.56. Docking analysis of compound **3.243**. Overall disposition of the ligand into LDH (A, residues 240-245 are hidden for a better visualization) and LDH-ligand interactions (B).

3.11. CELLULAR ASSAYS

During my PhD, I spent a research period (July 2009-December 2009) in the group of Prof. Paul J. Hergenrother at the Department of Chemistry at Urbana-Champaign (Illinois, USA). In this period, a series of derivatives, previously synthesized in our laboratory in Pisa, were tested under my supervision in cellular assays based on cytotoxicity on selected cell lines and then they were submitted to cell-based NMR studies to confirm their mechanism of action.

3.11.1. Cytotoxicity assays

The compounds were screened in triplicate on three different cell lines: HeLa (cervical cancer), U-937 (lymphoma) and CHO-K1 (chinese hamster ovary). They were assayed at two concentrations, 100 μM and 1 mM, and at two different incubation times, 24 h and 72 h. Briefly, compounds were dissolved in DMSO stock solutions (10 mM and 100 mM, with 1% DMSO final concentration in wells), cells were grown in 96-well plates in RPMI-1640 media supplemented with 10% FBS and 1% Penicillin/Streptomycin (for HeLa and U-937 cells) or in Ham's F-12K media (for CHO-K1 cells). Cells treated with compounds or controls were incubated under normoxia in a 37 ºC, 5% CO_2, 95% humidity incubator and in a hypoxic incubator (1% O_2). In each cytotoxicity experiment, the percentage of non-viable cells was calculated for each compound and the data were compared with those of control compounds that modulate glycolytic metabolism, such as 3-bromopyruvate (3-BP) and 2-deoxyglucose (2-DG) that inhibit the first glycolytic enzyme hexokinase. For assessing cell death, sulforhodamine B assay [142] was used in the HeLa and CHO-K1 cell-based experiments, while MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) protocol [143] was preferred for U-937 cells.

The best cytotoxicity data were obtained with HeLa cell line at 100 μM for 72 h, as shown in the two bar graphs below (Fig. **3.57-3.58**), since many compounds determined a decreased survival or the complete death of tumour cells. In the bar graphs, on the y axis it is reported the percentage of non-viable cells and on the x axis each compound is represented by two bars, the red one for normoxia and the blue one for hypoxia. The

smallest member of the NHI class, the unsubstituted derivative **3.89a** (Fig. **3.58**) showed a slight hypoxia-selective cytotoxic action and the 4-CF_3-6-phenyl-substituted derivative **3.139** (Fig. **3.57**) confirmed its inhibition property, reaching nearly a 50% activity if compared to that of control compounds 3-BP and 2-DG. The triazole-containing derivative **3.168a** (Fig. **3.57**), the carboxy-substituted compound **3.125c** and the 5-phenyl-substituted NHI **3.112** (Fig. **3.58**) showed more pronounced normoxia-selective action. Some compounds (**3.97** and **3.99** in Fig. **3.57** and **3.100** in Fig. **3.58**) showed unexpected high cytotoxic properties, which cannot be correlate to any inhibition of LDH-A and, therefore, is likely due to different mechanisms that are currently under investigation. The result generated by compound **3.120c** (Fig. **3.58**), which is also inactive as LDH-A inhibitor, may be explained by considering the increased permeability of this methyl ester derivative, when compared to its free carboxylic analogues, that may be followed by the hydrolysis of the ester group within the cell, thus generating the active compound **3.113** (Fig. **3.58**), which displayed a good cytotoxicity on the selected cell line (30-35% of non-viable cells). Hence, **3.120c** may be considered as a prodrug of **3.113**, although further studies are needed to definitely clarify this aspect.

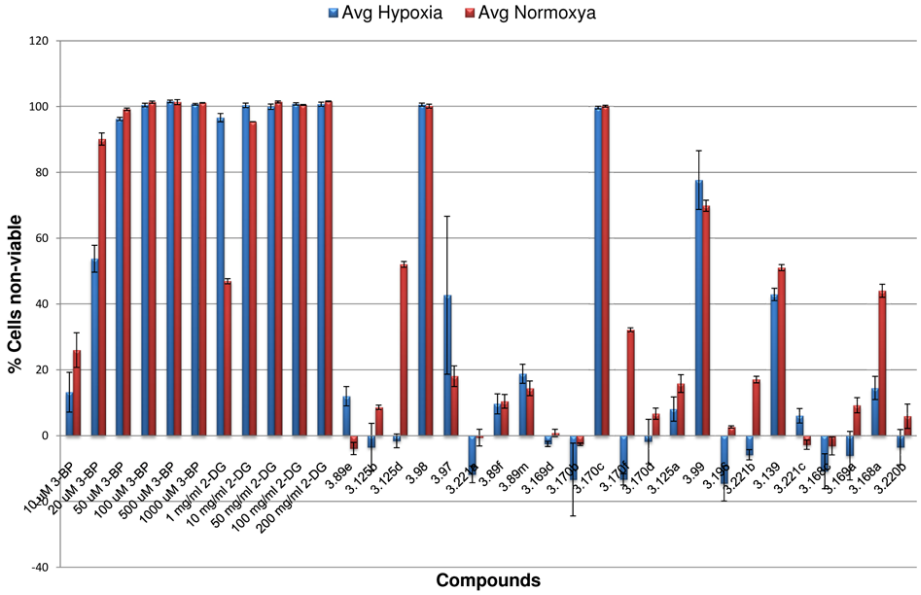


Figure 3.57. Cytotoxicity bar graph of the analyzed compounds tested on HeLa cell line at 100 μM for 72 h and of control compounds 3-bromopyruvate (3-BP) and 2-deoxyglucose (2-DG) tested at different concentrations, in hypoxic (blue bars) and normoxic (red bars) conditions.

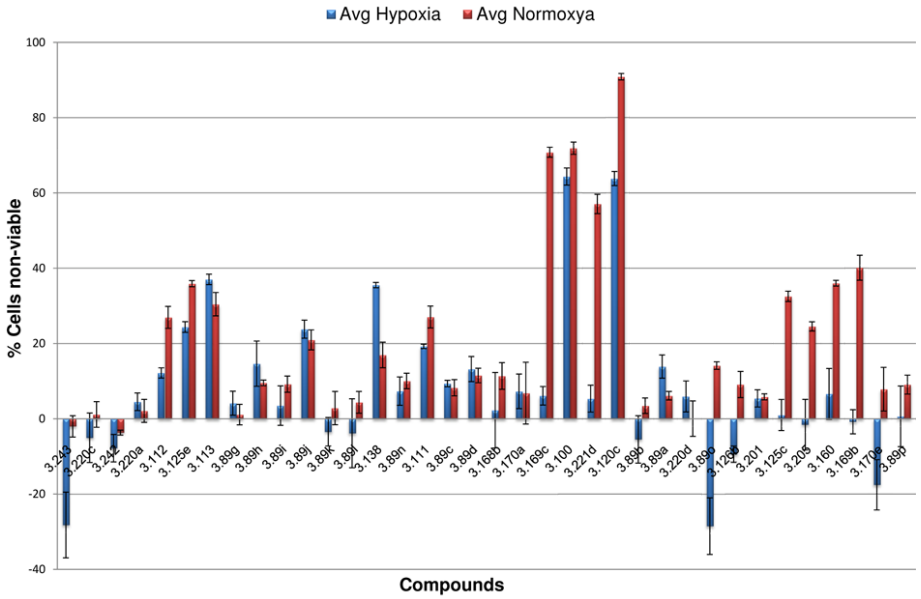


Figure 3.58. Cytotoxicity bar graph of the analyzed compounds tested on HeLa cell line at 100 μM for 72 h, in hypoxic (blue bars) and normoxic (red bars) conditions.

3.11.2. Cell-based NMR studies

On the basis of the enzymatic studies and cytotoxicity assays, we identified three compounds, phenyl-substituted NHI **3.112**, **3.113** and **3.139** (Fig. **3.59**), whose potency on the enzyme was accompanied by an adequate cytotoxic action *in vitro*. In order to confirm if their cytotoxic action might be related to LDH-A inhibition, ^{13}C-NMR studies were performed to discover if these molecules influence the hypoxic tumour metabolism and, in particular, to detect their ability to reduce the cellular production of lactate. This approach was recently reported as a noninvasive method to detect modifications of the cellular glucose metabolism in real time [144].

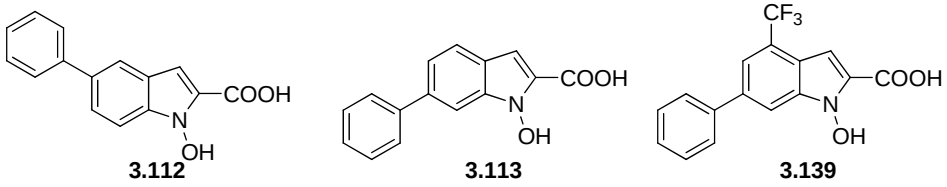


Figure 3.59. Structures of compounds **3.112**, **3.113** and **3.139**, chosen for further cellular assays.

Briefly, compounds were dissolved in DMSO stock solutions (10 mM and 50 mM, with 1% DMSO final concentration in wells), cells were grown in RPMI-1640 media supplemented with 10% FBS and 1% Penicillin/Streptomycin, glucose-free DMEM without phenol red was supplemented with D-[1,6-$^{13}C_2$]glucose to obtain 10 mM D-[1,6-$^{13}C_2$]-glucose-DMEM media to be used in the experiment. The three promising compounds were tested at 100 and 500 μM for 6 h and 12 h on HeLa cell line in the medium enriched with D-[1,6-$^{13}C_2$]glucose in normoxic conditions. Vehicle and gossypol at 50 μM, which is an unspecific lactate dehydrogenase inhibitor, were used as controls. After the incubation times, ^{13}C-spectra of each sample were acquired. For NMR experiments, 100 μL of deuterium oxide was added to each sample and ^{13}C-spectra were acquired with a 500 MHz Varian system.

D-[1,6-$^{13}C_2$]Glucose undergoes a glycolytic transformation by producing [3-^{13}C]pyruvate, which is then transformed by LDH into [3-^{13}C]lactate (Fig. **3.60**). The relative formation of lactate was measured by integrating the area corresponding to lactate-C3 peak at 20.28 ppm relative to the integration of the glucose-C6 peak at 60.95

ppm, so lactate/glucose ratio was calculated for each compound and compared to DMSO control. The vehicle peak at 38.87 ppm relative to the solvent DMSO was used as an internal standard.

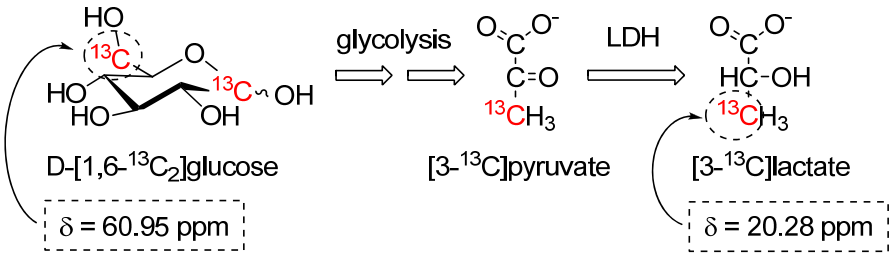


Figure 3.60. Formation of labeled [3-^{13}C]lactate from D-[1,6-$^{13}C_2$]glucose and relevant ^{13}C NMR peak frequencies.

If a compound influences the hypoxic tumour metabolism and in particular inhibits LDH-A, the ratio of carbon lactate peak over the carbon glucose peak should be reduced when compared to control experiments. Consequently, a confirmation of LDH-mediated cytotoxic activity may be found for those compounds showing low levels of [3-^{13}C]lactate compared with D-[1,6-$^{13}C_2$]glucose amount in tumour cells, corresponding to a low lactate/glucose ratio. In the bar graphs below, quantitative results relative to the experiments performed after 6 h (Fig. **3.61**) or 12 h (Fig. **3.62**) of incubation are shown. On the y axis it is reported the lactate/glucose ratio and on the x axis each compound is represented by two bars, the red one relative to 100 μM and the blue one relative to 500 μM concentration. It is evident that the ratios between lactate and glucose in HeLa cells incubated with compounds **3.139** and **3.113** at the concentration of 500 μM are markedly lower than the same ratio detected in DMSO control, whereas at 100 μM the effects are less important for compound **3.139**, showing lactate/glucose ratios slightly smaller than that of DMSO, or not detectable for compound **3.113**. In particular, compound **3.139** proved to be the most efficient inhibitor of cellular production of lactate, since no detectable amounts of lactate could be reported at any time interval when tested at 500 μM. Modest results were obtained for compound **3.112**, for which

the decrease in lactate production compared with the control values is less significant, in spite of its remarkable activity on the enzyme.

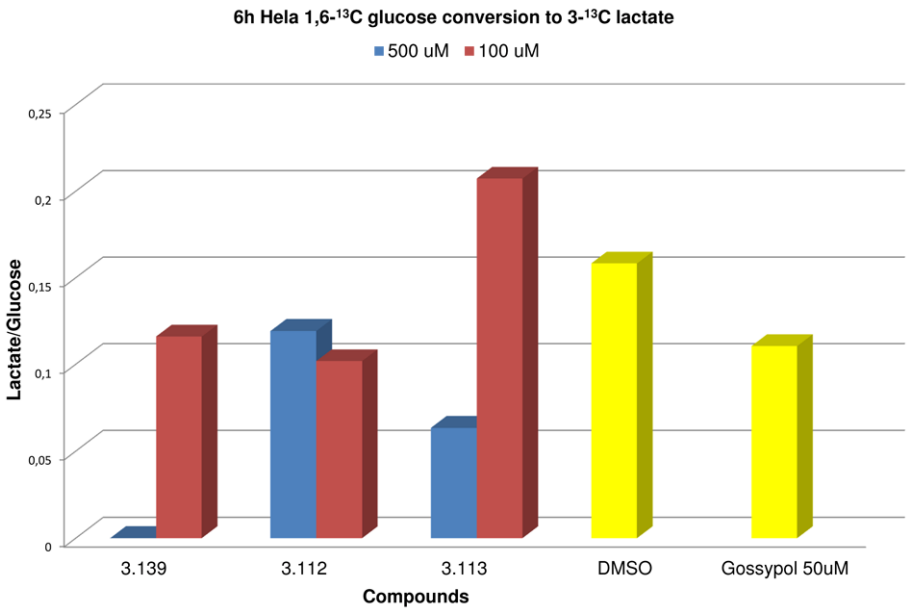


Figure 3.61. Effect of LDH inhibitors **3.139**, **3.112**, and **3.113** on the production of labeled [3-^{13}C]lactate from D-[1,6-$^{13}C_2$]glucose in HeLa cells after 6 h, at 500 μM (blue bars) and 100 μM (red bars), bars relative to controls (DMSO and gossypol) are in yellow.

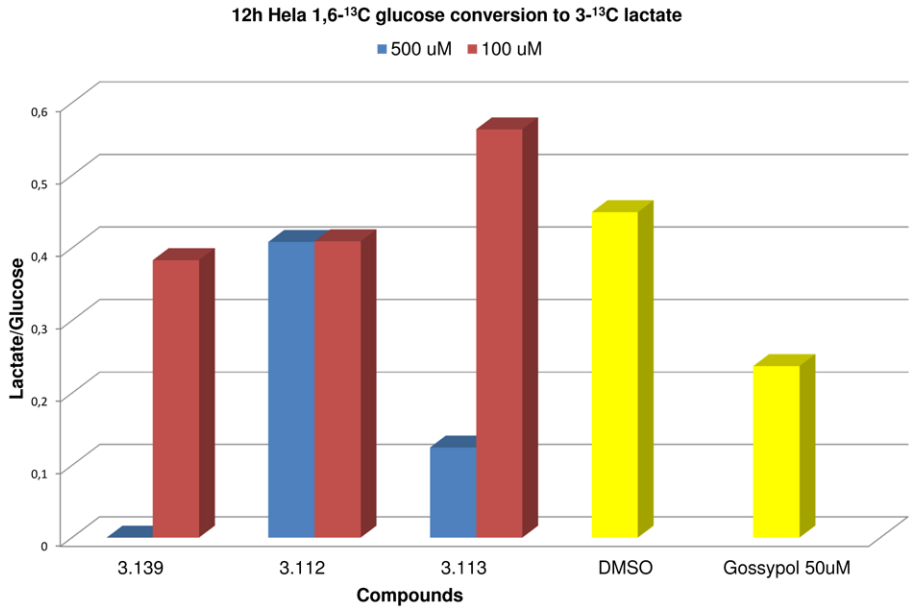


Figure 3.62. Effect of LDH inhibitors **3.139**, **3.112** and **3.113** on the production of labeled [3-^{13}C]lactate from D-[1,6-$^{13}C_2$]glucose in HeLa cells after 12 h, at 500 μM (blue bars) and 100 μM (red bars), bars relative to controls (DMSO and gossypol) are in yellow.

These results can be further explained by observing NMR spectra of the tested compounds (Fig. **3.63**). After addition of D-[1,6-$^{13}C_2$]glucose to the cell culture the lactate/glucose area ratio in untreated cells increased over time with a value of 0.16 after 6 h and a higher value of 0.45 after 12 h, thus showing a progressive conversion of glucose to lactate over that period of time (Fig. **3.63**, panels A and B). Treatment of cells with a 500 μM solution of compound **3.113** (panel C) caused a sensible reduction of that ratio (Lac/Glu = 0.124) after 12 h, whereas a remarkable drop of lactate production was caused by the same concentration of **3.139** (panel D), which showed basically no detectable amounts of lactate in the ^{13}C NMR spectrum (Lac/Glu < 0.01).

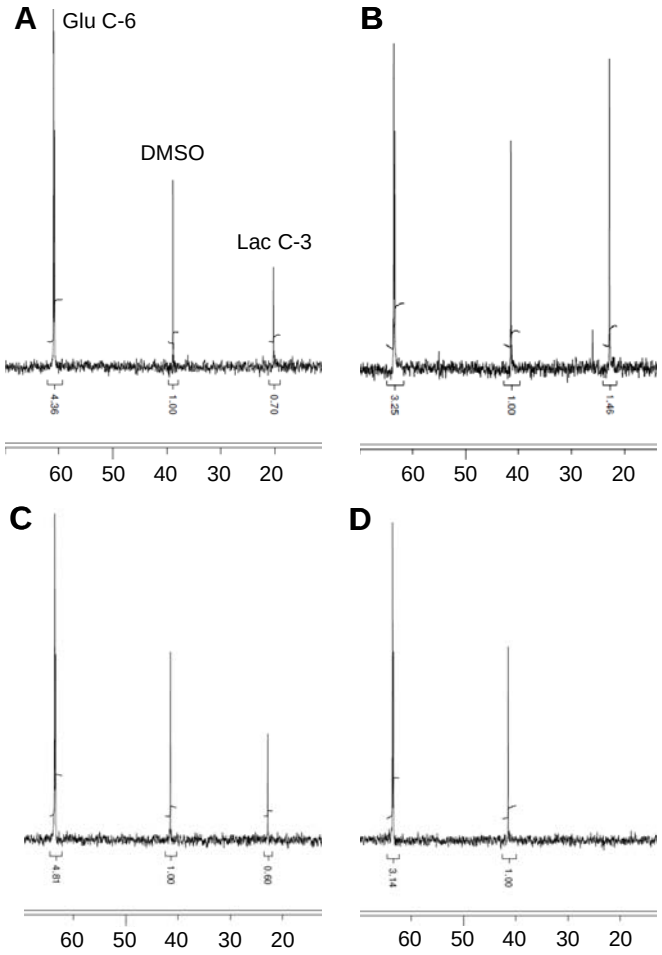


Figure 3.63. ^{13}C NMR spectra of HeLa cells fed with D-[1,6-$^{13}C_2$]glucose: (A) untreated (DMSO control) after 6 h; (B) untreated (DMSO control) after 12 h; (C) 12 h after addition of **3.113** (500 μM); (D) 12 h after addition of **3.139** (500 μM).

3.12. CONCLUSIONS

The classes of LDH-A inhibitors based on the *N*-hydroxyindole-2-carboxylic scaffold reported in this Chapter represent, to the best of our knowledge, the first example in Medicinal Chemistry of this type of small organic molecules specifically designed and synthesized to inhibit human LDH-A, in order to interfere with the peculiar metabolism of hypoxic tumours.

Enzymatic assays demonstrated the competitive behaviour against both the cofactor NADH and the substrate pyruvate for these derivatives, suggesting that they likely occupy both binding sites. Furthermore, their selectivity for the desired A isoform *versus* the B isoform of the enzyme appeared evident for almost all the compounds.

Molecular modeling studies suggested the possible binding mode of some representative examples of these derivatives in the LDH-A active site and they specified the main residues likely involved in the interactions with the inhibitors.

Furthermore, enzymatic assays demonstrated the importance of the OH-COOH moiety for the inhibitory activity of the *N*-hydroxy-2-carboxy-substituted indoles. The presence of a trifluoromethyl group in position 4 of the heterocycle together with an aryl ring in position 6 and even the substitution of the central scaffold with a *N*-methyl-*N*-arylsulfonamido group in position 6 led to the production of some of the most active LDH-A inhibitors. The promising enzymatic data for the best compounds were also supported by cellular experiments revealing their ability to reduce the production of lactate even in *in vitro* cell-based experiments, thus confirming their mechanism of action in living cells.

3.13. REFERENCES

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CHAPTER 4

“Experimental section”

4.1. EXPERIMENTAL SECTION

4.1.1. General details

Commercially available chemicals were purchased from Sigma-Aldrich or Alfa Aesar and used without further purification. NMR spectra were obtained with a Varian Gemini 200 MHz spectrometer. Chemical shifts (δ) are reported in parts per million downfield from tetramethylsilane and referenced from solvent references. Electron impact (EI, 70 eV) mass spectra were obtained on a HP-5988A mass spectrometer. Purity was routinely measured by HPLC on a Waters SunFire RP 18 (3.0 x 150 mm, 5 μm) column (Waters, Milford, MA, www.waters.com) using a Beckmann SystemGold instrument consisting of chromatography 125 Solvent Module and a 166 UV Detector. Mobile phases: 10 mM ammonium acetate in Millipore purified water (A) and HPLC grade acetonitrile (B). A gradient was formed from 5% to 80% of B in 10 minutes and held at 80% for 10 min; flow rate was 0.7 mL/min and injection volume was 30 μL; retention times (HPLC, t_R) are given in minutes. Compound HPLC purity was determined by monitoring at 254 and 300 nm and was found in the range 96-99%, unless otherwise noted. Chromatographic separations were performed on silica gel columns by flash (Kieselgel 40, 0.040–0.063 mm; Merck) or gravity column (Kieselgel 60, 0.063–0.200 mm; Merck) chromatography. Reactions were followed by thin-layer chromatography (TLC) on Merck aluminum silica gel (60 F_{254}) sheets that were visualized under a UV lamp. Evaporation was performed in vacuo (rotating evaporator). Sodium sulfate was always used as the drying agent. Microwave assisted reaction were run in a CEM or Biotage microwave synthesizer.

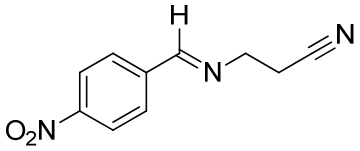
The following abbreviations, reagents, expressions or equipments, which are utilized in the following description, are explained as follows: RT (room temperature, 20-25 °C), molar equivalents (eq.), *N*,*N*-dimethylformamide (DMF), 1,2-dimetoxyethane (DME), dichloromethane (DCM), chloroform ($CHCl_3$), ethylacetate (EtOAc), tetrahydrofuran (THF), methanol (MeOH), diethylether (Et_2O), dimethylsulfoxide (DMSO), sodium hydride (NaH), dimethyl oxalate ("$(COOMe)_2$"), stannous chloride dihydrate ($SnCl_2$•$2H_2O$), sodium hypophosphite monohydrate (H_2PO_2Na•H_2O), palladium 10% on charcoal (Pd-C), lithium hydroxide (LiOH), hydrochloric acid (HCl),

acetic acid (AcOH), diethylamine (Et_2NH), triethylamine (Et_3N), sodium bicarbonate ($NaHCO_3$), normal concentration (N), millimoles (mmol), aqueous solution (aq.), thin layer chromatography (TLC), nuclear magnetic resonance (NMR), electronic impact mass spectrometry (EI/MS).

Compounds **3.89a-e**, **3.89k-n**, **3.111** and **3.138** were previously synthesized by undergraduate students during experimental thesis in the same research laboratory, therefore their preparation is not reported in this Thesis.

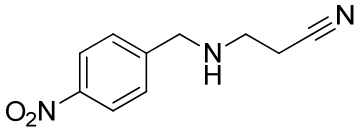
4.1.2. Synthetic procedures and characterization data

3-((4-Nitrobenzylidene)amino)propanenitrile (2.24)



A solution of commercially available 4-nitrobenzaldehyde **2.23** (0.65 g, 4.3 mmol) in anhydrous CH_2Cl_2 (30 mL) was treated with BAPN (0.95 g, 13 mmol) and stirred at RT overnight before being concentrated in vacuo. The crude product **2.24** was then used in the next step without any further purification.

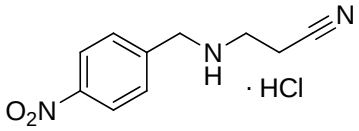
3-((4-Nitrobenzyl)amino)propanenitrile (2.25)



The crude product **2.24** was dissolved in CH_3OH (30 mL) and treated slowly and cautiously with $NaBH_4$ (1.2 g, 32 mmol) at 0 °C under nitrogen. After 1 h the reaction was diluted with ice water and extracted with CH_2Cl_2. The combined organic phase was washed with brine, dried over anhydrous sodium sulphate and concentrated in vacuo. The crude product was purified by flash chromatography (*n*-hexane/EtOAc, 4:6) affording 3-(4-nitrobenzylamino)propanenitrile **2.25** (0.76 g, 3.7 mmol, 86% yield). ^1H NMR ($CDCl_3$) δ (ppm): 2.55 (t, 2H, J = 6.4 Hz), 2.95 (t, 2H, J = 6.4 Hz), 3.96 (s, 2H),

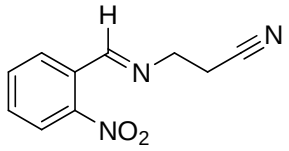
7.53 (AA'XX', 2H, J_{AX} = 8.8 Hz, $J_{AA'/XX'}$ = 2.1 Hz), 8.20 (AA'XX', 2H, J_{AX} = 8.6 Hz, $J_{AA'/XX'}$ = 2.1 Hz). ^{13}C NMR (50 MHz, $CDCl_3$) δ (ppm): 19.1, 44.6, 52.5, 118.6, 123.2, 123.9, 128.7, 147.2. MS *m/z* 206 ($M+H^+$).

3-((4-Nitrobenzyl)amino)propanenitrile hydrochloride (2.19)



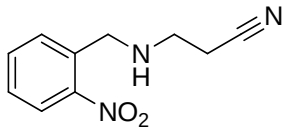
Compound **2.25** was dissolved in CH_3OH and treated with a saturated Et_2O·HCl solution. Compound **2.19** precipitated from the resulting mixture as a white solid (0.84 g, 3.5 mmol, 95% yield): ^{1}H NMR (D_2O) δ (ppm): 3.06 (t, 2H, *J* = 6.8 Hz), 3.53 (t, 2H, *J* = 6.8 Hz), 4.47 (s, 2H), 7.75 (AA'XX', 2H, J_{AX} = 8.8 Hz, $J_{AA'/XX'}$ = 2.2 Hz), 8.35 ppm (AA'XX', 2H, J_{AX} = 8.8 Hz, $J_{AA'/XX'}$ = 2.3 Hz). ^{13}C NMR (50 MHz, D_2O) δ (ppm): 15.5, 43.3, 51.0, 118.0, 124.9, 131.5, 138.0, 149.0. Anal. calcd for $C_{10}H_{12}ClN_3O_2$·H_2O: C 46.25, H 5.43, N 16.18, found: C 45.93, H 5.53, N 16.39.

3-((2-Nitrobenzylidene)amino)propanenitrile (2.27)



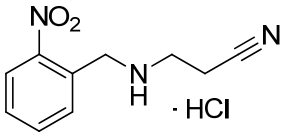
A solution of commercially available 2-nitrobenzaldehyde **2.26** (0.33 g, 2.2 mmol) in anhydrous CH_2Cl_2 (20 mL) was treated with BAPN (0.46 g, 6.6 mmol) and stirred at RT overnight before being concentrated in vacuo. The crude product **2.27** was then used in the next step without any further purification.

3-((2-Nitrobenzyl)amino)propanenitrile (2.28)



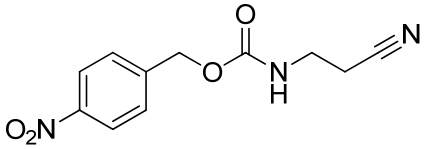
The crude product **2.27** was dissolved in CH_3OH (20 mL) and treated slowly and cautiously with $NaBH_4$ (0.62 g, 16 mmol) at 0 °C under nitrogen. After 1 h the reaction was diluted with ice water and extracted with CH_2Cl_2. The combined organic phase was washed with brine, dried over anhydrous sodium sulphate and concentrated in vacuo. The crude product was purified by flash chromatography (*n*-hexane/EtOAc, 1:1) affording 3-(2-nitrobenzylamino)propanenitrile **2.28** (0.39 g, 1.9 mmol, 86% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 2.54 (t, 2H, *J* = 6.6 Hz), 2.96 (t, 2H, *J* = 6.6 Hz), 4.10 (s, 2H), 7.44 (ddd, 1H, *J* = 8.0, 6.3, 2.5 Hz), 7.56-7.68 (m, 2H), 7.96 (dd, 1H, *J* = 7.8, 1.1 Hz). ^{13}C NMR (50 MHz, $CDCl_3$) δ (ppm): 19.0, 44.8, 50.2, 118.6, 123.1, 124.9, 128.3, 131.1, 133.4, 149.1. MS *m/z* 206 ($M+H^+$).

3-((2-Nitrobenzyl)amino)propanenitrile hydrochloride (2.20)



The intermediate **2.28** was dissolved in CH_3OH and treated with a saturated Et_2O·HCl solution. Compound **2.20** precipitated from the resulting mixture as a white solid (0.39 g, 1.6 mmol, 85% yield). 1H NMR (D_2O) δ (ppm): 3.11 (t, 2H, *J* = 6.9 Hz), 3.64 (t, 2H, *J* = 6.9 Hz), 4.60 (s, 2H), 7.71-7.88 (m, 3H), 8.32 (dd, 1H, *J* = 8.1, 1.2 Hz). ^{13}C NMR (50 MHz, D_2O) δ (ppm): 15.1, 43.4, 49.3, 117.7 125.7 126.3, 131.9 134.0, 135.5 148.4. Anal. calcd for $C_{10}H_{12}ClN_3O_2$·0.5H_2O: C 47.91, H 5.23, N 16.76, found: C 48.15, H 5.32, O 16.87.

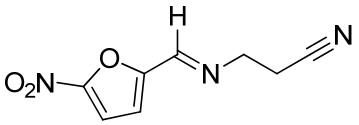
4-Nitrobenzyl (2-cyanoethyl)carbamate (2.21)



A solution of commercially available 4-nitrobenzylchloroformate **2.29** (1.95 g, 9.04 mmol) in anhydrous CH_2Cl_2 (50 mL) was treated at RT under nitrogen with BAPN (1.9 g, 27 mmol). After 1 h at RT the reaction mixture was diluted with water and extracted

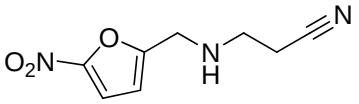
with CH_2Cl_2. The combined organic phase was washed with brine, dried over anhydrous sodium sulphate and concentrated in vacuo. The crude product was purified by flash chromatography (*n*-hexane/EtOAc, 4:6) to afford **2.21** as a white solid (2.0 g, 8.0 mmol, 89% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 2.64 (t, 2H, J = 6.2 Hz), 3.50 (q, 2H, J = 6.2 Hz), 5.22 (s, 2H), 5.30 (bs, 1H), 7.51 (AA'XX', 2H, J_{AX} = 8.8 Hz, $J_{AA'/XX'}$ = 2.1 Hz), 8.2 (AA'XX', 2H, J_{AX} = 8.8 Hz, $J_{AA'/XX'}$ = 2.2 Hz). ^{13}C NMR (50 MHz, $CDCl_3$) δ (ppm): 19.0, 37.5, 65.7, 117.9, 123.9, 128.3, 143.5, 147.8, 155.8. MS *m/z* 249 (M^+). Anal. calcd for $C_{11}H_{11}N_3O_4$: C 53.01, H 4.45, N 16.86, found: C 53.17, H 4.32, O 17.11.

3-(((5-Nitrofuran-2-yl)methylene)amino)propanenitrile (2.31)



A solution of commercially available 5-nitrofuran-1-carbaldehyde **2.30** (1.02 g, 7.23 mmol) in anhydrous CH_2Cl_2 (50 mL) was treated with BAPN (1.5 g, 22 mmol) and stirred at RT overnight before being concentrated in vacuo. The crude product **2.31** was then used in the next step without any further purification.

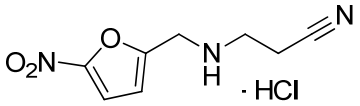
3-(((5-Nitrofuran-2-yl)methyl)amino)propanenitrile (2.32)



The crude product **2.31** was dissolved in CH_3OH (50 mL) and treated slowly and cautiously with $NaBH_4$ (2.0 g, 54 mmol) at 0 °C under nitrogen (caution: it is extremely important to maintain the temperature below 0 °C during the addition of $NaBH_4$, which must be very slow!). After 1 h the reaction was diluted with ice water and extracted with CH_2Cl_2. The combined organic phase was washed with brine, dried over anhydrous sodium sulphate and concentrated in vacuo. The crude product was purified by flash chromatography (*n*-hexane/EtOAc, 2:8) affording 3-((5-nitrofuran-2-yl)methylamino)propanenitrile **2.32** (0.83 g, 4.3 mmol, 59% yield). ^{1}H NMR ($CDCl_3$) δ

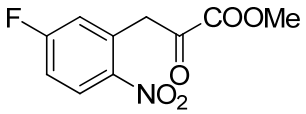
(ppm): 2.55 (t, 2H, *J* = 6.5 Hz), 2.98 (t, 2H, *J* = 6.5 Hz), 3.94 (s, 2H), 6.51 (d, 1H, *J* = 3.7 Hz), 7.28 (d, 1H, *J* = 3.7 Hz). MS *m/z* 196 ($M+H^+$).

3-(((5-Nitrofuran-2-yl)methyl)amino)propanenitrile hydrochloride (2.22)



Compound **2.32** was dissolved in CH_3OH and treated with a saturated Et_2O·HCl solution. Compound **2.22** precipitated from the resulting mixture as a white solid (0.88 g, 3.8 mmol, 88% yield). ^{1}H NMR (D_2O) δ (ppm): 3.09 (t, 2H, *J* = 6.8 Hz), 3.57 (t, 2H, *J* = 6.8 Hz), 4.57 (s, 2H), 7.02 (d, 1H, *J* = 3.8 Hz), 7.59 (d, 1H, *J* = 3.8 Hz). ^{13}C NMR (50 MHz, D_2O) δ (ppm): 15.2, 42.8, 43.3, 113.7, 116.6, 117.5, 147.7, 154.9. Anal. calcd for $C_8H_{10}ClN_3O3{\cdot}0.5H_2O$: C 39.93, H 4.61, N 17.46, found: C 39.64, H 4.82, O 17.34.

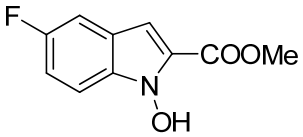
Methyl 3-(5-fluoro-2-nitrophenyl)-2-oxopropanoate (3.91f)



A stirred suspension of sodium hydride (60% dispersion in mineral oil, 722 mg, 18.0 mmol) in 18 mL of anhydrous DMF cooled to –15 °C under nitrogen was treated dropwise with a solution containing commercially available 5-fluoro-2-nitrotoluene **3.90f** (700 mg, 4.51 mmol) and dimethyl oxalate (2.66 g, 22.5 mmol) in 13 mL of anhydrous DMF. The mixture was left under stirring at the same temperature for 10 minutes and then it was slowly warmed to room temperature. The mixture was then left under stirring at RT for 1 h. Once the disappearance of the precursor was verified by TLC, the reaction mixture was slowly poured in an ice-water mixture; the water phase was acidified with 1N aqueous HCl and extracted several times with EtOAc. The combined organic phase was washed with saturated $NaHCO_3$ solution and brine, then dried over anhydrous sodium sulphate. Evaporation under vacuum of the organic solvent afforded a crude product which was purified by column chromatography over silica gel using *n*-hexane/EtOAc 7:3 as the eluent, to yield the nitroaryl-ketoester derivative **3.91f** (780 mg, 3.23 mmol, 72% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 3.95 (s,

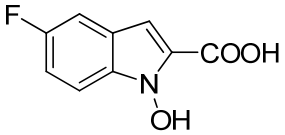
3H), 4.54 (s, 2H), 7.04 (dd, 1H, *J* = 8.4, 2.7 Hz), 7.19 (ddd, 1H, *J* = 9.2, 7.2, 2.7 Hz), 8.26 (dd, 1H, *J* = 9.2, 5.1 Hz); signals imputable to the enol form (~ 25%) δ (ppm): 3.96 (s, 3H), 6.99 (d, 1H, *J* = 1.1 Hz), 7.96-8.03 (m, 2H).

Methyl 5-fluoro-1-hydroxy-1*H*-indole-2-carboxylate (3.92f)



Ketoester **3.91f** (525 mg, 2.18 mmol) was dissolved in anhydrous DME (2.2 mL) and the resulting solution was added dropwise to a cooled (0 °C) solution of $SnCl_2 \cdot 2H_2O$ (1.10 g, 4.88 mmol) in DME (2.2 mL) containing 4Å molecular sieves, previously activated for 18 hours at 130 °C in oven and cooled to RT in a desiccator over anhydrous calcium chloride. The reaction mixture was stirred under nitrogen at RT for 3 h, then it was diluted with water and repeatedly extracted with EtOAc. The combined organic phase was dried over anhydrous sodium sulphate and evaporated to afford a crude residue that was purified by column chromatography over silica gel using $CHCl_3$ as the eluent, to give the *N*-hydroxyindol-ester derivative **3.92f** (169 mg, 0.808 mmol, 37% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 4.00 (s, 3H), 6.98 (d, 1H, *J* = 0.9 Hz), 7.13 (td, 1H, *J* = 9.0, 2.4 Hz), 7.27 (dd, 1H, *J* = 9.5, 2.0 Hz), 7.49 (dd, 1H, *J* = 9.2, 4.4 Hz), 10.27 (bs, 1H).

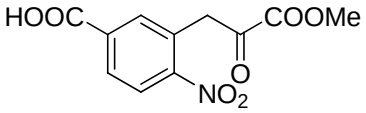
5-Fluoro-1-hydroxy-1*H*-indole-2-carboxylic acid (3.89f)



Methyl ester **3.92f** (150 mg, 0.717 mmol) was dissolved in a 1:1 mixture of THF/methanol (7.2 mL) and treated with 2.3 mL of 2N aqueous solution of LiOH. The reaction was kept under stirring in the dark at RT and it was monitored by TLC. After 1 h, the reaction mixture was concentrated, diluted with water and washed with Et_2O. Then, the aqueous phase was treated with 1N aqueous HCl and extracted several times

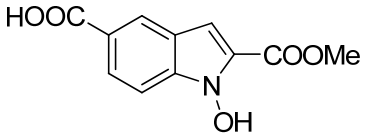
with EtOAc. The combined ethyl acetate organic phase was dried over anhydrous sodium sulphate and evaporated to afford the desired *N*-hydroxyindol-carboxylic acid product **3.89f** (140 mg, 0.717 .mmol, >99% yield) without any further purification. ^{1}H NMR (DMSO-d_6) δ (ppm): 6.98 (s, 1H), 7.18 (td, 1H, J = 9.2, 2.4), 7.39-7.48 (m, 2H). MS *m/z* 195 (M^+, 100%), 133 ($M^+ - CO_2 - H_2O$, 28%).

3-(3-Methoxy-2,3-dioxopropyl)-4-nitrobenzoic acid (3.91g)



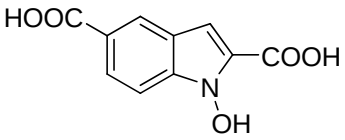
A stirred suspension of sodium hydride (60% dispersion in mineral oil, 926 mg, 23.2 mmol) in 16 mL of anhydrous DMF cooled to –15 °C under nitrogen was treated dropwise with a solution containing commercially available 3-methyl-4-nitrobenzoic acid **3.90g** (700 mg, 3.86 mmol) and dimethyl oxalate (2.74 g, 23.2 mmol) in 11 mL of anhydrous DMF. The mixture was left under stirring at the same temperature for 10 minutes and then it was slowly warmed to room temperature. The mixture was then left under stirring at RT for 5 h. Once the disappearance of the precursor was verified by TLC, the reaction mixture was slowly poured in an ice-water mixture; the water phase was acidified with 1N aqueous HCl and extracted several times with EtOAc. The combined organic phase was washed with saturated $NaHCO_3$ solution and brine, then dried over anhydrous sodium sulphate. Evaporation under vacuum of the organic solvent afforded a crude product which was purified by column chromatography over silica gel using $CHCl_3$/MeOH 9:1 with 0.05% trifluoroacetic acid as the eluent, to yield the nitroaryl-ketoester derivative **3.91g** (432 mg, 1.62 mmol, 42% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 3.95 (s, 3H), 4.61 (s, 2H), 7.90-8.21 (m, 3H); signals imputable to the enol form (~ 26%) δ (ppm): 3.91 (s, 3H), 6.85 (s, 1H), 8.97 (d, 1H, J = 1.8 Hz).

1-Hydroxy-2-(methoxycarbonyl)-1*H*-indole-5-carboxylic acid (3.92g)



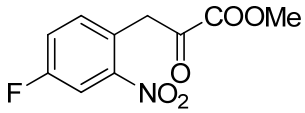
Ketoester **3.91g** (282 mg, 1.06 mmol) was dissolved in anhydrous DME (1.1 mL) and the resulting solution was added dropwise to a cooled (0 °C) solution of $SnCl_2 \cdot 2H_2O$ (572 mg, 2.53 mmol) in DME (1.1 mL) containing 4Å molecular sieves, previously activated for 18 hours at 130 °C in oven and cooled to RT in a desiccator over anhydrous calcium chloride. The reaction mixture was stirred under nitrogen at RT for 2 h, then it was diluted with water and repeatedly extracted with EtOAc. The combined organic phase was dried over anhydrous sodium sulphate and evaporated to afford a crude residue that was purified by column chromatography over silica gel using *n*-hexane/EtOAc 4:6 as the eluent to yield a mixture (38.5 mg) of the *N*-hydroxyindol-ester derivative **3.92g** (85%, 32.7 mg, 0.139 mmol, 13% yield) with its indole analogue (15%) ^{1}H NMR (acetone-d_6) δ (ppm): 3.91 (s, 3H), 7.27 (d, 1H, J = 0.9 Hz), 7.61 (dt, 1H, J = 8.8, 0.9 Hz), 8.04 (dd, 1H, J = 8.8, 1.5 Hz), 8.46 (dd, 1H, J = 1.5, 0.7 Hz), 10.66 (bs, 1H); signals imputable to the indole (~ 15%) δ (ppm): 3.92 (s, 3H), 7.35-7.37 (m, 1H), 7.98 (dd, 1H, J = 8.8, 1.5 Hz), 8.48-8.50 (m, 1H).

1-Hydroxy-1*H*-indole-2,5-dicarboxylic acid (3.89g)



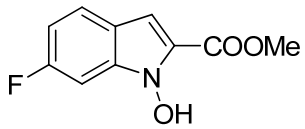
Methyl ester **3.92g** (35 mg, 0.149 mmol) was dissolved in a 1:1 mixture of THF/methanol (1.6 mL) and treated with 0.5 mL of 2N aqueous solution of LiOH. The reaction was kept under stirring in the dark at RT and it was monitored by TLC. After 3 h, the reaction mixture was concentrated, diluted with water and washed with Et_2O. Then, the aqueous phase was treated with 1N aqueous HCl and extracted several times with EtOAc. The combined ethyl acetate organic phase was dried over anhydrous sodium sulphate and evaporated to afford the desired *N*-hydroxyindol-carboxylic acid product **3.89g** (24.0 mg, 0.109 mmol, 73% yield) without any further purification. ^{1}H NMR (acetone-d_6) δ (ppm): 7.29 (d, 1H, J = 0.7 Hz), 7.61 (dt, 1H, J = 8.8, 0.7 Hz), 8.03 (dd, 1H, J = 8.8, 1.5 Hz), 8.47 (dd, 1H, J = 1.5, 0.7 Hz); signals imputable to the indole (~ 10%) δ (ppm): 7.36-7.38 (m, 1H), 7.96 (d, 1H, J = 1.5 Hz). MS *m/z* 221 (M^+, 78%), 205 (M^+ –O, 100%), 133 (M^+ -2 CO_2, 57%).

Methyl 3-(4-fluoro-2-nitrophenyl)-2-oxopropanoate (3.91h)



A stirred suspension of sodium hydride (60% dispersion in mineral oil, 721 mg, 18.0 mmol) in 18 mL of anhydrous DMF cooled to –15 °C under nitrogen was treated dropwise with a solution containing commercially available 4-fluoro-2-nitrotoluene **3.90h** (700 mg, 4.51 mmol) and dimethyl oxalate (2.66 mg, 22.5 mmol) in 13 mL of anhydrous DMF. The mixture was left under stirring at the same temperature for 10 minutes and then it was slowly warmed to room temperature. The mixture was then left under stirring at RT for 3 h. Once the disappearance of the precursor was verified by TLC, the reaction mixture was slowly poured in an ice-water mixture; the water phase was acidified with 1N aqueous HCl and extracted several times with EtOAc. The combined organic phase was washed with saturated $NaHCO_3$ solution and brine, then dried over anhydrous sodium sulphate. Evaporation under vacuum of the organic solvent afforded a crude product which was purified by column chromatography over silica gel using *n*-hexane/EtOAc 7:3 as the eluent, to yield the nitroaryl-ketoester derivative **3.91h** (961 mg, 3.98 mmol, 88% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 3.90 (s, 3H), 4.52 (s, 2H), 7.28-7.41 (m, 2H), 7.90 (dd, 1H, J = 8.2, 2.0 Hz); signals imputable to the enolic form (~ 13%) δ (ppm): 3.92 (m, 3H), 6.68 (bs, 1H, exchangeable), 6.87 (s, 1H), 7.64 (dd, 1H, J = 8.2, 2.7 Hz), 8.26 (dd, 1H, J = 9.0, 5.7 Hz).

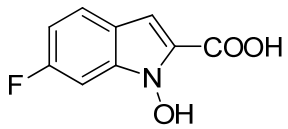
Methyl 6-fluoro-1-hydroxy-1*H*-indole-2-carboxylate (3.92h)



Ketoester **3.91h** (619 mg, 2.57 mmol) was dissolved in anhydrous DME (4.3 mL) and the resulting solution was added dropwise to a cooled (0 °C) solution of $SnCl_2 \cdot 2H_2O$ (1.30 g, 5.76 mmol) in DME (4.3 mL) containing 4Å molecular sieves, previously activated for 18 hours at 130 °C in oven and cooled to RT in a desiccator over anhydrous calcium chloride. The reaction mixture was stirred under nitrogen at RT

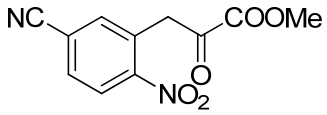
for 5 h, then it was diluted with water and repeatedly extracted with EtOAc. The combined organic phase was dried over anhydrous sodium sulphate and evaporated to afford a crude residue that was purified by column chromatography over silica gel using $CHCl_3$ as the eluent, to give the *N*-hydroxyindol-ester derivative **3.92h** (206 mg, 0.985 mmol, 38% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 3.98 (s, 3H), 6.90 (td, 1H, *J* = 9.0, 2.4 Hz), 7.02 (d, 1H, *J* = 0.9 Hz), 7.18 (dd, 1H, *J* = 9.2, 2.4 Hz), 7.57 (dd, 1H, *J* = 9.0, 5.1 Hz), 10.20 (bs, 1H).

6-Fluoro-1-hydroxy-1*H*-indole-2-carboxylic acid (3.89h)



Methyl ester **3.92h** (178 mg, 0.851 mmol) was dissolved in a 1:1 mixture of THF/methanol (8.6 mL) and treated with 2.7 mL of 2N aqueous solution of LiOH. The reaction was kept under stirring in the dark at RT and it was monitored by TLC. After 2 h, the reaction mixture was concentrated, diluted with water and washed with Et_2O. Then, the aqueous phase was treated with 1N aqueous HCl and extracted several times with EtOAc. The combined ethyl acetate organic phase was dried over anhydrous sodium sulphate and evaporated to afford the desired *N*-hydroxyindol-carboxylic acid product **3.89h** (144 mg, 0.738 mmol, 87% yield) without any further purification. ^{1}H NMR (DMSO-d_6) δ (ppm): δ 6.97 (ddd, 1H, *J* = 9.7, 8.8, 2.4 Hz), 7.04 (d, 1H, *J* = 0.7), 7.18 (ddd, 1H, *J* = 9.9, 1.8, 0.9 Hz), 7.67 (ddd, 1H, *J* = 8.6, 5.3, 0.4 Hz). MS *m/z* 195 (M^+, 100%), 177 ($M^+ - H_2O$, 43%), 133 ($M^+ - CO_2 - H_2O$, 72%).

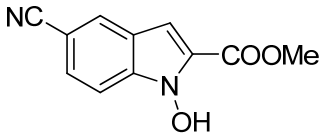
Methyl 3-(5-cyano-2-nitrophenyl)-2-oxopropanoate (3.91i)



A stirred suspension of sodium hydride (60% dispersion in mineral oil, 395 mg, 9.88 mmol) in 9.6 mL of anhydrous DMF cooled to –15 °C under nitrogen was treated dropwise with a solution containing commercially available 4-methyl-3-

nitrobenzonitrile **3.90i** (400 mg, 2 47 mmol) and dimethyl oxalate (1.46 g, 12.4 mmol) in 7.1 mL of anhydrous DMF. The mixture was left under stirring at the same temperature for 10 minutes and then it was slowly warmed to room temperature. The mixture was then left under stirring at RT for 4 h. Once the disappearance of the precursor was verified by TLC, the reaction mixture was slowly poured in an ice-water mixture; the water phase was acidified with 1N aqueous HCl and extracted several times with EtOAc. The combined organic phase was washed with saturated $NaHCO_3$ solution and brine, then dried over anhydrous sodium sulphate. Evaporation under vacuum of the organic solvent afforded a crude product which was purified by column chromatography over silica gel using *n*-hexane/EtOAc 7:3 as the eluent, to yield the nitroaryl-ketoester derivative **3.91i** (251 mg, 1.01 mmol, 41% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 3.96 (s, 3H), 4.60 (s, 2H), 7.65 (d, 1H, *J* = 2.0 Hz), 7.84 (dd, 1H, *J* = 8.1, 1.7 Hz), 8.27 (d, 1H, *J* = 8.6 Hz); signals imputable to the enol form (~ 22%) δ (ppm): 3.97 (s, 3H). 6.81 (s, 1H), 7.95 (d, 1H, *J* = 8.4 Hz), 8.64 (d, 1H, *J* = 1.6 Hz).

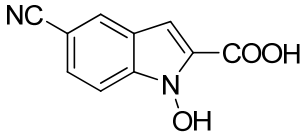
Methyl 5-cyano-1-hydroxy-1*H*-indole-2-carboxylate (3.92i)



Ketoester **3.91i** (90 mg, 0.363 mmol) was dissolved in anhydrous DME (0.4 mL) and the resulting solution was added dropwise to a cooled (0 °C) solution of $SnCl_2 \cdot 2H_2O$ (183 mg, 0.812 mmol) in DME (0.4 mL) containing 4Å molecular sieves, previously activated for 18 hours at 130 °C in oven and cooled to RT in a desiccator over anhydrous calcium chloride. The reaction mixture was stirred under nitrogen at RT for 10 h, then it was diluted with water and repeatedly extracted with EtOAc. The combined organic phase was dried over anhydrous sodium sulphate and evaporated to afford a crude residue that was purified by column chromatography over silica gel using *n*-hexane/EtOAc 7:3 as the eluent, to give a mixture (30.0 mg) of the *N*-hydroxyindol-ester derivative **3.92i** (70%, 21.0 mg, 0.0.971 mmol, 27% yield) with its indole analogue (30%). ^{1}H NMR ($CDCl_3$) δ (ppm): 4.03 (s, 3H), 7.12 (d, 1H, *J* = 0.7 Hz), 7.50-

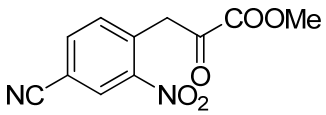
7.55 (m, 2H), 7.62 (d, 1H, *J* = 8.8 Hz), 8.03-8.08 (m, 1H), 10.66 (bs, 1H); signals imputable to the indole (~ 30%) δ (ppm): 3.98 (s, 3H), 7.27-7.28 (m, 1H), 9.25 (bs, 1H).

5-Cyano-1-hydroxy-1*H*-indole-2-carboxylic acid (3.89i)



Methyl ester **3.92i** (29.5 mg, 0.136 mmol) was dissolved in a 1:1 mixture of THF/methanol (1.4 mL) and treated with 0.44 mL of 2N aqueous solution of LiOH. The reaction was kept under stirring in the dark at RT and it was monitored by TLC. After 1.5 h, the reaction mixture was concentrated, diluted with water and washed with Et_2O. Then, the aqueous phase was treated with 1N aqueous HCl and extracted several times with EtOAc. The combined ethyl acetate organic phase was dried over anhydrous sodium sulphate and evaporated to afford the desired *N*-hydroxyindol-carboxylic acid product **3.89i** (28.0 mg, 0.138 mmol, >99% yield) without any further purification. ^{1}H NMR (DMSO-d_6) δ (ppm): 7.15 (s, 1H), 7.57-7.61 (m, 2H), 8.24 (s, 1H) ; signals imputable to the indole (~ 30%) δ (ppm): 7.22 (d, 1H, *J* = 0.8 Hz).

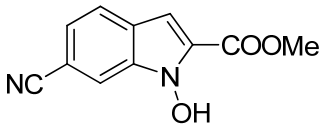
Methyl 3-(4-cyano-2-nitrophenyl)-2-oxopropanoate (3.91j)



A stirred suspension of sodium hydride (60% dispersion in mineral oil, 493 mg, 12.3 mmol) in 12 mL of anhydrous DMF cooled to –15 °C under nitrogen was treated dropwise with a solution containing commercially available 4-methyl-3-nitrobenzonitrile **3.90j** (500 mg, 3.08 mmol) and dimethyl oxalate (1.82 g, 15.4 mmol) in 8.9 mL of anhydrous DMF. The mixture was left under stirring at the same temperature for 10 minutes and then it was slowly warmed to room temperature. The mixture was then left under stirring at RT for 1 h. Once the disappearance of the precursor was verified by TLC, the reaction mixture was slowly poured in an ice-water mixture; the water phase was acidified with 1N aqueous HCl and extracted several

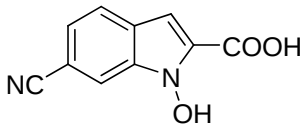
times with EtOAc. The combined organic phase was washed with saturated $NaHCO_3$ solution and brine, then dried over anhydrous sodium sulphate. Evaporation under vacuum of the organic solvent afforded a crude residue (382 mg) containing the desired nitroaryl-ketoester derivative **3.91j**, which was submitted to the following step without any further purification. ^{1}H NMR ($CDCl_3$) δ (ppm): 3.89 (s, 3H); 4.63 (s, 2H); 7.50 (d, 1H, *J* = 8.1 Hz); 7.90 (dd, 1H, *J* = 8.0, 1.7 Hz); 8.14 (d, 1H, *J* = 1.6 Hz); signals imputable to the enol form (~ 42%) δ (ppm): 3.93 (s, 3H); 6.86 (s, 1H); 7.80 (dd, 1H, *J* = 8.4, 1.3 Hz); 8.44-8.49 (m, 2H).

Methyl 6-cyano-1-hydroxy-1*H*-indole-2-carboxylate (3.92j)



The crude residue **3.91j** (382 mg) was dissolved in anhydrous DME (1.5 mL) and the resulting solution was added dropwise to a cooled (0 °C) solution of $SnCl_2 \cdot 2H_2O$ (778.3 mg, 3.45 mmol) in DME (1.5 mL) containing 4Å molecular sieves, previously activated for 18 hours at 130 °C in oven and cooled to RT in a desiccator over anhydrous calcium chloride. The reaction mixture was stirred under nitrogen at RT. Once the disappearance of the precursor is verified by TLC, the reaction mixture was diluted with water and repeatedly extracted with EtOAc. The combined organic phase was dried over anhydrous sodium sulphate and evaporated to afford a crude residue that was purified by column chromatography over silica gel using *n*-hexane/EtOAc 7:3 as the eluent, to give the *N*-hydroxyindol-ester derivative **3.92j** (34 mg, 0.157 mmol, 2 steps: 5% yield).

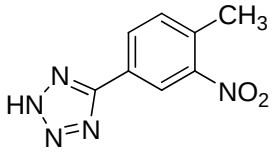
6-Cyano-1-hydroxy-1*H*-indole-2-carboxylic acid (3.89j)



Methyl ester **3.92j** (34 mg, 0.157 mmol) was dissolved in a 1:1 mixture of THF/methanol (1.6 mL) and treated with 0.5 mL of 2N aqueous solution of LiOH. The

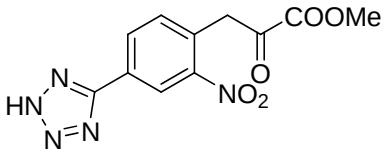
reaction was kept under stirring in the dark at RT and it was monitored by TLC. After 1 h, the reaction mixture was concentrated, diluted with water and washed with Et_2O. Then, the aqueous phase was treated with 1N aqueous HCl and extracted several times with EtOAc. The combined ethyl acetate organic phase was dried over anhydrous sodium sulphate and evaporated to afford the desired *N*-hydroxyindol-carboxylic acid product **3.89j** (31.8 mg, 0.157 mmol, >99% yield) without any further purification. ^{1}H NMR (DMSO-d_6) δ (ppm): 7.12 (d, 1H, J = 0.9 Hz), 7.41 (dd, 1H, J = 8.2, 1.5 Hz), 7.83 (dd, 1H, J = 8.2, 0.7 Hz), 7.97 (dt, 1H, J = 1.5, 0.7 Hz).

5-(4-Methyl-3-nitrophenyl)-2H-tetrazole (3.90o)



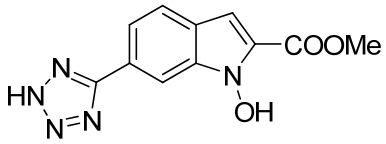
Commercially available 4-methyl-3-nitrobenzonitrile **3.90j** (500 mg, 3.08 mmol) was dissolved in water (6.2 mL) and treated with sodium azide (221 mg, 3.39 mmol) and zinc bromide hydrate (694 mg, 3.08 mmol). The resulting suspension was refluxed for 36 h, then treated with aqueous 3 N HCl (until pH 1) and EtOAc. Stirring was continued until all the white solid was dissolved. The organic phase was separated and the water phase was extracted again with EtOAc. The combined organic phase was concentrated and the residue was recovered with aqueous 0.25 N NaOH and stirred for 30 min. The suspension was filtered to remove $Zn(OH)_2$ and the resulting solution was acidified with 3N HCl to precipitate the tetrazole derivative. The solid was then dissolved in EtOAc and MeOH and the solution was dried and concentrated to give pure **3.90o** (571 mg, 90% yield); ^{1}H NMR (DMSO-d_6) δ (ppm): 2.61 (s, 3H), 7.76 (d, 1H, J = 7.9 Hz), 8.32 (dd, 1H, J = 8.1, 1.8 Hz), 8.67 (d, 1H, J = 1.6 Hz).

Methyl 3-(2-nitro-4-(2H-tetrazol-5-yl)phenyl)-2-oxopropanoate (3.91o)



A stirred suspension of sodium hydride (60% dispersion in mineral oil, 858 mg, 21.5 mmol) in 15 mL of anhydrous DMF cooled to –15 °C under nitrogen was treated dropwise with a solution containing the nitrotoluene precursor **3.90o** (550 mg, 2.68 mmol) and dimethyl oxalate (3.16 g, 26.8 mmol) in 5.1 mL of anhydrous DMF. The mixture was left under stirring at the same temperature for 10 minutes and then it was slowly warmed to room temperature. The mixture was then left under stirring at RT for 24 h. Once the disappearance of the precursor was verified by TLC, the reaction mixture was slowly poured in an ice-water mixture; the water phase was acidified with 1N aqueous HCl and extracted several times with EtOAc. The combined organic phase was washed with saturated $NaHCO_3$ solution and brine, then dried over anhydrous sodium sulphate. Evaporation under vacuum of the organic solvent afforded a crude product which was purified by column chromatography over silica gel using CH_2Cl_2/MeOH 9:1 as the eluent, to yield the nitroaryl-ketoester derivative **3.91o** (375 mg, 1.29 mmol, 48% yield). ^{1}H NMR (DMSO-d_6) δ (ppm): 3.80 (s, 3H), 4.71 (s, 2H), 8.34 (dd, 1H, J = 8.5, 1.7 Hz), 8.43 (d, 1H, J = 8.4 Hz), 8.57 (d, 1H, J = 1.6 Hz); signals imputable to the enol form (~ 40%) δ (ppm): 3.76 (s, 3H), 6.67 (s, 1H), 8.65 (dd, 1H, J = 8.4, 1.8 Hz), 8.74 (d, 1H, J = 1.8 Hz).

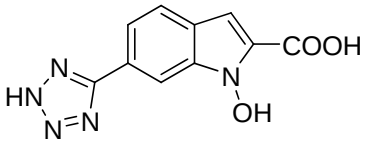
Methyl 1-hydroxy-6-(2*H*-tetrazol-5-yl)-1*H*-indole-2-carboxylate (3.92o)



Ketoester **3.91o** (300 mg, 1.03 mmol) was dissolved in THF (1.7 mL) and the resulting solution was added to an aqueous solution (1.7 mL) of sodium hypophosphite monohydrate (339 mg, 3.19 mmol). Then 10 mg of 10% palladium over charcoal was added and the resulting suspension was stirred ar RT. After 24 h the disappearance of the precursor is verified by TLC and the mixture was concentrated, diluted with water and repeatedly extracted with EtOAc. The combined organic phase was dried over anhydrous sodium sulphate and evaporated to afford a crude residue that was purified by column chromatography over over silica gel using CH_2Cl_2/MeOH 9:1 with 0.01%

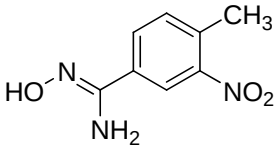
acetic acid as the eluent, to give the *N*-hydroxyindole **3.92o** (41.6 mg, 0.160 mmol, 16% yield). ^{1}H NMR (DMSO-d_6) δ (ppm): 3.88 (s, 3H); 7.13 (d, 1H, J = 0.9 Hz), 7.79-7-80 (m, 2H), 8.12-8.15 (m, 1H), 11.64 (bs, 1H).

1-Hydroxy-6-(2*H*-tetrazol-5-yl)-1*H*-indole-2-carboxylic acid (3.89o)



Methyl ester **3.92o** (35.0 mg, 0.135 mol) was dissolved in a 1:1 mixture of THF/methanol (1.4 mL) and treated with 0.4 mL of 2N aqueous solution of LiOH. The reaction was kept under stirring in the dark at RT and it was monitored by TLC. After 24 h, the reaction mixture was concentrated, diluted with water and washed with Et_2O. Then, the aqueous phase was treated with 1N aqueous HCl and extracted several times with EtOAc. The combined ethyl acetate organic phase was dried over anhydrous sodium sulphate and evaporated to afford the desired *N*-hydroxyindol-carboxylic acid product **3.89o** (32.1 mg, 0.131 mmol, 97% yield) without any further purification. ^{1}H NMR (DMSO-d_6) δ (ppm): 7.11 (d, 1H, J = 0.9 Hz), 7.79 (dd, 1H, J = 7.8, 1.5 Hz), 7.86 (d, 1H, J = 7.8 Hz), 8.17-8.19 (m, 1H). ^{13}C NMR (DMSO-d_6) δ (ppm): 104.63, 108.58, 118.82, 122.66, 123.28, 128.85, 135.40, 160.84. MS *m/z* 268 ($M+Na^+$, 10%), 252 ($M+Na^+ - O$, 9%), 181 ($M+Na^+ - CO_2 - HN_3$, 54%), 163 ($M+Na^+ - H_2O - CO_2 - HN_3$, 100%). HPLC, t_R 1.4 min.

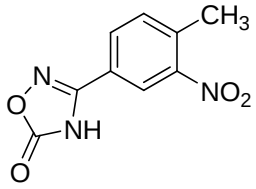
N'-hydroxy-4-methyl-3-nitrobenzimidamide (3.93)



Commercially available 4-methyl-3-nitrobenzonitrile **3.90j** (1.00 g, 6.17 mmol), DIEA (3.2 mL, 2.39 g, 18.5 mmol), hydroxylamine hydrochloride (644 mg, 9.26 mmol) and abs. EtOH (19 mL) were added in a vial. The resulting mixture was stirred at 80 °C. The reaction was monitored by TLC and, after 20 h, EtOH was removed under reduced

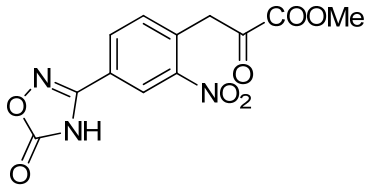
pressure. The residue was solubilized in AcOEt and then extracted with 1N aqueous HCl. 1M aqueous Na_2CO_3 solution was then added to the aqueous solution to pH 9-10. A solid precipitated, so it was filtered, washed with water and dried. The aqueous layer was further extracted with CH_2Cl_2, dried over anhydrous sodium sulphate and evaporated. Both the precipitate and the crude residue deriving from the extraction contained the amidoxime **3.93** (1.16 g, 5.94 mmol, yield 96%), which was used for the next step without any further purification. ^{1}H NMR (CD_3OD) δ (ppm): 2.57 (s, 3H), 7.46 (d, 1H, *J* = 8.1 Hz), 7.83 (dd, 1H, *J* = 8.2, 1.8 Hz), 8.23 (d, 1H, *J* = 1.8 Hz).

3-(4-Methyl-3-nitrophenyl)-1,2,4-oxadiazol-5(4*H*)-one (3.90p)



Amidoxime **3.93** (500 mg, 2.56), DBU (0.42 mL, 0.43 g, 2.8 mmol), CDI (623 mg, 3.84 mmol) and 13 mL of 1,4-dioxane were refluxed in a vial at 110 °C for 3 h. Then, dioxane was removed under reduced pressure. The residue was dissolved CH_2Cl_2 and washed several times with 1N aqueous HCl. The organic phase was dried over anhydrous sodium sulphate and evaporated to afford the desired nitrotoluene derivative **3.90p** (479 mg, 2.17, 85% yield), which was used for the next step without any further purification. ^{1}H NMR (CD_3OD) δ (ppm): 2.65 (s, 3H), 7.65 (d, 1H, *J* = 7.9 Hz), 7.98 (dd, 1H, *J* = 8.1, 1.8 Hz), 8.39 (d, 1H, *J* = 1.8 Hz).

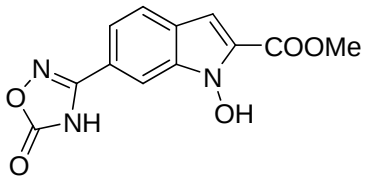
Methyl 3-(2-nitro-4-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)phenyl)-2-oxopropanoate (3.91p)



A stirred suspension of sodium hydride (60% dispersion in mineral oil, 435 mg, 10.9 mmol) in 7.5 mL of anhydrous DMF cooled to –15 °C under nitrogen was treated

dropwise with a solution containing the nitrotoluene precursor **3.90p** (300 mg, 1.36 mmol) and dimethyl oxalate (1.61 g, 13.6 mmol) in 3.9 mL of anhydrous DMF. The mixture was left under stirring at the same temperature for 10 minutes and then it was slowly warmed to room temperature. The mixture was then left under stirring at RT for 16 h. Once the disappearance of the precursor was verified by TLC, the reaction mixture was slowly poured in an ice-water mixture; the water phase was acidified with 1N aqueous HCl and extracted several times with EtOAc. The combined organic phase was washed with saturated $NaHCO_3$ solution and brine, then dried over anhydrous sodium sulphate. Evaporation under vacuum of the organic solvent afforded a crude product which was purified by column chromatography over silica gel using CH_2Cl_2/MeOH 95:5 as the eluent, to yield the nitroaryl-ketoester derivative **3.91p** (402 mg, 1.31 mmol, 96% yield). ^{1}H NMR (CD_3OD) δ (ppm): 3.90 (s, 3H), 4.86 (s, 2H), 8.01 (dd, 1H, *J* = 8.4, 1.6 Hz), 8.28 (d, 1H, *J* = 1.8 Hz), 8.51 (d, 1H, *J* = 8.4 Hz); signals imputable to the enol form (~ 30%) δ (ppm): 3.92 (s, 3H), 7.66 (d, 1H, *J* = 8.1 Hz), 8.10 (dd, 2H, *J* = 8.1, 1.8 Hz), 8.56 (d, 1H, *J* = 1.8 Hz).

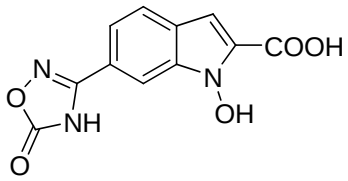
Methyl 1-hydroxy-6-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)-1*H*-indole-2-carboxylate (3.92p)



Ketoester **3.91p** (300 mg, 0.976 mmol) was dissolved in THF (1.6 mL) and the resulting solution was added to an aqueous solution (1.6 mL) of sodium hypophosphite monohydrate (322 mg, 3.04 mmol). Then 10 mg of 10% palladium over charcoal was added and the resulting suspension was stirred at RT for 24 h. Then it was monitored by TLC and $H_2PO_2Na{\cdot}H_2O$ (59.8 mg, 0.564 mmol) and Pd/C (4.5 mg) were added. After further 12h the disappearance of the precursor is verified by TLC and the mixture was concentrated, diluted with water and repeatedly extracted with EtOAc. The combined organic phase was dried over anhydrous sodium sulphate and evaporated to afford a crude residue that was purified by column chromatography over over silica gel using

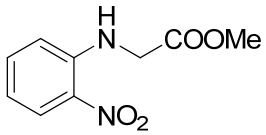
CHCl3/MeOH 95:5 as the eluent, to give the *N*-hydroxyindole **3.92p** (35.5 mg, 0.129 mmol, 13% yield). ^{1}H NMR (CD_3OD) δ (ppm): 3.94 (s, 3H); 7.14 (d, 1H, *J* = 0.7 Hz); 7.54 (dd, 1H, *J* = 8.4, 1.5 Hz); 7.77 (d, 1H, *J* = 8.6 Hz); 7.97 (s, 1H *J* = 0.7 Hz).

1-Hydroxy-6-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)-1*H*-indole-2-carboxylic acid (3.89p)



Methyl ester **3.92p** (25.0 mg, 0.0908 mmol) was dissolved in a 1:1 mixture of THF/methanol (0.91 mL) and treated with 0.27 mL of 2N aqueous solution of LiOH. The reaction was kept under stirring in the dark at RT and it was monitored by TLC. After 24 h, the reaction mixture was concentrated, diluted with water and washed with Et_2O. Then, the aqueous phase was treated with 1N aqueous HCl and extracted several times with EtOAc. The combined ethyl acetate organic phase was dried over anhydrous sodium sulphate and evaporated to afford the desired *N*-hydroxyindol-carboxylic acid product **3.89p** (23.5 mg, 0.0899 mmol, 99% yield) without any further purification. ^{1}H NMR (DMSO-d_6) δ (ppm): 7.22 (d, 1H, *J* = 0.9 Hz), 7.66 (dd, 1H, *J* = 8.4, 1.6 Hz), 7.88 (d, 1H, *J* = 8.4 Hz), 8.10 (s, 1H). ^{13}C NMR (DMSO-d_6) δ (ppm): 106.15, 109.19, 118.55, 120.84, 124.29, 124.69, 129.13, 131.70, 158.50, 160.20, 161.59.

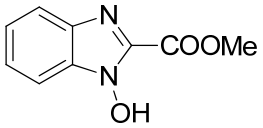
Methyl 2-((2-nitrophenyl)amino)acetate (3.95)



A solution containing methyl glycinate hydrochloride (890 mg, 7.09 mmol), 1-fluoro-2-nitrobenzene **3.94** (0.75 mL, 1.0 g, 7.1 mmol) and sodium bicarbonate (1.19 g, 14.2 mmol) in methanol (8.1 mL) was heated to reflux for 24 hours. Evaporation under vacuum of methanol afforded a crude product which is taken up with H_2O and EtOAc.

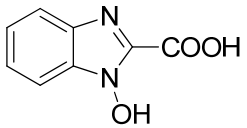
The organic phase was washed with brine and dried over anhydrous sodium sulphate. Evaporation under vacuum of the organic solvent afforded a crude product which was purified by column chromatography over silica gel using a 9:1 *n*-hexane/EtOAc mixture as the eluent, to yield the *N*-arylglycinic derivative **3.95** (357 mg, 1.70 mmol, 24% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 3.83 (s, 3H), 4.11 (d, 2H, *J* = 5.5 Hz), 6.69 (d, 1H, *J* = 8.6 Hz), 6.73 (td, 1H, *J* = 7.8, 1.3 Hz), 7.46 (td, 1H, *J* = 7.9, 1.6 Hz), 8.21 (dd, 1H, *J* = 8.5, 1.6 Hz), 8.39 (bs, 1H).

Methyl 1-hydroxy-1*H*-benzo[*d*]imidazole-2-carboxylate (3.96)



A freshly prepared solution of sodium methoxide (79.8 mg, 3.47 mmol) in MeOH (19.3 mL) was treated with the *N*-arylglycinic derivative **3.95** (270 mg, 1.28 mmol) prepared in the previous step. The resulting mixture was left under stirring at RT for 5 h. Once the disappearance of the glycinic precursor was verified by TLC, the reaction mixture was diluted with water and acidified with AcOH. The resulting suspension was extracted several times with Et_2O. The combined organic phase was washed with brine and dried over anhydrous sodium sulphate. Evaporation under vacuum of the organic solvent afforded a crude product which was purified by column chromatography over silica gel using a 95:5 mixture of EtOAc/MeOH as the eluent, to yield the *N*-hydroxybenzimidazol-ester derivative **3.96** (90.3 mg, 0.470 mmol, 37% yield). ^{1}H NMR (CD_3OD) δ (ppm): 4.04 (s, 3H), 7.29-7.51 (m, 2H), 7.63-7.74 (m, 2H). MS *m/z* 192 (M^+, 100%), 162 (M^+ –O –CH_2, 25%), 134 (M^+ –CO_2 –CH_2, 35%).

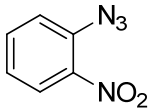
1-Hydroxy-1*H*-benzo[*d*]imidazole-2-carboxylic acid (3.97)



A solution containing the *N*-hydroxybenzimidazol-ester derivative **3.96** (80.0 mg, 0.416 mmol) in 4 mL of a 1:1 mixture of MeOH and THF was treated at RT with 1.3 of

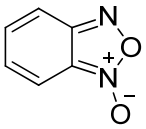
an aqueous 2N solution of LiOH. The reaction mixture was left under stirring in the dark at the same temperature for 2 h. Once the disappearance of the precursor was verified by TLC, most of the organic solvent was evaporated under vacuum and the reaction mixture was diluted with water, acidified with aqueous 1N solution of HCl and extracted several times with EtOAc. The combined organic phase was washed with brine and dried over anhydrous sodium sulphate. Evaporation under vacuum of the organic solvent afforded the final *N*-hydroxybenzimidazol-carboxylic acid product **3.97** (30.0 mg, 0.168 mmol, 40% yield). ^{1}H NMR (CD_3OD) δ (ppm): 7.36-7.49 (m, 2H), 7.67-7.70 (m, 2H), 8.65 (bs, 1H).

1-Azido-2-nitrobenzene (3.102)



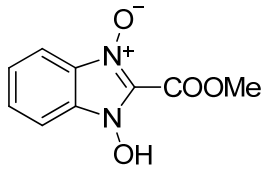
A solution of $NaNO_2$ (599 mg, 8.69 mmol) in H_2O (1.9 mL) was added dropwise to a stirred solution of commercially available 2-nitroaniline **3.101** (1.00 g, 7.24 mmol) in a mixture of concentrated HCl solution (3.8 mL) and H_2O (6.7 mL) at –5 °C. The mixture was stirred at 0 °C for 1 h. A solution of NaN_3 (471 mg, 7.24 mmol) in H_2O (1.9 mL) was added dropwise to the cooled mixture so that the temperature did not exceed 5 °C (vigorous foaming). The mixture was stirred for 1 h at 0-5 °C and then for 2 h at RT. The reaction was worked up by dilution with EtOAc. The organic layer was washed with saturated $NaHCO_3$ solution and brine, then dried over anhydrous sodium sulphate. Evaporation of the organic solvent yielded the azide derivative **3.102** (1.16 g, 7.07 mmol, 98% yield), which was directly used for the next step, without ^{1}H NMR analysis.

Benzo[*c*][1,2,5]oxadiazole 1-oxide (3.103)



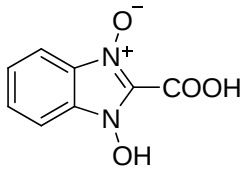
A solution of the azide **3.102** (1.15 g, 7.01 mmol) in toluene (17.5 mL) was added dropwise over boiling toluene (52.6 mL). The mixture was refluxed overnight. Removal of the solvent under vacuum afforded benzofuroxane **3.103** (974 mg, 7.16 mmol, >99% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 7.26-7.38 (m, 2H). 7.38-7.47 (m, 2H).

1-Hydroxy-2-(methoxycarbonyl)-1*H*-benzo[*d*]imidazole 3-oxide (3.105a)



The benzofurazan-oxide precursor **3.103** (900 mg, 6.61 mmol) was dissolved in MeOH (8.3 mL), methyl phenylsulfonylacetate (0.54 mL, 0.71 g, 3.3 mmol) was added and the crystals were dissolved by warming the mixture. Subsequently, 8% methanolic KOH solution (8.3 mL) was added and the solution was left at RT for 12 h. The solvent was evaporated to dryness, then water was added to the resulting residue and the aqueous phase was treated with 1N aqueous HCl and extracted several times with EtOAc. The combined ethyl acetate organic phase was dried over anhydrous sodium sulphate. After filtration, the solvent was removed in vacuo to give a crude which was purified by column chromatography over silica gel using *n*-hexane *n*-hexane/EtOAc 9:1 as the eluent, to give d the *N*-hydroxyindol-*N*-oxide-ester derivative **3.105a** (423 mg, 2.03 mmol, 31% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 4.72 (s, 3H), 7.20-7.55 (m, 4H).

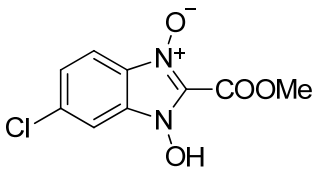
2-Carboxy-1-hydroxy-1*H*-benzo[*d*]imidazole 3-oxide (3.98)



Methyl ester **3.105a** (415 mg, 1.99 mmol) was dissolved in a 1:1 mixture of THF/methanol (20 mL) and treated with 6 mL of 2N aqueous solution of LiOH. The reaction was kept under stirring in the dark at RT and it was monitored by TLC. After 48 h, the reaction mixture was concentrated, diluted with water and washed with Et_2O.

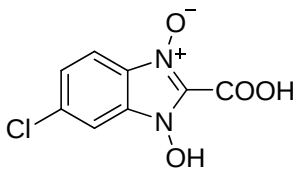
Then, the aqueous phase was treated with 1N aqueous HCl and extracted several times with EtOAc. The combined ethyl acetate organic phase was dried over anhydrous sodium sulphate and evaporated to afford a crude, that was purified by flash chromatography (*n*-hexane/ EtOAc 8:2) to yield the desired *N*-hydroxybenzimidazol-*N*-oxide-carboxylic acid product **3.98** (32.1 mg, 0.165 mmol, 8% yield) without any further purification. ^{1}H NMR (acetone-d_6) δ (ppm): 6.60-6.90 (m, 3H), 7.26 (bs, 1H), 11.64 (bs, 1H).

6-Chloro-1-hydroxy-2-(methoxycarbonyl)-1*H*-benzo[*d*]imidazole 3-oxide (3.105b)



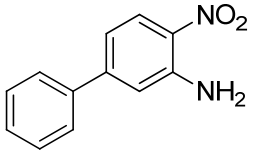
A solution containing commercially available 5-chlorobenzofurazan-3-oxide **3.104** (500 mg, 2.93 mmol) and methyl nitroacetate (0.32 mL, 0.42 g, 3.5 mmol) in THF (2.9 mL) was slowly treated at RT with Et_2NH (0.36 mL, 0.26 g, 3.5 mmol). After completion of the addition, the resulting mixture was left under stirring overnight. Then, the reaction mixture is diluted with water, acidified with a aqueous 1N solution of HCl, and extracted several times with EtOAc. The combined organic phase is washed with brine and dried over anhydrous sodium sulphate. Evaporation under vacuum of the organic solvent affords a crude product which is purified by column chromatography over silica gel using *n*-hexane/EtOAc 6:4 as the eluent, to yield the *N*-hydroxyindol-*N*-oxide-ester derivative **3.105b** (117 mg, 0.482 mmol, 16% yield). ^{1}H NMR ($CDCl_3$) δ (ppm); tautomer A: 4.08 (s, 3H), 7.42 (dd, 1H, J = 8.8, 1.6 Hz), 7.57 (d, 1H, J = 8.8 Hz), 7.78 (d, 1H, J = 1.8 Hz); tautomer B: 4.09 (s, 3H), 7.32 (dd, 1H, J = 8.8, 1.8 Hz), 7.64 (d, 1H, J = 1.8 Hz), 7.71 (d, 1H, J = 8.8 Hz).

2-Carboxy-6-chloro-1-hydroxy-1*H*-benzo[*d*]imidazole 3-oxide (3.99)



Methyl ester **3.105b** (100 mg, 0.412 mmol) was dissolved in a 1:1 mixture of THF/methanol (2.1 mL) and treated with 1.2 mL of 2N aqueous solution of LiOH. The reaction was kept under stirring in the dark at RT and it was monitored by TLC. After 3 h, the reaction mixture was concentrated, diluted with water and washed with Et_2O. Then, the aqueous phase was treated with 1N aqueous HCl and extracted several times with EtOAc. The combined ethyl acetate organic phase was dried over anhydrous sodium sulphate and evaporated to afford the final *N*-hydroxybenzimidazol-*N*-oxide-carboxylic acid product **3.99** (40.7 mg, 0.178 mmol, 43% yield) without any further purification. ^{1}H NMR (CD_3OD) δ (ppm); tautomer A: 7.35 (dd, 1H, *J* = 8.6, 1.9 Hz), 7.55 (d, 1H, *J* = 8.8 Hz), 8.33 (d, 1H, *J* = 2.4 Hz); tautomer B: 7.28 (dd, 1H, *J* = 8.6, 2.0 Hz), 7.61 (d, 1H, *J* = 8.9 Hz), 7.64 (d, 1H, *J* = 2.0 Hz). ^{13}C NMR (CD_3OD) δ (ppm): 110.33, 111.60, 119.36, 121.00, 124.49, 125.00, 129.61, 130.45.

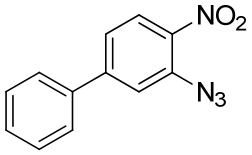
4-Nitro-[1,1'-biphenyl]-3-amine (3.107)



A solution of commercially available 5-chloro-2-nitroaniline **3.106** (1.00 g, 5.79 mmol) in dioxane (20.3 mL) was treated with cesium carbonate (3.21 g, 9.84 mmol), phenylboronic acid (1.13 g, 9.26 mmol), $Pd_2(dba)_3$ (170 mg, 0.185 mmol) and 0.8 mL of a 20% solution of tricyclohexylphosphine (0.446 mmol) in toluene, and the resulting mixture was heated to 80 °C in a sealed vial for 24 h. The reaction mixture was then cooled to RT, diluted with EtOAc and filtered through a Celite pad. The organic filtrate was concentrated under vacuum and the crude product was purified by flash chromatography (*n*-hexane/EtOAc 8:2) to yield pure **3.107** (212 mg, 0.990 mmol, 17%

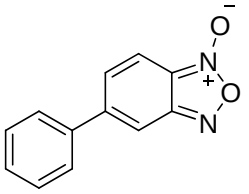
yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 6.15 (bs, 2H), 6.94 (dd, 1H, *J* = 8.8, 1.8 Hz), 6.98 (d, 1H, *J* = 1.3 Hz), 7.42-7.51 (m, 3H), 7.56-7.60 (m, 2H), 8.19 (d, 1H, *J* = 8.8 Hz).

3-Azido-4-nitro-1,1'-biphenyl (3.108)



A solution of $NaNO_2$ (77.3 mg, 1.12 mmol) in H_2O (0.2 mL) was added dropwise to a stirred solution of the synthesized amine derivative **3.107** (200 mg, 0.934 mmol) in a mixture of concentrated HCl solution (0.5 mL) and H_2O (0.9 mL) at –5 °C. The mixture was stirred at 0 °C for 1 h. A solution of NaN_3 (60.7 mg, 0.934 mmol) in H_2O (0.2 mL) was added dropwise to the cooled mixture so that the temperature did not exceed 5 °C (vigorous foaming). The mixture was stirred for 1 h at 0-5 °C and then for 12 h at RT. The reaction was worked up by dilution with EtOAc. The organic layer was washed with saturated $NaHCO_3$ solution and brine, then dried over anhydrous sodium sulphate. After evaporation of the organic solvent, the crude was purified by column chromatography over silica gel using *n*-hexane/EtOAc 9:1 as the eluent, to yield the azide derivative **3.108** (199 mg, 0.828 mmol, 89% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 7.41-7.63 (m, 7H), 8.05 (d, 1H, *J* = 8.4 Hz).

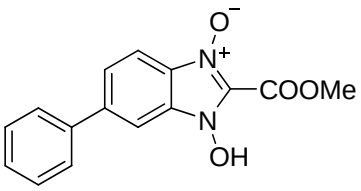
5-Phenylbenzo[*c*][1,2,5]oxadiazole 1-oxide (3.109)



A solution of the azide **3.108** (198 mg, 0.824 mmol) in toluene (2.1 mL) was added dropwise over boiling toluene (6.2 mL). The mixture was refluxed for 4 h. Removal of the solvent under vacuum afforded a crude product that was purified by column chromatography over silica gel using *n*-hexane/EtOAc 9:1 as the eluent, to yield

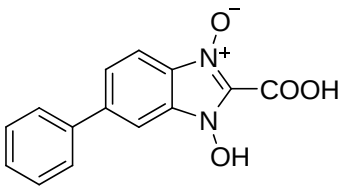
benzofuroxane **3.109** (91.2 mg, 0.430 mmol, 52% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 7.265-7.27 (m, 1H), 7.46-7.64 (m, 7H).

1-Hydroxy-2-(methoxycarbonyl)-6-phenyl-1*H*-benzo[*d*]imidazole 3-oxide (3.110)



A solution containing **3.109** (44.0 mg, 0.207 mmol) and methyl nitroacetate (0.02 mL, 0.030 g, 0.25 mmol) in THF (0.2 mL) was slowly treated at RT with Et_2NH (0.03 mL, 0.018 g, 0.25 mmol). After completion of the addition, the resulting mixture was left under stirring for 3 days. Then, the reaction mixture is diluted with water, acidified with a aqueous 1N solution of HCl, and extracted several times with EtOAc. The combined organic phase is washed with brine and dried over anhydrous sodium sulphate. Evaporation under vacuum of the organic solvent affords a crude product which is purified by column chromatography over silica gel using *n*-hexane/EtOAc 3:7 as the eluent, to yield the *N*-hydroxyindol-*N*-oxide-ester derivative **3.110** (5.0 mg, 0.018 mmol, 8% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 4.07 (s, 3H), 7.34-7.51 (m, 5H), 7.60-7.66 (m, 3H).

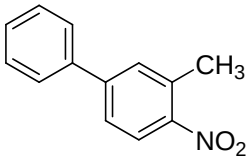
2-Carboxy-1-hydroxy-6-phenyl-1*H*-benzo[*d*]imidazole 3-oxide (3.100)



Methyl ester **3.110** (5.0 mg, 0.018 mmol) was dissolved in a 1:1 mixture of THF/methanol (0.4 mL) and treated with 0.1 mL of 2N aqueous solution of LiOH. The reaction was kept under stirring in the dark at RT and it was monitored by TLC. After 3 days, the reaction mixture was concentrated, diluted with water and washed with Et_2O. Then, the aqueous phase was treated with 1N aqueous HCl and extracted several times

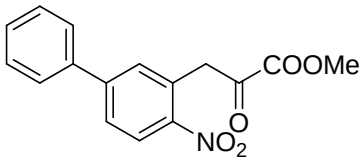
with EtOAc. The combined ethyl acetate organic phase was dried over anhydrous sodium sulphate and evaporated to afford the desired *N*-hydroxyindol-carboxylic acid product **3.100** (4.4 mg, 0.016 mmol, 90% yield) without any further purification. ^{1}H NMR (CD_3OD) δ (ppm): 7.40-7.53 (m, 3H), 7.66-7.75 (m, 2H), 7.85-8.02 (m, 3H), 9.20 (bs, 1H).

3-Methyl-4-nitrobiphenyl (3.118b)



A solution of $Pd(OAc)_2$ (31 mg, 0.14 mmol) and triphenylphosphine (183 mg, 0.699 mmol) in absolute ethanol (10 mL) and anhydrous toluene (10 mL) was stirred at RT under nitrogen for 10 min. After that period, commercially available 5-chloro-2-nitrotoluene **3.115** (800 mg, 4.66 mmol), 10 mL of 2M aqueous Na_2CO_3, and phenylboronic acid (910 mg, 7.46 mmol) were sequentially added. The resulting mixture was heated at 100 °C in a sealed vial under nitrogen overnight. After being cooled to RT, the mixture was diluted with water and extracted with EtOAc. The combined organic phase were dried and concentrated. The crude product was purified by flash chromatography (*n*-hexane) to yield **3.118b** (930 mg, 4.36 mmol, 94% yield). ^{1}H NMR (DMSO-d_6) δ (ppm): 2.69 (s, 3H); 7.40-7.63 (m, 7H); 8.09 (d, 1H, J = 10 Hz).

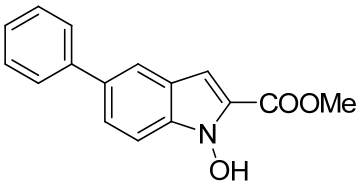
Methyl 3-(4-nitrobiphenyl-3-yl)-2-oxopropanoate (3.119b)



A stirred suspension of sodium hydride (60% dispersion in mineral oil, 563 mg, 14.1 mmol) in 9.9 mL of anhydrous DMF cooled to –15 °C under nitrogen was treated dropwise with a solution containing the nitrotoluene precursor **3.118b** (750 mg, 3.52 mmol) and dimethyl oxalate (2.08 g, 17.6 mmol) in 6.6 mL of anhydrous DMF. The

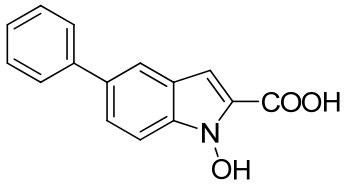
mixture was left under stirring at the same temperature for 10 minutes and then it was slowly warmed to room temperature. The mixture was then left under stirring at RT for 12 h. Once the disappearance of the precursor was verified by TLC, the reaction mixture was slowly poured in an ice-water mixture; the water phase was acidified with 1N aqueous HCl and extracted several times with EtOAc. The combined organic phase was washed with saturated $NaHCO_3$ solution and brine, then dried over anhydrous sodium sulphate. Evaporation under vacuum of the organic solvent afforded a crude product which was purified by column chromatography over silica gel using *n*-hexane/EtOAc 7:3 as the eluent, to yield the nitroaryl-ketoester derivative **3.119b** (814 mg, 2.72 mmol, 77% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 3.96 (s, 3H), 4.62 (s, 2H), 7.44-7.67 (m, 6H), 7.70 (dd, 1H, *J* = 8.6, 2.0 Hz), 8.28 (d, 1H, *J* = 8.6 Hz); signals imputable to the enol form (~ 30%) δ (ppm): 3.97 (s, 3H), 6.66 (d, 1H, exchangeable, *J* = 1.5 Hz), 7.06 (d, 1H, *J* = 1.3 Hz), 8.04 (d, 1H, *J* = 8.4 Hz), 8.46 (d, 1H, *J* = 2.0 Hz).

Methyl 1-hydroxy-5-phenyl-1*H*-indole-2-carboxylate (3.120b)



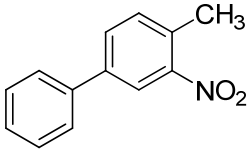
Ketoester **3.119b** (400 mg, 1.34 mmol) was dissolved in THF (2.2 mL) and the resulting solution was added to an aqueous solution (2.2 mL) of sodium hypophosphite monohydrate (439 mg, 4.14 mmol). Then 14 mg of 10% palladium over charcoal was added and the resulting suspension was stirred ar RT. After 4 h the disappearance of the precursor is verified by TLC and the mixture was concentrated, diluted with water and repeatedly extracted with EtOAc. The combined organic phase was dried over anhydrous sodium sulphate and evaporated to afford a crude residue that was purified by column chromatography over over silica gel using *n*-hexane/CH_2Cl_2 2:8 as the eluent, to give the *N*-hydroxyindole **3.120b** (69.0 mg, 0.258 mmol, 19% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 4.01 (s, 3H), 7.08 (s, 1H), 7.33-7.37 (m, 1H), 7.40-7.49 (m, 2H), 7.60-7.66 (m, 4H), 7.82-7.84 (m, 1H), 10.27 (bs, 1H).

1-Hydroxy-5-phenyl-1*H*-indole-2-carboxylic acid (3.112)



Methyl ester **3.120b** (60.0 mg, 0.224 mmol) was dissolved in a 1:1 mixture of THF/methanol (2.2 mL) and treated with 0.68 mL of 2N aqueous solution of LiOH. The reaction was kept under stirring in the dark at RT and it was monitored by TLC. After 24 h, the reaction mixture was concentrated, diluted with water and washed with Et_2O. Then, the aqueous phase was treated with 1N aqueous HCl and extracted several times with EtOAc. The combined ethyl acetate organic phase was dried over anhydrous sodium sulphate and evaporated to afford the desired *N*-hydroxyindol-carboxylic acid product **3.112** (51.1 mg, 0.202 mmol, 90% yield) without any further purification. ^{1}H NMR (DMSO-d_6) δ (ppm): 7.04 (d, 1H, J = 0.9 Hz), 7.32-7.36 (m, 1H), 7.42-7.53 (m, 2H), 7.60-7.70 (m, 4H), 7.90 (t, 1H, J = 0.9 Hz). ^{13}C NMR (DMSO-d_6) δ (ppm): 104.51, 110.10, 119.85, 119.93, 121.46, 124.10, 126.66 (2C), 127.34, 128.85 (2C), 132.67, 135.00, 140.94, 161.39. MS *m/z* 253 (M^+, 100%), 237 (M^+ –O, 40%), 190 (M^+ –H_2O –CO_2 –H, 62%), 165 (M^+ –H_2O –CO_2 –C_2H_2, 12%). HPLC, t_R 9.4 min.

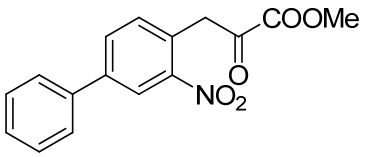
4-Methyl-3-nitrobiphenyl (3.118c)



A solution of $Pd(OAc)_2$ (31 mg, 0.14 mmol) and triphenylphosphine (182 mg, 0.694 mmol) in absolute ethanol (10 mL) and anhydrous toluene (10 mL) was stirred at RT under nitrogen for 10 min. After that period, commercially available 4-bromo-2-nitrotoluene **3.90d** (1.00 g, 4.63 mmol), 10 mL of 2M aqueous Na_2CO_3, and phenylboronic acid (904 mg, 7.41 mmol) were sequentially added. The resulting mixture was heated at 100 °C in a sealed vial under nitrogen overnight. After being cooled to RT, the mixture was diluted with water and extracted with EtOAc. The

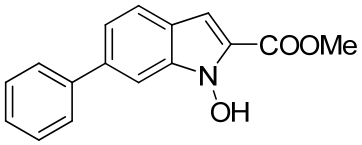
combined organic phase were dried and concentrated. The crude product was purified by flash chromatography (*n*-hexane/ EtOAc 95:5) to yield **3.118c** (966 mg, 4.53 mmol, 98% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 2.64 (s, 3H); 7.39-7.52 (m, 4H); 7.58-7.63 (m, 2H); 7.73 (dd, 1H, *J* = 7.4, 2.0 Hz); 8.21 (d, 1H, *J* = 1.8 Hz).

Methyl 3-(3-nitrobiphenyl-4-yl)-2-oxopropanoate (3.119c)



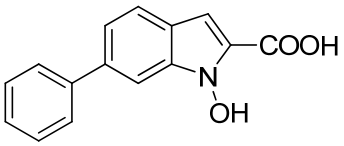
A stirred suspension of sodium hydride (60% dispersion in mineral oil, 375 mg, 9.38 mmol) in 9.2 mL of anhydrous DMF cooled to –15 °C under nitrogen was treated dropwise with a solution containing the nitrotoluene precursor **3.118c** (500 mg, 2.34 mmol) and dimethyl oxalate (1.38 g, 11.7 mmol) in 6.9 mL of anhydrous DMF. The mixture was left under stirring at the same temperature for 10 minutes and then it was slowly warmed to room temperature. The mixture was then left under stirring at RT for 4 h. Once the disappearance of the precursor was verified by TLC, the reaction mixture was slowly poured in an ice-water mixture; the water phase was acidified with 1N aqueous HCl and extracted several times with EtOAc. The combined organic phase was washed with saturated $NaHCO_3$ solution and brine, then dried over anhydrous sodium sulphate. Evaporation under vacuum of the organic solvent afforded a crude product which was purified by column chromatography over silica gel using *n*-hexane/EtOAc 7:3 as the eluent, to yield the nitroaryl-ketoester derivative **3.119c** (567 mg, 1.89 mmol, 81% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 3.96 (s, 3H), 4.58 (s, 2H), 7.40 (d, 1H, *J* = 8.1 Hz), 7.44-7.54 (m, 3H), 7.58-7.65 (m, 2H), 7.85 (dd, 1H, *J* = 7.9, 2.0 Hz), 8.40 (d, 1H, *J* = 2.0 Hz); signals imputable to the enol form (~ 20%) δ (ppm): 3.92 (s, 3H), 6.70 (bs, 1H, exchangeable), 6.97 (s, 1H), 7.83 (dd, 1H, *J* = 8.2, 1.9 Hz), 8.13 (d, 1H, *J* = 1.8 Hz), 8.40 (d, 1H, *J* = 8.4 Hz).

Methyl 1-hydroxy-6-phenyl-1*H*-indole-2-carboxylate (3.120c)



Triethylamine (0.93 mL, 0.68 g, 6.7 mmol) was added dropwise to a stirred solution of $SnCl_2 \cdot 2H_2O$ (453 mg, 2.01 mmol) and PhSH (0.62 mL, 0.66 g, 6.0 mmol) in acetonitrile (10.5 mL) at room temperature to generate a yellow precipitate over a period of 5 min. Then, **3.119c** (400 mg, 1.34 mmol) was transferred to the suspension and stirred at room temperature for 5 more min. After complete consumption of the starting material, the mixture was diluted with water and repeatedly extracted with EtOAc. The combined organic phase was dried over anhydrous sodium sulphate, diluted with toluene and a catalytic amount of *p*-toluensulfonic acid was added. Then, it was evaporated to afford a crude residue that was purified by column chromatography over silica gel using *n*-hexane/EtOAc 7:3 as the eluent, to give the *N*-hydroxyindole **3.120c** (182 mg, 0.681 mmol, 51% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 4.01 (s, 3H), 7.05 (d, 1H, J = 0.9 Hz), 7.36-7.51 (m, 4H), 7.67-7.75 (m, 4H), 10.26 (bs, 1H). ^{13}C NMR ($CDCl_3$) δ (ppm): 52.58, 103.89, 107.75, 120.48, 121.26, 121.72, 123.03, 127.47, 127.56 (2C), 128.91 (2C), 134.01, 139.23, 141.45, 164.54.

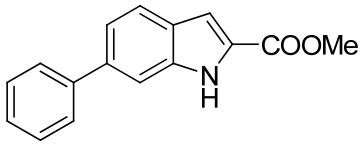
1-Hydroxy-6-phenyl-1*H*-indole-2-carboxylic acid (3.113)



Methyl ester **3.120c** (129 mg, 0.483 mmol) was dissolved in a 1:1 mixture of THF/methanol (4.8 mL) and treated with 1.4 mL of 2N aqueous solution of LiOH. The reaction was kept under stirring in the dark at RT and it was monitored by TLC. After 12 h, the reaction mixture was concentrated, diluted with water and washed with Et_2O. Then, the aqueous phase was treated with 1N aqueous HCl and extracted several times with EtOAc. The combined ethyl acetate organic phase was dried over anhydrous sodium sulphate and evaporated to afford the desired *N*-hydroxyindol-carboxylic acid

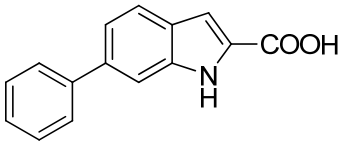
product **3.113** (121 mg, 0.478 mmol, 99% yield) without any further purification. ^{1}H NMR (DMSO-d_6) δ (ppm): 7.03 (s, 1H), 7.36-7.52 (m, 4H), 7.62-7.64 (m, 1H), 7.70-7.75 (m, 3H). ^{13}C NMR (acetone-d_6) δ (ppm): 106.08, 108.19, 121.37, 121.83, 123.63, 127.05, 127.96 (2C), 128.06, 129.68 (2C), 137.49, 139.25, 142.18, 162.05. MS *m/z* 253 (M^+, 100%), 191 (M^+ –H_2O –CO_2, 65%), 190 (M^+ –H_2O –CO_2 –H, 86%), 165 (M^+ –H_2O –CO_2 –C_2H_2, 32%). HPLC, t_R 9.2 min.

Methyl 6-phenyl-1*H*-indole-2-carboxylate (3.121)



Ketoester **3.119c** (300 mg, 1.00 mmol) was dissolved in anhydrous DME (1.0 mL) and the resulting solution was added dropwise to a cooled (0 °C) solution of $SnCl_2 \cdot 2H_2O$ (505 mg, 2.24 mmol) in DME (1.0 mL) containing 4Å molecular sieves, previously activated for 18 hours at 130 °C in oven and cooled to RT in a desiccator over anhydrous calcium chloride. The reaction mixture was stirred under nitrogen at RT for 24 h, then it was diluted with water and repeatedly extracted with EtOAc. The combined organic phase was dried over anhydrous sodium sulphate and evaporated to afford a 66:34 mixture of the indol-ester derivative **3.121** with its *N*-hydroxyindole analogue **3.120c** that was purified by column chromatography over silica gel using *n*-hexane/EtOAc 8:2 as the eluent, to give the indol-ester derivative **3.121** (40.0 mg, 0.159 mmol, 16% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 3.96 (s, 3H), 7.27 (dd, 1H, *J* = 2.0, 0.9 Hz), 7.36-7.51 (m, 4H), 7.61-7.68 (m, 3H), 7.75 (d, 1H, *J* = 8.4 Hz), 8.93 (bs, 1H).

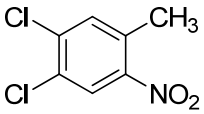
6-Phenyl-1*H*-indole-2-carboxylic acid (3.122)



Methyl ester **3.121** (40.0 mg, 0.159 mmol) was dissolved in a 1:1 mixture of THF/methanol (1.6 mL) and treated with 0.5 mL of 2N aqueous solution of LiOH. The

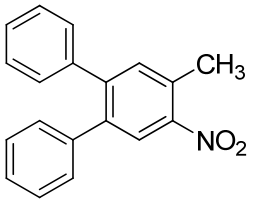
reaction was kept under stirring in the dark at RT and it was monitored by TLC. After 72 h, the reaction mixture was concentrated, diluted with water and washed with Et_2O. Then, the aqueous phase was treated with 1N aqueous HCl and extracted several times with EtOAc. The combined ethyl acetate organic phase was dried over anhydrous sodium sulphate and evaporated to afford the desired *N*-hydroxyindol-carboxylic acid product **3.122** (37.5 mg, 0.158 mmol, 99% yield) without any further purification. ^{1}H NMR (acetone-d_6) δ (ppm): 7.23 (dd, 1H, J = 2.2, 0.9 Hz), 7.34-7.52 (m, 4H), 7.67-7.80 (m, 4H), 10.94 (bs, 1H). ^{13}C NMR (acetone-d_6) δ (ppm): 108.70, 111.19, 121.00, 123.38, 126.10, 127.80, 127.89 (2C), 129.62 (2C), 129.65, 138.76, 142.60, 162.60. MS *m/z* 237 (M^+, 38%), 191 (M^+ –HCOOH, 40%), 190 (M^+ –HCOOH –H, 100%). HPLC, t_R = 9.2 min.

1,2-Dichloro-4-methyl-5-nitrobenzene (3.117)



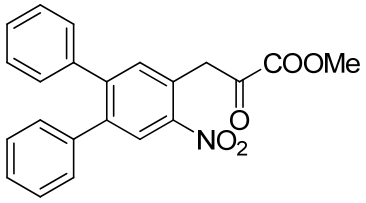
To a mixture of 3,4-dichlorotoluene **3.116** (0.8 mL, 1.00 g, 6.21 mmol) and concentrated H_2SO_4 (10 mL) was added portionwise KNO_3 (791 mg, 7.82 mmol) at 5 °C, and the resulting mixture was stirred at room temperature for 2 h. After cooling to 0 °C, the mixture was poured into ice-water. The resulting precipitate was collected with filtration and dried. Subsequently, the solid was dissolved in Et_2O, washed with brine, and dried over anhydrous sodium sulphate. After removal of the solvent, the residue was purified by column chromatography on silica gel using *n*-hexane/EtOAc 100:1 as the eluent to obtain 3, 4-dichloro-6-nitrotoluene **3.117** (465 mg, 2.26 mmol, 36% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 2.59 (s, 3H); 7.47 (s, 1H); 8.13 (s, 1H).

1,2-Diphenyl-4-methyl-5-nitrobenzene (3.118d)



Dichloro-aryl derivative **3.117** (460 mg, 2.23 mmol) was placed in a vial together with phenylboronic acid (1.09 g, 8.93 mmol), potassium phosphate (2.08 g, 9.81 mmol), $Pd(OAc)_2$ (13 mg, 0.059 mmol), tetrabutylammonium bromide (1.12 g, 34.6 mmol) and water (5 mL). The vial was sealed and heated under stirring at 125 °C for 48 h. The reaction mixture was cooled to RT and then diluted with water. The water phase was acidified with 1N aqueous HCl and repeatedly extracted with EtOAc. The combined organic phase was dried over anhydrous sodium sulphate and evaporated to afford a crude residue that was purified by column chromatography over silica gel using *n*-hexane as the eluent, to give pure **3.118d** (587 mg, 2.03 mmol, 91% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 2.70 (s, 3H), 7.10-7.18 (m, 4H), 7.22-7.30 (m, 6H), 7.41 (s, 1H), 8.11 (s, 1H).

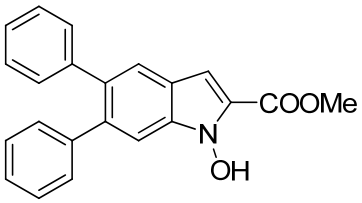
Methyl 3-(4,5-diphenyl-2-nitrophenyl)-2-oxopropanoate (3.119d)



A stirred suspension of sodium hydride (60% dispersion in mineral oil, 315 mg, 7.88 mmol) in 6 mL of anhydrous DMF cooled to –15 °C under nitrogen was treated dropwise with a solution containing the nitrotoluene precursor **3.118d** (570 mg, 1.97 mmol) and dimethyl oxalate (1.16 g, 9.85 mmol) in 6 mL of anhydrous DMF. The mixture was left under stirring at the same temperature for 10 minutes and then it was slowly warmed to room temperature. The mixture was then left under stirring at RT for 60 h. Once the disappearance of the precursor was verified by TLC, the reaction mixture

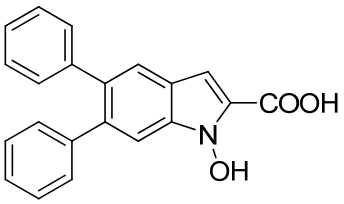
was slowly poured in an ice-water mixture; the water phase was acidified with 1N aqueous HCl and extracted several times with EtOAc. The combined organic phase was washed with saturated $NaHCO_3$ solution and brine, then dried over anhydrous sodium sulphate. Evaporation under vacuum of the organic solvent afforded a crude product which was purified by column chromatography over silica gel using *n*-hexane/EtOAc 8:2 as the eluent, to yield the nitroaryl-ketoester derivative **3.119d** (288 mg, 0.767 mmol, 38% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 3.97 (s, 3H), 4.62 (s, 2H), 7.11-7.20 (m, 3H), 7.23-7.30 (m, 7H), 7.36 (s, 1H), 8.28 (s, 1H), signals imputable to the enol form (~ 18%) δ (ppm): 6.53 (s, 1H); 7.06 (d, 1H, *J* = 1.3 Hz), 8.03 (s, 1H), 8.31 (s, 1H).

Methyl 1-hydroxy-5,6-diphenyl-1*H*-indole-2-carboxylate (3.120d)



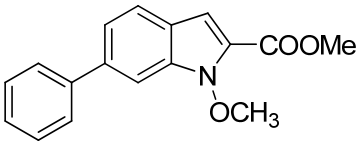
To a solution containing the ketoester **3.119d** (145 mg, 0.386 mmol) in 4 mL of MeOH, was added lead powder (400 mg, 1.93 mol) and 0.4 mL of HCO_2HNEt_3. The mixture was stirred at 55 °C for 12 h, cooled to RT, and filtered over a pad of Celite. The solvent was removed under reduced pressure and the residue purified by column chromatography over silica gel using *n*-hexane/EtOAc 1:1 as the eluent, to give the *N*-hydroxyindole **3.120d** (50.0 mg, 0.146 mmol, 38% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 4.01 (s, 3H), 7.09 (d, 1H, *J* = 0.5 Hz), 7.16-7.21 (m, 10H), 7.61 (s, 1H), 7.68 (s, 1H), 10.25 (bs, 1H).

1-Hydroxy-5,6-diphenyl-1*H*-indole-2-carboxylic acid (3.114)



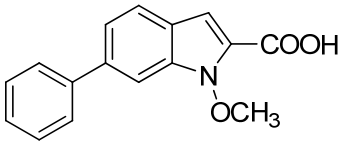
Methyl ester **3.120d** (48.0 mg, 0.140 mmol) was dissolved in a 1:1 mixture of THF/methanol (2 mL) and treated with 0.4 mL of 2N aqueous solution of LiOH. The reaction was kept under stirring in the dark at RT and it was monitored by TLC. After 3 h, the reaction mixture was concentrated, diluted with water and washed with Et_2O. Then, the aqueous phase was treated with 1N aqueous HCl and extracted several times with EtOAc. The combined ethyl acetate organic phase was dried over anhydrous sodium sulphate and evaporated to afford the desired *N*-hydroxyindol-carboxylic acid product **3.114** (37.0 mg, 0.112 mol, 80% yield) without any further purification. ^{1}H NMR (acetone-d_6) δ (ppm): 7.06-7.28 (m, 11H), 7.54 (s, 1H), 7.70 (s, 1H). ^{13}C NMR (acetone-d_6) δ (ppm): 106.28, 111.80, 121.88, 124.85, 126.80, 127.18, 127.51, 128.49 (2C), 128.56 (2C), 130.73 (2C), 130.84 (2C), 135.32, 136.47, 139.65, 142.93, 143.02, 162.01.

Methyl 1-methoxy-6-phenyl-1*H*-indole-2-carboxylate (3.123)



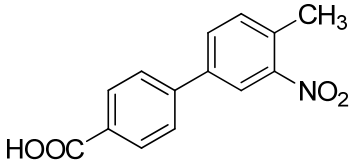
A solution of **3.120c** (250 mg, 0.935 mnol) in anhydrous THF was treated with DBU (0.5 mL) and iodomethane (0.2 mL), and the resulting mixture was stirred at RT for 1 h. The solvent was evaporated, then an aqueous 1N HCl solution was added and the mixture was extracted with AcOEt. The organic phase was dried over anhydrous sodium sulphate and evaporated to afford a crude residue that was purified by column chromatography (*n*-hexane/ethyl acetate 95:5, to give pure **3.123** (55.5 mg, 0.197 mmol, 21% yield); ^{1}H NMR ($CDCl_3$) δ (ppm): 3.96 (s, 3H), 4.24 (s, 3H), 7.13 (d, 1H, J = 0.7 Hz), 7.37-7.52 (m, 4H), 7.67-7.72 (m, 4H).

1-Methoxy-6-phenyl-1*H*-indole-2-carboxylic acid (3.124)



Methyl ester **3.123** (44.0 mg, 0.156 mmol) was dissolved in a 1:1 mixture of THF/methanol (1.6 mL) and treated with 0.46 mL of 2N aqueous solution of LiOH. The reaction was kept under stirring in the dark at RT and it was monitored by TLC. After 72 h, the reaction mixture was concentrated, diluted with water and washed with Et_2O. Then, the aqueous phase was treated with 1N aqueous HCl and extracted several times with EtOAc. The combined ethyl acetate organic phase was dried over anhydrous sodium sulphate and evaporated to afford the desired *N*-hydroxyindol-carboxylic acid product **3.124** (41.2 mg, 0.154 mmol, 99% yield) without any further purification. ^{1}H NMR ($CDCl_3$) δ (ppm): 4.26 (s, 3H), 7.31 (s, 1H), 7.39-7.53 (m, 4H), 7.67-7.76 (m, 4H). ^{13}C NMR ($CDCl_3$) δ (ppm): 66.40 (OMe), 107.66, 109.35, 121.19, 121.86, 123.37, 124.59, 127.62 (2C), 128.11, 128.98 (2C), 136.43, 140.16, 141.38, 164.60. MS *m/z* 267 (M^+, 50%), 253 (M^+ –CH_2, 30%), 237 (M^+ –O –CH_2, 9%), 191 (M^+ –CH_2 –H_2O –CO_2, 40%), 190 (M^+ –CH_2 –H_2O –CO_2 –H, 100%), 165 (M^+ –CH_2 –H_2O –CO_2 –C_2H_2, 21%). HPLC, t_R = 8.6 min (purity = 93%, major impurity accounting for >6% of residual peak area identified as **CG-277**).

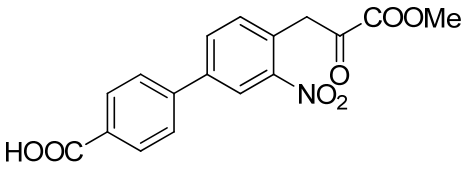
4'-Methyl-3'-nitrobiphenyl-4-carboxylic acid (3.130a)



A solution of $Pd(OAc)_2$ (20.8 mg, 0.0927 mmol) and triphenylphosphine (122 mg, 0.464 mmol) in absolute ethanol (6.9 mL) and anhydrous toluene (6.9 mL) was stirred at RT under nitrogen for 10 min in a vial. After that period, commercially available 4-bromo-2-nitrotoluene **3.90d** (667 mg, 3.09 mmol), 6.9 mL of 2M aqueous Na_2CO_3, and 4-carboxyphenylboronic acid **3.129a** (820 mg, 4.94 mmol) were sequentially added. The vial was sealed and heated under stirring at 105 °C in a microwave reactor for 20 min. The reaction mixture was then diluted with water and extracted with EtOAc. The combined organic phase were dried and concentrated. The crude product was purified by flash chromatography (*n*-hexane/EtOAc 1:1) to yield **3.130a** (388 mg, 1.51 mmol, 49% yield). ^{1}H NMR (DMSO-d_6) δ (ppm): 2.56 (s, 3H), 7.63 (d, 1H, J = 8.1 Hz), 7.89

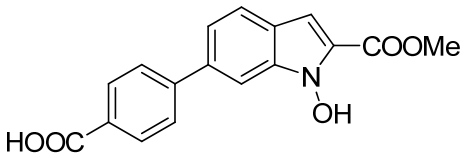
(AA'XX', 2H, J_{AX} = 8.6 Hz, $J_{AA'XX'}$ = 2.0 Hz), 8.03 (dd, 1H, J = 9.9, 2.0 Hz), 8.05 (AA'XX', 2H, J_{AX} = 8.6 Hz, $J_{AA'XX'}$ = 2.0 Hz), 8.31 (d, 1H, J = 2.0 Hz).

4'-(3-Methoxy-2,3-dioxopropyl)-3'-nitrobiphenyl-4-carboxylic acid (3.131a)



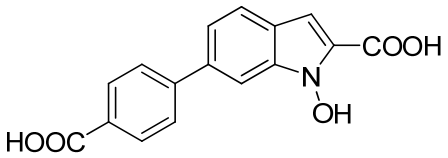
A stirred suspension of sodium hydride (60% dispersion in mineral oil, 350 mg, 8.76 mmol) in 5.7 mL of anhydrous DMF cooled to –15 °C under nitrogen was treated dropwise with a solution containing the nitrotoluene precursor **3.130a** (376 mg, 1.46 mmol) and dimethyl oxalate (1.04 g, 8.76 mmol) in 2.6 mL of anhydrous DMF. The mixture was left under stirring at the same temperature for 10 minutes and then it was slowly warmed to room temperature. The mixture was then left under stirring at RT for 18 h. Once the disappearance of the precursor was verified by TLC, the reaction mixture was slowly poured in an ice-water mixture; the water phase was acidified with 1N aqueous HCl and extracted several times with EtOAc. The combined organic phase was washed with saturated $NaHCO_3$ solution and brine, then dried over anhydrous sodium sulphate. Evaporation under vacuum of the organic solvent afforded a crude product which was purified by column chromatography over silica gel using *n*-hexane/EtOAc 2:8 with 0.01% trifluoroacetic acid as the eluent, to yield the nitroaryl-ketoester derivative **3.131a** (159 mg, 0.463 mmol, 32% yield). ^{1}H NMR (DMSO-d_6) δ (ppm): 3.86 (s, 3H), 4.66 (s, 2H), 7.50-7.66 (m, 2H), 7.93 (d, 1H, J = 8.1 Hz), 8.03-8.14 (m, 3H), 8.41 (d, 1H, J = 1.8 Hz); signals imputable to the enol form (~ 70%) δ (ppm): 3.84 (s, 3H), 6.62 (s, 1H), 8.30 (d, 1H, J =1.8 Hz), 8.36 (d, 1H, J = 8.4 Hz).

4-(1-Hydroxy-2-(methoxycarbonyl)-1*H*-indol-6-yl)benzoic acid (3.132a)



Ketoester **3.131a** (150 mg, 0.437 mmol) was dissolved in anhydrous DME (0.4 mL) and the resulting solution was added dropwise to a cooled (0 °C) solution of $SnCl_2 \cdot 2H_2O$ (221 mg, 0.979 mmol) in DME (0.4mL) containing 4Å molecular sieves, previously activated for 18 hours at 130 °C in oven and cooled to RT in a desiccator over anhydrous calcium chloride. The reaction mixture was stirred under nitrogen at RT for 8 h, then it was diluted with water and repeatedly extracted with EtOAc. The combined organic phase was dried over anhydrous sodium sulphate and evaporated to afford a crude residue that was purified by column chromatography over silica gel using *n*-hexane/EtOAc 4:6 with 0.01% trifluoroacetic acid as the eluent, to give a mixture (10.6 mg) of the *N*-hydroxyindol-ester derivative **3.132a** (80%, 8.5 mg, 0.027 mmol, 6% yield) with its indole analogue (20%) ^{1}H NMR (acetone-d_6) δ (ppm): 3.91 (s, 3H), 7.14 (d, 1H, *J* = 0.9 Hz), 7.53 (dd, 1H, *J* = 8.4, 1.6 Hz), 7.79 (dd, 1H, *J* = 8.4, 0.6 Hz), 7.86 (t, 1H, *J* = 0.9 Hz), 7.88-7.94 (m, 2H), 8.13-8.20 (m, 2H), 10.48 (bs, 1H); signals imputable to the indole (~ 20%) δ (ppm): 3.92 (s, 3H), 7.22-7.24 (m, 1H).

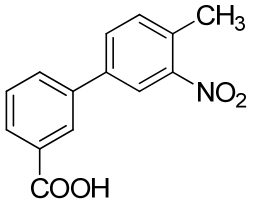
6-(4-Carboxyphenyl)-1-hydroxy-1*H*-indole-2-carboxylic acid (3.125a)



Methyl ester **3.132a** (10.6 mg, 0.0341 mmol) was dissolved in a 1:1 mixture of THF/methanol (0.3 mL) and treated with 0.1 mL of 2N aqueous solution of LiOH. The reaction was kept under stirring in the dark at RT. After 12 h, it was monitored by TLC and another portion of LiOH (0.2 mL) was added. After further 72 h, it was concentrated, diluted with water and washed with Et_2O. Then, the aqueous phase was treated with 1N aqueous HCl and extracted several times with EtOAc. The combined ethyl acetate organic phase was dried over anhydrous sodium sulphate and evaporated to afford the desired *N*-hydroxyindol-carboxylic acid product **3.125a** (10.0 mg, 0.0336 mmol, 99% yield) without any further purification. ^{1}H NMR (acetone-d_6) δ (ppm): 7.04 (s, 1H), 7.49 (dd, 1H, *J* = 8.4, 1.4 Hz), 7.76 (d, 1H, *J* = 8.6 Hz), 7.83 (d, 1H, *J* = 1.2

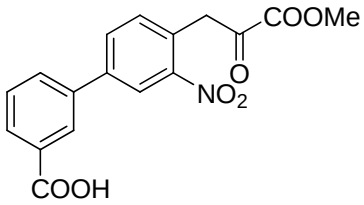
Hz), 7.88-7.92 (m, 2H), 8.13-8.17 (m, 2H); signals imputable to the indole (~ 30%) δ (ppm): 7.24-7.27 (m, 1H)

4'-Methyl-3'-nitrobiphenyl-3-carboxylic acid (3.130b)



A solution of $Pd(OAc)_2$ (16.2 mg, 0.0723 mmol) and triphenylphosphine (94.8 mg, 0.362 mmol) in absolute ethanol (5.4 mL) and anhydrous toluene (5.4 mL) was stirred at RT under nitrogen for 10 min in a vial. After that period, commercially available 4-bromo-2-nitrotoluene **3.90d** (520 mg, 2.41 mmol), 5.4 mL of 2M aqueous Na_2CO_3, and 3-carboxyphenylboronic acid **3.129b** (639 mg, 3.85 mmol) were sequentially added. The vial was sealed and heated under stirring at 105 °C in a microwave reactor for 20 min. The reaction mixture was then diluted with water and extracted with EtOAc. The combined organic phase were dried and concentrated. The crude product was purified by flash chromatography (*n*-hexane/EtOAc 1:1) to yield **3.130b** (469 mg, 1.82 mmol, 76% yield). ^{1}H NMR (acetone-d_6) δ (ppm): 2.62 (s, 3H), 7.47-7.76 (m, 3H), 7.96-8.13 (m, 2H), 8.29 (d, 1H, J = 1.8 Hz), 8.35 (t, 1H, J = 1.6 Hz).

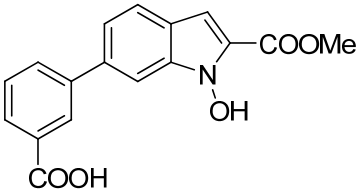
4'-(3-Methoxy-2,3-dioxopropyl)-3'-nitrobiphenyl-3-carboxylic acid (3.131b)



A stirred suspension of sodium hydride (60% dispersion in mineral oil, 131 mg, 3.27 mmol) in 2.1 mL of anhydrous DMF cooled to –15 °C under nitrogen was treated dropwise with a solution containing the nitrotoluene precursor **3.130b** (140 mg, 0.544 mmol) and dimethyl oxalate (386 mg, 3.27 mmol) in 1.0 mL of anhydrous DMF. The

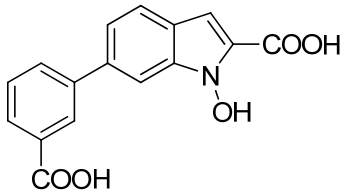
mixture was left under stirring at the same temperature for 10 minutes and then it was slowly warmed to room temperature. The mixture was then left under stirring at RT for 4 h. Once the disappearance of the precursor was verified by TLC, the reaction mixture was slowly poured in an ice-water mixture; the water phase was acidified with 1N aqueous HCl and extracted several times with EtOAc. The combined organic phase was washed with saturated $NaHCO_3$ solution and brine, then dried over anhydrous sodium sulphate. Evaporation under vacuum of the organic solvent afforded a crude product which was purified by column chromatography over silica gel using EtOAc as the eluent, to yield the nitroaryl-ketoester derivative **3.131b** (110 mg, 0.320 mmol, 59% yield). ^{1}H NMR (acetone-d_6) δ (ppm): 3.91 (s, 3H), 4.74 (s, 2H), 7.51-7.76 (m, 3H), 7.70-8.19 (m, 2H), 8.39-8.49 (m, 2H), 10.40 (bs, 1H); signals imputable to the enol form (~ 20%) δ (ppm): 6.81 (s, 1H).

3-(1-Hydroxy-2-(methoxycarbonyl)-1*H*-indol-6-yl)benzoic acid (3.132b)



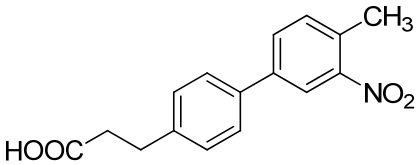
Ketoester **3.131b** (100 mg, 0.291 .mmol) was dissolved in anhydrous DME (0.2 mL) and the resulting solution was added dropwise to a cooled (0 °C) solution of $SnCl_2 \cdot 2H_2O$ (147 mg, 0.653 mmol) in DME (0.2 mL) containing 4Å molecular sieves, previously activated for 18 hours at 130 °C in oven and cooled to RT in a desiccator over anhydrous calcium chloride. The reaction mixture was stirred under nitrogen at RT for 6 h, then it was diluted with water and repeatedly extracted with EtOAc. The combined organic phase was dried over anhydrous sodium sulphate and evaporated to afford a crude residue that was purified by column chromatography over silica gel using $CHCl_3$/MeOH 95:5 as the eluent, to give the *N*-hydroxyindol-ester derivative **3.132b** (30 mg, 0.0964 , 33% yield). ^{1}H NMR (acetone-d_6) δ (ppm): 3.91 (s, 3H), 7.14 (d, 1H, J = 0.8 Hz), 7.52 (dd, 1H, J = 8.3, 1.6 Hz), 7.65 (t, 1H, J = 7.7 Hz), 7.78-7.82 (m, 2H), 8.01-8.08 (m, 2H), 8.39 (t, 1H, J = 1.7 Hz).

6-(3-Carboxyphenyl)-1-hydroxy-1*H*-indole-2-carboxylic acid (3.125b)



Methyl ester **3.132b** (30.0 mg, 0.0964 mmol) was dissolved in a 1:1 mixture of THF/methanol (1 mL) and treated with 0.3 mL of 2N aqueous solution of LiOH. The reaction was kept under stirring in the dark at RT and it was monitored by TLC. After 48 h, the reaction mixture was concentrated, diluted with water and washed with Et_2O. Then, the aqueous phase was treated with 1N aqueous HCl and extracted several times with EtOAc. The combined ethyl acetate organic phase was dried over anhydrous sodium sulphate and evaporated to afford the desired *N*-hydroxyindol-carboxylic acid product **3.125b** (26.8 mg, 0.0902 mmol, 94% yield) without any further purification. ^{1}H NMR (DMSO-d_6) δ (ppm): 7.06 (d, 1H, J = 0.7 Hz), 7.46 (dd, 1H, J = 8.4, 1.0 Hz), 7.62 (t, 1H, J = 7.7 Hz), 7.76 (d, 1H, J = 8.1 Hz), 7.92-8.02 (m, 3H), 8.24 (t, 1H, J = 3.0 Hz).

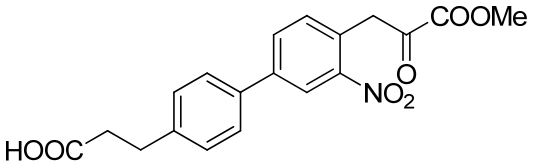
3-(4'-Methyl-3'-nitrobiphenyl-4-yl)propanoic acid (3.130c)



A solution of $Pd(OAc)_2$ (22 mg, 0.096 mmol) and triphenylphosphine (126 mg, 0.480 mmol) in absolute ethanol (7.2 mL) and anhydrous toluene (7.2 mL) was stirred at RT under nitrogen for 10 min. After that period, commercially available 4-bromo-2-nitrotoluene **3.90d** (696 mg, 3.22 mmol), 7.2 mL of 2M aqueous Na_2CO_3, and 4-(2-carboxyethyl)benzeneboronic acid **3.129c** (1.00 g, 5.15 mmol) were sequentially added. The resulting mixture was heated at 100 °C in a sealed vial under nitrogen overnight. After being cooled to RT, the mixture was diluted with water and extracted with EtOAc. The combined organic phase were dried and concentrated. The crude product was purified by flash chromatography ($CHCl_3$/MeOH 95:5) to yield **3.130c** (871 mg, 3.05 mmol, 95% yield). ^{1}H NMR (acetone-d_6) δ (ppm): 2.59 (s, 3H), 2.67 (t, 2H, J = 8.0 Hz),

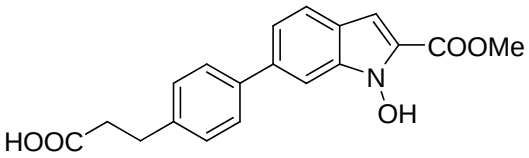
2.99 (t, 2H, J = 7.5 Hz), 7.40 (AA'XX', 2H, J_{AX} = 8.3 Hz, $J_{AA'/XX'}$ = 2.0 Hz), 7.56 (d, 1H, J = 7.9 HZ), 7.67 (AA'XX', 2H, J_{AX} = 8.4 Hz, $J_{AA'/XX'}$ = 2.0 Hz), 7.90 (dd, 1H, J = 7.9, 2.0 Hz), 8.20 (d, 1H, J = 2.0 Hz).

3-(4'-(3-Methoxy-2,3-dioxopropyl)-3'-nitrobiphenyl-4-yl)propanoic acid (3.131c)



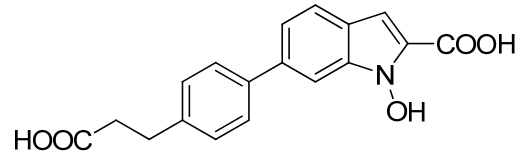
A stirred suspension of sodium hydride (60% dispersion in mineral oil, 506 mg, 12.6 mmol) in 8.7 mL of anhydrous DMF cooled to –15 °C under nitrogen was treated dropwise with a solution containing the nitrotoluene precursor **3.130c** (450 mg, 1.58 mmol) and dimethyl oxalate (1.87 g, 15.8 mmol) in 4.5 mL of anhydrous DMF. The mixture was left under stirring at the same temperature for 10 minutes and then it was slowly warmed to room temperature. The mixture was then left under stirring at RT for 18 h. Once the disappearance of the precursor was verified by TLC, the reaction mixture was slowly poured in an ice-water mixture; the water phase was acidified with 1N aqueous HCl and extracted several times with EtOAc. The combined organic phase was washed with saturated $NaHCO_3$ solution and brine, then dried over anhydrous sodium sulphate. Evaporation under vacuum of the organic solvent afforded a crude product which was purified by column chromatography over silica gel using *n*-hexane/EtOAc 4:6 as the eluent, to yield the nitroaryl-ketoester derivative **3.131c** (227 mg, 0.611 mmol, 39% yield). ^{1}H NMR (acetone-d_6) δ (ppm): 2.69 (t, 2H, J = 7.9 Hz), 2.96 (t, 2H, J = 7.7 Hz), 3.90 (s, 3H), 4.70 (s, 2H), 7.44 (AA'XX', 2H, J_{AX} = 8.1 Hz, $J_{AA'/XX'}$ = 2.2 Hz), 7.62 (d, 1H, J = 8.1 Hz), 7.73 (AA'XX', 2H, J_{AX} = 8.2 Hz, $J_{AA'/XX'}$ = 2.2 Hz), 8.02 (dd, 1H, J = 8.2, 1.8 Hz), 8.38 (d, 1H, J = 2.0 Hz); signals imputable to the enol form (~ 28%) δ (ppm): 6.80 (s, 1H), 8.01 (dd, 1H, J = 8.2, 2.0 Hz), 8.18 (d, 1H, J = 2.0 Hz), 8.45 (d, 1H, J = 8.2 Hz).

3-(4-(1-Hydroxy-2-(methoxycarbonyl)-1*H*-indol-6-yl)phenyl)propanoic acid (3.132c)



Ketoester **3.131c** (150 mg, 0.404 mmol) was dissolved in THF (0.7 mL) and the resulting solution was added to an aqueous solution (0.7 mL) of sodium hypophosphite monohydrate (133 mg, 1.25 mmol). Then 4.1 mg of 10% palladium over charcoal was added and the resulting suspension was stirred at RT for 21h. Then it was monitored by TLC and $H_2PO_2Na{\cdot}H_2O$ (133 mg, 1.25 mmol) and Pd/C (4.1 mg) were added. After further 26 h the disappearance of the precursor is verified by TLC and the mixture was concentrated, diluted with water and repeatedly extracted with EtOAc. The combined organic phase was dried over anhydrous sodium sulphate and evaporated to afford a crude residue that was purified by column chromatography over over silica gel using *n*-$CHCl_3$/MeOH 95:5 as the eluent, to give the *N*-hydroxyindole **3.132c** (21.2 mg, 0.0625 mmol, 15% yield). ^{1}H NMR (acetone-d_6) δ (ppm): 2.68 (t, 2H, J = 8.1 Hz), 2.98 (t, 2H, J = 7.5 Hz), 3.90 (s, 3H), 7.11 (d, 1H, J = 0.7 Hz), 7.39 (AA'XX', 2H, J_{AX} = 8.2 Hz, $J_{AA'/XX'}$ = 2.0 Hz), 7.67 (AA'XX', 2H, J_{AX} = 8.2 Hz, $J_{AA'/XX'}$ = 2.1 Hz), 7.71-7.75 (m, 2H), 10.50 (bs, 1H).

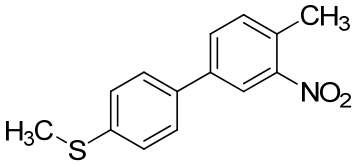
6-(4-(2-Carboxyethyl)phenyl)-1-hydroxy-1*H*-indole-2-carboxylic acid (3.125c)



Methyl ester **3.132c** (20.0 mg, 0.0589 mmol) was dissolved in a 1:1 mixture of THF/methanol (0.6 mL) and treated with 0.2 mL of 2N aqueous solution of LiOH. The reaction was kept under stirring in the dark at RT and it was monitored by TLC. After 48 h, the reaction mixture was concentrated, diluted with water and washed with Et_2O. Then, the aqueous phase was treated with 1N aqueous HCl and extracted several times with EtOAc. The combined ethyl acetate organic phase was dried over anhydrous

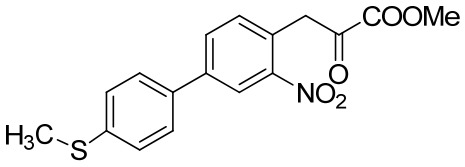
sodium sulphate and evaporated to afford the desired *N*-hydroxyindol-carboxylic acid product **3.125c** (19.0 mg, 0.0584 mmol, 99% yield) without any further purification. ^{1}H NMR (acetone-d_6) δ (ppm): 2.68 (t, 2H, J = 7.2 Hz), 2.99 (t, 2H, J = 7.5 Hz), 7.13 (d, 1H, J = 0.9 Hz), 7.39 (AA'/XX', 2H, J_{AX} = 8.1 Hz, $J_{AA'/XX'}$ = 2.0 Hz), 7.45 (dd, 1H, J = 8.8, 1.5 Hz), 7.68 (AA'/XX', 2H, J_{AX} = 8.2 Hz, $J_{AA'/XX'}$ = 1.9 Hz), 7.71-7.77 (m, 2H). ^{13}C NMR (acetone-d_6) δ (ppm): 32.44, 35.81, 106.13, 107.99, 121.34, 123.59, 127.64, 127.96 (2C), 129.75 (2C), 131.61, 131.90, 139.16, 140.04, 141.17, 160.83, 173.76. MS *m/z* 325 (M^+, 14%); 255 (M^+ –$C_3H_2O_2$, 32%); 175 (M^+ –$C_9H_{10}O_2$, 16%); 149 (M^+ – $C_9H_6O_3N$, 100%).

Methyl(4'-methyl-3'-nitrobiphenyl-4-yl)sulfane (3.133)



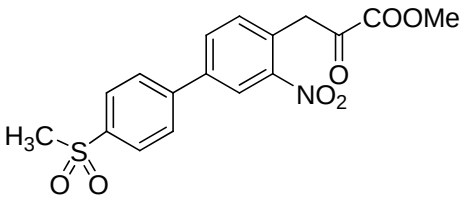
A solution of $Pd(OAc)_2$ (21.8 mg, 0.0972 mmol) and triphenylphosphine (128 mg, 0.486 mmol) in absolute ethanol (7.3 mL) and anhydrous toluene (7.3 mL) was stirred at RT under nitrogen for 10 min. After that period, commercially available 4-bromo-2-nitrotoluene **3.90d** (700 mg, 3.24 mmol), 7.3 mL of 2M aqueous Na_2CO_3, and 4-(methylthio)benzenboronic acid (871 mg, 5.18 mmol) were sequentially added. The resulting mixture was heated at 100 °C in a sealed vial under nitrogen overnight. After being cooled to RT, the mixture was diluted with water and extracted with EtOAc. The combined organic phase were dried and concentrated. The crude product was purified by flash chromatography (*n*-hexane/EtOAc 6:4) to yield **3.133** (770 mg, 2.97 mmol, 92% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 2.53 (s, 3H), 2.63 (s, 3H), 7.35 (AA'XX', 2H, J_{AX} = 8.4 Hz; $J_{AA'XX'}$ = 2.0 Hz), 7.39 (d, 1H, J = 8.1 Hz), 7.52 (AA'XX', 2H, J_{AX} = 6.8 Hz; $J_{AA'XX'}$ = 2.0 Hz), 7.70 (dd, 1H, J = 8.0, 1.9 Hz), 8.18 (d, 1H, J = 2.0 Hz).

Methyl 3-(4'-(methylthio)-3-nitrobiphenyl-4-yl)-2-oxopropanoate (3.134)



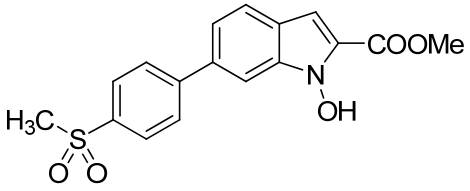
A stirred suspension of sodium hydride (60% dispersion in mineral oil, 432 mg, 10.8 mmol) in 7 mL of anhydrous DMF cooled to –15 °C under nitrogen was treated dropwise with a solution containing the nitrotoluene precursor **3.133** (700 mg, 2.70 mmol) and dimethyl oxalate (1.59 g, 13.5 mmol) in 5 mL of anhydrous DMF. The mixture was left under stirring at the same temperature for 10 minutes and then it was slowly warmed to room temperature. The mixture was then left under stirring at RT for 12 h. Once the disappearance of the precursor was verified by TLC, the reaction mixture was slowly poured in an ice-water mixture; the water phase was acidified with 1N aqueous HCl and extracted several times with EtOAc. The combined organic phase was washed with saturated $NaHCO_3$ solution and brine, then dried over anhydrous sodium sulphate. Evaporation under vacuum of the organic solvent afforded a crude product which was purified by column chromatography over silica gel using *n*-hexane/EtOAc 6:4 as the eluent, to yield the nitroaryl-ketoester derivative **3.134** (806 mg, 2.33 mmol, 86% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 2.53 (s, 3H), 3.95 (s, 3H), 4.56 (s, 2H), 7.31-7.40 (m, 3H), 7.51-7.59 (m, 2H), 7.82 (d, 1H, J = 7.9 Hz), 8.37 (d, 1H, J = 2.0 Hz); signals imputable to the enol form (~ 76%) δ (ppm): 3.96 (s, 3H), 6.68 (d, 1H, exchangeable, J = 1.5 Hz), 6.96 (d, 1H, J = 1.5 Hz), 7.35 (AA'XX', J_{AX} = 8.6 Hz, $J_{AA'XX'}$ = 1.7 Hz), 7.55 (AA'XX', J_{AX} = 8.8 Hz, $J_{AA'XX'}$ = 2.0 Hz), 7.80 (dd, 1H, J = 7.9, 1.9 Hz); 8.11 (d, 1H, J = 2.0 Hz), 8.33 (d, 1H, J = 8.2 Hz).

Methyl 3-(4'-(methylsulfonyl)-3-nitrobiphenyl-4-yl)-2-oxopropanoate (3.131d)



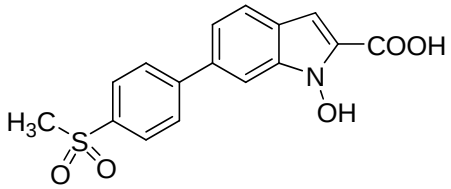
An aqueous solution (5.3 mL) of Oxone® (50% w/v, 2.67 g, 4.34 mmol) was added dropwise to a stirred solution of the methylthio-derivative **3.134** (500 mg, 1.45 mmol) in 1,4-dioxane (14.5 mL) at 0 °C, and the reaction was allowed to proceed with stirring at RT for 12 h. The reaction mixture was diluted with water, extracted with EtOAc, the organic phase was washed successively with water and brine, and dried over anhydrous sodium sulphate. After filtration, the solvent from the organic fraction was evaporated to give the desired crude product **3.131d** (556 mg, 1.47 mmol, >99% yield) which was used in the next reaction without any further purification. ^{1}H NMR ($CDCl_3$) δ (ppm): 3.12 (s, 3H), 3.96 (s, 3H), 4.62 (s, 2H), 7.47 (d, 1H, J = 7.9 Hz), 7.78-7.90 (m, 3H), 8.07 (AA'XX', 2H, J_{AX} = 8.6 Hz, $J_{AA'XX'}$ = 2.0 Hz), 8.42 (d, 1H J = 1.8 Hz); signals imputable to the enol form (~ 44%) δ (ppm): 3.97 (s, 3H), 6.80 (bs, 1H), 6.98 (s, 1H, exchangeable), 8.40 (d, 1H, J = 7.4 Hz).

Methyl 1-hydroxy-6-(4-(methylsulfonyl)phenyl)-1*H*-indole-2-carboxylate (3.132d)



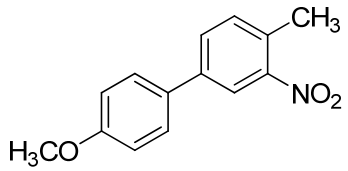
Ketoester **3.131d** (500 mg, 1.32 mmol) was dissolved in anhydrous DME (1.2 mL) and the resulting solution was added dropwise to a cooled (0 °C) solution of $SnCl_2 \cdot 2H_2O$ (670 mg, 2.97 mmol) in DME (1.2 mL) containing 4Å molecular sieves, previously activated for 18 hours at 130 °C in oven and cooled to RT in a desiccator over anhydrous calcium chloride. The reaction mixture was stirred under nitrogen at RT for 5 h, then it was diluted with water and repeatedly extracted with EtOAc. The combined organic phase was dried over anhydrous sodium sulphate and evaporated to afford a crude residue that was purified by column chromatography over silica gel using *n*-hexane/EtOAc 1:1 as the eluent, to give the *N*-hydroxyindol-ester derivative **3.132d** (25.8 mg, 0.0747 mmol, 6% yield). ^{1}H NMR (DMSO-d_6) δ (ppm): 3.27 (s, 3H), 3.88 (s, 3H), 7.14 (s, 1H), 7.53 (dd, 1H, J = 8.6, 1.5 Hz), 7.78-7.82 (m, 2H), 8.02 (s, 4H), 11.59 (bs, 1H).

1-Hydroxy-6-(4-(methylsulfonyl)phenyl)-1*H*-indole-2-carboxylic acid (3.125d)



Methyl ester **3.132d** (23.0 mg, 0.0666 mmol) was dissolved in a 1:1 mixture of THF/methanol (0.7 mL) and treated with 0.2 mL of 2N aqueous solution of LiOH. The reaction was kept under stirring in the dark at RT and it was monitored by TLC. After 12 h, the reaction mixture was concentrated, diluted with water and washed with Et_2O. Then, the aqueous phase was treated with 1N aqueous HCl and extracted several times with EtOAc. The combined ethyl acetate organic phase was dried over anhydrous sodium sulphate and evaporated to afford the desired *N*-hydroxyindol-carboxylic acid product **3.125d** (21.6 mg, 0.0652 mmol, 98% yield) without any further purification. ^{1}H NMR (DMSO-d_6) δ (ppm): 3.26 (s, 3H), 7.06 (s, 1H), 7.50 (dd, 1H, J = 8.2, 1.4 Hz), 7.76-7.80 (m, 2H), 8.01 (s, 4H). ^{13}C NMR (DMSO-d_6) δ (ppm): 43.65, 104.40, 107.95, 119.89, 121.15, 122.95, 127.65 (2C), 127.78 (2C), 131.42, 134.89, 136.17, 139.21, 145.49, 161.06.

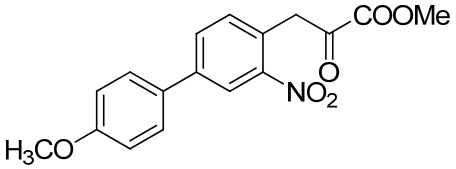
4'-Methoxy-4-methyl-3-nitrobiphenyl (3.130e)



A solution of $Pd(OAc)_2$ (15.6 mg, 0.0693 mmol) and triphenylphosphine (90.9 mg, 0.347 mmol) in absolute ethanol (5.2 mL) and anhydrous toluene (5.2 mL) was stirred at RT under nitrogen for 10 min in a vial. After that period, commercially available 4-bromo-2-nitrotoluene **3.90d** (500 mg, 2.31 mmol), 5.2 mL of 2M aqueous Na_2CO_3, and 4-methoxyphenylboronic acid **3.129e** (563 mg, 3.70 mmol) were sequentially added. The vial was sealed and heated under stirring at 105 °C in a microwave reactor for 20 min. The reaction mixture was then diluted with water and extracted with EtOAc. The combined organic phase were dried and concentrated. The crude product was purified

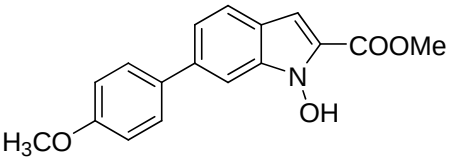
by flash chromatography (*n*-hexane/ EtOAc 9:1) to yield **3.130e** (513 mg, 2.11 mmol, 91% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 2.62 (s, 3H), 3.86 (s, 3H), 7.00 (AA'XX', 2H, J_{AX} = 8.8 Hz, $J_{AA'/XX'}$ = 2.7 Hz), 7.37 (d, 1H, J = 7.9 Hz), 7.54 (AA'XX', 2H, J_{AX} = 8.8 Hz, $J_{AA'/XX'}$ = 2.7 Hz), 7.68 (dd, 1H, J = 7.9, 2.0 Hz), 8.16 (d, 1H, J = 2.0 Hz).

Methyl 3-(4'-methoxy-3-nitrobiphenyl-4-yl)-2-oxopropanoate (3.131e)



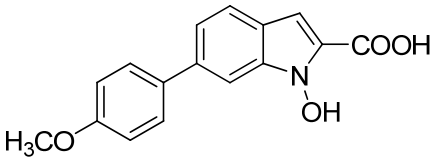
A stirred suspension of sodium hydride (60% dispersion in mineral oil, 263 mg, 6.58 mmol) in 3.9 mL of anhydrous DMF cooled to –15 °C under nitrogen was treated dropwise with a solution containing the nitrotoluene precursor **3.130e** (400 mg, 1.64 mmol) and dimethyl oxalate (968 mg, 8.20 mmol) in 2.9 mL of anhydrous DMF. The mixture was left under stirring at the same temperature for 10 minutes and then it was slowly warmed to room temperature. The mixture was then left under stirring at RT for 6 h. Once the disappearance of the precursor was verified by TLC, the reaction mixture was slowly poured in an ice-water mixture; the water phase was acidified with 1N aqueous HCl and extracted several times with EtOAc. The combined organic phase was washed with saturated $NaHCO_3$ solution and brine, then dried over anhydrous sodium sulphate. Evaporation under vacuum of the organic solvent afforded a crude product which was purified by column chromatography over silica gel using *n*-hexane/EtOAc 7:3 as the eluent, to yield the nitroaryl-ketoester derivative **3.131e** (432 mg, 1.31 mmol, 80% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 3.87 (s, 3H), 3.95 (s, 3H), 4.55 (s, 2H), 7.00 (AA'XX', 2H, J_{AX} = 9.0 Hz, $J_{AA'/XX'}$ = 2.6 Hz), 7.36 (d, 1H, J = 7.9 Hz), 7.56 (AA'XX', 2H, J_{AX} = 8.8 Hz, $J_{AA'/XX'}$ = 2.6 Hz), 7.79 (dd, 1H, J = 7.9, 2.0 Hz), 8.35 (d, 1H, J = 2.0 Hz); signals imputable to the enol form (~ 19%) δ (ppm): 6.67 (bs, 1H), 8.08 (d, 1H, J = 1.8 Hz), 8.31 (d, 1H, J = 8.4 Hz).

Methyl 1-hydroxy-6-(4-methoxyphenyl)-1*H*-indole-2-carboxylate (3.132e)



Ketoester **3.131e** (280 mg, 0.850 mmol) was dissolved in anhydrous DME (0.9 mL) and the resulting solution was added dropwise to a cooled (0 °C) solution of $SnCl_2 \cdot 2H_2O$ (430 mg, 1.90 mmol) in DME (0.9 mL) containing 4Å molecular sieves, previously activated for 18 hours at 130 °C in oven and cooled to RT in a desiccator over anhydrous calcium chloride. The reaction mixture was stirred under nitrogen at RT for 5 h, then it was monitored by TLC and another portion of $SnCl_2 \cdot 2H_2O$ (430 mg, 1.90 mmol) was added. After further 2.5 h, another portion of $SnCl_2 \cdot 2H_2O$ (430 mg, 1.90 mmol mmol) was added. 4.5 h Later, the mixture was diluted with water and repeatedly extracted with EtOAc. The combined organic phase was dried over anhydrous sodium sulphate and evaporated to afford a crude residue that was purified by column chromatography over silica gel using *n*-hexane/EtOAc 7:3 as the eluent, to give the *N*-hydroxyindol-ester derivative **3.132e** (48.6 mg, 0.163 mmol, 19% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 3.87 (s, 3H), 4.00 (s, 3H), 7.00 (AA'XX', 2H, J_{AX} = 8.4 Hz, $J_{AA'/XX'}$ = 2.6 Hz), 7.04 (d, 1H, J = 0.7 Hz), 7.37 (dd, 1H, J = 8.4, 1.6 Hz), 7.59-7.69 (m, 4H), 10. 26 (bs, 1H).

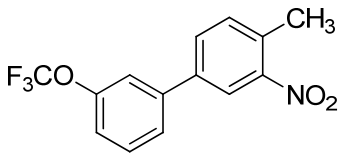
1-Hydroxy-6-(4-methoxyphenyl)-1*H*-indole-2-carboxylic acid (3.125e)



Methyl ester **3.132e** (45.0 mg, 0.151 mmol) was dissolved in a 1:1 mixture of THF/methanol (1.5 mL) and treated with 0.5 mL of 2N aqueous solution of LiOH. The reaction was kept under stirring in the dark at RT and it was monitored by TLC. After 3 h, the reaction mixture was concentrated, diluted with water and washed with Et_2O. Then, the aqueous phase was treated with 1N aqueous HCl and extracted several times

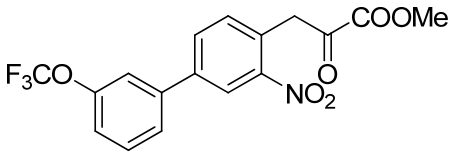
with EtOAc. The combined ethyl acetate organic phase was dried over anhydrous sodium sulphate and evaporated to afford the desired *N*-hydroxyindol-carboxylic acid product **3.125e** (42.3 mg, 0.149 mmol, 99% yield) without any further purification. ^{1}H NMR (DMSO-d_6) δ (ppm): 3.81 (s, 3H), 7.02-7.06 (m, 3H), 7.38 (dd, 1H, J = 8.3, 1.6 Hz), 7.57 (d, 1H, J = 1.4 Hz), 7.64-7.70 (m, 3H). ^{13}C NMR (DMSO-d_6) δ (ppm): 55.18, 104.61, 106.34, 114.36, 119.71, 119.91, 122.53, 127.03, 127.88, 132.93, 136.73, 141.67, 158.71, 161.03. MS *m/z* 283 (M^+, 21%), 267 (M^+ –O, 100%).

4-Methyl-3-nitro-3'-(trifluoromethoxy)biphenyl (3.130f)



Commercially available 1-bromo-3-trifluoromethoxybenzene **3.128f** (0.260 mL, 424 mg, 1.76 mmol) was placed in a vial together with 4-methyl-3-nitrobenzeneboronic acid **3.127** (350 mg, 1.94 mmol), sodium carbonate (560 mg, 5.28 mmol), $Pd(OAc)_2$ (1.6 mg, 0.0070 mmol), tetrabutylammonium bromide (567 mg, 1.76 mmol) and water (5.5 mL). The vial was sealed and heated under stirring at 150 °C in a microwave reactor for 5 min. The reaction mixture was then diluted with water and repeatedly extracted with EtOAc. The combined organic phase was dried over anhydrous sodium sulphate and evaporated to afford a crude residue that was purified by column chromatography over silica gel using *n*-hexane/EtOAc 95:5 as the eluent, to give pure **3.130f** (482 mg, 1.62 mmol, 92% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 2.65 (s, 3H), 7.27-7.28 (m, 1H), 7.42-7.54 (m, 4H), 7.71 (dd, 1H, J = 8.2, 2.1 Hz), 8.19 (d, 1H, J = 2.0 Hz)

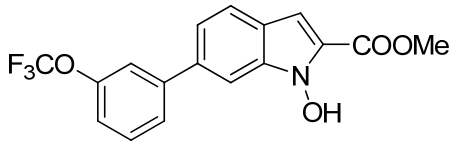
Methyl 3-(3-nitro-3'-(trifluoromethoxy)biphenyl-4-yl)-2-oxopropanoate (3.131f)



A stirred suspension of sodium hydride (60% dispersion in mineral oil, 259 mg, 6.32 mmol) in 4.4 mL of anhydrous DMF cooled to –15 °C under nitrogen was treated

dropwise with a solution containing the nitrotoluene precursor **3.130f** (470 mg, 1.58 mmol) and dimethyl oxalate (933 mg, 7.90 mmol) in 3.0 mL of anhydrous DMF. The mixture was left under stirring at the same temperature for 10 minutes and then it was slowly warmed to room temperature. The mixture was then left under stirring at RT for 72 h, then it was heated at 40 °C for 12 h. Once the disappearance of the precursor was verified by TLC, the reaction mixture was slowly poured in an ice-water mixture; the water phase was acidified with 1N aqueous HCl and extracted several times with EtOAc. The combined organic phase was washed with saturated $NaHCO_3$ solution and brine, then dried over anhydrous sodium sulphate. Evaporation under vacuum of the organic solvent afforded a crude product which was purified by column chromatography over silica gel using *n*-hexane/EtOAc 7:3 as the eluent, to yield the nitroaryl-ketoester derivative **3.131f** (548 mg, 1.43 mmol, 90% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 3.96 (s, 3H), 4.59 (s, 2H), 7.28-7.33 (m, 1H), 7.40-7.59 (m, 4H), 7.83 (dd, 1H, *J* = 7.9, 2.0 Hz), 8.38 (d, 1H, *J* = 1.8 Hz); signals imputable to the enol form (~ 20%) δ (ppm): 3.97 (s, 3H), 6.73 (d, 1H, *J* = 1.5 Hz), 6.97 (d, 1H, *J* = 1.5 Hz), 8.11 (d, 1H, *J* = 1.8 Hz), 8.36 (d, 1H, *J* = 7.1 Hz).

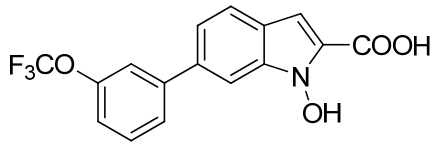
Methyl 1-hydroxy-6-(3-(trifluoromethoxy)phenyl)-1*H*-indole-2-carboxylate (3.132f)



Triethylamine (0.18 mL, 0.13 g, 1.3 mmol) was added dropwise to a stirred solution of $SnCl_2 \cdot 2H_2O$ (88.3 mg, 0.391 mmol) and PhSH (0.12 mL, 0.13 g, 1.2 mmol) in acetonitrile (2.1 mL) at room temperature to generate a yellow precipitate over a period of 5 min. Then, **3.131f** (100 mg, 0.261 mmol) was transferred to the suspension and stirred at room temperature for 5 more min. After complete consumption of the starting material, the mixture was diluted with water and repeatedly extracted with EtOAc. The combined organic phase was dried over anhydrous sodium sulphate, diluted with toluene and a catalytic amount of *p*-toluensulfonic acid was added. Then it was

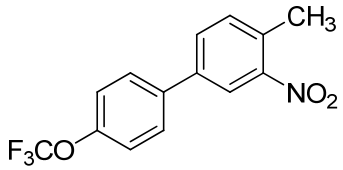
evaporated to afford a crude residue that was purified by column chromatography over silica gel using *n*-hexane/EtOAc 7:3 as the eluent, to give the *N*-hydroxyindole **3.132f** (55.9 mg, 0.159 mmol, 61% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 4.01 (s, 3H), 7.06 (d, 1H, *J* = 0.9 Hz), 7.18-7.25 (m, 1H), 7.36 (dd, 1H, *J* = 8.3, 1.7 Hz), 7.48 (t, 1H, *J* = 8.1 Hz), 7.52-7.54 (m, 1H), 7.62 (dt, 1H, *J* = 7.7, 2.8 Hz), 7.69-7.73 (m, 2H), 10.33 (bs, 1H).

1-Hydroxy-6-(3-(trifluoromethoxy)phenyl)-1*H*-indole-2-carboxylic acid (3.125f)



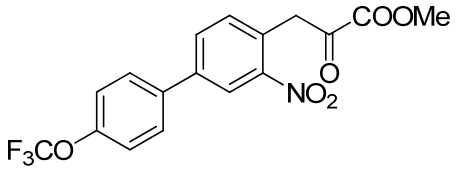
Methyl ester **3.132f** (50.0 mg, 0.142 mmol) was dissolved in a 1:1 mixture of THF/methanol (1.4 mL) and treated with 0.43 mL of 2N aqueous solution of LiOH. The reaction was kept under stirring in the dark at RT and it was monitored by TLC. After 3 h, the reaction mixture was concentrated, diluted with water and washed with Et_2O. Then, the aqueous phase was treated with 1N aqueous HCl and extracted several times with EtOAc. The combined ethyl acetate organic phase was dried over anhydrous sodium sulphate and evaporated to afford the desired *N*-hydroxyindol-carboxylic acid product **3.125f** (39.6 mg, 0.117 mmol, 83% yield) without any further purification. ^{1}H NMR (acetone-d_6) δ (ppm): 7.16 (d, 1H, *J* = 0.7 Hz), 7.31-7.38 (m, 1H), 7.49 (dd, 1H, *J* = 8.4, 1.6 Hz), 7.63 (t, 1H, *J* = 7.9 Hz), 7.68-7.70 (m, 1H), 7.77-7.83 (m, 3H). ^{13}C NMR (acetone-d_6): δ 105.95, 108.72, 120.34, 120.55, 121.23, 121.62 (q, *J* = 253.4 Hz), 122.41, 123.94, 126.94, 127.53, 131.48, 137.31, 137.49, 144.81, 150.62, 162.27. MS *m/z* 337 (M^+, 56%), 321 (M^+ –O, 63%), 293 (M^+ –CO_2, 5%), 275 (M^+ –CO_2 –H_2O, 8%), 249 (M^+ –CO_2 –H_2O –C_2H_2, 13%), 190 (M^+ –$C_6H_4F_3O$ +H, 100%), 177 (M^+ –$C_7H_4F_3O$ +H, 20%). HPLC, t_R = 10.4 min.

4-Methyl-3-nitro-4'-(trifluoromethoxy)biphenyl (3.130g)



Commercially available 1-bromo-4-trifluoromethoxybenzene **3.128g** (0.150 mL, 250 mg, 1.04 mmol) was placed in a vial together with 4-methyl-3-nitrobenzeneboronic acid **3.127** (207 mg, 1.14 mmol), sodium carbonate (331 mg, 3.12 mmol), $Pd(OAc)_2$ (0.9 mg, 0.004 mmol), tetrabutylammonium bromide (335 mg, 1.04 mmol) and water (1.8 mL). The vial was sealed and heated under stirring at 150 °C in a microwave reactor for 5 min. The reaction mixture was then diluted with water and repeatedly extracted with EtOAc. The combined organic phase was dried over anhydrous sodium sulphate and evaporated to afford a crude residue that was purified by column chromatography over silica gel using *n*-hexane/EtOAc 95:5 as the eluent, to give pure **3.130g** (134 mg, 0.451 mmol, 43% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 2.65 (s, 3H), 7.28-7.36 (m, 2H), 7.43 (d, 1H, J = 7.9 Hz), 7.62 (AA'XX', 2H, J_{AX} = 8.4 Hz, $J_{AA'/XX'}$ = 2.2 Hz), 7.69 (dd, 1H, J = 8.0, 1.7 Hz), 8.18 (d, 1H, J = 1.8 Hz).

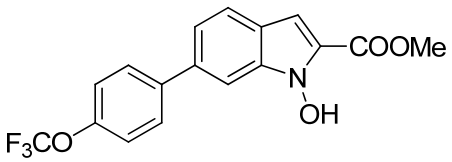
Methyl 3-(3-nitro-4'-(trifluoromethoxy)biphenyl-4-yl)-2-oxopropanoate (3.131g)



A stirred suspension of sodium hydride (60% dispersion in mineral oil, 70.1 mg, 1.75 mmol) in 1.2 mL of anhydrous DMF cooled to –15 °C under nitrogen was treated dropwise with a solution containing the nitrotoluene precursor **3.130g** (130 mg, 0.437 mmol) and dimethyl oxalate (259 mg, 2.19 mmol) in 0.8 mL of anhydrous DMF. The mixture was left under stirring at the same temperature for 10 minutes and then it was slowly warmed to room temperature. The mixture was then left under stirring at RT for 24 h. Once the disappearance of the precursor was verified by TLC, the reaction mixture was slowly poured in an ice-water mixture; the water phase was acidified with 1N

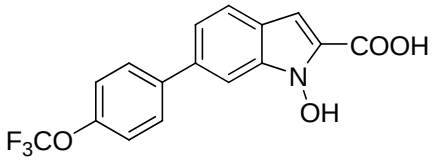
aqueous HCl and extracted several times with EtOAc. The combined organic phase was washed with saturated $NaHCO_3$ solution and brine, then dried over anhydrous sodium sulphate. Evaporation under vacuum of the organic solvent afforded a crude product which was purified by column chromatography over silica gel using *n*-hexane/EtOAc 7:3 as the eluent, to yield the nitroaryl-ketoester derivative **3.131g** (94.6 mg, 0.247 mmol, 56% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 3.96 (s, 3H), 4.59 (s, 2H), 7.30-7.38 (m, 2H), 7.42 (d, 1H, *J* = 8.1 Hz), 7.60-7.68 (m, 2H), 7.81 (dd, 1H, *J* = 7.8, 2.0 Hz), 8.37 (d, 1H, *J* = 2.2 Hz); signals imputable to the enol form (~ 22%) δ (ppm): 6.72 (bs, 1H), 6.97 (bs, 1H), 8.10 (d, 1H, *J* = 2.0 Hz).

Methyl 1-hydroxy-6-(4-(trifluoromethoxy)phenyl)-1*H*-indole-2-carboxylate (3.132g)



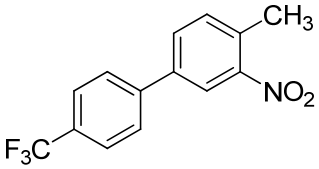
Triethylamine (0.16 mL, 0.12 g, 1.2 mmol) was added dropwise to a stirred solution of $SnCl_2 \cdot 2H_2O$ (79.4 mg, 0.352 mmol) and PhSH (0.11 mL, 0.12 g, 1.1 mmol) in acetonitrile (1.8 mL) at room temperature to generate a yellow precipitate over a period of 5 min. Then, **3.131g** (90.0 mg, 0.235 mmol) was transferred to the suspension and stirred at room temperature for 5 more min. After complete consumption of the starting material, the mixture was diluted with water and repeatedly extracted with EtOAc. The combined organic phase was dried over anhydrous sodium sulphate, diluted with toluene and a catalytic amount of *p*-toluensulfonic acid was added. Then it was evaporated to afford a crude residue that was purified by column chromatography over silica gel using *n*-hexane/EtOAc 8:2 as the eluent, to give a mixture (48.3 mg) of the *N*-hydroxyindol-ester derivative **3.132g** (92%, 44.4 mg, 0.126 mmol, 54% yield) with its indole analogue (8%) ^{1}H NMR ($CDCl_3$) δ (ppm): 4.01 (s, 3H), 7.06 (s, 1H), 7.24-7.38 (m, 3H), 7.65-7.73 (m, 4H), 10.32 (bs, 1H); signals imputable to the indole (~ 8%) δ (ppm): 3.97 (s, 3H), 7.18 (s, 1H), 110.06 (bs, 1H).

1-Hydroxy-6-(4-(trifluoromethoxy)phenyl)-1*H*-indole-2-carboxylic acid (3.125g)



Methyl ester **3.132g** (43.0 mg, 0.122 mmol) was dissolved in a 1:1 mixture of THF/methanol (1.2 mL) and treated with 0.37 mL of 2N aqueous solution of LiOH. The reaction was kept under stirring in the dark at RT and it was monitored by TLC. After 4 h, the reaction mixture was concentrated, diluted with water and washed with Et_2O. Then, the aqueous phase was treated with 1N aqueous HCl and extracted several times with EtOAc. The combined ethyl acetate organic phase was dried over anhydrous sodium sulphate and evaporated to afford the desired *N*-hydroxyindol-carboxylic acid product **3.125g** (22.6 mg, 0.0670 mmol, 55% yield) without any further purification. ^{1}H NMR (acetone-d_6) δ (ppm): 7.16 (d, 1H, J = 0.9 Hz), 7.42-7.50 (m, 3H), 7.75-7.80 (m, 2H), 7.88 (AA´XX´, 2H, J_{AX} = 8.9 Hz, $J_{AA'/XX'}$ = 2.6 Hz); signals imputable to the indole (~ 10%) δ (ppm): 7.19-7.21 (m, 1H). ^{13}C NMR (acetone-d_6) δ (ppm): 106.02, 108.48, 121.23, 121.43 (q, J = 256.5 Hz), 122.06, 122.23 (2C), 123.81, 127.34, 129.66 (2C), 137.36, 137.60, 141.42, 149.21, 161.98. MS *m/z* 337 (M^+, 82%), 321 (M^+ –O, 61%), 293 (M^+ –CO_2, 6%), 275 (M^+ –CO_2 –H_2O, 18%), 249 (M^+ –CO_2 –H_2O –C_2H_2, 21%), 190 (M^+ –$C_6H_4F_3O$ +H, 100%).

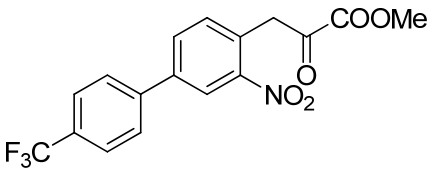
4-Methyl-3-nitro-4'-(trifluoromethyl)biphenyl (3.130h)



Commercially available 4-bromo-2-nitrotoluene **3.90d** (540 mg, 2.50 mmol) was placed in a vial together with 4-trifluoromethylphenylboronic acid **3.129h** (617 mg, 3.25 mmol), potassium phosphate (902 mg, 4.25 mmol), $Pd(OAc)_2$ (7.4 mg, 0.033 mmol), tetrabutylammonium bromide (5.0 g, 15 mmol) and water (0.5 mL). The vial was sealed and heated under stirring at 125 °C for 24 h. The reaction mixture was

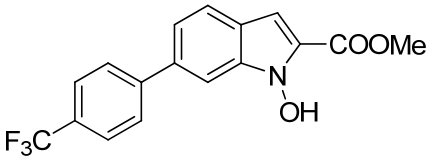
cooled to RT and then diluted with water. The water phase was acidified with 1N aqueous HCl and repeatedly extracted with EtOAc. The combined organic phase was dried over anhydrous sodium sulphate and evaporated to afford a crude residue that was purified by column chromatography over silica gel using *n*-hexane/EtOAc 88:2 as the eluent, to give pure **3.130h** (510 mg, 1.81 mmol, 73% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 2.66 (s, 3H), 7.46 (d, 1H, *J* = 8.2 Hz), 7.73 (s, 4H), 7-73-7.77 (m, 1H), 8.22 (d, 1H, *J* = 2.0 Hz).

Methyl 3-(3-nitro-4'-(trifluoromethyl)biphenyl-4-yl)-2-oxopropanoate (3.131h)



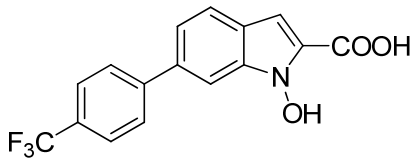
A stirred suspension of sodium hydride (60% dispersion in mineral oil, 280 mg, 7.00 mmol) in 5 mL of anhydrous DMF cooled to –15 °C under nitrogen was treated dropwise with a solution containing the nitrotoluene precursor **3.130h** (492 mg, 1.75 mmol) and dimethyl oxalate (1.03 g, 8.8 mmol) in 5 mL of anhydrous DMF. The mixture was left under stirring at the same temperature for 10 minutes and then it was slowly warmed to room temperature. The mixture was then left under stirring at RT. Once the disappearance of the precursor was verified by TLC, the reaction mixture was slowly poured in an ice-water mixture; the water phase was acidified with 1N aqueous HCl and extracted several times with EtOAc. The combined organic phase was washed with saturated $NaHCO_3$ solution and brine, then dried over anhydrous sodium sulphate. Evaporation under vacuum of the organic solvent afforded a crude product which was purified by column chromatography over silica gel using *n*-hexane/EtOAc 7:3 as the eluent, to yield the nitroaryl-ketoester derivative **3.131h** (400 mg, 1.09 mmol, 62% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 3.96 (s, 3H), 4.60 (s, 2H), 7.45 (d, 1H, *J* = 8.1 Hz), 7.75 (s, 4H), 7.86 (dd, 1H, *J* = 7.9, 1.8 Hz), 8.41 (d, 1H, *J* = 1.8 Hz).

Methyl 1-hydroxy-6-(4-(trifluoromethyl)phenyl)-1*H*-indole-2-carboxylate (3.132h)



Triethylamine (0.42 mL, 0.30 g, 3.0 mmol) was added dropwise to a stirred solution of $SnCl_2 \cdot 2H_2O$ (203 mg, 0.898 mmol) and PhSH (0.28 mL, 0.30 g, 2.7 mmol) in acetonitrile (5 mL) at room temperature to generate a yellow precipitate over a period of 5 min. Then, **3.131h** (220 mg, 0.599 mmol) was transferred to the suspension and stirred at room temperature for 5 more min. After complete consumption of the starting material, the mixture was diluted with water and repeatedly extracted with EtOAc. The combined organic phase was dried over anhydrous sodium sulphate, diluted with toluene and a catalytic amount of *p*-toluensulfonic acid was added. Then it was evaporated to afford a crude residue that was purified by column chromatography over silica gel using CH_2Cl_2/MeOH 98:2 as the eluent, to give the *N*-hydroxyindole **3.132h** (101 mg, 0.301 mmol, 50% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 4.02 (s, 3H), 7.07 (d, 1H, J = 0.7 Hz), 7.39 (dd, 1H, J = 8.6, 1.6 Hz), 7.69-7.82 (m, 6H), 10.36 (bs, 1H).

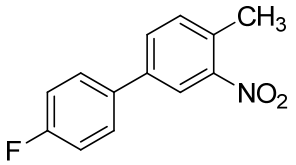
1-Hydroxy-6-(4-(trifluoromethyl)phenyl)-1*H*-indole-2-carboxylic acid (3.125h)



Methyl ester **3.132h** (80.0 mg, 0.241 mmol) was dissolved in a 1:1 mixture of THF/methanol (2.4 mL) and treated with 0.72 mL of 2N aqueous solution of LiOH. The reaction was kept under stirring in the dark at RT and it was monitored by TLC. After 2 h, the reaction mixture was concentrated, diluted with water and washed with Et_2O. Then, the aqueous phase was treated with 1N aqueous HCl and extracted several times with EtOAc. The combined ethyl acetate organic phase was dried over anhydrous sodium sulphate and evaporated to afford the desired *N*-hydroxyindol-carboxylic acid product **3.125h** (69.0 mg, 0.215 mmol, 89% yield) without any further purification. ^{1}H

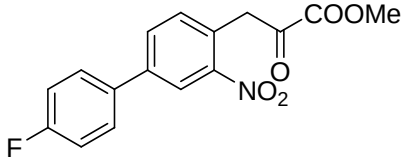
NMR (acetone-d_6) δ (ppm): 7.17 (d, 1H, J = 0.7 Hz), 7.52 (dd, 1H, J = 8.4, 1.6 Hz), 7.81 (dd, 1H, J = 8.4, 0.7 Hz), 7.82-7.90 (m, 3H), 7.96-8.03 (m, 2H). ^{13}C NMR (acetone-d_6) δ (ppm): 106.00, 108.83, 121.23, 122.45, 123.94, 125.48 (q, J = 269.7 Hz), 126.54 (q, 2C, J = 3.7 Hz), 127.60, 128.62 (2C), 129.35 (q, J = 34.8 Hz), 137.36, 137.38, 146.10, 161.99. MS *m/z* 321 (M^+, 100%), 305 (M^+ –O, 18%).

4'-Fluoro-4-methyl-3-nitrobiphenyl (3.130i)



Commercially available 4-bromo-2-nitrotoluene **3.90d** (540 mg, 2.50 mmol) was placed in a vial together with 4-fluorophenylboronic acid **3.129i** (455 mg, 3.25 mmol), potassium phosphate (902 mg, 4.25 mmol), $Pd(OAc)_2$ (7.4 mg, 0.033 mmol), tetrabutylammonium bromide (5.0 g, 15 mmol) and water (0.5 mL). The vial was sealed and heated under stirring at 125 °C for 24 h. The reaction mixture was cooled to RT and then diluted with water. The water phase was acidified with 1N aqueous HCl and repeatedly extracted with EtOAc. The combined organic phase was dried over anhydrous sodium sulphate and evaporated to afford a crude residue that was purified by column chromatography over silica gel using *n*-hexane/EtOAc 88:2 as the eluent, to give pure **3.130i** (400 mg, 1.73 mmol, 69% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 2.64 (s, 3H), 7.10-7.23 (m, 2H), 7.41 (d, 1H, J = 7.9 Hz), 7.50-7.62 (m, 2H), 7.68 (dd, 1H, J = 8.0, 1.9 Hz), 8.16 (d, 1H, J = 1.8 Hz).

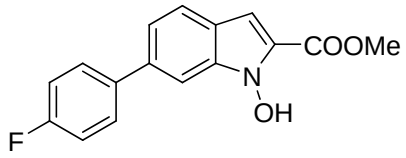
Methyl 3-(4'-fluoro-3-nitrobiphenyl-4-yl)-2-oxopropanoate (3.131i)



A stirred suspension of sodium hydride (60% dispersion in mineral oil, 272 mg, 6.76 mmol) in 5 mL of anhydrous DMF cooled to –15 °C under nitrogen was treated

dropwise with a solution containing the nitrotoluene precursor **3.130i** (390 mg, 1.69 mmol) and dimethyl oxalate (998 mg, 8.45 mmol) in 5 mL of anhydrous DMF. The mixture was left under stirring at the same temperature for 10 minutes and then it was slowly warmed to room temperature. The mixture was then left under stirring at RT. Once the disappearance of the precursor was verified by TLC, the reaction mixture was slowly poured in an ice-water mixture; the water phase was acidified with 1N aqueous HCl and extracted several times with EtOAc. The combined organic phase was washed with saturated $NaHCO_3$ solution and brine, then dried over anhydrous sodium sulphate. Evaporation under vacuum of the organic solvent afforded a crude product which was purified by column chromatography over silica gel using *n*-hexane/EtOAc 8:2 as the eluent, to yield the nitroaryl-ketoester derivative **3.131i** (300 mg, 0.946 mmol, 56% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 3.96 (s, 3H), 4.58 (s, 2H), 7.13-7.24 (m, 2H), 7.39 (d, 1H, J = 7.9 Hz), 7.54-7.64 (m, 2H), 7.80 (dd, 1H, J = 7.9, 2.0 Hz), 8.35 (d, 1H, J = 2.0 Hz); signals imputable to the enol form (~ 24%) δ (ppm): 3.94 (s, 3H), 6.96 (s, 1H), 8.08 (d, 1H, J = 2.0 Hz).

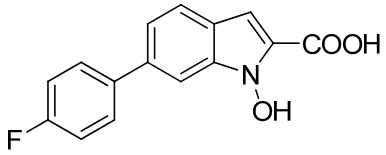
Methyl 6-(4-fluorophenyl)-1-hydroxy-1*H*-indole-2-carboxylate (3.132i)



Triethylamine (0.28 mL, 0.20 g, 2.0 mmol) was added dropwise to a stirred solution of $SnCl_2 \cdot 2H_2O$ (135 mg, 0.600 mmol) and PhSH (0.19 mL, 0.20 g, 1.8 mmol) in acetonitrile (5 mL) at room temperature to generate a yellow precipitate over a period of 5 min. Then, **3.131i** (127 mg, 0.400 mmol) was transferred to the suspension and stirred at room temperature for 5 more min. After complete consumption of the starting material, the mixture was diluted with water and repeatedly extracted with EtOAc. The combined organic phase was dried over anhydrous sodium sulphate, diluted with toluene and a catalytic amount of *p*-toluensulfonic acid was added. Then it was evaporated to afford a crude residue that was purified by column chromatography over silica gel using CH_2Cl_2/MeOH 95:2 as the eluent, to give the *N*-hydroxyindole **3.132i**

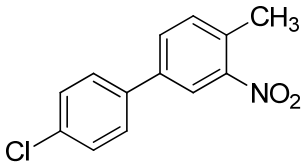
(50.0 mg, 0.175 mmol, 44% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 4.01 (s, 3H), 7.05 (d, 1H, *J* = 0.9 Hz), 7.10-7.20 (m, 2H), 7.34 (dd, 1H, *J* = 8.5, 1.6 Hz), 7.60-7.71 (m, 4H), 10.31 (bs, 1H).

6-(4-fluorophenyl)-1-hydroxy-1*H*-indole-2-carboxylic acid (3.125i)



Methyl ester **3.132i** (35.0 mg, 0.123 mmol) was dissolved in a 1:1 mixture of THF/methanol (2.0 mL) and treated with 0.37 mL of 2N aqueous solution of LiOH. The reaction was kept under stirring in the dark at RT and it was monitored by TLC. After 3 h, the reaction mixture was concentrated, diluted with water and washed with Et_2O. Then, the aqueous phase was treated with 1N aqueous HCl and extracted several times with EtOAc. The combined ethyl acetate organic phase was dried over anhydrous sodium sulphate and evaporated to afford the desired *N*-hydroxyindol-carboxylic acid product **3.125i** (31.0 mg, 0.114 mmol, 93% yield) without any further purification. ^{1}H NMR (acetone-d_6) δ (ppm): 7.15 (d, 1H, *J* = 0.6 Hz), 7.18-7.32 (m, 2H), 7.42 (dd, 1H, *J* = 8.6, 1.6 Hz), 7.67-7.86 (m, 4H). ^{13}C NMR (acetone-d_6) δ (ppm): 106.19, 108.17, 116.32 (d, 2C, *J* = 21.0 Hz), 121.26, 121.77, 123.68, 127.13, 129.79 (d, 2C, *J* = 8.2 Hz), 137.50, 138.14, 138.52 (d, *J* = 3.7 Hz), 161.90, 163.15 (d, *J* = 244.5 Hz). MS *m/z* 271 (M^+, 100%), 255 (M^+ –O, 33%), 208 (M^+ –CO_2 –F, 55%).

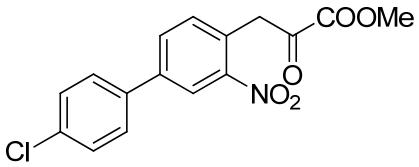
4'-Chloro-4-methyl-3-nitrobiphenyl (3.130j)



Commercially available 4-bromo-2-nitrotoluene **3.90d** (432 mg, 2.00 mmol) was placed in a vial together with 4-chlorobenzeneboronic acid **3.129j** (313 mg, 2.00 mmol), sodium carbonate (636 mg, 6.00 mmol), $Pd(OAc)_2$ (1.8 mg, 0.0080 mmol),

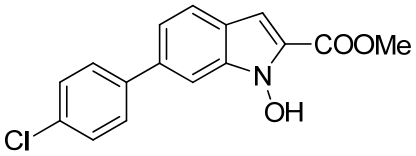
tetrabutylammonium bromide (644 mg, 2.00 mmol) and water (4 mL). The vial was sealed and heated under stirring at 150 °C in a microwave reactor for 5 min. The reaction mixture was then diluted with water and repeatedly extracted with EtOAc. The combined organic phase was dried over anhydrous sodium sulphate and evaporated to afford a crude residue that was purified by column chromatography over silica gel using *n*-hexane/EtOAc 88:2 as the eluent, to give pure **3.130j** (440 mg, 1.78 mmol, 89% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 2.64 (s, 3H), 7.40-7.56 (m, 5H), 7.69 (dd, 1H, J = 7.7, 1.8 Hz), 8.17 (d, 1H, J = 2.0 Hz).

Methyl 3-(4'-chloro-3-nitrobiphenyl-4-yl)-2-oxopropanoate (3.131j)



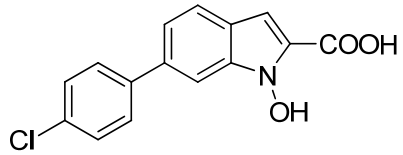
A stirred suspension of sodium hydride (60% dispersion in mineral oil, 240 mg, 6.00 mmol) in 10 mL of anhydrous DMF cooled to –15 °C under nitrogen was treated dropwise with a solution containing the nitrotoluene precursor **3.130j** (372 mg, 1.50 mmol) and dimethyl oxalate (886 mg, 7.51 mmol) in 5 mL of anhydrous DMF. The mixture was left under stirring at the same temperature for 10 minutes and then it was slowly warmed to room temperature. The mixture was then left under stirring at RT. Once the disappearance of the precursor was verified by TLC, the reaction mixture was slowly poured in an ice-water mixture; the water phase was acidified with 1N aqueous HCl and extracted several times with EtOAc. The combined organic phase was washed with saturated $NaHCO_3$ solution and brine, then dried over anhydrous sodium sulphate. Evaporation under vacuum of the organic solvent afforded a crude product which was purified by column chromatography over silica gel using *n*-hexane/EtOAc 8:2 as the eluent, to yield the nitroaryl-ketoester derivative **3.131j** (260 mg, 0.779 mmol, 52% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 3.95 (s, 3H), 4.58 (s, 2H), 7.40-7.60 (m, 5H), 7.81 (dd, 1H, J = 8.0, 1.9 Hz), 8.36 (d, 1H, J = 1.8 Hz); signals imputable to the enol form (~ 23%) δ (ppm): 3.96 (s, 3H), 6.72 (d, 1H, exchangeable, J = 1.5 Hz), 6.96 (d, 1H, J = 1.5 Hz), 8.09 (d, 1H, J = 2.0 Hz).

Methyl 6-(4-chlorophenyl)-1-hydroxy-1*H*-indole-2-carboxylate (3.132j)



Triethylamine (0.45 mL, 0.32 g, 3.2 mmol) was added dropwise to a stirred solution of $SnCl_2 \cdot 2H_2O$ (216 mg, 0.962 mmol) and PhSH (0.31 mL, 0.32 g, 2.9 mmol) in acetonitrile (5 mL) at room temperature to generate a yellow precipitate over a period of 5 min. Then, **3.131j** (214 mg, 0.641 mmol) was transferred to the suspension and stirred at room temperature for 5 more min. After complete consumption of the starting material, the mixture was diluted with water and repeatedly extracted with EtOAc. The combined organic phase was dried over anhydrous sodium sulphate, diluted with toluene and a catalytic amount of *p*-toluensulfonic acid was added. Then it was evaporated to afford a crude residue that was purified by column chromatography over silica gel using *n*-hexane/EtOAc 8:2 as the eluent, to give the *N*-hydroxyindole **3.132j** (66.0 mg, 0.219 mmol, 34% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 4.01 (s, 3H), 7.05 (d, 1H, J = 0.9 Hz), 7.35 (dd, 1H, J = 8.6, 1.5 Hz), 7.43 (AA'XX', 2H, J_{AX} = 8.8 Hz, $J_{AA'XX'}$ = 2.3 Hz), 7.62 (AA'XX', 2H, J = J_{AX} = 8.8 Hz, $J_{AA'XX'}$ = 2.2 Hz), 7.66-7.72 (m, 2H), 10.33 (bs, 1H).

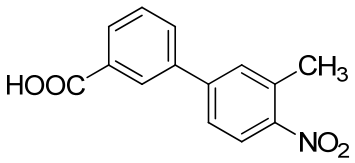
6-(4-Chlorophenyl)-1-hydroxy-1*H*-indole-2-carboxylic acid (3.125j)



Methyl ester **3.132j** (62.0 mg, 0.205 mmol) was dissolved in a 1:1 mixture of THF/methanol (3 mL) and treated with 0.62 mL of 2N aqueous solution of LiOH. The reaction was kept under stirring in the dark at RT and it was monitored by TLC. After complete consumption of the starting material, the reaction mixture was concentrated, diluted with water and washed with Et_2O. Then, the aqueous phase was treated with 1N aqueous HCl and extracted several times with EtOAc. The combined ethyl acetate organic phase was dried over anhydrous sodium sulphate and evaporated to afford the

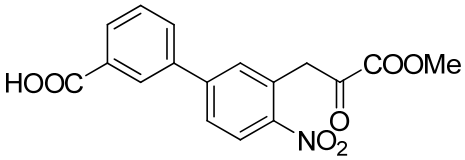
desired *N*-hydroxyindol-carboxylic acid product **3.125j** (55.0 mg, 0.191 mmol, 93% yield) without any further purification. ^{1}H NMR (DMSO-d_6) δ (ppm): 7.04 (d, 1H, J = 0.8 Hz), 7.41 (dd, 1H, J = 8.4, 1.4 Hz), 7.52 (AA´XX´, 2H, J_{AX} = 8.4 Hz, $J_{AA'XX'}$ = 2.0 Hz), 7.65 (s, 1H), 7.68-7.82 (m, 3H). ^{13}C NMR (DMSO-d_6) δ (ppm): 104.49, 107.16, 119.71, 120.58, 122.77, 127.52, 128.58 (2C), 128.85 (2C), 132.04, 135.53, 136.31, 139.39, 161.08. MS *m/z* 289 (^{37}Cl: M^+, 15%), 287 (^{35}Cl: M^+, 30%), 271 (^{35}Cl: M^+ –O, 55%), 190 (^{35}Cl: M^+ –Cl –H_2O –CO_2, 100%). HPLC, t_R = 10.2 min.

3'-Methyl-4'-nitrobiphenyl-3-carboxylic acid (3.135a)



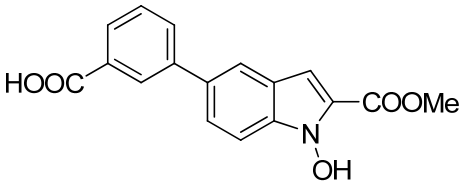
A solution of $Pd(OAc)_2$ (25.2 mg, 0.112 mmol) and triphenylphosphine (147 mg, 0.560 mmol) in absolute ethanol (3.8 mL) and anhydrous toluene (3.8 mL) was stirred at RT under nitrogen for 10 min. After that period, commercially available 5-chloro-2-nitrotoluene **3.115** (646 mg, 3.76 mmol), 3.8 mL of 2M aqueous Na_2CO_3, and 3-carboxybenzeneboronic acid **3.129b** (1.00 g, 6.03 mmol) were sequentially added. The resulting mixture was heated at 100 °C in a sealed vial under nitrogen overnight. After being cooled to RT, the mixture was diluted with water and extracted with EtOAc. The combined organic phase were dried and concentrated. The crude product was purified by flash chromatography ($CHCl_3$/MeOH 95:5) to yield **3.135a** (1.18 g, 4.59 mmol, >99% yield). ^{1}H NMR (acetone-d_6) δ (ppm): 2.69 (s, 3H), 7.67 (t, 1H, J = 7.8 Hz), 7.80 (dd, 1H, J = 8.4, 2.0 Hz), 7.84 (dt, 1H, J = 8.8, 2.0 Hz), 8.02 (dt, 1H, J = 7.8, 1.4 Hz), 8.10 (d, 1H, J = 8.4 Hz), 8.14 (d, 1H, J = 2.0 Hz), 8.36 (t, 1H, J = 2.0 Hz).

3'-(3-Methoxy-2,3-dioxopropyl)-4'-nitrobiphenyl-3-carboxylic acid (3.136a)



A stirred suspension of sodium hydride (60% dispersion in mineral oil, 870 mg, 21.8 mmol) in 15.0 mL of anhydrous DMF cooled to –15 °C under nitrogen was treated dropwise with a solution containing the nitrotoluene precursor **3.135a** (700 mg, 2.72 mmol) and dimethyl oxalate (3.21 g, 27 mmol) in 7.75 mL of anhydrous DMF. The mixture was left under stirring at the same temperature for 10 minutes and then it was slowly warmed to room temperature. The mixture was then left under stirring at RT for 21 h. Once the disappearance of the precursor was verified by TLC, the reaction mixture was slowly poured in an ice-water mixture; the water phase was acidified with 1N aqueous HCl and extracted several times with EtOAc. The combined organic phase was washed with saturated $NaHCO_3$ solution and brine, then dried over anhydrous sodium sulphate. Evaporation under vacuum of the organic solvent afforded a crude product which was purified by column chromatography over silica gel using $CHCl_3$/MeOH 95:5 as the eluent, to yield the nitroaryl-ketoester derivative **3.136a** (739 mg, 2.15 mmol, 79% yield). ^{1}H NMR (acetone-d_6) δ (ppm): 3.90 (s, 3H), 4.82 (s, 2H), 7.69 (t, 1H, J = 7.6 Hz), 8.03-8.08 (m, 2H), 8.13 (dt, 1H, J = 7.7, 1.4 Hz), 8.27-8.33 (m, 2H), 8.38 (t, 1H, J = 1.4 Hz); signals imputable to the enol form (~ 12%) δ (ppm): 3.91 (s, 3H), 6.92 (bs, 1H), 8.00 (t, 1H, J = 2.2 Hz).

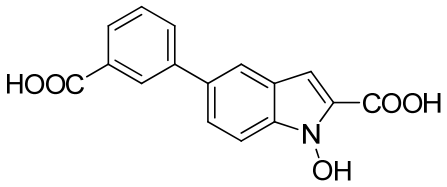
3-(1-Hydroxy-2-(methoxycarbonyl)-1*H*-indol-5-yl)benzoic acid (3.137a)



Ketoester **3.136a** (300 mg, 0.874 mmol) was dissolved in THF (1.5 mL) and the resulting solution was added to an aqueous solution (1.5 mL) of sodium hypophosphite monohydrate (286 mg, 2.70 mmol). Then 8.8 mg of 10% palladium over charcoal was added and the resulting suspension was stirred ar RT. After 26 h the disappearance of the precursor is verified by TLC and the mixture was concentrated, diluted with water and repeatedly extracted with EtOAc. The combined organic phase was dried over anhydrous sodium sulphate and evaporated to afford a crude residue that was purified by column chromatography over silica gel using $CHCl_3$/MeOH 95:5 as the eluent, to

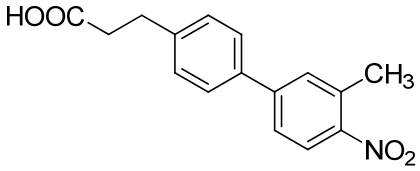
give the *N*-hydroxyindole **3.137a** (57.7 mg, 0.185 mmol, 21% yield). ^{1}H NMR (acetone-d_6) δ (ppm): 3.90 (s, 3H); 7.19 (s, 1H), 7.60 (t, 1H, J = 7.3 Hz), 7.72 (dd, 1H, J = 8.4, 1.4 Hz), 7.74 (dt, 1H, J = 7.3, 1.4 Hz), 7.93-8.02 (m, 3H), 8.33 (t, 1H, J = 1.4 Hz).

5-(3-Carboxyphenyl)-1-hydroxy-1*H*-indole-2-carboxylic acid (3.126a)



Methyl ester **3.137a** (32.0 mg, 0.103 mmol) was dissolved in a 1:1 mixture of THF/methanol (1.03 mL) and treated with 0.31 mL of 2N aqueous solution of LiOH. The reaction was kept under stirring in the dark at RT. After 23h. it was monitored by TLC and another portion of LiOH (0.31 mL) was added. After further 24 h, the reaction mixture was concentrated, diluted with water and washed with Et_2O. Then, the aqueous phase was treated with 1N aqueous HCl and extracted several times with EtOAc. The combined ethyl acetate organic phase was dried over anhydrous sodium sulphate and evaporated to afford the desired *N*-hydroxyindol-carboxylic acid product **3.126a** (25.8 mg, 0.0868 mmol, 84% yield) without any further purification. ^{1}H NMR (acetone-d_6) δ (ppm): 7.18 (d, 1H, J = 0.9 Hz), 7.58 (td, 1H, J = 7.5, 0.4 Hz), 7.62 (dt, 1H, J = 8.8, 0.7 Hz), 7.71 (dd, 1H, J = 8.6, 1.6 Hz), 7.91 (dd, 1H, J = 2.0, 1.3 Hz), 7.94-8.00 (m, 2H), 8.31 (t, 1H, J = 1.6 Hz).

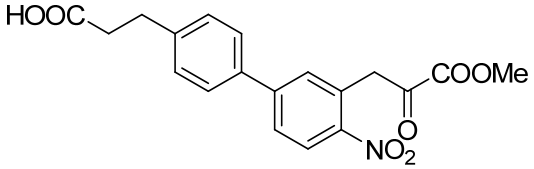
3-(3'-Methyl-4'-nitrobiphenyl-4-yl)propanoic acid (3.135b)



A solution of $Pd(OAc)_2$ (11 mg, 0.048 mmol) and triphenylphosphine (63 mg, 0.24 mmol) in absolute ethanol (3.6 mL) and anhydrous toluene (3.6 mL) was stirred at RT under nitrogen for 10 min. After that period, commercially available 5-chloro-2-

nitrotoluene **3.115** (276 mg, 1.61 mmol), 3.6 mL of 2M aqueous Na_2CO_3, and 4-(2-carboxyethyl)benzeneboronic acid **3.129c** (500 mg, 2.58 mmol) were sequentially added. The resulting mixture was heated at 100 °C in a sealed vial under nitrogen overnight. After being cooled to RT, the mixture was diluted with water and extracted with EtOAc. The combined organic phase were dried and concentrated. The crude product was purified by flash chromatography ($CHCl_3$/MeOH 95:5) to yield **3.135b** (427 mg, 1.50 mmol, 93% yield). ^{1}H NMR (acetone-d_6) δ (ppm): 2.66 (s, 3H), 2.68 (t, 2H, J = 7.6 Hz), 2.99 (t, 2H, J = 7.6 Hz), 7.28 (d, 1H, J = 1.5 Hz), 7.43 (AA'XX', 2H, J_{AX} = 8.6 Hz, $J_{AA'XX'}$ = 2.0 Hz), 7.66-7.71 (m, 3H), 8.08 (d, 1H, J = 8.4 Hz).

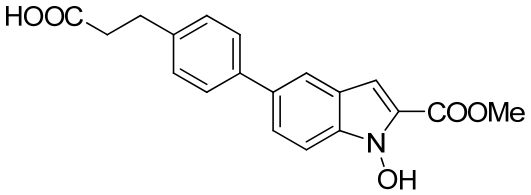
3-(3'-(3-Methoxy-2,3-dioxopropyl)-4'-nitrobiphenyl-4-yl)propanoic acid (3.136b)



A stirred suspension of sodium hydride (60% dispersion in mineral oil, 467 mg, 11.7 mmol) in 8.1 mL of anhydrous DMF cooled to –15 °C under nitrogen was treated dropwise with a solution containing the nitrotoluene precursor **3.135b** (416 mg, 1.46 mmol) and dimethyl oxalate (1.72 g, 14.6 mmol) in 4.2 mL of anhydrous DMF. The mixture was left under stirring at the same temperature for 10 minutes and then it was slowly warmed to room temperature. The mixture was then left under stirring at RT for 16 h. Once the disappearance of the precursor was verified by TLC, the reaction mixture was slowly poured in an ice-water mixture; the water phase was acidified with 1N aqueous HCl and extracted several times with EtOAc. The combined organic phase was washed with saturated $NaHCO_3$ solution and brine, then dried over anhydrous sodium sulphate. Evaporation under vacuum of the organic solvent afforded a crude product which was purified by column chromatography over silica gel using *n*-hexane/EtOAc 4:6 as the eluent, to yield the nitroaryl-ketoester derivative **3.136b** (247 mg, 0.665 mmol, 46% yield). ^{1}H NMR (acetone-d_6) δ (ppm): 2.68 (t, 2H, J = 7.7 Hz), 2.99 (t, 2H, J = 7.8 Hz), 3.90 (s, 3H), 4.77 (s, 2H), 7.44 (AA'XX', 2H, J_{AX} = 8.6 Hz, $J_{AA'XX'}$ = 1.8 Hz), 7.72 (AA'XX', 2H, J_{AX} = 8.4 Hz, $J_{AA'XX'}$ = 1.8 Hz), 7.86-7.92 (m, 2H), 8.26 (d, 1H,

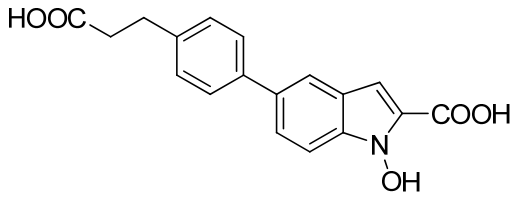
J = 8.2 Hz); signals imputable to the enol form (~ 26%) δ (ppm): 3.83 (s, 3H), 6.07 (bs, 1H, exchangeable), 6.92 (s, 1H), 8.57 (d, 1H, *J* = 1.8 Hz).

3-(4-(1-Hydroxy-2-(methoxycarbonyl)-1*H*-indol-5-yl)phenyl)propanoic acid (3.137b)



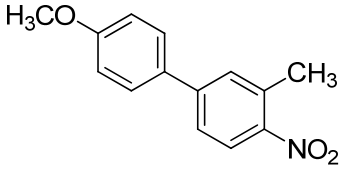
Ketoester **3.136b** (100 mg, 0.269 mmol) was dissolved in THF (0.5 mL) and the resulting solution was added to an aqueous solution (0.5 mL) of sodium hypophosphite monohydrate (88.4 g, 0.834 mmol). Then 2.7 mg of 10% palladium over charcoal was added and the resulting suspension was stirred at RT for 22h. Then it was monitored by TLC and $H_2PO_2Na{\cdot}H_2O$ (88.4 g, 0.834 mmol) and Pd/C (2.7 mg) were added. After further 72 h, another portion of $H_2PO_2Na{\cdot}H_2O$ (44.2 mg, 0.417 mmol) and Pd/C (1.4 mg) was added. The disappearance of the precursor is verified by TLC after 8 h and the mixture was concentrated, diluted with water and repeatedly extracted with EtOAc. The combined organic phase was dried over anhydrous sodium sulphate and evaporated to afford a crude residue that was purified by column chromatography over silica gel using *n*-hexane/EtOAc 6:4 with 0.01% trifluoroacetic acid as the eluent, to give a mixture (19.8 mg) of the *N*-hydroxyindol-ester derivative **3.137b** (85%, 16.8 mg, 0.0496 mmol, 18% yield) with its indole analogue (15%). ^{1}H NMR (acetone-d_6) δ (ppm): 2.66 (t, 2H, *J* = 7.3 Hz), 2.97 (t, 2H, *J* = 7.7 Hz), 3.90 (s,3H), 7.14 (s, 1H), 7.32-7.40 (m, 2H), 7.59-7.71 (m, 4H), 7.89-7.92 (m, 1H); 10.50 (bs, 1H), signals imputable to the indole (~ 15%) δ (ppm): 7.24-7.25 (m, 1H), 7.93-7.95 (m, 1H), 11.00 (bs, 1H).

5-(4-(2-Carboxyethyl)phenyl)-1-hydroxy-1*H*-indole-2-carboxylic acid (3.126b)



Methyl ester **3.137b** (19.8 mg, 0.0583 mmol) was dissolved in a 1:1 mixture of THF/methanol (0.6 mL) and treated with 0.2 mL of 2N aqueous solution of LiOH. The reaction was kept under stirring in the dark at RT and it was monitored by TLC. After 24 h, the reaction mixture was concentrated, diluted with water and washed with Et_2O. Then, the aqueous phase was treated with 1N aqueous HCl and extracted several times with EtOAc. The combined ethyl acetate organic phase was dried over anhydrous sodium sulphate and evaporated to afford the desired *N*-hydroxyindol-carboxylic acid product **3.126b** (17.5 mg, 0.538 mmol, 92% yield) without any further purification. ^{1}H NMR (acetone-d_6) δ (ppm): 2.67 (t, 2H, J = 7.8 Hz), 2.97 (t, 2H, J = 7.6 Hz), 7.17 (s, 1H), 7.36 (d, 2H, J = 8.0 Hz), 7.58-7.69 (m, 4H), 7.91-7.92 (m, 1H), 10.85 (bs, 1H); signals imputable to the indole (~ 15%) δ (ppm): 7.24-7.27 (m, 1H), 7.69-7.71 (m, 1H), 7.93-7.95 (m, 1H). ^{13}C NMR (acetone-d_6) δ (ppm): 31.15, 35.85, 106.57, 110.81, 120.97, 122.83, 125.80, 127.78 (2C), 129.64 (2C), 134.77, 140.27, 140.46, 161.85, 173.78. MS *m/z* 325 (M^+, 12%), 255 ($M^+ - C_3H_2O_2$, 100%), 175 ($M^+ - C_9H_{10}O_2$, 18%), 149 ($M^+ - C_9H_6O_3N$, 31%).

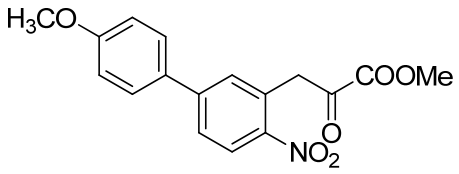
4'-Methoxy-3-methyl-4-nitrobiphenyl (3.135c)



Commercially available 5-chloro-2-nitrotoluene **3.115** (500 mg, 2.91 mmol) was placed in a vial together with 4-methoxyphenylboronic acid **3.129e** (532 mg, 3.50 mmol), sodium carbonate (928 mg, 8.76 mmol), $Pd(OAc)_2$ (2.6 mg, 0.011 mmol), tetrabutylammonium bromide (940 mg, 2.91 mmol) and water (5 mL). The vial was

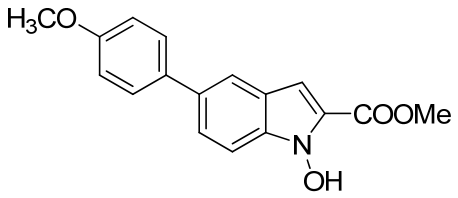
sealed and heated under stirring at 175 °C in a microwave reactor for 5 min. The reaction mixture was then diluted with water and repeatedly extracted with EtOAc. The combined organic phase was dried over anhydrous sodium sulphate and evaporated to afford a crude residue that was purified by column chromatography over silica gel using *n*-hexane/EtOAc 9:1 as the eluent, to give pure **3.135c** (705 mg, 2.90 mmol, 99% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 2.69 (s, 3H), 3.87 (s, 3H), 7.01 (AA'XX', 2H, J_{AX} = 8.8 Hz, $J_{AA'/XX'}$ = 2.5 Hz), 7.46-7.60 (m, 4H), 8.08 (d, 1H, J = 9.2 Hz).

Methyl 3-(4'-methoxy-4-nitrobiphenyl-3-yl)-2-oxopropanoate (3.136c)



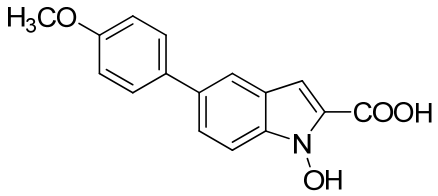
A stirred suspension of sodium hydride (60% dispersion in mineral oil, 400 mg, 10.0 mmol) in 10 mL of anhydrous DMF cooled to –15 °C under nitrogen was treated dropwise with a solution containing the nitrotoluene precursor **3.135c** (608 mg, 2.50 mmol) and dimethyl oxalate (1.48 g, 12.5 mmol) in 10 mL of anhydrous DMF. The mixture was left under stirring at the same temperature for 10 minutes and then it was slowly warmed to room temperature. The mixture was then left under stirring at RT. Once the disappearance of the precursor was verified by TLC, the reaction mixture was slowly poured in an ice-water mixture; the water phase was acidified with 1N aqueous HCl and extracted several times with EtOAc. The combined organic phase was washed with saturated $NaHCO_3$ solution and brine, then dried over anhydrous sodium sulphate. Evaporation under vacuum of the organic solvent afforded a crude product which was purified by column chromatography over silica gel using *n*-hexane/EtOAc 7:3 as the eluent, to yield the nitroaryl-ketoester derivative **3.136c** (548 mg, 1.66 mmol, 67% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 3.87 (s, 3H), 3.95 (s, 3H), 4.60 (s, 2H), 6.96-7.05 (m, 2H), 7.46 (d, 1H, J = 2.0 Hz), 7.56 (AA'XX', 2H, J_{AX} = 8.8 Hz, $J_{AA'/XX'}$ = 2.5 Hz), 7.64 (dd, 1H, J = 8.6, 2.0 Hz), 8.24 (d, 1H, J = 8.6 Hz); signals imputable to the enol form (~ 13%) δ (ppm): 6.70 (bs, 1H), 8.02 (d, 1H, J = 8.4 Hz), 8.57 (d, 1H, J = 2.0 Hz).

Methyl 1-hydroxy-5-(4-methoxyphenyl)-1*H*-indole-2-carboxylate (3.137c)



Ketoester **3.136c** (329 mg, 1.00 mmol) was dissolved in anhydrous DME (1.5 mL) and the resulting solution was added dropwise to a cooled (0 °C) solution of $SnCl_2 \cdot 2H_2O$ (562 mg, 2.49 mmol) in DME (1.5 mL) containing 4Å molecular sieves, previously activated for 18 hours at 130 °C in oven and cooled to RT in a desiccator over anhydrous calcium chloride. The reaction mixture was stirred under nitrogen at RT for 24 h, then it was diluted with water and repeatedly extracted with EtOAc. The combined organic phase was dried over anhydrous sodium sulphate and evaporated to afford a crude residue that was purified by column chromatography over silica gel using CH_2Cl_2 as the eluent, to give the *N*-hydroxyindol-ester derivative **3.137c** (30 mg, 0.101 mmol, 10% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 3.86 (s, 3H), 4.01 (s, 3H), 6.99 (AA'XX', 2H, J_{AX} = 8.8 Hz, $J_{AA'/XX'}$ = 2.2 Hz), 7.06 (s, 1H), 7.52-7.56 (m, 4H), 7.77 (t, 1H, J = 1.3 Hz), 10.25 (bs, 1H).

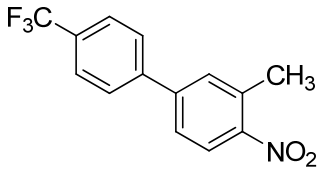
1-Hydroxy-5-(4-methoxyphenyl)-1*H*-indole-2-carboxylic acid (3.126c)



Methyl ester **3.137c** (28.0 mg, 0.0942 mmol) was dissolved in a 1:1 mixture of THF/methanol (2 mL) and treated with 0.3 mL of 2N aqueous solution of LiOH. The reaction was kept under stirring in the dark at RT and it was monitored by TLC. After 3 h, the reaction mixture was concentrated, diluted with water and washed with Et_2O. Then, the aqueous phase was treated with 1N aqueous HCl and extracted several times with EtOAc. The combined ethyl acetate organic phase was dried over anhydrous sodium sulphate and evaporated to afford the desired *N*-hydroxyindol-carboxylic acid

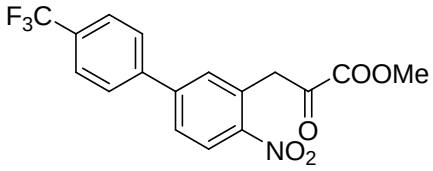
product **3.126c** (26.0 mg, 0.0918 mmol, 97% yield) without any further purification. ^{1}H NMR (DMSO-d_6) δ (ppm): 3.79 (s, 3H), 6.96-7.08 (m, 3H), 7.47 (d, 1H, J = 8.6 Hz), 7.52-7.66 (m, 3H), 7.83 (s, 1H). ^{13}C NMR (DMSO-d_6) δ (ppm): 55.16, 104.90, 110,06, 114.28, 119.20, 121.53, 124.10, 127.19, 127.70, 132.58, 133.31, 135.11, 158.27, 161.20. MS *m/z* 284 ($M+H^+$, 20%), 283 (M^+, 100%), 267 (M^+ –O, 99%), 252 (M^+ – CH_3O, 19%).

3-Methyl-4-nitro-4'-(trifluoromethyl)biphenyl (3.135d)



Commercially available 5-chloro-2-nitrotoluene **3.115** (429 mg, 2.50 mmol) was placed in a vial together with 4-trifluoromethylphenylboronic acid **3.129h** (475 mg, 2.50 mmol), sodium carbonate (794 mg, 7.50 mmol), $Pd(OAc)_2$ (2.2 mg, 0.010 mmol), tetrabutylammonium bromide (805 mg, 2.50 mmol) and water (5 mL). The vial was sealed and heated under stirring at 175 °C in a microwave reactor for 5 min. The reaction mixture was then diluted with water and repeatedly extracted with EtOAc. The combined organic phase was dried over anhydrous sodium sulphate and evaporated to afford a crude residue that was purified by column chromatography over silica gel using *n*-hexane/EtOAc 99:1 as the eluent, to give pure **3.135d** (478 mg, 1.70 mmol, 68% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 2.70 (s, 3H), 7.52-7.59 (m, 2H), 7.67-7.78 (m, 4H), 8.11 (d, 1H, J = 9.0 Hz).

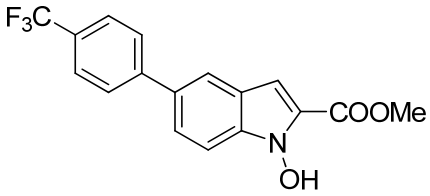
Methyl 3-(4-nitro-4'-(trifluoromethyl)biphenyl-3-yl)-2-oxopropanoate (3.136d)



A stirred suspension of sodium hydride (60% dispersion in mineral oil, 256 mg, 6.40 mmol) in 6 mL of anhydrous DMF cooled to –15 °C under nitrogen was treated

dropwise with a solution containing the nitrotoluene precursor **3.135d** (450 mg, 1.60 mmol) and dimethyl oxalate (944 mg, 8.00 mmol) in 4 mL of anhydrous DMF. The mixture was left under stirring at the same temperature for 10 minutes and then it was slowly warmed to room temperature. The mixture was then left under stirring at RT. Once the disappearance of the precursor was verified by TLC, the reaction mixture was slowly poured in an ice-water mixture; the water phase was acidified with 1N aqueous HCl and extracted several times with EtOAc. The combined organic phase was washed with saturated $NaHCO_3$ solution and brine, then dried over anhydrous sodium sulphate. Evaporation under vacuum of the organic solvent afforded a crude product which was purified by column chromatography over silica gel using *n*-hexane/EtOAc 8:2 as the eluent, to yield the nitroaryl-ketoester derivative **3.136d** (339 mg, 0.923 mmol, 58% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 3.96 (s, 3H), 4.63 (s, 2H), 7.52 (d, 1H, J = 2.0 Hz), 7.68-7.80 (m, 5H), 8.31 (d, 1H, J = 8.6 Hz); signals imputable to the enol form (~ 8%) δ (ppm): 3.97 (s, 3H), 6.71 (bs, 1H, exchangeable), 7.04 (bs, 1H), 7.60 (dd, 1H, J = 8.6, 2.2 Hz), 8.05 (d, 1H, J = 8.4 Hz), 8.48 (d, 1H, J = 2.0 Hz).

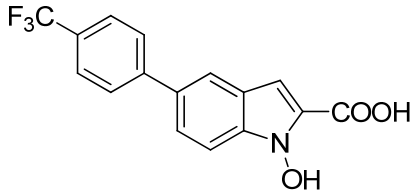
Methyl 1-hydroxy-5-(4-(trifluoromethyl)phenyl)-1*H*-indole-2-carboxylate (3.137d)



Triethylamine (0.46 mL, 0.33 g, 3.30 mmol) was added dropwise to a stirred solution of $SnCl_2 \cdot 2H_2O$ (224 mg, 0.988 mmol) and PhSH (0.30 mL, 0.33 g, 3.0 mmol) in acetonitrile (5 mL) at room temperature to generate a yellow precipitate over a period of 5 min. Then, **3.136d** (242 mg, 0.659 mmol) was transferred to the suspension and stirred at room temperature for 5 more min. After complete consumption of the starting material, the mixture was diluted with water and repeatedly extracted with EtOAc. The combined organic phase was dried over anhydrous sodium sulphate, diluted with toluene and a catalytic amount of *p*-toluensulfonic acid was added. Then it was evaporated to afford a crude residue that was purified by column chromatography over

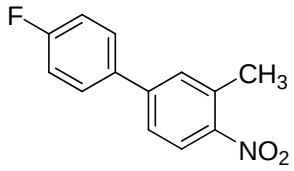
silica gel using *n*-hexane/EtOAc 8:2 as the eluent, to give the *N*-hydroxyindole **3.137d** (69.0 mg, 0.206 mmol, 31% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 4.02 (s, 3H), 7.10 (s, 1H), 7.61-7.77 (m, 6H), 7.85 (t, 1H, *J* = 1.3 Hz), 10.35 (bs, 1H).

1-Hydroxy-5-(4-(trifluoromethyl)phenyl)-1*H*-indole-2-carboxylic acid (3.126d)



Methyl ester **3.137d** (60.0 mg, 0.179 mmol) was dissolved in a 1:1 mixture of THF/methanol (2 mL) and treated with 0.54 mL of 2N aqueous solution of LiOH. The reaction was kept under stirring in the dark at RT and it was monitored by TLC. Once the disappearance of the precursor was verified, the reaction mixture was concentrated, diluted with water and washed with Et_2O. Then, the aqueous phase was treated with 1N aqueous HCl and extracted several times with EtOAc. The combined ethyl acetate organic phase was dried over anhydrous sodium sulphate and evaporated to afford the desired *N*-hydroxyindol-carboxylic acid product **3.126d** (52.0 mg, 0.162 mmol, 90% yield) without any further purification. ^{1}H NMR (DMSO-d_6) δ (ppm): 7.10 (s, 1H), 7.56 (d, 1H, *J* = 8.6 Hz), 7.70 (dd, 1H, *J* = 8.6, 1.6 Hz), 7.80 (d, 2H, *J* = 8.4 Hz), 7.92 (d, 2H, *J* = 8.2 Hz), 8.02 (s, 1H). ^{13}C NMR (DMSO-d_6) δ (ppm): 105.11, 110.31, 120.67, 121.42, 124.10, 124.41 (q, *J* = 271.0 Hz), 125.61 (q, 2C, *J* = 3.6 Hz), 126.93 (q, *J* = 30.7 Hz), 127.25 (2C), 127.59, 131.02, 135.62, 144.84, 161.00.

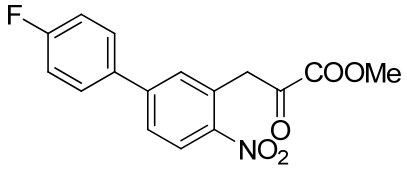
4'-Fluoro-3-methyl-4-nitrobiphenyl (3.135e)



Commercially available 5-chloro-2-nitrotoluene **3.115** (429 mg, 2.50 mmol) was placed in a vial together with 4-fluorobenzeneboronic acid **3.129i** (350 mg, 2.50 mmol),

sodium carbonate (795 mg, 7.50 mmol), $Pd(OAc)_2$ (2.2 mg, 0.010 mmol), tetrabutylammonium bromide (806 mg, 2.50 mmol) and water (5 mL). The vial was sealed and heated under stirring at 175 °C in a microwave reactor for 5 min. The reaction mixture was then diluted with water and repeatedly extracted with EtOAc. The combined organic phase was dried over anhydrous sodium sulphate and evaporated to afford a crude residue that was purified by column chromatography over silica gel using *n*-hexane/EtOAc 99:1 as the eluent, to give pure **3.135e** (500 mg, 2.16 mmol, 86% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 2.69 (s, 3H), 7.10-7.23 (m, 2H), 7.46-7.53 (m, 2H), 7.53-7.63 (m, 2H), 8.08 (d, 1H, J = 9.0 Hz).

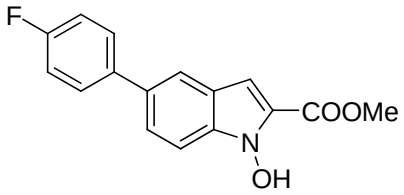
Methyl 3-(4'-fluoro-4-nitrobiphenyl-3-yl)-2-oxopropanoate (3.136e)



A stirred suspension of sodium hydride (60% dispersion in mineral oil, 280 mg, 7.00 mmol) in 6 mL of anhydrous DMF cooled to –15 °C under nitrogen was treated dropwise with a solution containing the nitrotoluene precursor **3.135e** (404 mg, 1.75 mmol) and dimethyl oxalate (1.03 g, 8.75 mmol) in 4 mL of anhydrous DMF. The mixture was left under stirring at the same temperature for 10 minutes and then it was slowly warmed to room temperature. The mixture was then left under stirring at RT overnight. Once the disappearance of the precursor was verified by TLC, the reaction mixture was slowly poured in an ice-water mixture; the water phase was acidified with 1N aqueous HCl and extracted several times with EtOAc. The combined organic phase was washed with saturated $NaHCO_3$ solution and brine, then dried over anhydrous sodium sulphate. Evaporation under vacuum of the organic solvent afforded a crude product which was purified by column chromatography over silica gel using *n*-hexane/EtOAc 8:2 as the eluent, to yield the nitroaryl-ketoester derivative **3.136e** (369 mg, 1.16 mmol, 66% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 3.96 (s, 3H), 4.61 (s, 2H), 7.12-7.24 (m, 2H), 7.47 (d, 1H; J = 2.0 Hz), 7.55-7.69 (m, 3H), 8.28 (d, 1H, J = 8.6 Hz);

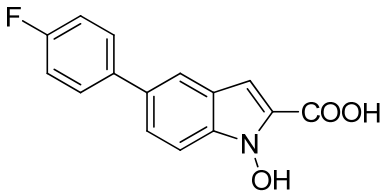
signals imputable to the enol form (~ 19%) δ (ppm): 3.97 (s, 3H), 6.67 (bs, 1H, exchangeable), 7.05 (s, 1H), 8.04 (d, 1H, *J* = 8.4 Hz), 8.41 (d, 1H, *J* = 2.0 Hz).

Methyl 5-(4-fluorophenyl)-1-hydroxy-1*H*-indole-2-carboxylate (3.137e)



Triethylamine (0.66 mL, 0.48 g, 4.7 mmol) was added dropwise to a stirred solution of $SnCl_2 \cdot 2H_2O$ (318 mg, 1.41 mmol) and PhSH (0.44 mL, 0.47 g, 4.3 mmol) in acetonitrile (5 mL) at room temperature to generate a yellow precipitate over a period of 5 min. Then, **3.136e** (300 mg, 0.946 mmol) was transferred to the suspension and stirred at room temperature for 5 more min. After complete consumption of the starting material, the mixture was diluted with water and repeatedly extracted with EtOAc. The combined organic phase was dried over anhydrous sodium sulphate, diluted with toluene and a catalytic amount of *p*-toluensulfonic acid was added. Then it was evaporated to afford a crude residue that was purified by column chromatography over silica gel using CH_2Cl_2/MeOH 9:1 as the eluent, to give the *N*-hydroxyindole **3.137e** (86.0 mg, 0.301 mmol, 32% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 4.01 (s, 3H), 7.07 (s, 1H), 7.08-7.19 (m, 2H), 7.52-7.64 (m, 4H), 7.77 (t, 1H, *J* = 1.3 Hz), 10.30 (bs, 1H).

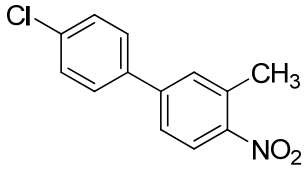
5-(4-Fluorophenyl)-1-hydroxy-1*H*-indole-2-carboxylic acid (3.126e)



Methyl ester **3.137e** (75.0 mg, 0.263 mmol) was dissolved in a 1:1 mixture of THF/methanol (2.6 mL) and treated with 0.8 mL of 2N aqueous solution of LiOH. The reaction was kept under stirring in the dark at RT and it was monitored by TLC. Then, the reaction mixture was concentrated, diluted with water and washed with Et_2O. The

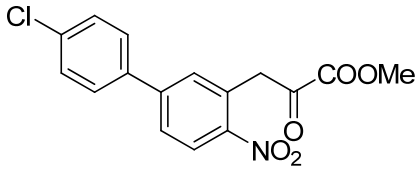
aqueous phase was treated with 1N aqueous HCl and extracted several times with EtOAc. The combined ethyl acetate organic phase was dried over anhydrous sodium sulphate and evaporated to afford the desired *N*-hydroxyindol-carboxylic acid product **3.126e** (65.0 mg, 0.240 mmol, 91% yield) without any further purification. ^{1}H NMR (acetone-d_6) δ (ppm): 7.17 (d, 1H, J = 0.7 Hz), 7.18-7.28 (m, 2H), 7.56-7.63 (m, 4H), 7.91 (dd, 1H, J = 1.5, 0.9 Hz). ^{13}C NMR (DMSO-d_6) δ (ppm): 101.65, 109.80, 115.48 (d, 2C, J = 21.1 Hz), 119.71, 121.26, 122.95, 127.41, 128.39 (d, 2C, J = 7.3 Hz), 130.96, 133.06, 137.65 (d, J = 2.7 Hz), 161.21 (d, J = 242.6 Hz), 162.50. MS *m/z* 271 (M^+, 60%), 255 (M^+ –O, 100%), 208 (M^+ –CO_2 –F, 88%).

4'-Chloro-3-methyl-4-nitrobiphenyl (3.135f)



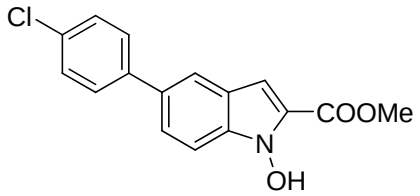
Commercially available 5-chloro-2-nitrotoluene **3.115** (429 mg, 2.50 mmol) was placed in a vial together with 4-chlorobenzeneboronic acid **3.129j** (391 mg, 2.50 mmol), sodium carbonate (795 mg, 7.50 mmol), $Pd(OAc)_2$ (2.2 mg, 0.010 mmol), tetrabutylammonium bromide (806 mg, 2.50 mmol) and water (5 mL). The vial was sealed and heated under stirring at 175 °C in a microwave reactor for 5 min. The reaction mixture was then diluted with water and repeatedly extracted with EtOAc. The combined organic phase was dried over anhydrous sodium sulphate and evaporated to afford a crude residue that was purified by column chromatography over silica gel using *n*-hexane/EtOAc 99:1 as the eluent, to give pure **3.135f** (522 mg, 2.11 mmol, 84% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 2.69 (s, 3H), 7.41-7.58 (m, 6H), 8.09 (d, 1H, J = 9.2 Hz).

Methyl 3-(4'-chloro-4-nitrobiphenyl-3-yl)-2-oxopropanoate (3.136f)



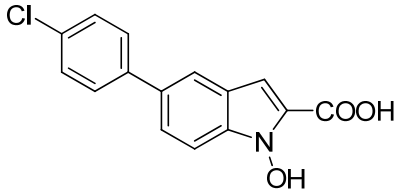
A stirred suspension of sodium hydride (60% dispersion in mineral oil, 240 mg, 6.00 mmol) in 5 mL of anhydrous DMF cooled to –15 °C under nitrogen was treated dropwise with a solution containing the nitrotoluene precursor **3.135f** (372 mg, 1.50 mmol) and dimethyl oxalate (885 mg, 7.50 mmol) in 5 mL of anhydrous DMF. The mixture was left under stirring at the same temperature for 10 minutes and then it was slowly warmed to room temperature. The mixture was then left under stirring at RT overnight. Once the disappearance of the precursor was verified by TLC, the reaction mixture was slowly poured in an ice-water mixture; the water phase was acidified with 1N aqueous HCl and extracted several times with EtOAc. The combined organic phase was washed with saturated $NaHCO_3$ solution and brine, then dried over anhydrous sodium sulphate. Evaporation under vacuum of the organic solvent afforded a crude product which was purified by column chromatography over silica gel using *n*-hexane/EtOAc 8:2 as the eluent, to yield the nitroaryl-ketoester derivative **3.136f** (428 mg, 1.28 mmol, 86% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 3.96 (s, 3H), 4.61 (s, 2H), 7.42-7.51 (m, 3H), 7.51-7.60 (m, 3H), 8.28 (d, 1H, J = 8.4 Hz); signals imputable to the enol form (~ 68%) δ (ppm): 3.97 (s, 3H), 6.69 (d, 1H, exchangeable, J = 1.5 Hz), 7.04 (d, 1H, J = 1.5 Hz), 7.65 (dd, 1H, J = 8.5, 2.1 Hz), 8.04 (d, 1H, J = 8.6 Hz), 8.43 (d, 1H, J = 2.0 Hz).

Methyl 5-(4-chlorophenyl)-1-hydroxy-1*H*-indole-2-carboxylate (3.137f)



Triethylamine (0.45 mL, 0.32 g, 3.2 mmol) was added dropwise to a stirred solution of $SnCl_2 \cdot 2H_2O$ (216 mg, 0.962 mmol) and PhSH (0.30 mL, 0.32 g, 2.9 mmol) in acetonitrile (5 mL) at room temperature to generate a yellow precipitate over a period of 5 min. Then, **3.136f** (214 mg, 0.641 mmol) was transferred to the suspension and stirred at room temperature for 5 more min. After complete consumption of the starting material, the mixture was diluted with water and repeatedly extracted with EtOAc. The combined organic phase was dried over anhydrous sodium sulphate, diluted with toluene and a catalytic amount of *p*-toluensulfonic acid was added. Then it was evaporated to afford a crude residue that was purified by column chromatography over silica gel using *n*-hexane/EtOAc 8:2 as the eluent, to give the *N*-hydroxyindole **3.137f** (55.0 mg, 0.182 mmol, 28% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 4.01 (s, 3H), 7.08 (s, 1H), 7.41 (AA'XX', 2H, J_{AX} = 8.4 Hz, $J_{AA'/XX'}$ = 2.0 Hz), 7.53-7.59 (m, 4H), 7.79 (t, 1H, J = 1.1 Hz), 10.32 (bs, 1H).

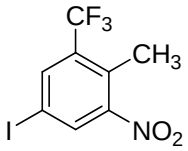
5-(4-Chlorophenyl)-1-hydroxy-1*H*-indole-2-carboxylic acid (3.126f)



Methyl ester **3.137f** (50.0 mg, 0.165 mmol) was dissolved in a 1:1 mixture of THF/methanol (3 mL) and treated with 0.5 mL of 2N aqueous solution of LiOH. The reaction was kept under stirring in the dark at RT and it was monitored by TLC. After 3 h, the reaction mixture was concentrated, diluted with water and washed with Et_2O. The aqueous phase was treated with 1N aqueous HCl and extracted several times with EtOAc. The combined ethyl acetate organic phase was dried over anhydrous sodium sulphate and evaporated to afford the desired *N*-hydroxyindol-carboxylic acid product **3.126f** (48.0 mg, 0.167 mmol, >99% yield) without any further purification. ^{1}H NMR (DMSO-d_6) δ (ppm): 7.07 (s, 1H), 7.44-7.56 (m, 3H), 7.63 (dd, 1H, J = 8.7, 1.6 Hz), 7.71 (AA'/XX', 2H, J_{AX} = 8.6 Hz, $J_{AA'/XX'}$ = 1.5 Hz), 7.93 (d, 1H, J = 0.8 Hz). ^{13}C NMR (DMSO-d_6) δ (ppm): 105.11, 110.24, 120.07, 121.44, 124.08, 127.43, 128.38 (2C),

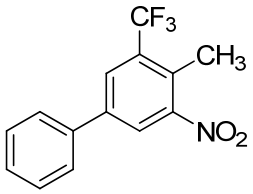
128.78 (2C), 131.42, 135.46, 139.70, 161.10. MS *m/z* 289 (^{37}Cl: M^+, 40%), 287 (^{35}Cl: M^+, 100%), 271 (^{35}Cl: M^+ –O, 85%). HPLC, t_R = 9.9 min

5-Iodo-2-methyl-1-nitro-3-(trifluoromethyl)benzene (3.147)



To H_2SO_4 (4.4 mL, 96%) stirring under a nitrogen atmosphere at 0-5 °C was added *N*-iodosuccinimide (1.27 g, 5.66 mmol) portionwise. The resulting dark red coloured mixture was stirred at 0-5 °C for 40 min., after which time 2-methyl-3-nitrobenzotrifluoride **3.90l** (1.00 g, 3.77 mmol), in H_2SO_4 (3.0 mL, 96%), was added dropwise over 1h. The solution was stirred at 5-10 °C for 5 h and warmed slowly to RT over 16 h. The resulting solution was poured carefully into ice and extracted with EtOAc. The extracts were combined, washed with saturated aqueous sodium hydrogensulphite and water. The organic phase was then dried over anhydrous sodium sulphate. After filtration, the solvent was removed in vacuo to give a crude which was purified by column chromatography over silica gel using *n*-hexane as the eluent, to give the iodo-derivative **3.147** (798 mg, 2.41 mmol, 64% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 2.50 (q, 3H, *J* = 1.5 Hz), 8.17 (d, 2H, *J* = 1.5 Hz).

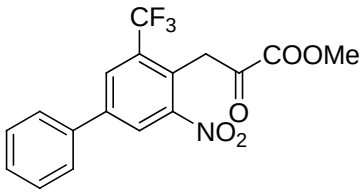
4-Methyl-3-nitro-5-(trifluoromethyl)biphenyl (3.148a)



Iodo-aryl derivative **3.147** (500 mg, 1.51 mmol) was placed in a vial together with phenylboronic acid (203 mg, 1.66 mmol), sodium carbonate (480 mg, 4.53 mmol), $Pd(OAc)_2$ (1.4 mg, 0.006 mmol), tetrabutylammonium bromide (486 mg, 1.51 mmol) and water (3 mL). The vial was sealed and heated under stirring at 150 °C in a microwave reactor for 5 min. The reaction mixture was then diluted with water and

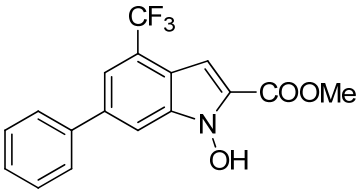
repeatedy extracted with EtOAc. The organic phase was dried and evaporated to afford a crude residue that was purified by column chromatography (*n*-hexane/EtOAc 9:1) to give pure **3.148a** (407 mg, 1.45 mmol, 96% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 2.60 (q, 3H, *J* = 1.5 Hz), 7.44-7.62 (m, 5H), 8.07 (d, 1H, *J* = 1.5 Hz), 8.11 (d, 1H, *J* = 1.5 Hz). MS *m/z* 281 (M^+, 58%), 264 (M^+ –OH, 100%).

Methyl 3-(3-nitro-5-(trifluoromethyl)biphenyl-4-yl)-2-oxopropanoate (3.149a)



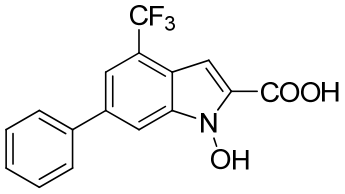
Potassium *tert*-butoxide (142 mg, 1.20 mmol) was dispersed in anhydrous diethyl ether (2.0 mL) at 0 °C under nitrogen, and dry methanol was added (0.2 mL) until complete dissolution was obtained. Next, dimethyl oxalate (142 mg, 1.20 mmol) was added and stirring was continued at 0 °C for 15 min. Finally, a solution of the nitrotoluene derivative **3.148a** (281 mg, 1.00 mmol) in dry Et_2O (1.5 mL) was slowly added at the same temperature and the resulting reddish suspension was then left under stirring at RT for 48 h. The mixture was diluted with EtOAc and 1N aqueous HCl. The combined organic phase was washed with saturated $NaHCO_3$ solution and brine, then dried over anhydrous sodium sulphate. Evaporation under vacuum of the organic solvent afforded a crude product which was purified by column chromatography over silica gel using *n*-hexane/EtOAc 9:1 as the eluent, to yield the nitroaryl-ketoester derivative **3.149a** (311 mg, 0.847 mmol, 85% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 3.98 (s, 3H), 4.71 (s, 2H), 7.48-7.65 (m, 5H), 8.19 (d, 1H, *J* = 1.8 Hz), 8.44 (d, 1H, *J* = 1.8 Hz); signals imputable to the enol form (~ 10%) δ (ppm): 3.95 (s, 3H), 6.19 (d, 1H, exchangeable, *J* = 1.6 Hz), 6.80-6.82 (m, 1H). MS *m/z* 367 (M^+, 5%), 308 (M^+ – $COOCH_3$, 17%), 280 (M^+ –$COOCH_3$ –CO, 100%).

Methyl 1-hydroxy-6-phenyl-4-(trifluoromethyl)-1*H*-indole-2-carboxylate (3.150a)



Ketoester **3.149a** (311 mg, 0.847 mmol) was dissolved in anhydrous DME (1.5 mL) and the resulting solution was added dropwise to a cooled (0 °C) solution of $SnCl_2 \cdot 2H_2O$ (428 mg, 1.90 mmol) in DME (1.5 mL) containing 4Å molecular sieves, previously activated for 18 hours at 130 °C in oven and cooled to RT in a desiccator over anhydrous calcium chloride. The reaction mixture was stirred under nitrogen at RT for 72 h, then it was diluted with water and repeatedly extracted with EtOAc. The combined organic phase was dried over anhydrous sodium sulphate and evaporated to afford a crude residue that was purified by column chromatography over silica gel using *n*-hexane/EtOAc 8:2 as the eluent, to give the *N*-hydroxyindol-ester derivative **3.150a** (114 mg, 0.340 mmol, 40% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 4.04 (s, 3H), 7.21 (qd, 1H, J = 1.6, 1.0 Hz), 7.40-7.54 (m, 3H), 7.66-7.71 (m, 3H), 7.91-7.94 (m, 1H), 10.52 (bs, 1H). ^{13}C NMR (acetone-d_6) δ (ppm): 52.85, 102.34, 111.30, 119.10 (q, J = 4.6 Hz, III), 122.55, 125.08 (q, J = 32.8 Hz), 124.44 (q, J = 270.4 Hz), 125.14, 127.51 (2C), 128.05, 129.13 (2C), 134.02, 135.30, 138.43, 140.16, 164.27. MS *m/z* 335 (M^+, 100%), 321 ($M^+ - CH_2$, 90%), 319 ($M^+ - O$, 7%), 305 ($M^+ - CH_2 - O$, 9%), 259 ($M^+ - CH_2 - H_2O - CO_2$, 81%), 190 ($M^+ - CH_2 - H_2O - CO_2 - CF_3$, 81%). HPLC, t_R 12.1 min.

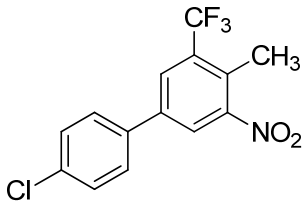
1-Hydroxy-6-phenyl-4-(trifluoromethyl)-1*H*-indole-2-carboxylic acid (3.139)



Methyl ester **3.150a** (14.0 mg, 0.0418 mmol) was dissolved in a 1:1 mixture of THF/methanol (0.42 mL) and treated with 0.13 mL of 2N aqueous solution of LiOH. The reaction was kept under stirring in the dark at RT and it was monitored by TLC.

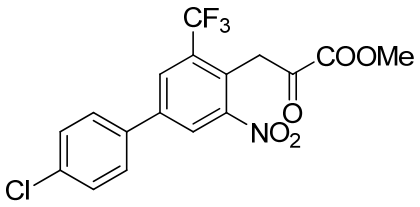
After 12 h, the reaction mixture was concentrated, diluted with water and washed with Et_2O. Then, the aqueous phase was treated with 1N aqueous HCl and extracted several times with EtOAc. The combined ethyl acetate organic phase was dried over anhydrous sodium sulphate and evaporated to afford the desired *N*-hydroxyindol-carboxylic acid product **3.139** (12.6 mg, 0.0392 mmol, 94% yield) without any further purification. ^{1}H NMR (acetone-d_6) δ (ppm): 7.20 (qd, 1H, J = 1.8, 0.7 Hz), 7.43-7.58 (m, 3H), 7.80-7.85 (m, 3H), 8.04-8.06 (m, 1H). ^{13}C NMR (acetone-d_6) δ (ppm): 103.43, 112.32, 117.22, 119.01 (q, J = 4.8 Hz), 123.81 (q, J = 33.0 Hz), 125.47 (q, J = 262.8 Hz), 128.07, 128.71 (2C), 129.53, 129.89 (2C), 133.64, 137.54, 138.52, 140.71, 161.45. MS *m/z* 321 (M^+, 100%), 305 (M^+ –O, 10%), 259 (M^+ –H_2O –CO_2, 41%), 190 (M^+ –H_2O –CO_2 –CF_3, 38%). HPLC, t_R = 10.5 min.

4'-Chloro-4-methyl-3-nitro-5-(trifluoromethyl)biphenyl (3.148b)



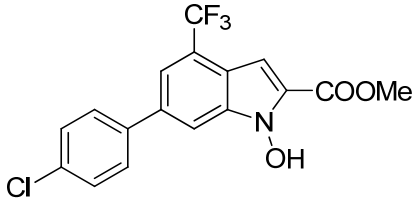
Iodo-aryl derivative **3.147** (500 mg, 1.51 mmol) was placed in a vial together with 4-chlorobenzeneboronic acid **3.129j** (260 mg, 1.66 mmol), sodium carbonate (480 mg, 4.53 mmol), $Pd(OAc)_2$ (1.4 mg, 0.0060 mmol), tetrabutylammonium bromide (486 mg, 1.51 mmol) and water (3 mL). The vial was sealed and heated under stirring at 150 °C in a microwave reactor for 5 min. The reaction mixture was then diluted with water and repeatedly extracted with EtOAc. The combined organic phase was dried over anhydrous sodium sulphate and evaporated to afford a crude residue that was purified by column chromatography over silica gel using *n*-hexane as the eluent, to give pure **3.148b** (260 mg, 0.824 mmol, 55% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 2.60 (q, 3H, J = 1.6 Hz), 7.45-7.56 (m, 4H), 8.03 (d, 1H, J = 2.0 Hz), 8.07 (d, 1H, J = 1.5 Hz).

Methyl 3-(4'-chloro-3-nitro-5-(trifluoromethyl)biphenyl-4-yl)-2-oxopropanoate (3.149b)



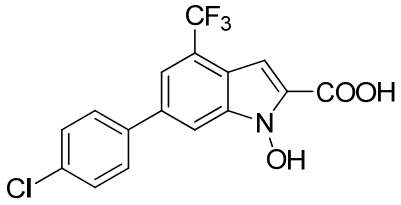
Potassium *tert*-butoxide (170 mg, 1.52 mmol) was dispersed in anhydrous diethyl ether (1.50 mL) at 0 °C under nitrogen, and dry methanol was added (0.05 mL) until complete dissolution was obtained. Next, dimethyl oxalate (180 mg, 1.52 mmol) was added and stirring was continued at 0 °C for 15 min. Finally, a solution of the nitrotoluene derivative **3.148b** (240 mg, 0.760 mmol) in dry Et_2O (0.50 mL) was slowly added at the same temperature and the resulting reddish suspension was then left under stirring at RT for 48 h. The mixture was diluted with EtOAc and 1N aqueous HCl. The combined organic phase was washed with saturated $NaHCO_3$ solution and brine, then dried over anhydrous sodium sulphate. Evaporation under vacuum of the organic solvent afforded a crude product which was purified by column chromatography over silica gel using *n*-hexane/EtOAc 9:1 as the eluent, to yield the nitroaryl-ketoester derivative **3.149b** (215 mg, 0.535 mmol, 70% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 3.97 (s, 3H), 4.71 (s, 2H), 7.46-7.60 (m, 4H), 8.15 (d, 1H, J = 1.8 Hz), 8.41 (d, 1H, J = 1.8 Hz); signals imputable to the enol form (~ 15%) δ (ppm): 3.96 (s, 3H), 6.23 (d, 1H, exchangeable, J = 1.6 Hz), 6.80 (dq, 1H, J = 1.8, 1.1 Hz), 8.08 (d, 1H, J = 1.8 Hz), 8.19 (d, 1H, J = 1.6 Hz).

Methyl 6-(4-chlorophenyl)-1-hydroxy-4-(trifluoromethyl)-1*H*-indole-2-carboxylate (3.150b)



Ketoester **3.149b** (100 mg, 0.249 mmol) was dissolved in anhydrous DME (0.6 mL) and the resulting solution was added dropwise to a cooled (0 °C) solution of $SnCl_2 \cdot 2H_2O$ (119 mg, 0.625 mmol) in DME (0.6 mL) containing 4Å molecular sieves, previously activated for 18 hours at 130 °C in oven and cooled to RT in a desiccator over anhydrous calcium chloride. The reaction mixture was stirred under nitrogen at RT overnight, then it was diluted with water and repeatedly extracted with EtOAc. The combined organic phase was dried over anhydrous sodium sulphate and evaporated to afford a crude residue that was purified by column chromatography over silica gel using *n*-hexane/EtOAc 8:2 as the eluent, to give the *N*-hydroxyindol-ester derivative **3.150b** (52.7 mg, 0.143 mmol, 57% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 4.04 (s, 3H), 7.21 (qd, 1H, J = 1.6, 1.1 Hz), 7.46 (AA'XX', 2H, J_{AX} = 8.8 Hz, $J_{AA'/XX'}$ = 2.2 Hz), 7.62 (AA'XX', 2H, J_{AX} = 8.8 Hz, $J_{AA'/XX'}$ = 2.2 Hz), 7.65-7.67 (m, 1H), 7.87-7.90 (m, 1H), 10.55 (bs, 1H).

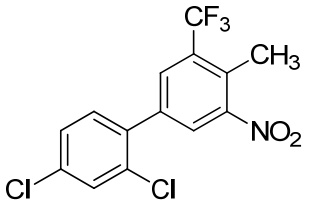
6-(4-Chlorophenyl)-1-hydroxy-4-(trifluoromethyl)-1*H*-indole-2-carboxylic acid (3.141)



Methyl ester **3.150b** (32.0 mg, 0.0866 mmol) was dissolved in a 1:1 mixture of THF/methanol (1.0 mL) and treated with 0.26 mL of 2N aqueous solution of LiOH. The reaction was kept under stirring in the dark at RT and it was monitored by TLC. After 3 h, the reaction mixture was concentrated, diluted with water and washed with Et_2O. Then, the aqueous phase was treated with 1N aqueous HCl and extracted several times with EtOAc. The combined ethyl acetate organic phase was dried over anhydrous sodium sulphate and evaporated to afford the desired *N*-hydroxyindol-carboxylic acid product **3.141** (30.5 mg, 0.0857 mmol, 99% yield) without any further purification. ^{1}H NMR (DMSO-d_6) δ (ppm): 7.00 (qd, 1H, J = 1.8, 0.7 Hz), 7.55 (d, 2H, J = 8.4 Hz), 7.76 (s, 1H), 7.84 (d, 2H, J = 8.6 Hz), 7.98 (s, 1H). ^{13}C NMR (DMSO-d_6) δ (ppm): 101.19,

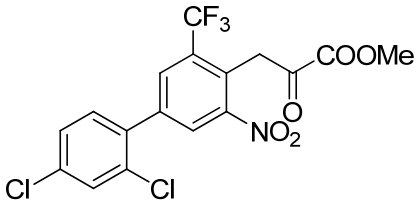
111.55, 115.81, 115.83, 117.43 (q, J = 5.5 Hz), 121.93 (q, J = 33.0 Hz), 124.38 (q, J = 271.9 Hz), 128.87 (2C), 128.98 (2C), 132.73, 134.82, 136.11, 137.92, 160.68. MS *m/z* 357 (^{37}Cl: M^+, 32%), 355 (^{35}Cl: M^+, 100%), 341 (^{37}Cl: M^+ –O, 10%), 339 (^{35}Cl: M^+ –O, 93%), 295 (^{37}Cl: M^+ –H_2O –CO_2, 36%), 293 (^{35}Cl: M^+ –H_2O –CO_2, 72%), 258 (M^+ – H_2O –CO_2 –Cl, 77%). HPLC, t_R = 11.0 min.

2,4-Dichloro-4'-methyl-3'-nitro-5'-(trifluoromethyl)biphenyl (3.148c)



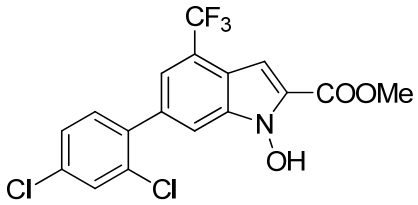
A solution of $Pd(OAc)_2$ (10 mg, 0.045 mmol) and triphenylphosphine (59.0 mg, 0.225 mmol) in absolute ethanol (3.4 mL) and anhydrous toluene (3.4 mL) was stirred at RT under nitrogen for 10 min. After that period, compound **3.147** (497 mg, 1.50 mmol), 3.4 mL of 2M aqueous Na_2CO_3, and 2,4-dichlorobenzeneboronic acid (458 mg, 2.40 mmol) were sequentially added. The resulting mixture was heated at 100 °C in a sealed vial under nitrogen overnight. After being cooled to RT, the mixture was diluted with water and extracted with EtOAc. The combined organic phase were dried and concentrated. The crude product was purified by flash chromatography (*n*-hexane) to yield **3.148c** (280 mg, 0.800 mmol, 53% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 2.62 (q, 3H, J = 1.5 Hz), 7.33 (d, 1H, J = 8.2 Hz), 7.38 (dd, 1H, J = 8.4, 2.0 Hz), 7.55 (d, 1H, J = 2.0 Hz), 7.92 (d, 1H, J = 1.8 Hz), 7.98 (d, 1H, J = 1.8 Hz).

Methyl 3-(2',4'-dichloro-3-nitro-5-(trifluoromethyl)biphenyl-4-yl)-2-oxopropanoate (3.149c)



Potassium *tert*-butoxide (96.0 mg, 0.857 mmol) was dispersed in anhydrous diethyl ether (1.0 mL) at 0 °C under nitrogen, and dry methanol was added (0.04 mL) until complete dissolution was obtained. Next, dimethyl oxalate (101 mg, 0.857 mmol) was added and stirring was continued at 0 °C for 15 min. Finally, a solution of the nitrotoluene derivative **3.148c** (250 mg, 0.714 mmol) in dry Et_2O (1.0 mL) was slowly added at the same temperature and the resulting reddish suspension was then left under stirring at RT for 48 h. The mixture was diluted with EtOAc and 1N aqueous HCl. The combined organic phase was washed with saturated $NaHCO_3$ solution and brine, then dried over anhydrous sodium sulphate. Evaporation under vacuum of the organic solvent afforded a crude product which was purified by column chromatography over silica gel using *n*-hexane/EtOAc 9:1 as the eluent, to yield the nitroaryl-ketoester derivative **3.149c** (88.0 mg, 0.202 mmol, 28% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 3.98 (s, 3H), 4.74 (s, 2H), 7.33 (d, 1H, *J* = 8.4 Hz), 7.41 (dd, 1H, *J* = 8.2, 2.0 Hz), 7.57 (d, 1H, *J* = 1.8 Hz), 8.06 (d, 1H, *J* = 1.6 Hz), 8.33 (d, 1H, *J* = 1.8 Hz); signals imputable to the enol form (~ 30%) δ (ppm): 3.96 (s, 3H), 7.10 (s, 1H).

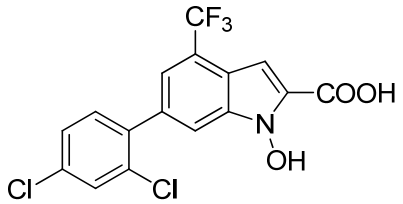
Methyl 6-(2,4-dichlorophenyl)-1-hydroxy-4-(trifluoromethyl)-1*H*-indole-2-carboxylate (3.150c)



Ketoester **3.149c** (85.0 mg, 0.194 mmol) was dissolved in anhydrous DME (0.75 mL) and the resulting solution was added dropwise to a cooled (0 °C) solution of $SnCl_2{\cdot}2H_2O$ (92.0 mg, 0.487 mmol) in DME (0.75 mL) containing 4Å molecular sieves, previously activated for 18 hours at 130 °C in oven and cooled to RT in a desiccator over anhydrous calcium chloride. The reaction mixture was stirred under nitrogen at RT overnight, then it was diluted with water and repeatedly extracted with EtOAc. The combined organic phase was dried over anhydrous sodium sulphate and evaporated to afford a crude residue that was purified by column chromatography over silica gel using

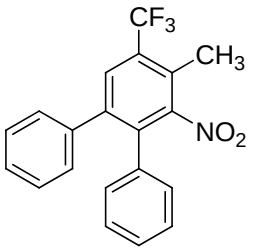
n-hexane/EtOAc 9:1 as the eluent, to give the *N*-hydroxyindol-ester derivative **3.150c** (18.0 mg, 0.0445 mmol, 23% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 4.05 (s, 3H), 7.23 (qd, 1H, *J* = 1.6, 0.9 Hz), 7.35 (d, 1H, *J* = 1.1 Hz), 7.49-7.55 (m, 2H), 7.78 (t, 1H, *J* = 1.1 Hz), 10.53 (bs, 1H).

6-(2,4-Dichlorophenyl)-1-hydroxy-4-(trifluoromethyl)-1*H*-indole-2-carboxylic acid (3.142)



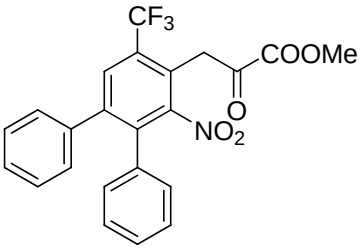
Methyl ester **3.150c** (18.0 mg, 0.0445 mmol) was dissolved in a 1:1 mixture of THF/methanol (0.50 mL) and treated with 0.14 mL of 2N aqueous solution of LiOH. The reaction was kept under stirring in the dark at RT and it was monitored by TLC. After 3 h, the reaction mixture was concentrated, diluted with water and washed with Et_2O. Then, the aqueous phase was treated with 1N aqueous HCl and extracted several times with EtOAc. The combined ethyl acetate organic phase was dried over anhydrous sodium sulphate and evaporated to afford the desired *N*-hydroxyindol-carboxylic acid product **3.142** (15.3 mg, 0.0392 mmol, 88% yield) without any further purification. ^{1}H NMR (acetone-d_6) δ (ppm): 7.20 (qd, 1H, *J* = 1.6, 0.8 Hz), 7.53 (dd, 1H, *J* = 8.6, 2.0 Hz), 7.58-7.63 (m, 2H), 7.68 (d, 1H, *J* = 1.8 Hz), 7.89 (s, 1H). ^{13}C NMR (acetone-d_6) δ (ppm): 103.06, 115.42, 117.49, 120.90 (q, *J* = 4.8 Hz), 123.10 (q, *J* = 32.5 Hz), 125.41 (q, *J* = 272.9 Hz), 128.47, 129.17, 130.31, 133.79, 133.92, 134.79, 135.05, 136.45, 139.07, 161.70. MS *m/z* 393 ($^{37/37}$Cl: M^+, 11%), 391 ($^{37/35}$Cl: M^+ , 64%), 389 ($^{35/35}$Cl: M^+, 100%), 377 ($^{37/37}$Cl: M^+ –O, 4%), 375 ($^{37/35}$Cl: M^+ –O, 24%), 373 ($^{35/35}$Cl: M^+ –O, 37%), 331 ($^{37/37}$Cl: M^+ –H_2O –CO_2, 13%), 329 ($^{37/35}$Cl: M^+ –H_2O –CO_2, 55%), 327 ($^{35/35}$Cl: M^+ –H_2O –CO_2, 82%), 294 (^{37}Cl: M^+ –H_2O –CO_2 –Cl, 38%), 292 (^{35}Cl: M^+ –H_2O –CO_2 –Cl, 92%). HPLC, t_R = 11.9 min.

6-Phenyl-3-methyl-2-nitro-4-(trifluoromethyl)biphenyl (3.152)



Commercially available 3,4-dichloro-2-nitro-6-trifluoromethyltoluene **3.151** (274 mg, 1.00 mmol) was placed in a vial together with phenylboronic acid (366 mg, 3.00 mmol), sodium carbonate (636 mg, 6.00 mmol), $Pd(OAc)_2$ (2.5 mg, 0.01 mmol), tetrabutylammonium bromide (660 mg, 2.00 mmol) and water (3 mL). The vial was sealed and heated under stirring at 175 °C in a microwave reactor for 10 min. The reaction mixture was then diluted with water and repeatedly extracted with EtOAc. The combined organic phase was dried over anhydrous sodium sulphate and evaporated to afford a crude residue that was purified by column chromatography over silica gel using *n*-hexane/EtOAc 95:5 as the eluent, to give pure **3.152** (296 mg, 0.828 mmol, 83% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 2.47 (q, 3H, J = 1.5 Hz), 7.01-7.11 (m, 5H), 7.16-7.28 (m, 5H), 7.82 (s, 1H).

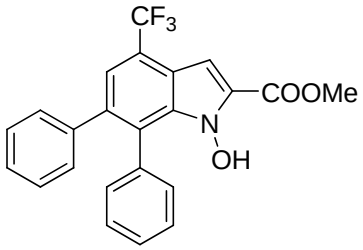
Methyl 3-(6-phenyl-2-nitro-4-(trifluoromethyl)biphenyl-3-yl)-2-oxopropanoate (3.153)



A stirred suspension of sodium hydride (60% dispersion in mineral oil, 130 mg, 3.25 mmol) in 5 mL of anhydrous DMF cooled to –15 °C under nitrogen was treated dropwise with a solution containing the nitrotoluene precursor **3.152** (290 mg, 0.812 mmol) and dimethyl oxalate (479 mg, 4.06 mmol) in 5 mL of anhydrous DMF. The

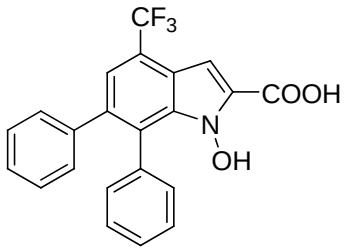
mixture was left under stirring at the same temperature for 10 minutes and then it was slowly warmed to room temperature. The mixture was then left under stirring at RT for 12 h. Once the disappearance of the precursor was verified by TLC, the reaction mixture was slowly poured in an ice-water mixture; the water phase was acidified with 1N aqueous HCl and extracted several times with EtOAc. The combined organic phase was washed with saturated $NaHCO_3$ solution and brine, then dried over anhydrous sodium sulphate. Evaporation under vacuum of the organic solvent afforded a crude product which was purified by column chromatography over silica gel using *n*-hexane/EtOAc 8:2 as the eluent, to yield the nitroaryl-ketoester derivative **3.153** (342 mg, 0.771 mmol, 95% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 3.95 (s, 3H), 4.42 (s, 2H), 7.05-7.09 (m, 5H), 7.21-7.25 (m, 5H), 7.91 (s, 1H); signals imputable to the enol form (~ 11%) δ (ppm): 3.92 (s, 3H), 6.15 (d, 1H, exchangeable, J = 1.2 Hz), 6.54-6.56 (m, 1H).

Methyl 1-hydroxy-6,7-diphenyl-4-(trifluoromethyl)-1*H*-indole-2-carboxylate (3.154)



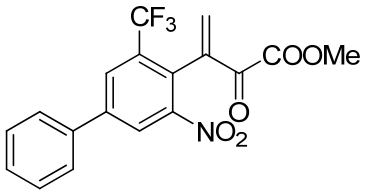
A stirred suspension of Zn dust (82.0 mg, 1.25 mmol) and iodine (16.0 mg, 0.0625 mmol) in anhydrous THF (1 mL) was heated to reflux for approximately 3 h and then allowed to cool to RT. A solution of 1N aqueous NH_4Cl (2.0 mL) and ketoester **3.153** (111 mg, 0.270 mmol) in anhydrous THF (1 mL) was added to the activated Zn suspension and stirring was continued until the complete consumption of starting material in the absence of light at RT. The crude reaction mixture was purified directly by column chromatography over silica gel using *n*-hexane/EtOAc 8:2 as the eluent, to give the *N*-hydroxyindole **3.154** (16.0 mg, 0.0389 mmol, 14% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 4.00 (s, 3H), 7.09-7.31 (m, 11H), 7.54 (s, 1H), 10.26 (bs, 1H).

1-Hydroxy-6,7-diphenyl-4-(trifluoromethyl)-1*H*-indole-2-carboxylic acid (3.140)



Methyl ester **3.154** (12.0 mg, 0.0292 mmol) was dissolved in a 1:1 mixture of THF/methanol (1.0 mL) and treated with 0.1 mL of 2N aqueous solution of LiOH. The reaction was kept under stirring in the dark at RT and it was monitored by TLC. After 2 h, the reaction mixture was concentrated, diluted with water and washed with Et_2O. Then, the aqueous phase was treated with 1N aqueous HCl and extracted several times with EtOAc. The combined ethyl acetate organic phase was dried over anhydrous sodium sulphate and evaporated to afford the desired *N*-hydroxyindol-carboxylic acid product **3.140** (10.0 mg, 0.0252 mmol, 86% yield) without any further purification. ^{1}H NMR (DMSO-d_6) δ (ppm): 7.02-7.22 (m, 11H), 7.41 (d, 1H, J = 0.8 Hz). ^{13}C NMR (DMSO-d_6) δ (ppm): 101.08, 116.65, 119.73, 120.40 (q, J = 4.0 Hz), 123.92 (q, J = 32.3 Hz), 124.09 (q, J = 269.3 Hz), 126.48, 126.70 (2C), 127.59 (2C), 128.67, 129.36, 129.87 (2C), 130.85 (2C), 133.20, 135.62, 137.12, 140.06, 160.60. MS *m/z* 397 (M^+, 14%), 381 (M^+ –O, 38%), 335 (M^+ –H_2O –CO_2, 100%). HPLC, t_R = 11.2 min.

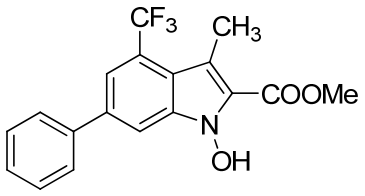
Methyl 3-(3-nitro-5-(trifluoromethyl)biphenyl-4-yl)-2-oxobut-3-enoate (3.157a)



Potassium *tert*-butoxide (320 mg, 2.85 mmol) was dispersed in anhydrous diethyl ether (4.6 mL) at 0 °C under nitrogen, and dry methanol was added (0.5 mL) until complete dissolution was obtained. Next, dimethyl oxalate (336 mg, 2.85 mmol) was added and stirring was continued at 0 °C for 15 min. Finally, a solution of the nitrotoluene derivative **3.148a** (400 mg, 1.42 mmol) in dry Et_2O (1.8 mL) was slowly

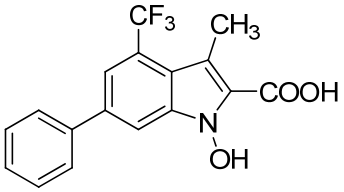
added at the same temperature and the resulting reddish suspension was then left under stirring at RT for 48 h. The reaction mixture was then filtered to obtain a reddish solid, corresponding to the potassium salt of the nitroaryl-ketoester derivative **3.149a** (304 mg). To a solution of derivative **3.149a** (304 mg) in THF (25 mL) at 0 °C was added dimethylmethylene ammonium chloride (211 mg, 2.25 mmol) and the reaction mixture stirred for 12 h at RT. After cooling to 0 °C, the reaction mixture was quenched with saturated aqueous NH_4Cl solution, diluted with EtOAc, washed with H_2O and dried over anhydrous sodium sulphate. After concentration, the residue was subjected to flash column chromatography using *n*-hexane/EtOAc 9:1 as the eluent to give the α,β-unsaturated ketoester **3.157a** (159 mg, 0.419 mmol, 2 steps: 30% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 3.93 (s, 3H), 6.18 (s, 1H), 6.83 (s, 1H), 7.50-7.60 (m, 3H), 7.63-7.68 (m, 2H), 8.22 (d, 1H, J = 1.8 Hz), 8.46 (d, 1H, J = 1.8 Hz).

Methyl 1-hydroxy-3-methyl-6-phenyl-4-(trifluoromethyl)-1*H*-indole-2-carboxylate (3.158a)



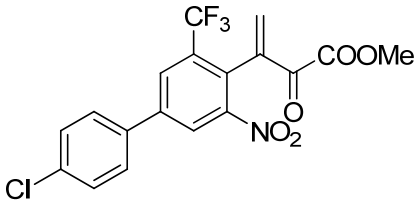
Triethylsilane (0.13 mL, 92.0 mg, 0.792 mmol) and α,β-unsaturated ketoester **3.157a** (60.0 mg, 0.158 mmol) were added at room temperature to a solution of $SnCl_2 \cdot 2H_2O$ (89.3 mg, 0.396 mmol) in DME (1.0 mL) containing 4Å molecular sieves, previously activated for 18 hours at 130 °C in oven and cooled to RT in a desiccator over anhydrous calcium chloride. The reaction mixture was stirred under nitrogen at 40 °C for 3 h, then it was diluted with water and repeatedly extracted with EtOAc. The combined organic phase was dried over anhydrous sodium sulphate and evaporated to afford a crude residue that was purified by column chromatography over silica gel using *n*-hexane/EtOAc 85:15 as the eluent, to give the *N*-hydroxyindol-ester derivative **3.158a** (8.3 mg, 0.024 mmol, 15% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 2.68 (s, 3H), 4.07 (s, 3H), 7.36-7.54 (m, 3H), 7.66-7.78 (m, 3H), 7.90-7.94 (m, 1H), 10.72 (bs, 1H).

1-Hydroxy-3-methyl-6-phenyl-4-(trifluoromethyl)-1*H*-indole-2-carboxylic acid (3.155)



Methyl ester **3.158a** (8.3 mg, 0.0238 mmol) was dissolved in a 1:1 mixture of THF/methanol (0.4 mL) and treated with 0.10 mL of 2N aqueous solution of LiOH. The reaction was kept under stirring in the dark at RT and it was monitored by TLC. After 4 h, the reaction mixture was concentrated, diluted with water and washed with Et_2O. Then, the aqueous phase was treated with 1N aqueous HCl and extracted several times with EtOAc. The combined ethyl acetate organic phase was dried over anhydrous sodium sulphate and evaporated to afford the desired *N*-hydroxyindol-carboxylic acid product **3.155** (4.5 mg, 0.0134 mmol, 56% yield) without any further purification. ^{1}H NMR (acetone-d_6) δ (ppm): 2.67 (q, 3H, J = 1.8 Hz), 7.38-7.58 (m, 3H), 7.78-7.84 (m, 3H), 8.03 (dq, 1H, J = 1.5, 0.7 Hz). ^{13}C NMR (DMSO-d_6) δ (ppm): 10.63 (q, J = 5.2 Hz), 111.43, 111.63, 116.18, 117.76 (q, J = 4.6 Hz), 121.60 (q, J = 32.0 Hz), 124.37 (q, J = 269.9 Hz), 126.96 (2C), 127.67, 127.88, 129.16 (2C), 135.50, 136.50, 139.08, 162.02. MS *m/z* 335 (M^+, 18%), 320 (M^+ –CH_3, 18%), 319 (M^+ –O, 100%), 318 (M^+ –OH, 6%), 291 (M^+ –CO_2, 5%), 275 (M^+ –CO_2 –H_2O, 46%).

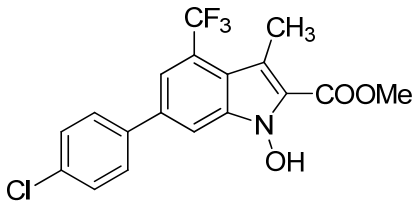
Methyl 3-(4'-chloro-3-nitro-5-(trifluoromethyl)biphenyl-4-yl)-2-oxobut-3-enoate (3.157b)



Potassium *tert*-butoxide (224 mg, 2.00 mmol) was dispersed in anhydrous diethyl ether (2.0 mL) at 0 °C under nitrogen, and dry methanol was added (0.05 mL) until complete dissolution was obtained. Next, dimethyl oxalate (236 mg, 2.00 mmol) was

added and stirring was continued at 0 °C for 15 min. Finally, a solution of the nitrotoluene derivative **3.148b** (316 mg, 1.00 mmol) in dry Et_2O (2.0 mL) was slowly added at the same temperature and the resulting reddish suspension was then left under stirring at RT for 48 h. The reaction mixture was then filtered to obtain a reddish solid, corresponding to the potassium salt of the nitroaryl-ketoester derivative **3.149b** (331 mg). To a solution of derivative **3.149b** (331 mg) in THF (25 mL) at 0 °C was added dimethylmethylene ammonium chloride (211 mg, 2.25 mmol) and the reaction mixture stirred for 12 h at RT. After cooling to 0 °C, the reaction mixture was quenched with saturated aqueous NH_4Cl solution, diluted with EtOAc, washed with H_2O and dried over anhydrous sodium sulphate. After concentration, the residue was subjected to flash column chromatography using *n*-hexane/EtOAc 9:1 as the eluent to give the α,β-unsaturated ketoester **3.157b** (84.0 mg, 0.203 mmol, 2 steps: 20% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 3.93 (s, 3H), 6.17 (s, 1H), 6.83 (s, 1H), 7.49-7.62 (m, 4H), 8.18 (d, 1H, J = 1.8 Hz), 8.42 (d, 1H, J = 1.8 Hz).

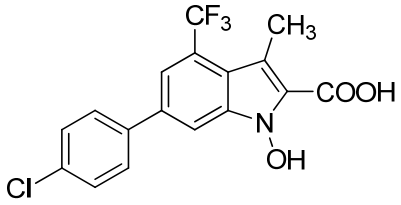
Methyl 6-(4-chlorophenyl)-1-hydroxy-3-methyl-4-(trifluoromethyl)-1*H*-indole-2-carboxylate (3.158b)



Triethylsilane (0.14 mL, 105 mg, 0.900 mmol) and α,β-unsaturated ketoester **3.157b** (75.0 mg, 0.181 mmol) were added at room temperature to a solution of $SnCl_2{\cdot}2H_2O$ (102 mg, 0.452 mmol) in DME (1.13 mL) containing 4Å molecular sieves, previously activated for 18 hours at 130 °C in oven and cooled to RT in a desiccator over anhydrous calcium chloride. The reaction mixture was stirred under nitrogen at 40 °C for 3 h, then it was diluted with water and repeatedly extracted with EtOAc. The combined organic phase was dried over anhydrous sodium sulphate and evaporated to afford a crude residue that was purified by column chromatography over silica gel using *n*-hexane/EtOAc 9:1 as the eluent, to give the *N*-hydroxyindol-ester derivative **3.158b**

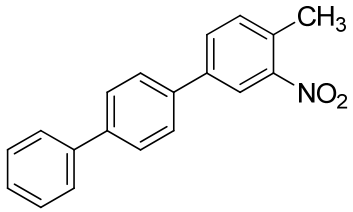
(15.0 mg, 0.0391 mmol, 22% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 2.67 (q, 3H, J = 1.6 Hz), 4.08 (s, 3H), 7.45 (AA'XX', 2H, J_{AX} = 8.6 Hz, $J_{AA'/XX'}$ = 2.3 Hz), 7.62 (AA'XX', 2H, J_{AX} = 8.6 Hz, $J_{AA'/XX'}$ = 2.2 Hz), 7.70 (s, 1H), 7.89 (s, 1H), 10.74 (bs, 1H).

6-(4-Chlorophenyl)-1-hydroxy-3-methyl-4-(trifluoromethyl)-1*H*-indole-2-carboxylic acid (3.156)



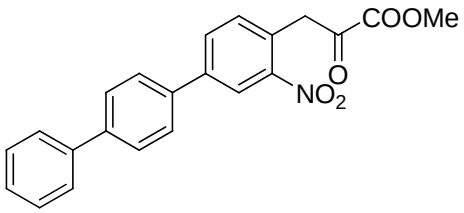
Methyl ester **3.158b** (15.0 mg, 0.0391 mmol) was dissolved in a 1:1 mixture of THF/methanol (1.0 mL) and treated with 0.12 mL of 2N aqueous solution of LiOH. The reaction was kept under stirring in the dark at RT and it was monitored by TLC. After 3 h, the reaction mixture was concentrated, diluted with water and washed with Et_2O. Then, the aqueous phase was treated with 1N aqueous HCl and extracted several times with EtOAc. The combined ethyl acetate organic phase was dried over anhydrous sodium sulphate and evaporated to afford the desired *N*-hydroxyindol-carboxylic acid product **3.156** (14.0 mg, 0.0379 mmol, 97% yield) without any further purification. ^{1}H NMR (acetone-d_6) δ (ppm): 2.67 (q, 3H, J = 1.6 Hz), 7.55 (AA′XX′, 2H, J_{AX} = 8.6 Hz, $J_{AA'/XX'}$ = 2.4 Hz), 7.81 (s, 1H), 7.85 (AA′XX′, 2H, J_{AX} = 8.8 Hz, $J_{AA'/XX'}$ = 2.2 Hz), 8.04 (s, 1H). ^{13}C NMR (acetone-d_6) δ (ppm): 11.24 (q, J = 5.5 Hz), 112.62, 114.86, 117.81, 119.04 (q, J = 6.4 Hz), 123.63 (q, J = 33.6 Hz), 125.35 (q, J = 271.0 Hz), 127.12, 129.63 (2C), 129.89 (2C), 134.27, 136.37, 137.61, 139.39, 163.04. MS *m/z* 371 (^{37}Cl: M^+, 20%), 369 (^{35}Cl: M^+, 58%), 355 (^{37}Cl: M^+ –O, 37%), 353 (^{35}Cl: M^+ –O, 100%), 309 (^{37}Cl: M^+ –H_2O –CO_2, 46%), 307 (^{35}Cl: M^+ –H_2O –CO_2, 74%). HPLC, t_R 11.6 min.

4'-Phenyl-4-methyl-3-nitrobiphenyl (3.162a)



Commercially available 4-bromo-2-nitrotoluene **3.90d** (500 mg, 2.31 mmol) was placed in a vial together with biphenyl-4-boronic acid **3.161a** (503 mg, 2.54 mmol), sodium carbonate (735 mg, 6.93 mmol), $Pd(OAc)_2$ (3.8 mg, 0.017 mmol), tetrabutylammonium bromide (746 mg, 2.31 mmol) and water (3 mL). The vial was sealed and heated under stirring at 150 °C in a microwave reactor for 5 min. The reaction mixture was then diluted with water and repeatedly extracted with EtOAc. The combined organic phase was dried over anhydrous sodium sulphate and evaporated to afford a crude residue that was purified by column chromatography over silica gel using *n*-hexane/EtOAc 95:5 as the eluent, to give pure **3.162a** (558 mg, 1.93 .mmol, 84% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 2.66 (s, 3H), 7.38-7.52 (m, 4H), 7.65 (AA'XX', 2H, J_{AX} = 7.0 Hz, $J_{AA'/XX'}$ = 1.5 Hz), 7.70 (s, 4H), 7.78 (dd, 1H, J = 8.0, 1.9 Hz), 8.26 (d, 1H, J = 2.0 Hz).

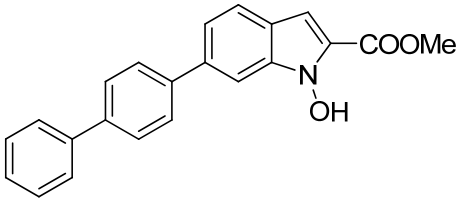
Methyl 3-(4'-phenyl-3-nitrobiphenyl-4-yl)-2-oxopropanoate (3.163a)



A stirred suspension of sodium hydride (60% dispersion in mineral oil, 304 mg, 7.60 mmol) in 5.3 mL of anhydrous DMF cooled to –15 °C under nitrogen was treated dropwise with a solution containing the nitrotoluene precursor **3.162a** (550 mg, 1.90 mmol) and dimethyl oxalate (1.12 g, 9.50 mmol) in 3.6 mL of anhydrous DMF. The mixture was left under stirring at the same temperature for 10 minutes and then it was slowly warmed to room temperature. The mixture was then left under stirring at RT for

5.5h. Once the disappearance of the precursor was verified by TLC, the reaction mixture was slowly poured in an ice-water mixture; the water phase was acidified with 1N aqueous HCl and extracted several times with EtOAc. The combined organic phase was washed with saturated $NaHCO_3$ solution and brine, then dried over anhydrous sodium sulphate. Evaporation under vacuum of the organic solvent afforded a crude product which was purified by column chromatography over silica gel using *n*-hexane/EtOAc 6:4 as the eluent, to yield the nitroaryl-ketoester derivative **3.163a** (555 mg, 1.48 mmol, 78% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 3.96 (s, 3H), 4.59 (s, 2H), 7.38-7.53 (m, 4H), 7.65 (AA'XX', 2H, J_{AX} = 6.8 Hz, $J_{AA'/XX'}$ = 1.8 Hz), 7.72 (s, 4H), 7.90 (dd, 1H, *J* = 8.0, 1.9 Hz), 8.45 (d, 1H, *J* = 1.8 Hz); signals imputable to the enol form (~ 40%) δ (ppm): 3.97 (s, 3H), 6.70 (d, 1H, exchangeable, *J* = 1.5 Hz), 6.99 (d, 1H, *J* = 1.3 Hz), 7.72 (s, 4H), 8.19 (d, 1H, *J* = 2.0 Hz), 8.36 (d, 1H, *J* = 8.4 Hz).

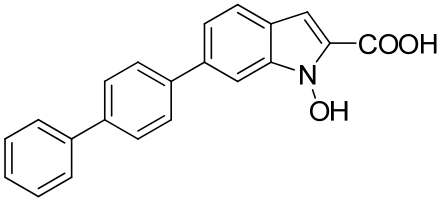
Methyl 6-(biphenyl-4-yl)-1-hydroxy-1*H*-indole-2-carboxylate (3.164a)



Triethylamine (0.28 mL, 0.20 g, 2.0 mmol) was added dropwise to a stirred solution of $SnCl_2 \cdot 2H_2O$ (135 mg, 0.60 mmol) and PhSH (0.18 mL, 0.20 g, 1.8 mmol) in acetonitrile (3.1 mL) at room temperature to generate a yellow precipitate over a period of 5 min. Then, **3.163a** (150 mg, 0.400 mmol) was transferred to the suspension and stirred at room temperature for 5 more min. After complete consumption of the starting material, the mixture was diluted with water and repeatedly extracted with EtOAc. The combined organic phase was dried over anhydrous sodium sulphate, diluted with toluene and a catalytic amount of *p*-toluensulfonic acid was added. Then it was evaporated to afford a crude residue that was purified by column chromatography over silica gel using *n*-hexane/EtOAc 7:3 as the eluent, to give a mixture (93.1 mg) of the *N*-hydroxyindol-ester derivative **3.164a** (80%, 74.5 mg, 0.217 mmol, 54% yield) with its indole analogue (20%) . ^{1}H NMR ($CDCl_3$) δ (ppm): 4.01 (s, 3H), 7.06 (s, 1H), 7.33-7.51

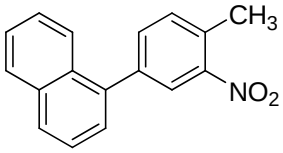
(m, 4H), 7.65-7.80 (m, 8H), 10.31 (bs, 1H); signals imputable to the indole (~ 20%) δ (ppm): 3.97 (s, 3H); 7.18 (s, 1H).

6-(Biphenyl-4-yl)-1-hydroxy-1*H*-indole-2-carboxylic acid (3.159a)



Methyl ester **3.164a** (50.0 mg, 0.146 mmol) was dissolved in a 1:1 mixture of THF/methanol (1.5 mL) and treated with 0.44 mL of 2N aqueous solution of LiOH. The reaction was kept under stirring in the dark at RT and it was monitored by TLC. After 5h, the reaction mixture was concentrated, diluted with water and washed with Et_2O. Then, the aqueous phase was treated with 1N aqueous HCl and extracted several times with EtOAc. The combined ethyl acetate organic phase was dried over anhydrous sodium sulphate and evaporated to afford the desired *N*-hydroxyindol-carboxylic acid product **3.159a** (44.5 mg, 0.135 mmol, 93% yield) without any further purification. ^{1}H NMR (acetone-d_6) δ (ppm): 7.16 (d, 1H, J = 0.7 Hz), 7.34-7.56 (m, 3H), 7.72-7.90 (m, 9H); signals imputable to the indole (~ 10%) δ (ppm): 7.21 (s, 1H). ^{13}C NMR (acetone-d_6) δ (ppm): 106.10, 108.23, 121.37, 122.12, 123.78, 126.29, 127.65 (2C), 128.18, 128.26 (2C), 128.51 (2C), 129.77 (2C), 137.54, 138.85, 140.84, 141.33, 141.44, 162.40. MS *m/z* 329 (M^+, 7%), 313 (M^+ –O, 100%), 267 (M^+ –OH –COOH, 41%), 190 (M^+ – OH –COOH –C_6H_5, 27%). HPLC, t_R = 10.4 min.

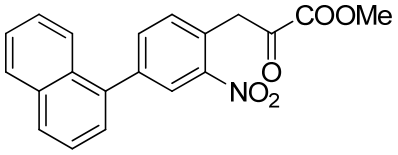
1-(4-Methyl-3-nitrophenyl)naphthalene (3.162b)



Commercially available 4-bromo-2-nitrotoluene **3.90d** (570 mg, 2.64 mmol) was placed in a vial together with 1-naphtaleneboronic acid **3.161b** (500 mg, 2.91 mmol), sodium carbonate (839 mg, 7.92 mmol), $Pd(OAc)_2$ (2.4 mg, 0.011 mmol),

tetrabutylammonium bromide (852 mg, 2.64 mmol) and water (3 mL). The vial was sealed and heated under stirring at 150 °C in a microwave reactor for 5 min. The reaction mixture was then diluted with water and repeatedly extracted with EtOAc. The combined organic phase was dried over anhydrous sodium sulphate and evaporated to afford a crude residue that was purified by column chromatography over silica gel using *n*-hexane/EtOAc 95:5 as the eluent, to give pure **3.162b** (642 mg, 2.44 mmol, 92% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 2.71 (s, 3H), 7.40-7.58 (m, 5H), 7.65 (dd, 1H, *J* = 7.9, 1.8 Hz), 7.81 (ddd, 1H, *J* = 8.2, 1.5, 0.7 Hz), 7.89-7.96 (m, 2H), 8.12 (d, 1H, *J* = 1.6 Hz).

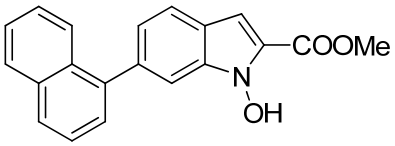
Methyl 3-(4-(naphthalen-1-yl)-2-nitrophenyl)-2-oxopropanoate (3.163b)



A stirred suspension of sodium hydride (60% dispersion in mineral oil, 377 mg, 9.43 mmol) in 6.6 mL of anhydrous DMF cooled to –15 °C under nitrogen was treated dropwise with a solution containing the nitrotoluene precursor **3.162b** (620 mg, 2.36 mmol) and dimethyl oxalate (1.39 g, 11.8 mmol) in 4.4 mL of anhydrous DMF. The mixture was left under stirring at the same temperature for 10 minutes and then it was slowly warmed to room temperature. The mixture was then left under stirring at RT for 12 h. Once the disappearance of the precursor was verified by TLC, the reaction mixture was slowly poured in an ice-water mixture; the water phase was acidified with 1N aqueous HCl and extracted several times with EtOAc. The combined organic phase was washed with saturated $NaHCO_3$ solution and brine, then dried over anhydrous sodium sulphate. Evaporation under vacuum of the organic solvent afforded a crude product which was purified by column chromatography over silica gel using *n*-hexane/EtOAc 8:2 as the eluent, to yield the nitroaryl-ketoester derivative **3.163b** (526 mg, 1.51 mmol, 64% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 3.98 (s, 3H), 4.64 (s, 2H), 7.42-7.60 (m, 5H), 7.74-7.88 (m, 2H), 7.90-7.98 (m, 2H), 8.33 (d, 1H, *J* = 1.8 Hz); signals imputable to the

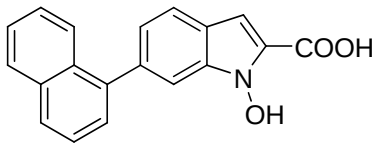
enol form (~ 25%) δ (ppm): 6.73 (d, 1H, exchangeable, *J* = 1.3 Hz), 7.03 (bs, 1H), 8.06 (d, 1H, *J* = 1.8 Hz), 8.39 (d, 1H, *J* = 8.2 Hz).

Methyl 1-hydroxy-6-(naphthalen-1-yl)-1*H*-indole-2-carboxylate (3.164b)



Triethylamine (0.30 mL, 0.22 g, 2.1 mmol) was added dropwise to a stirred solution of $SnCl_2 \cdot 2H_2O$ (136 mg, 0.601 mmol) and PhSH (0.20 mL, 0.21 g, 1.9 mmol) in acetonitrile (3.4 mL) at room temperature to generate a yellow precipitate over a period of 5 min. Then, **3.163b** (150 mg, 0.429 mmol) was transferred to the suspension and stirred at room temperature for 5 more min. After complete consumption of the starting material, the mixture was diluted with water and repeatedly extracted with EtOAc. The combined organic phase was dried over anhydrous sodium sulphate, diluted with toluene and a catalytic amount of *p*-toluensulfonic acid was added. Then it was evaporated to afford a crude residue that was purified by column chromatography over silica gel using *n*-hexane/EtOAc 8:2 as the eluent, to give a mixture (104 mg) of the *N*-hydroxyindol-ester derivative **3.164b** (91%, 94.6 mg, 0.298 mmol, 70% yield) with its indole analogue (9%) ^{1}H NMR ($CDCl_3$) δ (ppm):4.02 (s, 3H), 7.12 (d, 1H, *J* = 0.9 Hz), 7.29 (dd, 1H, *J* = 8.3, 1.6 Hz), 7.39-7.59 (m, 4H), 7.67 (t, 1H, *J* = 0.6 Hz), 7.73 (dd, 1H, *J* = 8.4, 0.7 Hz), 7.86-7.98 (m, 3H), 10.26 (bs, 1H); signals imputable to the indole (~ 9%) δ (ppm): 3.98 (s, 3H), 7.22 (s, 1H), 11.05 (bs, 1H).

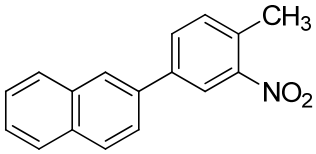
1-Hydroxy-6-(naphthalen-1-yl)-1*H*-indole-2-carboxylic acid (3.159b)



Methyl ester **3.164b** (93.0 mg, 0.293 mmol) was dissolved in a 1:1 mixture of THF/methanol (2.9 mL) and treated with 0.9 mL of 2N aqueous solution of LiOH. The reaction was kept under stirring in the dark at RT and it was monitored by TLC. After

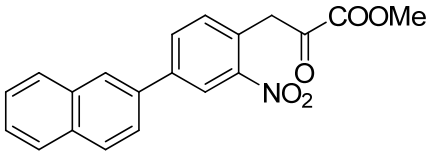
1.5 h, the reaction mixture was concentrated, diluted with water and washed with Et_2O. Then, the aqueous phase was treated with 1N aqueous HCl and extracted several times with EtOAc. The combined ethyl acetate organic phase was dried over anhydrous sodium sulphate and evaporated to afford the desired *N*-hydroxyindol-carboxylic acid product **3.159b** (72.9 mg, 0.240 mmol, 82% yield) without any further purification. ^{1}H NMR (DMSO-d_6) δ (ppm): 7.11 (d, 1H, J = 0.9 Hz), 7.21 (dd, 1H, J = 8.2, 1.6 Hz), 7.45-7.64 (m, 5H), 7.77 (dd, 1H, J = 8.2, 0.6 Hz), 7.86 (dd, 1H, J = 7.9, 1.3 Hz), 7.97 (d, 1H, J = 8.6 Hz), 8.02 (dd, 1H, J = 7.9, 1.6 Hz); signals imputable to the indole (~ 9%) δ (ppm): 7.16 (s, 1H). ^{13}C NMR (DMSO-d_6) δ (ppm): 104.65, 110.28, 120.26, 121.97, 122.84, 125.30, 125.50, 125.83, 126.24, 126.99, 127.34, 127.48, 128.28, 130.94, 133.38, 135.99, 136.66, 139.86, 161.06. MS *m/z* 303 (M^+, 53%), 287 (M^+ –O, 87%), 241 (M^+ –OH –COOH, 100%). HPLC, t_R 10.3 min.

2-(4-Methyl-3-nitrophenyl)naphthalene (3.162c)



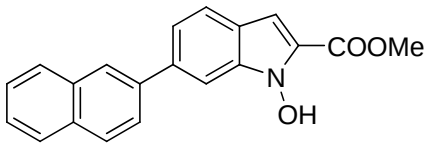
Commercially available 4-bromo-2-nitrotoluene **3.90d** (570 mg, 2.64 mmol) was placed in a vial together with 2-naphtaleneboronic acid **3.161c** (500 mg, 2.91 mmol), sodium carbonate (839 mg, 7.92 mmol), $Pd(OAc)_2$ (2.4 mg, 0.011 mmol), tetrabutylammonium bromide (852 mg, 2.64 mmol) and water (3 mL). The vial was sealed and heated under stirring at 150 °C in a microwave reactor for 5 min. The reaction mixture was then diluted with water and repeatedly extracted with EtOAc. The combined organic phase was dried over anhydrous sodium sulphate and evaporated to afford a crude residue that was purified by column chromatography over silica gel using *n*-hexane/EtOAc 98:2 as the eluent, to give pure **3.162c** (677 mg, 2.57 mmol, 97% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 2.67 (s, 3H), 7.45 (d, 1H, J = 7.7 Hz), 7.50-7.58 (m, 2H), 7.73 (dd, 1H, J = 8.4, 1.8 Hz), 7.83-7.98 (m, 4H), 8.07 (t, 1H, J = 1.0 Hz), 8.35 (d, 1H, J = 2.0 Hz).

Methyl 3-(4-(naphthalen-2-yl)-2-nitrophenyl)-2-oxopropanoate (3.163c)



A stirred suspension of sodium hydride (60% dispersion in mineral oil, 377 mg, 9.43 mmol) in 6.6 mL of anhydrous DMF cooled to −15 °C under nitrogen was treated dropwise with a solution containing the nitrotoluene precursor **3.162c** (620 mg, 2.36 mmol) and dimethyl oxalate (1.39 g, 11.8 mmol) in 4.4 mL of anhydrous DMF. The mixture was left under stirring at the same temperature for 10 minutes and then it was slowly warmed to room temperature. The mixture was then left under stirring at RT for 12 h. Once the disappearance of the precursor was verified by TLC, the reaction mixture was slowly poured in an ice-water mixture; the water phase was acidified with 1N aqueous HCl and extracted several times with EtOAc. The combined organic phase was washed with saturated $NaHCO_3$ solution and brine, then dried over anhydrous sodium sulphate. Evaporation under vacuum of the organic solvent afforded a crude product which was purified by column chromatography over silica gel using *n*-hexane/EtOAc 7:3 as the eluent, to yield the nitroaryl-ketoester derivative **3.163c** (644 mg, 1.84 mmol, 78% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 3.96 (s, 3H), 4.60 (s, 2H), 7.44 (d, 1H, J = 8.1 Hz), 7.51-7.57 (m, 2H), 7.77 (t, 1H, J = 1.8 Hz), 7.87-8.11 (m, 5H), 8.53 (d, 1H, J = 1.8 Hz); signals imputable to the enol form (~ 42%) δ (ppm): 3.97 (s, 3H), 6.72 (d, 1H, exchangeable, J = 1.5 Hz), 7.00 (d, 1H, J = 1.5 Hz), 7.72 (t, 1H, J = 1.9 Hz), 8.27 (d, 1H, J = 1.8 Hz), 8.39 (d, 1H, J = 8.4 Hz).

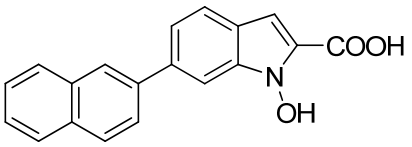
Methyl 1-hydroxy-6-(naphthalen-2-yl)-1*H*-indole-2-carboxylate (3.164c)



Triethylamine (0.40 mL, 0.29 g, 2.9 mmol) was added dropwise to a stirred solution of $SnCl_2 \cdot 2H_2O$ (181 mg, 0.802 mmol) and PhSH (0.30 mL, 0.29 g, 2.6 mmol) in acetonitrile (4.5 mL) at room temperature to generate a yellow precipitate over a period

of 5 min. Then, **3.163c** (200 mg, 0.573 mmol) was transferred to the suspension and stirred at room temperature for 5 more min. After complete consumption of the starting material, the mixture was diluted with water and repeatedly extracted with EtOAc. The combined organic phase was dried over anhydrous sodium sulphate, diluted with toluene and a catalytic amount of *p*-toluensulfonic acid was added. Then it was evaporated to afford a crude residue that was purified by column chromatography over silica gel using *n*-hexane/EtOAc 8:2 as the eluent, to give a mixture (110 mg) of the *N*-hydroxyindol-ester derivative **3.164c** (91%, 100 mg, 0.315 mmol, 55% yield) with its indole analogue (9%). ^{1}H NMR ($CDCl_3$) δ (ppm): 4.02 (s, 3H), 7.08 (d, 1H, *J* = 0.9 Hz), 7.47-7.58 (m, 3H), 7.74 (dd, 1H, *J* = 8.4, 0.7 Hz), 7.82-7.97 (m, 5H), 8.15 (d, 1H, *J* = 1.6 Hz), 10.33 (bs, 1H); signals imputable to the indole (~ 9%) δ (ppm): 3.98 (s, 3H), 7.19 (s, 1H).

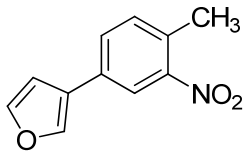
1-Hydroxy-6-(naphthalen-2-yl)-1*H*-indole-2-carboxylic acid (3.159c)



Methyl ester **3.164c** (70.0 mg, 0.221 mmol) was dissolved in a 1:1 mixture of THF/methanol (2.2 mL) and treated with 0.7 mL of 2N aqueous solution of LiOH. The reaction was kept under stirring in the dark at RT and it was monitored by TLC. After 3.5 h, the reaction mixture was concentrated, diluted with water and washed with Et_2O. Then, the aqueous phase was treated with 1N aqueous HCl and extracted several times with EtOAc. The combined ethyl acetate organic phase was dried over anhydrous sodium sulphate and evaporated to afford the desired *N*-hydroxyindol-carboxylic acid product **3.159c** (66.5 mg, 0.219 mmol, 99% yield) without any further purification. ^{1}H NMR (acetone-d_6) δ (ppm): 7.17 (d, 1H, *J* = 0.9 Hz), 7.51-7.57 (m, 2H), 7.63 (dd, 1H, *J* = 8.5, 1.6 Hz), 7.81 (dd, 1H, *J* = 8.4, 0.6 Hz), 7.92-7.97 (m, 3H), 7.99-8.06 (m, 2H), 8.29 (d, 1H, *J* = 1.5 Hz); signals imputable to the indole (~ 13%) δ (ppm): 3.98 (s, 3H), 7.21 (s, 1H). ^{13}C NMR (DMSO-d_6) δ (ppm): 104.52, 107.40, 120.09, 120.40, 120.49, 122.68, 125.23 (2C), 125.95, 126.30, 127.37, 128.14, 128.39, 132.09, 133.35, 136.50,

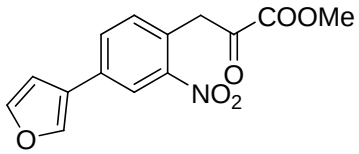
136.68, 137.86, 161.08. MS *m/z* 303 (M^+, 44%), 287 (M^+ –O, 100%), 241 (M^+ –OH – COOH, 57%). HPLC, t_R = 10.1 min.

3-(4-Methyl-3-nitrophenyl)furan (3.162d)



A solution of $Pd(OAc)_2$ (20.6 mg, 0.0918 mmol) and triphenylphosphine (120 mg, 0.459 mmol) in absolute ethanol (6.9 mL) and anhydrous toluene (6.9 mL) was stirred at RT under nitrogen for 10 min. After that period, commercially available 3-bromofuran **3.161d** (0.28 mL, 450 mg, 3.06 mmol), 6.9 mL of 2M aqueous Na_2CO_3, and 4-methyl-3-nitrobenzeneboronic acid **3.127** (886 mg, 4.90 mmol) were sequentially added. The resulting mixture was heated at 100 °C in a sealed vial under nitrogen overnight. After being cooled to RT, the mixture was diluted with water and extracted with EtOAc. The combined organic phase were dried and concentrated. The crude product was purified by flash chromatography (*n*-hexane/ EtOAc 95:5) to yield **3.162d** (437 mg, 2.15 mmol, 70% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 2.60 (s, 3H), 6.72 (dd, 1H, *J* = 1.9, 1.0 Hz), 7.34 (d, 1H, *J* = 7.7 Hz), 7.51 (*pseudo*-t, 1H, *J* = 1.7 Hz), 7.60 (dd, 1H, *J* = 7.9, 1.8 Hz), 7.79 (t, 1H, *J* = 0.7 Hz), 8.07 (d, 1H, *J* = 1.8 Hz).

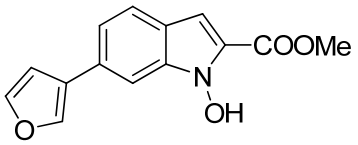
Methyl 3-(4-(furan-3-yl)-2-nitrophenyl)-2-oxopropanoate (3.163d)



A stirred suspension of sodium hydride (60% dispersion in mineral oil, 296 mg, 7.40 mmol) in 5.2 mL of anhydrous DMF cooled to –15 °C under nitrogen was treated dropwise with a solution containing the nitrotoluene precursor **3.162d** (376 mg, 1.85 mmol) and dimethyl oxalate (1.09 g, 9.25 mmol) in 3.5 mL of anhydrous DMF. The mixture was left under stirring at the same temperature for 10 minutes and then it was slowly warmed to room temperature. The mixture was then left under stirring at RT for

4h. Once the disappearance of the precursor was verified by TLC, the reaction mixture was slowly poured in an ice-water mixture; the water phase was acidified with 1N aqueous HCl and extracted several times with EtOAc. The combined organic phase was washed with saturated $NaHCO_3$ solution and brine, then dried over anhydrous sodium sulphate. Evaporation under vacuum of the organic solvent afforded a crude product which was purified by column chromatography over silica gel using *n*-hexane/EtOAc 7:3 as the eluent, to yield the nitroaryl-ketoester derivative **3.163d** (431 mg, 1.49 mmol, 81% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 3.94 (s, 3H), 4.54 (s, 2H), 6.74 (dd, 1H, *J* = 1.7, 0.8 Hz), 7.32 (d, 1H, *J* = 8.1 Hz), 7.53 (dd, 1H, *J* = 2.9, 1.6 Hz), 7.72 (dd, 1H, *J* = 7.8, 1.9 Hz), 7.82-7.85 (m, 1H), 8.26 (d, 1H, *J* = 1.6 Hz); signals imputable to the enol form (~ 28%) δ (ppm): 3.95 (s, 3H), 6.66 (d, 1H, exchangeable, *J* = 1.6 Hz), 6.94 (d, 1H, *J* = 1.1 Hz), 7.70 (dd, 1H, *J* = 8.2, 2.0 Hz), 7.99 (d, 1H, *J* = 1.8 Hz), 8.28 (d, 1H, *J* = 8.6 Hz).

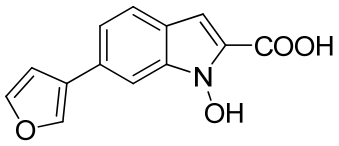
Methyl 6-(furan-3-yl)-1-hydroxy-1*H*-indole-2-carboxylate (3.164d)



Triethylamine (0.24 mL, 0.17 g, 1.7 mmol) was added dropwise to a stirred solution of $SnCl_2 \cdot 2H_2O$ (117 mg, 0.519 mmol) and PhSH (0.16 mL, 0.17 g, 1.6 mmol) in acetonitrile (2.7 mL) at room temperature to generate a yellow precipitate over a period of 5 min. Then, **3.163d** (100 mg, 0.346 mmol) was transferred to the suspension and stirred at room temperature for 5 more min. After complete consumption of the starting material, the mixture was diluted with water and repeatedly extracted with EtOAc. The combined organic phase was dried over anhydrous sodium sulphate, diluted with toluene and a catalytic amount of *p*-toluensulfonic acid was added. Then it was evaporated to afford a crude residue that was purified by column chromatography over silica gel using *n*-hexane/EtOAc 7:3 as the eluent, to give a mixture (70.4 mg) of the *N*-hydroxyindol-ester derivative **3.164d** (90%, 63.4 mg, 0.246 mmol, 71% yield) with its indole analogue (10%). ^{1}H NMR ($CDCl_3$) δ (ppm): 4.00 (s, 3H), 6.80 (dd, 1H, *J* = 1.8,

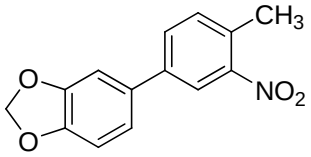
0.9 Hz), 7.02 (d, 1H, *J* = 0.9 Hz), 7.29 (dd, 1H, *J* = 8.5, 1.4 Hz), 7.51 (*pseudo*-t, 1H, *J* = 1.6 Hz), 7.60-7.66 (m, 2H), 7.82 (dd, 1H, *J* = 1.5, 0.9 Hz), 10.32 (bs, 1H); signals imputable to the indole (~ 10%) δ (ppm): 3.96 (s, 3H), 7.16 (d, 1H, *J* = 1.5 Hz), 11.07 (bs, 1H).

6-(Furan-3-yl)-1-hydroxy-1*H*-indole-2-carboxylic acid (3.159d)



Methyl ester **3.164d** (30.0 mg, 0.117 mmol) was dissolved in a 1:1 mixture of THF/methanol (1.2 mL) and treated with 0.35 mL of 2N aqueous solution of LiOH. The reaction was kept under stirring in the dark at RT and it was monitored by TLC. After 12h, the reaction mixture was concentrated, diluted with water and washed with Et_2O. Then, the aqueous phase was treated with 1N aqueous HCl and extracted several times with EtOAc. The combined ethyl acetate organic phase was dried over anhydrous sodium sulphate and evaporated to afford the desired *N*-hydroxyindol-carboxylic acid product **3.159d** (27.7 mg, 0.114 mmol, 97% yield) without any further purification. ^{1}H NMR (acetone-d_6) δ (ppm): 7.01 (dd, 1H, *J* = 1.8, 0.9 Hz), 7.10 (d, 1H, *J* = 0.9 Hz), 7.42 (dd, 1H, *J* = 8.4, 1.5 Hz), 7.65-7.72 (m, 3H), 8.13 (dd, 1H, *J* = 1.5, 0.9 Hz); signals imputable to the indole (~ 15%) δ (ppm): 7.18 (d, 1H, *J* = 2.2 Hz), 7.48 (dd, 1H, *J* = 8.0, 1.2 Hz). ^{13}C NMR (acetone-d_6) δ (ppm): 106.24, 106.75, 109.61, 120.33, 121.52, 123.59, 127.75, 129.64, 130.64, 137.34, 140.02, 144.86, 162.14. MS *m/z* 243 (M^+, 56%), 227 (M^+ –O, 100%), 180 (M^+ –CO_2 –H_2O, 26%). HPLC, t_R = 8.6 min.

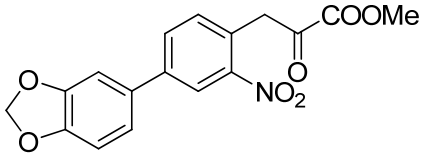
5-(4-Methyl-3-nitrophenyl)benzo[*d*][1,3]dioxole (3.162e)



Commercially available 4-bromo-1,2-(methylenedioxy)benzene **3.161e** (0.30 mL, 505 mg, 2.51 mmol) was placed in a vial together with 4-methyl-3-nitrobenzeneboronic

acid **3.127** (500 mg, 2.76 mmol), sodium carbonate (798 mg, 7.53 mmol), $Pd(OAc)_2$ (2.3 mg, 0.010 mmol), tetrabutylammonium bromide (809 mg, 2.51 mmol) and water (5 mL). The vial was sealed and heated under stirring at 150 °C in a microwave reactor for 5 min. The reaction mixture was then diluted with water and repeatedly extracted with EtOAc. The combined organic phase was dried over anhydrous sodium sulphate and evaporated to afford a crude residue that was purified by column chromatography over silica gel using *n*-hexane/EtOAc 95:5 as the eluent, to give pure **3.162e** (574 mg, 2.23 mmol, 89% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 2.62 (s, 3H), 6.02 (s, 2H), 6.90 (d, 1H, *J* = 8.6 Hz), 7.04-7-10 (m, 2H), 7.37 (d, 1H, *J* = 7.7 Hz), 7.64 (dd, 1H, *J* = 8.0, 2.1 Hz), 8.12 (d, 1H, *J* = 1.8 Hz).

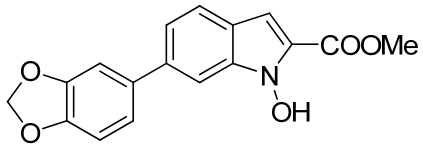
Methyl 3-(4-(benzo[*d*][1,3]dioxol-5-yl)-2-nitrophenyl)-2-oxopropanoate (3.163e)



A stirred suspension of sodium hydride (60% dispersion in mineral oil, 354 mg, 8.85 mmol) in 8.3 mL of anhydrous DMF cooled to –15 °C under nitrogen was treated dropwise with a solution containing the nitrotoluene precursor **3.162e** (569 mg, 2.21 mmol) and dimethyl oxalate (1.30 g, 11.1 mmol) in 4.1 mL of anhydrous DMF. The mixture was left under stirring at the same temperature for 10 minutes and then it was slowly warmed to room temperature. The mixture was then left under stirring at RT for 12h. Once the disappearance of the precursor was verified by TLC, the reaction mixture was slowly poured in an ice-water mixture; the water phase was acidified with 1N aqueous HCl and extracted several times with EtOAc. The combined organic phase was washed with saturated $NaHCO_3$ solution and brine, then dried over anhydrous sodium sulphate. Evaporation under vacuum of the organic solvent afforded a crude product which was purified by column chromatography over silica gel using *n*-hexane/EtOAc 7:3 as the eluent, to yield the nitroaryl-ketoester derivative **3.163e** (566 mg, 1.65 mmol, 75% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 3.95 (s, 3H), 4.56 (s, 2H), 6.04 (s, 2H), 6.92 (d, 1H, *J* = 8.6 Hz), 7.08-7.13 (m, 2H), 7.35 (d, 1H, *J* = 7.9 Hz), 7.76 (dd, 1H, *J* = 7.9, 2.0

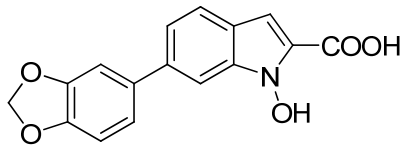
Hz), 8.31 (d, 1H, *J* = 2.0 Hz); signals imputable to the enol form (~ 36%) δ (ppm): 6.69 (d, 1H, *J* = 1.5 Hz), 7.74 (dd, 1H, *J* = 8.6, 2.6 Hz), 8.05 (d, 1H, *J* = 2.0 Hz), 8.30 (d, 1H, *J* = 7.5 Hz).

Methyl 6-(benzo[*d*][1,3]dioxol-5-yl)-1-hydroxy-1*H*-indole-2-carboxylate (3.164e)



Triethylamine (0.20 mL, 0.15 g, 1.5 mmol) was added dropwise to a stirred solution of $SnCl_2 \cdot 2H_2O$ (98.6 mg, 0.437 mmol) and PhSH (0.13 mL, 0.14 g, 1.3 mmol) in acetonitrile (2.3 mL) at room temperature to generate a yellow precipitate over a period of 5 min. Then, **3.163e** (100 mg, 0.291 mmol) was transferred to the suspension and stirred at room temperature for 5 more min. After complete consumption of the starting material, the mixture was diluted with water and repeatedly extracted with EtOAc. The combined organic phase was dried over anhydrous sodium sulphate, diluted with toluene and a catalytic amount of *p*-toluensulfonic acid was added. Then it was evaporated to afford a crude residue that was purified by column chromatography over silica gel using *n*-hexane/EtOAc 6:4 as the eluent, to give the *N*-hydroxyindole **3.164e** (57.8 mg, 0.186 mmol, 64% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 4.00 (s, 3H), 6.02 (s, 2H), 6.91 (d, 1H, *J* = 8.6 Hz), 7.04 (s, 1H), 7.15-7.18 (m, 2H), 7.32 (dd, 1H, *J* = 8.8, 1.5 Hz), 7.63-7.67 (m, 2H), 10.29 (bs, 1H).

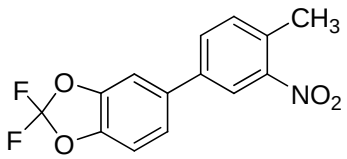
6-(Benzo[*d*][1,3]dioxol-5-yl)-1-hydroxy-1*H*-indole-2-carboxylic acid (3.159e)



Methyl ester **3.164e** (50.0 mg, 0.161 mmol) was dissolved in a 1:1 mixture of THF/methanol (1.6 mL) and treated with 0.48 mL of 2N aqueous solution of LiOH. The reaction was kept under stirring in the dark at RT and it was monitored by TLC. After 24h, the reaction mixture was concentrated, diluted with water and washed with Et_2O.

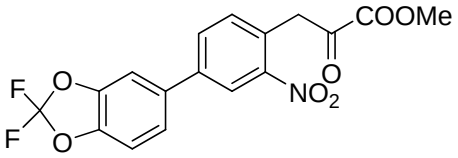
Then, the aqueous phase was treated with 1N aqueous HCl and extracted several times with EtOAc. The combined ethyl acetate organic phase was dried over anhydrous sodium sulphate and evaporated to afford the desired *N*-hydroxyindol-carboxylic acid product **3.159e** (47.4 mg, 0.159 mmol, 99% yield) without any further purification. ^{1}H NMR (DMSO-d_6) δ (ppm): 6.07 (s, 2H), 7.01 (d, 1H J = 8.1 Hz), 7.02 (s, 1H), 7.19 (dd, 1H, J = 8.5, 1.3 Hz), 7.29 (d, 1H, J = 1.5 Hz), 7.36 (dd, 1H, J = 8.6, 1.5 Hz), 7.54-7.58 (m, 1H), 7.67 (d, 1H, J = 8.8 Hz). ^{13}C NMR (DMSO-d_6) δ (ppm): 101.14, 104.65, 106.78, 107.29, 108.69, 119.98, 120.11, 120.46, 122.53, 127.16, 134.97, 136.50, 136.86, 146.69, 147.95, 161.13. MS *m/z* 297 (M^+, 19%), 281 (M^+ –O, 100%), 235 (M^+ – O –CH_2O_2, 36%).

2,2-Difluoro-5-(4-methyl-3-nitrophenyl)benzo[*d*][1,3]dioxole (3.162f)



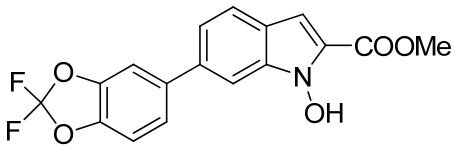
Commercially available 5-bromo-2,2-difluoro-1,3-benzodioxole **3.161f** (0.34 mL, 595 mg, 2.51 mmol) was placed in a vial together with 4-methyl-3-nitrobenzeneboronic acid **3.127** (500 mg, 2.76 mmol), sodium carbonate (798 mg, 7.53 mmol), $Pd(OAc)_2$ (2.3 mg, 0.010 mmol), tetrabutylammonium bromide (810 mg, 2.51 mmol) and water (5 mL). The vial was sealed and heated under stirring at 150 °C in a microwave reactor for 5 min. The reaction mixture was then diluted with water and repeatedly extracted with EtOAc. The combined organic phase was dried over anhydrous sodium sulphate and evaporated to afford a crude residue that was purified by column chromatography over silica gel using *n*-hexane/EtOAc 9:1 as the eluent, to give pure **3.162f** (798 mg, 2.72 mmol, >99% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 2.64 (s, 3H), 7.16 (d, 1H, J = 8.8 Hz), 7.29 (d, 1H, J = 1.6 Hz), 7.31 (dd, 1H, J = 6.8, 1.6 Hz), 7.42 (d, 1H, J = 7.9 Hz), 7.65 (dd, 1H, J = 8.0, 1.9 Hz), 8.13 (d, 1H, J = 1.8 Hz).

Methyl 3-(4-(2,2-difluorobenzo[*d*][1,3]dioxol-5-yl)-2-nitrophenyl)-2-oxopropanoate (3.163f)



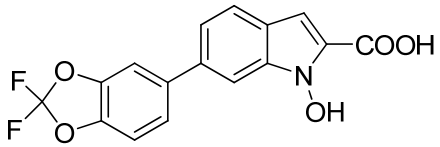
A stirred suspension of sodium hydride (60% dispersion in mineral oil, 431 mg, 10.8 mmol) in 7.6 mL of anhydrous DMF cooled to –15 °C under nitrogen was treated dropwise with a solution containing the nitrotoluene precursor **3.162f** (790 mg, 2.69 mmol) and dimethyl oxalate (1.59 g, 13.5 mmol) in 5 mL of anhydrous DMF. The mixture was left under stirring at the same temperature for 10 minutes and then it was slowly warmed to room temperature. The mixture was then left under stirring at RT for 12h. Once the disappearance of the precursor was verified by TLC, the reaction mixture was slowly poured in an ice-water mixture; the water phase was acidified with 1N aqueous HCl and extracted several times with EtOAc. The combined organic phase was washed with saturated $NaHCO_3$ solution and brine, then dried over anhydrous sodium sulphate. Evaporation under vacuum of the organic solvent afforded a crude product which was purified by column chromatography over silica gel using *n*-hexane/EtOAc 6:4 as the eluent, to yield the nitroaryl-ketoester derivative **3.163f** (749 mg, 1.96 mmol, 73% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 3.95 (s, 3H), 4.58 (s, 2H), 7.14-7.20 (m, 1H), 7.31-7.36 (m, 2H), 7.41 (d, 1H, J = 7.9 Hz), 7.77 (dd, 1H, J = 7.9, 2.0 Hz), 8.32 (d, 1H, J = 1.6 Hz); signals imputable to the enol form (~ 44%) δ (ppm): 3.96 (s, 3H), 6.74 (d, 1H, exchangeable, J = 1.5 Hz), 6.95 (d, 1H, J = 1.1 Hz), 7.74 (dd, 1H, J = 8.5, 2.0 Hz), 8.05 (d, 1H, J = 2.0 Hz), 8.34 (d, 1H, J = 8.1 Hz).

Methyl 6-(2,2-difluorobenzo[*d*][1,3]dioxol-5-yl)-1-hydroxy-1*H*-indole-2-carboxylate (3.164f)



Triethylamine (0.37 mL, 0.27 g, 2.6 mmol) was added dropwise to a stirred solution of $SnCl_2 \cdot 2H_2O$ (178 mg, 0.791 mmol) and PhSH (0.24 mL, 0.26 g, 2.4 mmol) in acetonitrile (4.1 mL) at room temperature to generate a yellow precipitate over a period of 5 min. Then, **3.163f** (200 mg, 0.527 mmol) was transferred to the suspension and stirred at room temperature for 5 more min. After complete consumption of the starting material, the mixture was diluted with water and repeatedly extracted with EtOAc. The combined organic phase was dried over anhydrous sodium sulphate, diluted with toluene and a catalytic amount of *p*-toluensulfonic acid was added. Then it was evaporated to afford a crude residue that was purified by column chromatography over silica gel using *n*-hexane/EtOAc 6:4 as the eluent, to give the *N*-hydroxyindole **3.164f** (94.9 mg, 0.273 mmol, 52% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 4.01 (s, 3H), 7.05 (d, 1H, J = 0.9 Hz), 7.14 (d, 1H, J = 8.8 Hz), 7.30 (dd, 1H, J = 8.4, 1.6 Hz), 7.37 (d, 1H, J = 1.8 Hz), 7.38 (dd, 1H, J = 6.2, 1.8 Hz), 7.66 (t, 1H, J = 0.8 Hz), 7.69 (dd, 1H, J = 8.4, 0.7 Hz), 10.34 (bs, 1H).

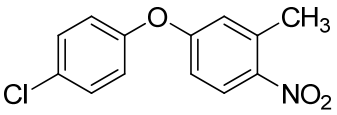
6-(2,2-Difluorobenzo[*d*][1,3]dioxol-5-yl)-1-hydroxy-1*H*-indole-2-carboxylic acid (3.159f)



Methyl ester **3.164f** (60.0 mg, 0.173 mmol) was dissolved in a 1:1 mixture of THF/methanol (1.7 mL) and treated with 0.5 mL of 2N aqueous solution of LiOH. The reaction was kept under stirring in the dark at RT. After 36 h it was monitored by TLC and another portion of LiOH (0.25 mL) was added. After further 6 h, the reaction mixture was concentrated, diluted with water and washed with Et_2O. Then, the aqueous phase was treated with 1N aqueous HCl and extracted several times with EtOAc. The combined ethyl acetate organic phase was dried over anhydrous sodium sulphate and evaporated to afford the desired *N*-hydroxyindol-carboxylic acid product **3.159f** (55.6 mg, 0.167 mmol, 96% yield) without any further purification. ^{1}H NMR (DMSO-d_6) δ (ppm): 7.04 (d, 1H, J = 0.7 Hz), 7.41 (dd, 1H, J = 8.3, 1.0 Hz), 7.49 (d, 1H, J = 8.4 Hz),

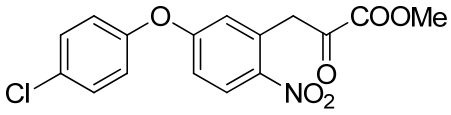
7.57 (dd, 1H, J = 8.4, 1.1 Hz), 7.66 (d, 1H, J = 0.9 Hz), 7.72 (d, 1H, J = 8.4 Hz), 7.82 (d, 1H, J = 1.6 Hz). ^{13}C NMR (DMSO-d_6) δ (ppm): 104.58, 107.53, 108.89, 110.35, 120.04, 120.56, 122.73, 123.11, 127.54, 131.23 (t, J = 262 Hz), 135.66, 136.33, 137.81, 142.05, 143.43, 161.06. MS *m/z* 333 (M^+, 26%), 317 (M^+ -O, 12%), 289 (M^+ -CO_2, 5%), 271 (M^+ -CO_2 -H_2O, 7%), 245 (M^+ -CO_2 -H_2O -C_2H_2, 14%), 177 (M^+ –$C_7H_3F_2O_2$ +H, 100%). HPLC, t_R = 10.5 min.

4-(4-Chlorophenoxy)-2-methyl-1-nitrobenzene (3.165)



p-Chlorophenol (995 mg, 7.74 mmol), potassium carbonate (1.07 g, 7.74 mmol) and dimethylsulfoxide (12.5 mL) were stirred at RT for 15 min in a vial. After that period, commercially available 5-fluoro-2-nitrotoluene **3.90f** (800 mg, 5.16 mmol) was added. The resulting mixture was heated at 80 °C in a sealed vial for 24 h. After being cooled to RT, the mixture was diluted with water and extracted with EtOAc. The combined organic phase was washed with brine and then dried over anhydrous sodium sulphate and concentrated. The crude product was purified by flash chromatography (*n*-hexane/ EtOAc 8:2) to yield **3.165** (1.05 g, 3.98 mmol, 77% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 2.55 (s, 3H), 6.97 (dd, 1H, J = 9.1, 2.8 Hz), 7.06 (d, 1H, J = 2.7 Hz),7.20 (AA'XX', 2H, J_{AX} = 8.8 Hz, $J_{AA'/XX'}$ = 2.7 Hz), 7.53 (AA'XX', 2H, J_{AX} = 8.8 Hz, $J_{AA'/XX'}$ = 2.7 Hz), 8.08 (d, 1H, J = 9.0 Hz).

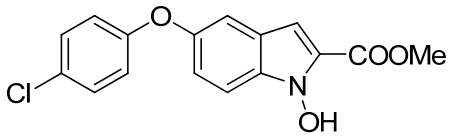
Methyl 3-(5-(4-chlorophenoxy)-2-nitrophenyl)-2-oxopropanoate (3.166)



A stirred suspension of sodium hydride (60% dispersion in mineral oil, 425 mg, 10.6 mmol) in 9 mL of anhydrous DMF cooled to –15 °C under nitrogen was treated dropwise with a solution containing the nitrotoluene precursor **3.165** (700 mg, 2.66 mmol) and dimethyl oxalate (1.57 g, 13.3 mmol) in 6 mL of anhydrous DMF. The

mixture was left under stirring at the same temperature for 10 minutes and then it was slowly warmed to room temperature. The mixture was then left under stirring at RT for 5 h. Once the disappearance of the precursor was verified by TLC, the reaction mixture was slowly poured in an ice-water mixture; the water phase was acidified with 1N aqueous HCl and extracted several times with EtOAc. The combined organic phase was washed with saturated $NaHCO_3$ solution and brine, then dried over anhydrous sodium sulphate. Evaporation under vacuum of the organic solvent afforded a crude product which was purified by column chromatography over silica gel using *n*-hexane/EtOAc 8:2 as the eluent, to yield the nitroaryl-ketoester derivative **3.166** (747 mg, 2.14 mmol, 80% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 3.94 (s, 3H), 4.49 (s, 2H), 6.82 (d, 1H, J = 2.7 Hz), 6.89 (dd, 1H, J = 9.1, 2.7 Hz), 7.05 (AA'XX', 2H, J_{AX} = 9.0 Hz, $J_{AA'/XX'}$ = 2.7 Hz), 7.40 (AA'XX', 2H, J_{AX} = 9.0 Hz, $J_{AA'/XX'}$ = 2.7 Hz), 8.21 (d, 1H, J = 9.2 Hz); signals imputable to the enol form (~ 12%) δ (ppm): 6.91 (dd, 1H, J = 9.2, 2.7 Hz), 7.78 (d, 1H, J = 2.7 Hz), 8.00 (d, 1H, J = 9.2 Hz).

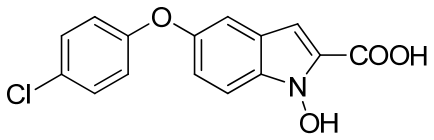
Methyl 5-(4-chlorophenoxy)-1-hydroxy-1*H*-indole-2-carboxylate (3.167)



Ketoester **3.166** (700 mg, 2.00 mmol) was dissolved in anhydrous DME (3.5 mL) and the resulting solution was added dropwise to a cooled (0 °C) solution of $SnCl_2 \cdot 2H_2O$ (1.01 g, 4.48 mmol) in DME (3.5 mL) containing 4Å molecular sieves, previously activated for 18 hours at 130 °C in oven and cooled to RT in a desiccator over anhydrous calcium chloride. The reaction mixture was stirred under nitrogen at RT for 3 h, then it was diluted with water and repeatedly extracted with EtOAc. The combined organic phase was dried over anhydrous sodium sulphate and evaporated to afford a crude residue that was purified by column chromatography over silica gel using *n*-hexane/EtOAc 8:2 as the eluent, to give the *N*-hydroxyindol-ester derivative **3.167** (25.0 mg, 0.0787 mmol, 4% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 4.00 (s, 3H), 6.90 (AA'XX', 2H, J_{AX} = 9.0 Hz, $J_{AA'/XX'}$ = 2.8 Hz), 6.96 (d, 1H, J = 0.9 Hz), 7.11 (dd, 1H, J

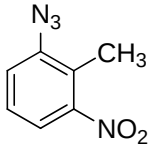
= 9.0, 2.2 Hz), 7.22 (d, 1H, J = 2.4 Hz), 7.26 (AA'XX', 2H, J_{AX} = 9.0 Hz, $J_{AA'XX'}$ = 2.8 Hz), 7.54 (d, 1H, J = 9.0 Hz).

5-(4-Chlorophenoxy)-1-hydroxy-1*H*-indole-2-carboxylic acid (3.160)



Methyl ester **3.167** (25.0 mg, 0.0787 mmol) was dissolved in a 1:1 mixture of THF/methanol (1.6 mL) and treated with 0.24 mL of 2N aqueous solution of LiOH. The reaction was kept under stirring in the dark at RT and it was monitored by TLC. After 2 h, the reaction mixture was concentrated, diluted with water and washed with Et_2O. Then, the aqueous phase was treated with 1N aqueous HCl and extracted several times with EtOAc. The combined ethyl acetate organic phase was dried over anhydrous sodium sulphate and evaporated to afford the desired *N*-hydroxyindol-carboxylic acid product **3.160** (19.0 mg, 0.0626 mmol, 79% yield) without any further purification. ^{1}H NMR (acetone-d_6) δ (ppm): 6.98 (AA'XX', 2H, J_{AX} = 9.1 Hz, $J_{AA'XX'}$ = 2.8 Hz), 7.10 (d, 1H, J = 0.9 Hz), 7.14 (dd, 1H, J = 9.0, 2.2 Hz), 7.33 (d, 1H, J = 2.2 Hz), 7.36 (AA'XX', 2H, J_{AX} = 9.0 Hz, $J_{AA'XX'}$ = 2.8 Hz), 7.59 (dt, 1H, J = 9.0, 0.8 Hz).

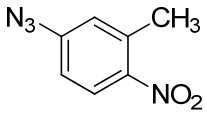
1-Azido-2-methyl-3-nitrobenzene (3.174)



A solution of $NaNO_2$ (1.36 g, 19.7 mmol) in H_2O (4.3 mL) was added dropwise to a stirred solution of commercially available 2-methyl-3-nitroaniline **3.171** (2.50 g, 16.4 mmol) in a mixture of concentrated HCl solution (8.6 mL) and H_2O (15 mL) at –5 °C. The mixture was stirred at 0 °C for 1 h. A solution of NaN_3 (1.07 g, 16.4 mmol) in H_2O (4.3 mL) was added dropwise to the cooled mixture so that the temperature did not exceed 5 °C (vigorous foaming). The mixture was stirred for 1 h at 0-5 °C and then for 12 h at RT. The reaction was worked up by dilution with EtOAc. The organic layer was

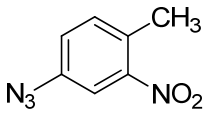
washed with saturated $NaHCO_3$ solution and brine, then dried over anhydrous sodium sulphate. After evaporation of the organic solvent, the crude was purified by column chromatography over silica gel using *n*-hexane/EtOAc 8:2 as the eluent, to yield the azide derivative **3.174** (2.39 g, 13.4 mmol, 82% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 2.36 (s, 3H), 7.32-7.41 (m, 2H), 7.54-7.64 (m, 1H). MS *m/z* 178 (M^+, 33%), 162 (M^+ –O, 76%), 104 (M^+ –O_2 –N_3, 100%).

4-Azido-2-methyl-1-nitrobenzene (3.175)



A solution of $NaNO_2$ (1.73 g, 25.2 mmol) in H_2O (6.6 mL) was added dropwise to a stirred solution of commercially available 3-methyl-4-nitroaniline **3.172** (3.19 g, 21.0 mmol) in a mixture of concentrated HCl solution (10 mL) and H_2O (20 mL) at –5 °C. The mixture was stirred at 0 °C for 1 h. A solution of NaN_3 (1.37 g, 21.0 mmol) in H_2O (6.6 mL) was added dropwise to the cooled mixture so that the temperature did not exceed 5 °C (vigorous foaming). The mixture was stirred for 1 h at 0-5 °C and then for 3 h at RT. The reaction was worked up by dilution with EtOAc. The organic layer was washed with saturated $NaHCO_3$ solution and brine, then dried over anhydrous sodium sulphate. After evaporation of the organic solvent, the crude was purified by column chromatography over silica gel using *n*-hexane/EtOAc 7:3 as the eluent, to yield the azide derivative **3.175** (3.17 g, 17.8 mmol, 85% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 2.63 (s, 3H), 6.92-6.70 (m, 2H), 8.07 (d, 1H, J = 8.2 Hz).

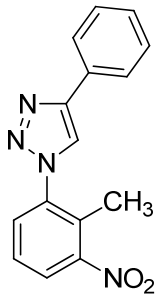
4-Azido-1-methyl-2-nitrobenzene (3.176)



A solution of $NaNO_2$ (1.63 g, 23.6 mmol) in H_2O (5.1 mL) was added dropwise to a stirred solution of commercially available 4-methyl-3-nitroaniline **3.173** (3.00 g, 19.7 mmol) in a mixture of concentrated HCl solution (10.4 mL) and H_2O (18.1 mL) at –5

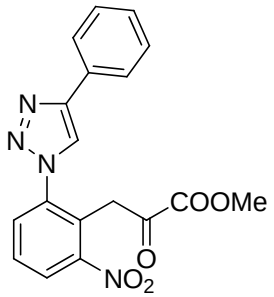
°C. The mixture was stirred at 0 °C for 1 h. A solution of NaN_3 (1.28 g, 19.7 mmol) in H_2O (5.2 mL) was added dropwise to the cooled mixture so that the temperature did not exceed 5 °C (vigorous foaming). The mixture was stirred for 1 h at 0-5 °C and then for 15 h at RT. The reaction was worked up by dilution with EtOAc. The organic layer was washed with saturated $NaHCO_3$ solution and brine, then dried over anhydrous sodium sulphate. After evaporation of the organic solvent, the crude was purified by column chromatography over silica gel using *n*-hexane/EtOAc 8:2 as the eluent, to yield the azide derivative **3.176** (3.15 g, 17.7 mmol, 90% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 2.57 (s, 3H), 7.16 (dd, 1H, *J* = 8.2, 2.4 Hz), 7.33 (d, 1H, *J* = 8.2 Hz), 7.64 (d, 1H, *J* = 2.4 Hz).

1-(2-Methyl-3-nitrophenyl)-4-phenyl-1*H*-1,2,3-triazole (3.180a)



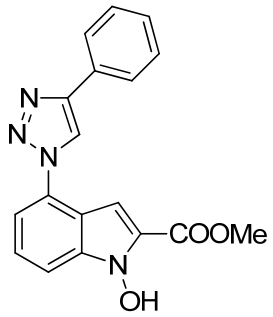
Phenylacetylene **3.177** (1.3 mL, 1.2 g, 12 mmol) and azide **3.174** (1.07 g, 6.00 mmol) were suspended in a 1:1 mixture of water and tert-butyl alcohol (24 mL). A freshly prepared sodium ascorbate aqueous solution (119 mg, 0.600 mmol, in 0.6 mL of water) was added, followed by copper (II) sulfate pentahydrate aqueous solution (15 mg, 0.060 mmol, in 0.2 mL of water). The heterogeneous mixture was stirred vigorously in the dark overnight. The reaction mixture was diluted with water, cooled in ice and the precipitate was collected by filtration. After washing the precipitate with cold water, it was dried and then subjected to flash chromatography using *n*-hexane/EtOAc 7:3 as the eluent, to yield the triazole derivative **3.180a** (831 mg, 2.96 mmol, 49% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 2.36 (s, 3H), 7.36-7.59 (m, 4H), 7.68 (dd, 1H, *J* = 7.9, 1.5 Hz), 7.88-7.94 (m, 2H), 8.01 (s, 1H), 8.06 (dd, 1H, *J* = 8.1, 1.5 Hz).

Methyl 3-(2-nitro-6-(4-phenyl-1*H*-1,2,3-triazol-1-yl)phenyl)-2-oxopropanoate (3.181a)



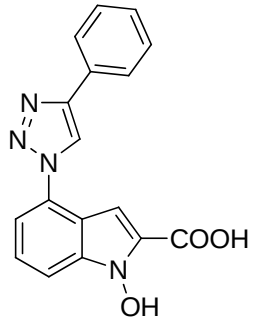
A stirred suspension of sodium hydride (60% dispersion in mineral oil, 457 mg, 11.4 mmol) in 10 mL of anhydrous DMF cooled to –15 °C under nitrogen was treated dropwise with a solution containing the nitrotoluene precursor **3.180a** (800 mg, 2.85 mmol) and dimethyl oxalate (1.68 g, 14.3 mmol) in 6.8 mL of anhydrous DMF. The mixture was left under stirring at the same temperature for 10 minutes and then it was slowly warmed to room temperature. The mixture was then left under stirring at RT for 4 h. Once the disappearance of the precursor was verified by TLC, the reaction mixture was slowly poured in an ice-water mixture; the water phase was acidified with 1N aqueous HCl and extracted several times with EtOAc. The combined organic phase was washed with saturated $NaHCO_3$ solution and brine, then dried over anhydrous sodium sulphate. Evaporation under vacuum of the organic solvent afforded a crude product which was purified by column chromatography over silica gel using *n*-hexane/EtOAc 6:4 as the eluent, to yield the nitroaryl-ketoester derivative **3.181a** (848 mg, 2.31 mmol, 81% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 3.91 (s, 3H), 4.35 (s, 2H), 7.35-7.52 (m, 3H), 7.68-7.90 (m, 3H), 7.87 (dd, 1H, J = 8.1, 1.6 Hz), 8.04 (s, 1H), 8.32 (dd, 1H, J = 7.7, 2.0 Hz).

Methyl 1-hydroxy-4-(4-phenyl-1*H*-1,2,3-triazol-1-yl)-1*H*-indole-2-carboxylate (3.182a)



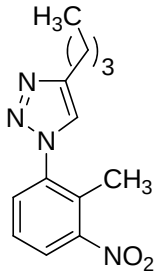
Ketoester **3.181a** (500 mg, 1.37 mmol) was dissolved in anhydrous DME (1.4 mL) and the resulting solution was added dropwise to a cooled (0 °C) solution of $SnCl_2 \cdot 2H_2O$ (692 mg, 3.07 mmol) in DME (1.4 mL) containing 4Å molecular sieves, previously activated for 18 hours at 130 °C in oven and cooled to RT in a desiccator over anhydrous calcium chloride. The reaction mixture was stirred under nitrogen at RT for 6.5 h, then it was monitored by TLC and another portion of $SnCl_2 \cdot 2H_2O$ (116 mg, 0.514 mmol) was added. After further 12 h, it was diluted with water and repeatedly extracted with EtOAc. The combined organic phase was dried over anhydrous sodium sulphate and evaporated to afford a crude residue that was purified by column chromatography over silica gel using *n*-hexane/EtOAc 6:4 as the eluent, to give the *N*-hydroxyindol-ester derivative **3.182a** (268 mg, 0.802 mmol, 59% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 4.01 (s, 3H), 7.34-7.53 (m, 6H), 7.66 (dt, 1H, J = 7.9, 1.1 Hz), 7.92-7.99 (m, 2H), 8.29 (s, 1H), 10.50 (bs, 1H).

1-Hydroxy-4-(4-phenyl-1*H*-1,2,3-triazol-1-yl)-1*H*-indole-2-carboxylic acid (3.168a)



Methyl ester **3.182a** (260 mg, 0.778 mmol) was dissolved in a 1:1 mixture of THF/methanol (7.8 mL) and treated with 2.3 mL of 2N aqueous solution of LiOH. The reaction was kept under stirring in the dark at RT and it was monitored by TLC. After 1 h, the reaction mixture was concentrated, diluted with water and washed with Et_2O. Then, the aqueous phase was treated with 1N aqueous HCl and extracted several times with EtOAc. The combined ethyl acetate organic phase was dried over anhydrous sodium sulphate and evaporated to afford the desired *N*-hydroxyindol-carboxylic acid product **3.168a** (247 mg, 0.771 mmol, 99% yield) without any further purification. ^{1}H NMR (DMSO-d_6) δ (ppm): 7.35-7.44 (m, 2H), 7.48-7.56 (m, 3H), 7.59-7.65 (m, 2H), 8.00-8.05 (m, 2H), 9.35 (s, 1H). ^{13}C NMR (DMSO-d_6) δ (ppm): 103.25, 110.51, 112.86, 113.41, 120.82, 124.84, 125.44 (2C), 127.88, 128.21, 128.92 (2C), 129.78, 130.20, 136.99, 146.77, 160.77. MS *m/z* 321 ($M+H^+$).

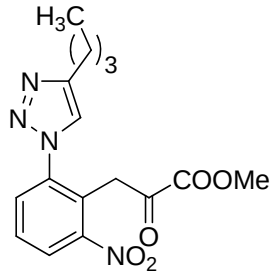
4-Butyl-1-(2-methyl-3-nitrophenyl)-1*H*-1,2,3-triazole (3.180b)



1-Hexyne **3.178** (0.77 mL, 0.55 g, 6.7 mmol) and azide **3.174** (1.20 g, 6.74 mmol) were suspended in a 1:1 mixture of water and tert-butyl alcohol (27 mL). A freshly

prepared sodium ascorbate aqueous solution (134 mg, 0.674 mmol, in 0.8 mL of water) was added, followed by copper (II) sulfate pentahydrate aqueous solution (16.8 mg, 0.0674 mmol, in 0.2 mL of water). The heterogeneous mixture was stirred vigorously in the dark at RT for 4 h, then it was checked by TLC and heated to 80 °C for 72 h. The reaction mixture was diluted with water, cooled in ice and the precipitate was collected by filtration. After washing the precipitate with cold water, it was dried and then subjected to flash chromatography using *n*-hexane/EtOAc 7:3 as the eluent, to yield the triazole derivative **3.180b** (263 mg, 1.01 mmol, 15% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 0.96 (t, 3H, *J* = 7.3 Hz), 1.35-1.53 (sext, 2H, *J* = 7.3 Hz), 1.63-1.82 (m, 2H), 2.29 (s, 3H), 2.83 (t, 2H, *J* = 7.7 Hz), 7.50 (s, 1H), 7.51 (t, 1H, *J* = 8.0 Hz), 7.61 (dd, 1H, *J* = 7.9, 1.5 Hz), 8.02 (dd, 1H, *J* = 8.1, 1.6 Hz).

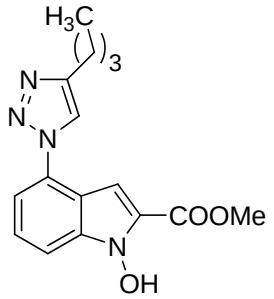
Methyl 3-(2-(4-butyl-1*H*-1,2,3-triazol-1-yl)-6-nitrophenyl)-2-oxopropanoate (3.181b)



A stirred suspension of sodium hydride (60% dispersion in mineral oil, 154 mg, 3.84 mmol) in 2.5 mL of anhydrous DMF cooled to –15 °C under nitrogen was treated dropwise with a solution containing the nitrotoluene precursor **3.180b** (250 mg, 0.961 mmol) and dimethyl oxalate (567 mg, 4.81 mmol) in 1.7 mL of anhydrous DMF. The mixture was left under stirring at the same temperature for 10 minutes and then it was slowly warmed to room temperature. The mixture was then left under stirring at RT for 24 h, then it was heated to 60 °C overnight. Once the disappearance of the precursor was verified by TLC, the reaction mixture was slowly poured in an ice-water mixture; the water phase was acidified with 1N aqueous HCl and extracted several times with EtOAc. The combined organic phase was washed with saturated $NaHCO_3$ solution and brine, then dried over anhydrous sodium sulphate. Evaporation under vacuum of the

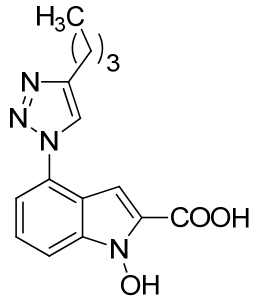
organic solvent afforded a crude product which was purified by column chromatography over silica gel using *n*-hexane/EtOAc 1:1 as the eluent, to yield the nitroaryl-ketoester derivative **3.181b** (181 mg, 0.523 mmol, 54% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 0.95 (t, 3H, *J* = 7.2 Hz), 1.22-1.49 (m, 2H), 1.60-1.77 (m, 2H), 2.78 (t, 2H, *J* = 7.7 Hz), 3.92 (s, 3H), 4.29 (s, 2H), 7.53 (s, 1H), 7.63-7.73 (m, 2H), 8.27 (dd, 1H, *J* = 6.7, 3.0 Hz); signals imputable to the enol form (~ 4%) δ (ppm): 6.49 (s, 1H).

Methyl 4-(4-butyl-1*H*-1,2,3-triazol-1-yl)-1-hydroxy-1*H*-indole-2-carboxylate (3.182b)



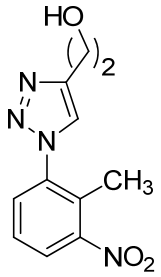
Ketoester **3.181b** (175 mg, 0.505 mmol) was dissolved in anhydrous DME (0.5 mL) and the resulting solution was added dropwise to a cooled (0 °C) solution of $SnCl_2 \cdot 2H_2O$ (255 mg, 1.13 mmol) in DME (0.5 mL) containing 4Å molecular sieves, previously activated for 18 hours at 130 °C in oven and cooled to RT in a desiccator over anhydrous calcium chloride. The reaction mixture was stirred under nitrogen at RT for 3 h, then it was diluted with water and repeatedly extracted with EtOAc. The combined organic phase was dried over anhydrous sodium sulphate and evaporated to afford a crude residue that was purified by column chromatography over silica gel using *n*-hexane/EtOAc 6:4 as the eluent, to give the *N*-hydroxyindol-ester derivative **3.182b** (99.5 mg, 0.317 mmol, 63% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 0.97 (t, 3H, *J* = 7.2 Hz), 1.22-1.54 (m, 2H), 1.62.1.82 (m, 2H), 2.83 (t, 2H, *J* = 7.7 Hz), 3.99 (s, 3H), 7.29 (dd, 1H, *J* = 7.4, 0.9 Hz), 7.37-7.45 (m, 2H), 7.62 (dt, 1H, *J* = 8.4, 0.9 Hz), 7.81 (s, 1H), 10.64 (bs, 1H).

4-(4-Butyl-1*H*-1,2,3-triazol-1-yl)-1-hydroxy-1*H*-indole-2-carboxylic acid (3.168b)



Methyl ester **3.182b** (90.0 mg, 0.286 mmol) was dissolved in a 1:1 mixture of THF/methanol (2.9 mL) and treated with 0.9 mL of 2N aqueous solution of LiOH. The reaction was kept under stirring in the dark at RT and it was monitored by TLC. After 2 h, the reaction mixture was concentrated, diluted with water and washed with Et_2O. Then, the aqueous phase was treated with 1N aqueous HCl and extracted several times with EtOAc. The combined ethyl acetate organic phase was dried over anhydrous sodium sulphate and evaporated to afford the desired *N*-hydroxyindol-carboxylic acid product **3.168b** (81.0 mg, 0.270 mmol, 94% yield) without any further purification. ^{1}H NMR (DMSO-d_6) δ (ppm): 0.93 (t, 3H, J = 7.2 Hz), 1.39 (sext., 2H, J = 7.3 Hz), 1.69 (quint., 2H, J = 7.5 Hz), 2.75 (t, 2H, J = 7.6 Hz), 7.32 (d, 1H, J = 0.7 Hz), 7.42-7.53 (m, 2H), 7.57 (ddd, 1H, J = 7.1, 2.2, 0.6 Hz), 8.62 (s, 1H). ^{13}C NMR (DMSO-d_6) δ (ppm): 13.76, 21.77, 24.68, 31.00, 103.41, 110.08, 112.48, 113.30, 121.29, 124.86, 127.68, 129.96, 137.01, 147.53, 160.73. MS *m/z* 301 (M +H$^+$), 285 (M+H$^+$ –O).

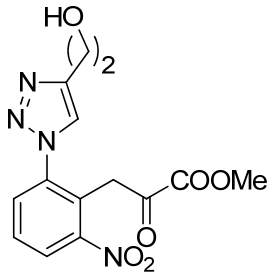
2-(1-(2-Methyl-3-nitrophenyl)-1*H*-1,2,3-triazol-4-yl)ethanol (3.180c)



3-Butyn-1-ol **3.179** (0.47 mL, 0.43 g, 6.2 mmol) and azide **3.174** (1.10 g, 6.18 mmol) were suspended in a 1:1 mixture of water and tert-butyl alcohol (24.7 mL). A

freshly prepared sodium ascorbate aqueous solution (122 mg, 0.618 mmol, in 0.6 mL of water) was added, followed by copper (II) sulfate pentahydrate aqueous solution (15.4 mg, 0.0618 mmol, in 0.2 mL of water). The heterogeneous mixture was stirred vigorously in the dark at 80 °C overnight. The reaction mixture was diluted with water and repeatedly extracted with EtOAc. The combined organic phase was dried over anhydrous sodium sulphate and evaporated to afford a crude residue, that was purified by flash chromatography using EtOAc as the eluent, to yield the triazole derivative **3.180c** (1.52 g, 6.12 mmol, 99% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 2.29 (s, 3H), 3.08 (t, 2H, *J* = 5.8 Hz), 4.04 (t, 2H, *J* = 5.8 Hz), 7.47-7.64 (m, 2H), 7.67 (s, 1H), 8.03 (dd, 1H, *J* = 8.0, 1.0 Hz).

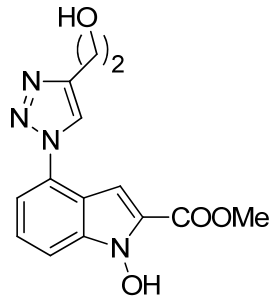
Methyl 3-(2-(4-(2-hydroxyethyl)-1*H*-1,2,3-triazol-1-yl)-6-nitrophenyl)-2-oxopropanoate (3.181c)



A stirred suspension of sodium hydride (60% dispersion in mineral oil, 774 mg, 19.3 mmol) in 12.8 mL of anhydrous DMF cooled to –15 °C under nitrogen was treated dropwise with a solution containing the nitrotoluene precursor **3.180c** (1.20 g, 4.84 mmol) and dimethyl oxalate (2.86 g, 24.2 mmol) in 8.4 mL of anhydrous DMF. The mixture was left under stirring at the same temperature for 10 minutes and then it was slowly warmed to room temperature. The mixture was then left under stirring at RT for 1 h. Once the disappearance of the precursor was verified by TLC, the reaction mixture was slowly poured in an ice-water mixture; the water phase was acidified with 1N aqueous HCl and extracted several times with EtOAc. The combined organic phase was washed with saturated $NaHCO_3$ solution and brine, then dried over anhydrous sodium sulphate. Evaporation under vacuum of the organic solvent afforded a crude product which was purified by column chromatography over silica gel using EtOAc as the

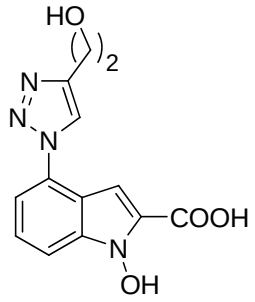
eluent, to yield the nitroaryl-ketoester derivative **3.181c** (1.11 g, 3.32 mmol, 69% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 3.03 (t, 2H, *J* = 5.9 Hz), 3.92 (s, 3H), 4.00 (t, 2H, *J* = 5.9 Hz), 4.30 (s, 2H), 7.68-7.74 (m, 2H), 7.71 (s, 1H), 8.27 (dd, 1H, *J* = 6.9, 2.8 Hz); signals imputable to the enol form (~ 17%) δ (ppm): 3.85 (s, 3H), 6.51 (s, 1H)

Methyl 1-hydroxy-4-(4-(2-hydroxyethyl)-1*H*-1,2,3-triazol-1-yl)-1*H*-indole-2-carboxylate (3.182c)



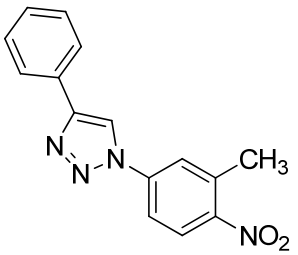
Ketoester **3.181c** (500 mg, 1.50 mmol) was dissolved in anhydrous DME (1.6 mL) and the resulting solution was added dropwise to a cooled (0 °C) solution of $SnCl_2 \cdot 2H_2O$ (759 mg, 3.37 mmol) in DME (1.6 mL) containing 4Å molecular sieves, previously activated for 18 hours at 130 °C in oven and cooled to RT in a desiccator over anhydrous calcium chloride. The reaction mixture was stirred under nitrogen at RT for 45 min., then it was diluted with water and repeatedly extracted with EtOAc. The combined organic phase was dried over anhydrous sodium sulphate and evaporated to afford a crude residue that was purified by column chromatography over silica gel using EtOA/acetone 9:1 as the eluent, to give the *N*-hydroxyindol-ester derivative **3.182c** (229 mg, 0.758 mmol, 51% yield). ^{1}H NMR (acetone-d_6) δ (ppm): 3.01 (t, 2H, *J* = 6.0 Hz), 3.90 (t, 2H, *J* = 5.9 Hz), 3.92 (s, 3H), 7.49 (d, 1H, *J* = 0.6 Hz), 7.51-7.58 (m, 2H), 7.67 (ddd, 1H, *J* = 5.2, 4.0, 0.9 Hz), 8.41 (s, 1H), 10.71 (bs, 1H).

1-Hydroxy-4-(4-(2-hydroxyethyl)-1*H*-1,2,3-triazol-1-yl)-1*H*-indole-2-carboxylic acid (3.168c)



Methyl ester **3.182c** (85.0 mg, 0.281 mmol) was dissolved in a 1:1 mixture of THF/methanol (2.8 mL) and treated with 0.8 mL of 2N aqueous solution of LiOH. The reaction was kept under stirring in the dark at RT and it was monitored by TLC. After 2 h, the reaction mixture was concentrated, diluted with water and washed with Et_2O. Then, the aqueous phase was treated with 1N aqueous HCl and extracted several times with EtOAc. The combined ethyl acetate organic phase was dried over anhydrous sodium sulphate and evaporated to afford the desired *N*-hydroxyindol-carboxylic acid product **3.168c** (80.2 mg, 0.278 mmol, 99% yield) without any further purification. ^{1}H NMR (DMSO-d_6) δ (ppm): 2.90 (t, 2H, J = 7.0 Hz), 3.74 (t, 2H, J = 6.9 Hz), 7.32 (d, 1H, J = 0.7 Hz), 7.46-7.52 (m, 2H), 7.56-7.60 (m, 1H), 8.60 (s, 1H). ^{13}C NMR (DMSO-d_6) δ (ppm): 29.18, 60.22, 103.39, 110.13, 112.54, 113.34, 121.95, 124.88, 127.70, 129.94, 137.04, 145.05, 160.70. MS *m/z* 287 (M^+ –H).

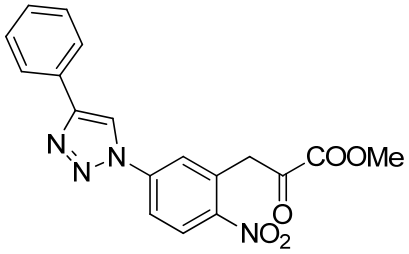
1-(3-Methyl-4-nitrophenyl)-4-phenyl-1*H*-1,2,3-triazole (3.184a)



Phenylacetylene **3.177** (1.3 mL, 1.2 g, 12 mmol) and azide **3.175** (1.06 g, 5.93 mmol) were suspended in a 1:1 mixture of water and tert-butyl alcohol (23.6 mL). A

freshly prepared sodium ascorbate aqueous solution (117 mg, 0.593 mmol, in 0.6 mL of water) was added, followed by copper (II) sulfate pentahydrate aqueous solution (14.9 mg, 0.0593 mmol, in 0.2 mL of water). The heterogeneous mixture was stirred vigorously in the dark for 24 h. The reaction mixture was diluted with water, cooled in ice and the precipitate was collected by filtration. After washing the precipitate with cold water, it was dried and then subjected to flash chromatography using *n*-hexane/EtOAc 7:3 as the eluent, to yield the triazole derivative **3.184a** (617 mg, 2.20 mmol, 37% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 2.75 (s, 3H), 7.40-7.54 (m, 3H), 7.81 (dd, 1H, J = 9.0, 2.6 Hz), 7.87-7.95 (m, 3H), 8.22 (d, 1H, J = 8.8 Hz), 8.27 (s, 1H).

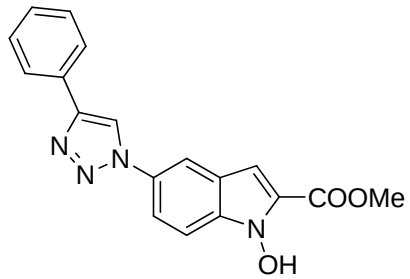
Methyl 3-(2-nitro-5-(4-phenyl-1*H*-1,2,3-triazol-1-yl)phenyl)-2-oxopropanoate (3.185a)



A stirred suspension of sodium hydride (60% dispersion in mineral oil, 352 mg, 8.81 mmol) in 5.8 mL of anhydrous DMF cooled to –15 °C under nitrogen was treated dropwise with a solution containing the nitrotoluene precursor **3.184a** (617 mg, 2.20 mmol) and dimethyl oxalate (1.30 g, 11.0 mmol) in 3.9 mL of anhydrous DMF. The mixture was left under stirring at the same temperature for 10 minutes and then it was slowly warmed to room temperature. The mixture was then left under stirring at RT overnight. Once the disappearance of the precursor was verified by TLC, the reaction mixture was slowly poured in an ice-water mixture; the water phase was acidified with 1N aqueous HCl and extracted several times with EtOAc. The combined organic phase was washed with saturated $NaHCO_3$ solution and brine, then dried over anhydrous sodium sulphate. Evaporation under vacuum of the organic solvent afforded a crude product which was purified by column chromatography over silica gel using *n*-hexane/EtOAc 7:3 as the eluent, to yield the nitroaryl-ketoester derivative **3.185a** (335

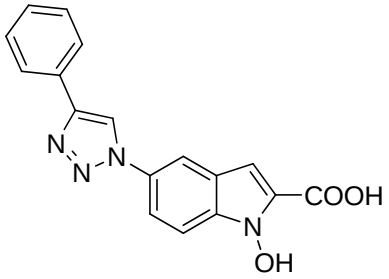
mg, 0.914 mmol, 42% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 3.97 (s, 3H), 4.68 (s, 2H), 7.40-7.54 (m, 3H), 7.88-7.98 (m, 4H), 8.29 (s, 1H), 8.41 (d, 1H, *J* = 8.2 Hz).

Methyl 1-hydroxy-5-(4-phenyl-1*H*-1,2,3-triazol-1-yl)-1*H*-indole-2-carboxylate (3.186a)



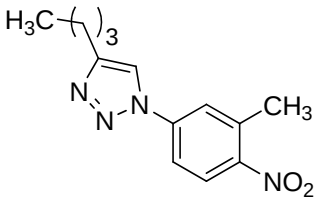
Ketoester **3.185a** (325 mg, 0.887 mmol) was dissolved in anhydrous DME (1.3 mL) and the resulting solution was added dropwise to a cooled (0 °C) solution of $SnCl_2 \cdot 2H_2O$ (448 mg, 1.99 mmol) in DME (1.3 mL) containing 4Å molecular sieves, previously activated for 18 hours at 130 °C in oven and cooled to RT in a desiccator over anhydrous calcium chloride. The reaction mixture was stirred under nitrogen at RT overnight, then it was diluted with water and repeatedly extracted with EtOAc. The combined organic phase was dried over anhydrous sodium sulphate and evaporated to afford a crude residue that was purified by column chromatography over silica gel using *n*-hexane/EtOAc 4:6 as the eluent, to give the *N*-hydroxyindol-ester derivative **3.186a** (31.0 mg, 0.0927 mmol, 10% yield). ^{1}H NMR (DMSO-d_6) δ (ppm): 3.90 (s, 3H); 7.25 (d, 1H, *J* = 0.7 Hz), 7.38 (tt, 1H, *J* = 7.4, 1.3 Hz), 7.46-7.55 (m, 2H), 7.71 (d, 1H, *J* = 9.0 Hz), 7.89-7.99 (m, 3H), 8.23 (dd, 1H, *J* = 2.0, 1.5 Hz), 9.30 (s, 1H), 11.74 (bs, 1H).

1-Hydroxy-5-(4-phenyl-1*H*-1,2,3-triazol-1-yl)-1*H*-indole-2-carboxylic acid (3.169a)



Methyl ester **3.186a** (28.0 mg, 0.0837 mmol) was dissolved in a 1:1 mixture of THF/methanol (0.8 mL) and treated with 0.3 mL of 2N aqueous solution of LiOH. The reaction was kept under stirring in the dark at RT and it was monitored by TLC. After 1.5 h, the reaction mixture was concentrated, diluted with water and washed with Et_2O. Then, the aqueous phase was treated with 1N aqueous HCl and extracted several times with EtOAc. The combined ethyl acetate organic phase was dried over anhydrous sodium sulphate and evaporated to afford the desired *N*-hydroxyindol-carboxylic acid product **3.169a** (25.7 mg, 0.0802 mmol, 96% yield) without any further purification. ^{1}H NMR (DMSO-d_6) δ (ppm): 7.16 (s, 1H), 7.34-7.54 (m, 3H), 7.67 (d, 1H, J = 8.8 Hz), 7.88 (dd, 1H, J = 8.8, 2.0 Hz), 7.95-7.99 (m, 2H), 8.20 (d, 1H, J = 1.8 Hz), 9.29 (s, 1H). ^{13}C NMR (DMSO-d_6) δ (ppm): 104.52, 110.70, 113.57, 117.76, 119.73, 120.56, 125.12 (2C), 127.85, 128.45, 128.70 (2C), 129.00, 130.31, 134.68, 146.89, 160.77. MS *m/z* 321 ($M+H^+$, 10%), 287 (M^+ –O –OH, 100%).

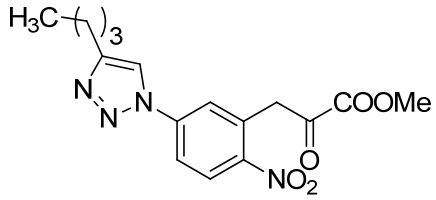
4-Butyl-1-(3-methyl-4-nitrophenyl)-1*H*-1,2,3-triazole (3.184b)



1-Hexyne **3.178** (0.68 mL, 0.49 g, 5.9 mmol) and azide **3.175** (1.06 g, 5.93 mmol) were suspended in a 1:1 mixture of water and tert-butyl alcohol (23.6 mL). A freshly prepared sodium ascorbate aqueous solution (117 mg, 0.593 mmol, in 0.6 mL of water) was added, followed by copper (II) sulfate pentahydrate aqueous solution (14.9 mg,

0.0593 mmol, in 0.2 mL of water). The heterogeneous mixture was stirred vigorously in the dark overnight. The reaction mixture was diluted with water, cooled in ice and the precipitate was collected by filtration. After washing the precipitate with cold water, it was dried and then subjected to flash chromatography using *n*-hexane/EtOAc 7:3 as the eluent, to yield the triazole derivative **3.184b** (813 mg, 3.12 mmol, 53% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 0.96 (t, 3H, *J* = 7.1 Hz), 1.43 (sext., 2H, *J* = 7.1 Hz), 1.64-1.80 (m, 2H), 2.71 (s, 3H), 2.81 (t, 2H, *J* = 7.3 Hz), 7.71 (dd, 1H, *J* = 9.0, 2.6 Hz), 7.79 (s, 1H), 7.80 (d, 1H, *J* = 2.6 Hz), 8.17 (d, 1H, *J* = 8.8 Hz).

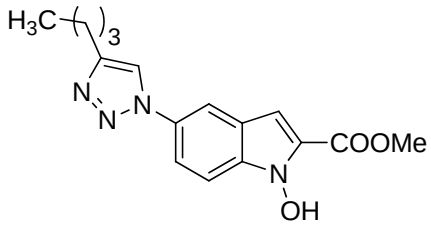
Methyl 3-(5-(4-butyl-1*H*-1,2,3-triazol-1-yl)-2-nitrophenyl)-2-oxopropanoate (3.185b)



A stirred suspension of sodium hydride (60% dispersion in mineral oil, 493 mg, 12.3 mmol) in 8 mL of anhydrous DMF cooled to –15 °C under nitrogen was treated dropwise with a solution containing the nitrotoluene precursor **3.184b** (802 mg, 3.08 mmol) and dimethyl oxalate (1.82 g, 15.4 mmol) in 5.5 mL of anhydrous DMF. The mixture was left under stirring at the same temperature for 10 minutes and then it was slowly warmed to room temperature. The mixture was then left under stirring at RT for 2.5 h. Once the disappearance of the precursor was verified by TLC, the reaction mixture was slowly poured in an ice-water mixture; the water phase was acidified with 1N aqueous HCl and extracted several times with EtOAc. The combined organic phase was washed with saturated $NaHCO_3$ solution and brine, then dried over anhydrous sodium sulphate. Evaporation under vacuum of the organic solvent afforded a crude product which was purified by column chromatography over silica gel using *n*-hexane/EtOAc 7:3 as the eluent, to yield the nitroaryl-ketoester derivative **3.185b** (840 mg, 2.43 mmol, 79% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 0.96 (t, 3H, *J* = 7.2 Hz), 1.42 (sext., 2H, *J* = 7.1 Hz), 1.64-1.81 (m, 2H), 2.82 (t, 2H, *J* = 7.6 Hz), 3.96 (s, 3H), 4.65 (s,

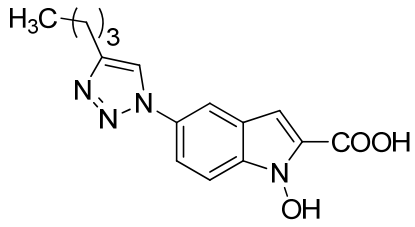
2H), 7.80-7.91 (m, 3H), 8.36 (d, 1H, *J* = 9.7 Hz); signals imputable to the enol form (~ 20%) δ (ppm): 6.91 (s, 1H, exchangeable), 7.03 (s, 1H), 8.09 (d, 1H, *J* = 9.0 Hz), 8.62 (d, 1H, *J* = 2.4 Hz).

Methyl 5-(4-butyl-1*H*-1,2,3-triazol-1-yl)-1-hydroxy-1*H*-indole-2-carboxylate (3.186b)



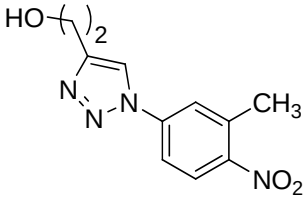
Ketoester **3.185b** (500 mg, 1.44 mmol) was dissolved in anhydrous DME (2 mL) and the resulting solution was added dropwise to a cooled (0 °C) solution of $SnCl_2 \cdot 2H_2O$ (733 mg, 3.25 mmol) in DME (2 mL) containing 4Å molecular sieves, previously activated for 18 hours at 130 °C in oven and cooled to RT in a desiccator over anhydrous calcium chloride. The reaction mixture was stirred under nitrogen at RT for 5 h, then it was diluted with water and repeatedly extracted with EtOAc. The combined organic phase was dried over anhydrous sodium sulphate and evaporated to afford a crude residue that was purified by column chromatography over silica gel using *n*-hexane/EtOAc 6:4 as the eluent, to give the *N*-hydroxyindol-ester derivative **3.186b** (33.2 mg, 0.106 mmol, 7% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 0.95 (t, 3H, *J* = 7.3 Hz), 1.40 (sext., 2H, *J* = 7.1 Hz), 1.71 (quint., 2H, *J* = 7.5 Hz), 2.80 (t, 2H, *J* = 7.9 Hz), 4.01 (s, 3H), 7.10 (s, 1H), 7.64-7.72 (m, 3H), 7.91 (t, 1H, *J* = 1.2 Hz).

5-(4-Butyl-1*H*-1,2,3-triazol-1-yl)-1-hydroxy-1*H*-indole-2-carboxylic acid (3.169b)



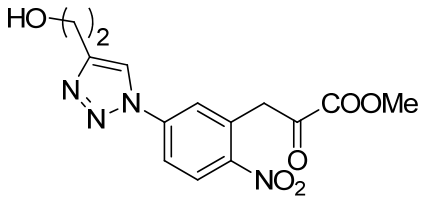
Methyl ester **3.186b** (31.2 mg, 0.0993 mmol) was dissolved in a 1:1 mixture of THF/methanol (1 mL) and treated with 0.3 mL of 2N aqueous solution of LiOH. The reaction was kept under stirring in the dark at RT and it was monitored by TLC. After 3 h, the reaction mixture was concentrated, diluted with water and washed with Et_2O. Then, the aqueous phase was treated with 1N aqueous HCl and extracted several times with EtOAc. The combined ethyl acetate organic phase was dried over anhydrous sodium sulphate and evaporated to afford the desired *N*-hydroxyindol-carboxylic acid product **3.169b** (29.4 mg, 0.0979 mmol, 99% yield) without any further purification. ^{1}H NMR (DMSO-d_6) δ (ppm): 0.93 (t, 3H, J = 7.3 Hz), 1.38 (sext., 2H, J = 7.5 Hz), 1.66 (quint., 2H, J = 7.5 Hz), 2.70 (t, 2H, J = 7.6 Hz), 7.12 (s, 1H), 7.61 (d, 1H, J = 9.0 Hz), 7.81 (dd, 1H, J = 9.1, 1.9 Hz), 8.11 (d, 1H, J = 1.6 Hz), 8.53 (s, 1H).

2-(1-(3-Methyl-4-nitrophenyl)-1*H*-1,2,3-triazol-4-yl)ethanol (3.184c)



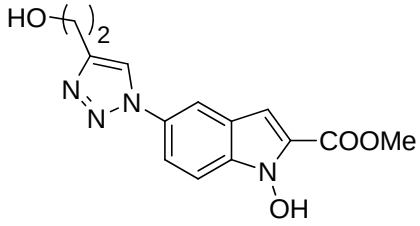
3-Butyn-1-ol **3.179** (0.47 mL, 0.44 g, 6.3 mmol) and azide **3.175** (1.11 g, 6.26 mmol) were suspended in a 1:1 mixture of water and tert-butyl alcohol (25.3 mL). A freshly prepared sodium ascorbate aqueous solution (124 mg, 0.626 mmol, in 0.7 mL of water) was added, followed by copper (II) sulfate pentahydrate aqueous solution (15.6 mg, 0.0626 mmol, in 0.2 mL of water). The heterogeneous mixture was stirred vigorously in the dark at 80 °C for 3.5 h. The reaction mixture was diluted with water and repeatedly extracted with EtOAc. The combined organic phase was dried over anhydrous sodium sulphate and evaporated to afford a crude residue, that was purified by flash chromatography using EtOAc as the eluent, to yield the triazole derivative **3.184c** (1.04 g, 4.19 mmol, 67% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 2.72 (s, 3H), 3.06 (t, 2H, J = 5.9 Hz), 4.00 (t, 2H, J = 5.7 Hz), 7.73 (dd, 1H, J = 8.8, 2.2 Hz), 7.80 (d, 1H, J = 2.0 Hz), 7.96 (s, 1H), 8.18 (d, 1H, J = 8.8 Hz).

Methyl 3-(5-(4-(2-hydroxyethyl)-1*H*-1,2,3-triazol-1-yl)-2-nitrophenyl)-2-oxopropanoate (3.185c)



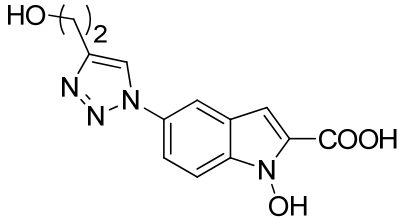
A stirred suspension of sodium hydride (60% dispersion in mineral oil, 322 mg, 8.04 mmol) in 5.2 mL of anhydrous DMF cooled to −15 °C under nitrogen was treated dropwise with a solution containing the nitrotoluene precursor **3.184c** (500 mg, 2.01 mmol) and dimethyl oxalate (1.19 g, 10.1 mmol) in 3.5 mL of anhydrous DMF. The mixture was left under stirring at the same temperature for 10 minutes and then it was slowly warmed to room temperature. The mixture was then left under stirring at RT for 1 h. Once the disappearance of the precursor was verified by TLC, the reaction mixture was slowly poured in an ice-water mixture; the water phase was acidified with 1N aqueous HCl and extracted several times with EtOAc. The combined organic phase was washed with saturated $NaHCO_3$ solution and brine, then dried over anhydrous sodium sulphate. Evaporation under vacuum of the organic solvent afforded a crude product which was purified by column chromatography over silica gel using EtOAc as the eluent, to yield the nitroaryl-ketoester derivative **3.185c** (392 mg, 1.17 mmol, 58% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 1.25 (t, 2H, J = 7.1 Hz), 3.06 (t, 2H, J = 5.7 Hz), 3.96 (s, 3H), 4.65 (s, 2H), 7.81-7.94 (m, 2H), 8.35 (d, 1H, J = 8.1 Hz); signals imputable to the enol form (~ 22%) δ (ppm): 3.97 (s, 3H), 7.01 (s, 1H), 8.10 (d, 1H, J = 8.8 Hz), 8.64 (d, 1H, J = 2.4 Hz).

Methyl 1-hydroxy-5-(4-(2-hydroxyethyl)-1*H*-1,2,3-triazol-1-yl)-1*H*-indole-2-carboxylate (3.186c)



Ketoester **3.185c** (375 mg, 1.12 mmol) was dissolved in anhydrous DME (1.2 mL) and the resulting solution was added dropwise to a cooled (0 °C) solution of $SnCl_2 \cdot 2H_2O$ (567 mg, 2.51 mmol) in DME (1.6 mL) containing 4Å molecular sieves, previously activated for 18 hours at 130 °C in oven and cooled to RT in a desiccator over anhydrous calcium chloride. The reaction mixture was stirred under nitrogen at RT for 2 h, then it was diluted with water and repeatedly extracted with EtOAc. The combined organic phase was dried over anhydrous sodium sulphate and evaporated to afford a crude residue that was purified by column chromatography over silica gel using $CHCl_3$/MeOH 95:5 as the eluent, to give the *N*-hydroxyindol-ester derivative **3.186c** (101 mg, 0.334 mmol, 30% yield). ^{1}H NMR (DMSO-d_6) δ (ppm): 2.86 (t, 2H, J = 7.1 Hz), 3.71 (td, 2H, J = 6.9, 5.3 Hz), 3.88 (s, 3H), 4.77 (t, 1H, J = 5.3 Hz), 7.20 (d, 1H, J = 0.7 Hz), 7.64 (d, 1H, J = 9.0 Hz), 7.83 (dd, 1H, J = 8.8, 2.0 Hz), 8.13 (d, 1H, J = 1.5 Hz), 8.53 (s, 1H), 11.70 (bs, 1H).

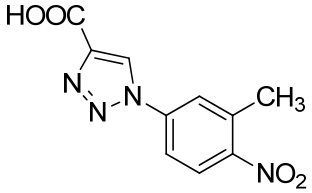
1-Hydroxy-5-(4-(2-hydroxyethyl)-1*H*-1,2,3-triazol-1-yl)-1*H*-indole-2-carboxylic acid (3.169c)



Methyl ester **3.186c** (36.3 mg, 0.120 mmol) was dissolved in a 1:1 mixture of THF/methanol (1.3 mL) and treated with 0.36 mL of 2N aqueous solution of LiOH. The reaction was kept under stirring in the dark at RT and it was monitored by TLC. After

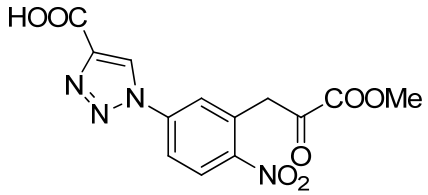
2.5 h, the reaction mixture was concentrated, diluted with water and washed with Et_2O. Then, the aqueous phase was treated with 1N aqueous HCl and extracted several times with EtOAc. The combined ethyl acetate organic phase was dried over anhydrous sodium sulphate and evaporated to afford the desired *N*-hydroxyindol-carboxylic acid product **3.169c** (33.3 mg, 0.116 mmol, 96% yield) without any further purification. ^{1}H NMR (DMSO-d_6) δ (ppm): 2.86 (t, 2H, J = 6.9 Hz), 3.71 (t, 2H, J = 6.9 Hz), 7.12 (s, 1H), 7.61 (d, 1H, J = 9.0 Hz), 7.80 (dd, 1H, J = 9.0, 1.9 Hz), 8.10 (d, 1H, J = 1.7 Hz), 8.52 (s, 1H). MS *m/z* 288 (M^+ 47%), 272 (M^+ –O, 50%), 226 (M^+ –C_2H_5O, –OH 52%), 181 (M^+ –OH, –COOH, –C_2H_5O, 100%).

1-(3-Methyl-4-nitrophenyl)-1*H*-1,2,3-triazole-4-carboxylic acid (3.184d)



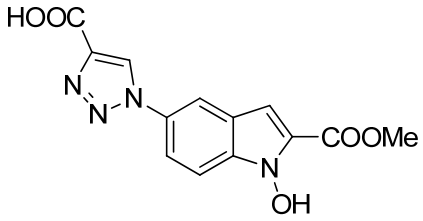
Propiolic acid **3.183** (0.35 mL, 0.39 g, 5.6 mmol) and azide **3.175** (1.00 g, 5.62 mmol) were suspended in a 1:1 mixture of water and tert-butyl alcohol (22.5 mL). A freshly prepared sodium ascorbate aqueous solution (111 mg, 0.562 mmol, in 0.6 mL of water) was added, followed by copper (II) sulfate pentahydrate aqueous solution (15 mg, 0.056 mmol, in 0.2 mL of water). The heterogeneous mixture was stirred vigorously in the dark at RT overnight, then it was heated to 80 °C for 12 h. The reaction mixture was diluted with water, cooled in ice and the precipitate was collected by filtration. After washing the precipitate with cold water, it was dried and then subjected to flash chromatography using CH_2Cl_2/MeOH/CH_3COOH 90:8:2 as the eluent, to yield the triazole derivative **3.184d** (992 mg, 4.00 mmol, 71% yield), which was directly used for the next step, without ^{1}H NMR analysis.

1-(3-(3-Methoxy-2,3-dioxopropyl)-4-nitrophenyl)-1*H*-1,2,3-triazole-4-carboxylic acid (3.185d)



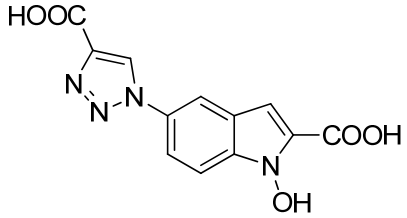
A stirred suspension of sodium hydride (60% dispersion in mineral oil, 773 mg, 19.3 mmol) in 12.6 mL of anhydrous DMF cooled to –15 °C under nitrogen was treated dropwise with a solution containing the nitrotoluene precursor **3.184d** (799 mg, 3.22 mmol) and dimethyl oxalate (2.28 g, 19.3 mmol) in 15.7 mL of anhydrous DMF. The mixture was left under stirring at the same temperature for 10 minutes and then it was slowly warmed to room temperature. The mixture was then left under stirring at RT for 7 days. Once the disappearance of the precursor was verified by TLC, the reaction mixture was slowly poured in an ice-water mixture; the water phase was acidified with 1N aqueous HCl and extracted several times with EtOAc. The combined organic phase was washed with saturated $NaHCO_3$ solution and brine, then dried over anhydrous sodium sulphate. Evaporation under vacuum of the organic solvent afforded a crude product which was purified by column chromatography over silica gel using CH_2Cl_2/MeOH/CH_3COOH 90:8:2 as the eluent, to yield the nitroaryl-ketoester derivative **3.185d** (452 mg, 1.35 mmol, 42% yield). ^{1}H NMR (DMSO-d_6) δ (ppm): 3.87 (s, 3H), 4.72 (s, 2H), 8.01 (m, 1H), 8.16-8.29 (m, 2H), 8.37 (d, 1H, J = 8.8 Hz), 9.53 (s, 1H); signals imputable to the enol form (~ 64%) δ (ppm): 3.83 (s, 3H), 6.68 (s, 1H), 8.00 (dd, 1H, J = 9.1, 2.2 Hz), 8.97 (s, 1H), 9.48 (s, 1H).

1-(1-Hydroxy-2-(methoxycarbonyl)-1*H*-indol-5-yl)-1*H*-1,2,3-triazole-4-carboxylic acid (3.186d)



Ketoester **3.185d** (401 mg, 1.20 mmol) was dissolved in anhydrous DME (1.5 mL) and the resulting solution was added dropwise to a cooled (0 °C) solution of $SnCl_2 \cdot 2H_2O$ (606 mg, 2.69 mmol) in DME (1.7 mL) containing 4Å molecular sieves, previously activated for 18 hours at 130 °C in oven and cooled to RT in a desiccator over anhydrous calcium chloride. The reaction mixture was stirred under nitrogen at RT for 5 h, then it was diluted with water and repeatedly extracted with EtOAc. The combined organic phase was dried over anhydrous sodium sulphate and evaporated to afford a crude residue that was purified by column chromatography over silica gel using CH_2Cl_2/MeOH/CH_3COOH 90:8:2 as the eluent, to give a mixture (85.9 mg) of the *N*-hydroxyindol-ester derivative **3.186d** (78%, 67.0 mg, 0.222 mmol, 18% yield) with its indole analogue (22%). ^{1}H NMR (DMSO-d_6) δ (ppm): 3.88 (s, 3H), 7.20 (s, 1H), 7.66 (d, 1H, J = 8.8 Hz), 7.88 (dd, 1H, J = 8.3, 2.0 Hz), 8.22 (d, 1H, J = 2.0 Hz), 9.07 (s, 1H); signals imputable to the indole (~ 22%) δ (ppm): 3.90 (s, 3H), 7.28 (s, 1H).

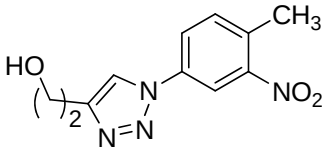
5-(4-Carboxy-1*H*-1,2,3-triazol-1-yl)-1-hydroxy-1*H*-indole-2-carboxylic acid (3.169d)



Methyl ester **3.186d** (78.8 mg, 0.261 mmol) was dissolved in a 1:1 mixture of THF/methanol (3 mL) and treated with 0.8 mL of 2N aqueous solution of LiOH. The reaction was kept under stirring in the dark at RT and it was monitored by TLC. After 3

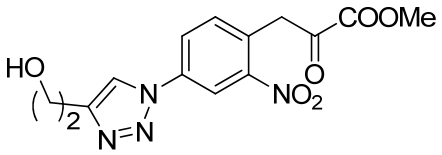
h, the reaction mixture was concentrated, diluted with water and washed with Et_2O. Then, the aqueous phase was treated with 1N aqueous HCl and extracted several times with EtOAc. The combined ethyl acetate organic phase was dried over anhydrous sodium sulphate and evaporated to afford the desired *N*-hydroxyindol-carboxylic acid product **3.169d** (74.4 mg, 0.258 mmol, 99% yield) without any further purification. ^{1}H NMR (DMSO-d_6) δ (ppm): 7.14 (d, 1H, J = 0.7 Hz), 7.65 (d, 1H, J = 9.0 Hz), 7.87 (dd, 1H, J = 9.0, 2.2 Hz), 8.23 (d, 1H, J = 2.0 Hz), 9.32 (s, 1H), 12.13 (bs, 1H); signals imputable to the indole (~ 37%) δ (ppm): 7.22 (d, 1H, J = 1.3 Hz), 7.61 (d, 1H, J = 8.8 Hz), 7.81 (dd, 1H, J = 9.0, 2.2 Hz), 9.31 (s, 1H).

2-(1-(4-Methyl-3-nitrophenyl)-1*H*-1,2,3-triazol-4-yl)ethanol (3.190a)



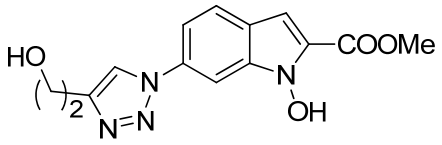
3-Butyn-1-ol **3.179** (0.42 mL, 0.39 g, 5.6 mmol) and azide **3.176** (1.00 g, 5.62 mmol) were suspended in a 1:1 mixture of water and tert-butyl alcohol (22.5 mL). A freshly prepared sodium ascorbate aqueous solution (111 mg, 0.562 mmol, in 0.6 mL of water) was added, followed by copper (II) sulfate pentahydrate aqueous solution (14.0 mg, 0.0562 mmol, in 0.2 mL of water). The heterogeneous mixture was stirred vigorously in the dark at 80 °C for 4 h. The reaction mixture was diluted with water and repeatedly extracted with EtOAc. The combined organic phase was dried over anhydrous sodium sulphate and evaporated to afford a crude residue, that was purified by flash chromatography using EtOAc as the eluent, to yield the triazole derivative **3.190a** (1.21 g, 4.87 mmol, 87% yield). ^{1}H NMR (DMSO-d_6) δ (ppm): 2.57 (s, 3H), 2.86 (t, 2H, J = 6.8 Hz), 3.71 (q, 2H, J = 6.3 Hz), 4.78 (t, 1H, J = 5.5 Hz), 7.74 (d, 1H, J = 8.4 Hz), 8.18 (dd, 1H, J = 8.2, 2.4 Hz), 8.50 (d, 1H, J = 2.4 Hz), 8.74 (s, 1H).

Methyl 3-(4-(4-(2-hydroxyethyl)-1*H*-1,2,3-triazol-1-yl)-2-nitrophenyl)-2-oxopropanoate (3.191a)



A stirred suspension of sodium hydride (60% dispersion in mineral oil, 387 mg, 9.67 mmol) in 6.3 mL of anhydrous DMF cooled to –15 °C under nitrogen was treated dropwise with a solution containing the nitrotoluene precursor **3.190a** (600 mg, 2.42 mmol) and dimethyl oxalate (1.43 g, 12.1 mmol) in 4.3 mL of anhydrous DMF. The mixture was left under stirring at the same temperature for 10 minutes and then it was slowly warmed to room temperature. The mixture was then left under stirring at RT for 1.5 h. Once the disappearance of the precursor was verified by TLC, the reaction mixture was slowly poured in an ice-water mixture; the water phase was acidified with 1N aqueous HCl and extracted several times with EtOAc. The combined organic phase was washed with saturated $NaHCO_3$ solution and brine, then dried over anhydrous sodium sulphate. Evaporation under vacuum of the organic solvent afforded a crude product which was purified by column chromatography over silica gel using EtOAc as the eluent, to yield the nitroaryl-ketoester derivative **3.191a** (432 mg, 1.29 mmol, 53% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 3.07 (t, 2H, *J* = 5.9 Hz), 3.97 (s, 3H), 4.03 (t, 2H, *J* = 5.9 Hz), 4.63 (s, 2H), 7.99 (s, 1H), 8.12 (dd, 1H, *J* = 8.4, 2.2 Hz), 8.31 (d, 1H, *J* = 2.4 Hz), 8.47 (d, 1H, *J* = 8.8 Hz); signals imputable to the enol form (~ 59%) δ (ppm): 3.96 (s, 3H), 6.95 (s, 1H), 7.52 (d, 1H, *J* = 8.2 Hz), 8.54 (d, 1H, *J* = 2.4 Hz).

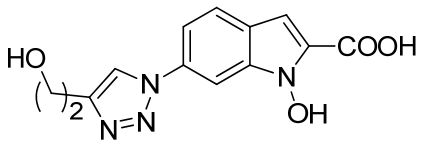
Methyl 1-hydroxy-6-(4-(2-hydroxyethyl)-1*H*-1,2,3-triazol-1-yl)-1*H*-indole-2-carboxylate (3.192a)



Ketoester **3.191a** (250 mg, 0.748 mmol) was dissolved in anhydrous DME (0.7 mL) and the resulting solution was added dropwise to a cooled (0 °C) solution of

$SnCl_2 \cdot 2H_2O$ (378 mg, 1.68 mmol) in DME (0.7 mL) containing 4Å molecular sieves, previously activated for 18 hours at 130 °C in oven and cooled to RT in a desiccator over anhydrous calcium chloride. The reaction mixture was stirred under nitrogen at RT for 24 h, then it was diluted with water and repeatedly extracted with EtOAc. The combined organic phase was dried over anhydrous sodium sulphate and evaporated to afford a crude residue that was purified by column chromatography over silica gel using CH_2Cl_2/MeOH 95:5 as the eluent, to give a mixture (23 mg) of the *N*-hydroxyindol-ester derivative **3.192a** (81%, 18.6 mg, 0.0616 mmol, 8% yield) with its indole analogue (19%). ^{1}H NMR ($CDCl_3$) δ (ppm): 3.01 (t, 2H, *J* = 6.6 Hz), 3.90 (t, 2H, *J* = 6.6 Hz), 3.94 (s, 3H), 7.16 (d, 1H, *J* = 0.9 Hz), 7.59 (dd, 1H, *J* = 8.7, 1.9 Hz), 7.81 (dd, 1H, *J* = 8.2, 0.6 Hz), 7.93-7.95 (m, 1H), 8.41 (s, 1H); signals imputable to the indole (~ 19%) δ (ppm): 3.95 (s, 3H), 7.25 (d, 1H; *J* = 0.9 Hz), 7.84 (dd, 1H, *J* = 8.6, 0.6 Hz), 7.89-7.91 (m, 1H), 8.34 (s, 1H).

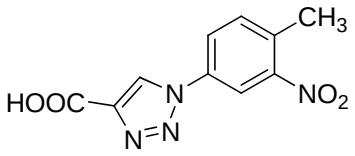
1-Hydroxy-6-(4-(2-hydroxyethyl)-1*H*-1,2,3-triazol-1-yl)-1*H*-indole-2-carboxylic acid (3.170a)



Methyl ester **3.192a** (23.0 mg, 0.0761 mmol) was dissolved in a 1:1 mixture of THF/methanol (0.8 mL) and treated with 0.2 mL of 2N aqueous solution of LiOH. The reaction was kept under stirring in the dark at RT and it was monitored by TLC. After 2 h, the reaction mixture was concentrated, diluted with water and washed with Et_2O. Then, the aqueous phase was treated with 1N aqueous HCl and extracted several times with EtOAc. The combined ethyl acetate organic phase was dried over anhydrous sodium sulphate and evaporated to afford the desired *N*-hydroxyindol-carboxylic acid product **3.170a** (21.7 mg, 0.0753 mmol, 99% yield) without any further purification. ^{1}H NMR (DMSO-d_6) δ (ppm): 2.87 (t, 2H, *J* = 6.4 Hz), 3.72 (t, 2H, *J* = 6.9 Hz), 7.11 (d, 1H, *J* = 0.8 Hz), 7.65 (dd, 1H, *J* = 8.6, 2.0 Hz), 7.83 (d, 1H, *J* = 8.8 Hz), 7.88-7.91 (m, 1H), 8.69 (s, 1H); signals imputable to the indole (~ 19%) δ (ppm): 7.18 (s, 1H), 7.86

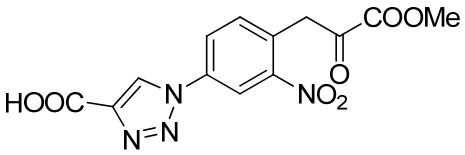
(d, 1H, J = 8.6 Hz), 8.57 (s, 1H). ^{13}C NMR (DMSO-d_6) δ (ppm): 29.27, 60.20, 100.48, 104.87, 110.77, 113.15, 120.49, 120.89, 123.70, 128.85, 135.53, 145.53, 160.80. MS m/z 288 (M^+).

1-(4-Methyl-3-nitrophenyl)-1*H*-1,2,3-triazole-4-carboxylic acid (3.190b)



Propiolic acid **3.183** (0.37 mL, 0.41 g, 5.9 mmol) and azide **3.176** (1.05 g, 5.89 mmol) were suspended in a 1:1 mixture of water and tert-butyl alcohol (23.6 mL). A freshly prepared sodium ascorbate aqueous solution (117 mg, 0.589 mmol, in 0.6 mL of water) was added, followed by copper (II) sulfate pentahydrate aqueous solution (14.7 mg, 0.0589 mmol, in 0.2 mL of water). The heterogeneous mixture was stirred vigorously in the dark for 24 h. The reaction mixture was diluted with water, cooled in ice and the precipitate was collected by filtration. After washing the precipitate with cold water, it was dried and then subjected to flash chromatography using CH_2Cl_2/MeOH 85:15 as the eluent, to yield the triazole derivative **3.190b** (777 mg, 3.13 mmol, 53% yield). ^{1}H NMR (DMSO-d_6) δ (ppm): 2.58 (s, 3H), 7.76 (d, 1H, J = 8.4 Hz), 8.26 (dd, 1H, J = 8.4, 2.2 Hz), 8.60 (d, 1H, J = 2.2 Hz), 9.44 (s, 1H).

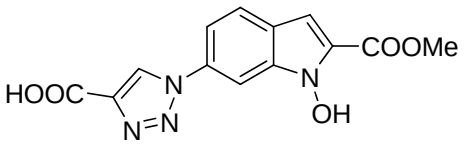
1-(4-(3-Methoxy-2,3-dioxopropyl)-3-nitrophenyl)-1*H*-1,2,3-triazole-4-carboxylic acid (3.191b)



A stirred suspension of sodium hydride (60% dispersion in mineral oil, 677 mg, 16.9 mmol) in 11 mL of anhydrous DMF cooled to –15 °C under nitrogen was treated dropwise with a solution containing the nitrotoluene precursor **3.190b** (700 mg, 2.82 mmol) and dimethyl oxalate (2.00 g, 16.9 mmol) in 5 mL of anhydrous DMF. The mixture was left under stirring at the same temperature for 10 minutes and then it was

slowly warmed to room temperature. The mixture was then left under stirring at RT overnight. Once the disappearance of the precursor was verified by TLC, the reaction mixture was slowly poured in an ice-water mixture; the water phase was acidified with 1N aqueous HCl and extracted several times with EtOAc. The combined organic phase was washed with saturated $NaHCO_3$ solution and brine, then dried over anhydrous sodium sulphate. Evaporation under vacuum of the organic solvent afforded a crude product which was purified by column chromatography over silica gel using $CHCl_3$/MeOH 9:1 with 0.05% trifluoroacetic acid as the eluent, to yield the nitroaryl-ketoester derivative **3.191b** (1.10 g, 3.46 mmol, > 99% yield). ^{1}H NMR (DMSO-d_6) δ (ppm): 3.86 (s, 3H), 4.70 (s, 2H), 7.77 (d, 1H, J = 8.4 Hz), 8.35-8.40 (m, 1H), 8.72 (d, 1H, J = 2.4 Hz), 9.59 (s, 1H), 10.56 (bs, 1H); signals imputable to the enol form (~ 63%) δ (ppm): 3.85 (s, 3H), 6.63 (s, 1H), 8.43 (d, 1H, J = 9.0 Hz), 8.60 (d, 1H, J = 2.2 Hz), 9.55 (s, 1H).

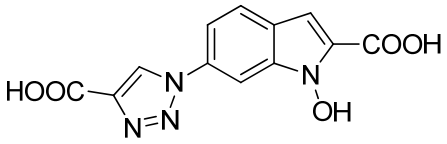
1-(1-Hydroxy-2-(methoxycarbonyl)-1*H*-indol-6-yl)-1*H*-1,2,3-triazole-4-carboxylic acid (3.192b)



Ketoester **3.191b** (500 mg, 1.50 mmol) was dissolved in anhydrous DME (1.5 mL) and the resulting solution was added dropwise to a cooled (0 °C) solution of $SnCl_2 \cdot 2H_2O$ (756 mg, 3.35 mmol) in DME (1.5 mL) containing 4Å molecular sieves, previously activated for 18 hours at 130 °C in oven and cooled to RT in a desiccator over anhydrous calcium chloride. The reaction mixture was stirred under nitrogen at RT for 5 h, then it was diluted with water and repeatedly extracted with EtOAc. The combined organic phase was dried over anhydrous sodium sulphate and evaporated to afford a crude residue that was purified by crystallization using EtOAc and distilled *n*-hexane, to give a mixture (28.5 mg) of the *N*-hydroxyindol-ester derivative **3.192b** (84%, 23.9 mg, 0.0792 mmol, 5% yield) with its indole analogue (16%).^{1}H NMR (DMSO-d_6) δ (ppm): 3.89 (s, 3H), 7.20 (d, 1H, J = 0.9 Hz), 7.74 (dd, 1H, J = 8.7, 1.9

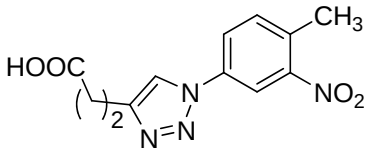
Hz), 7.89 (d, 1H, *J* = 8.8 Hz), 8.07 (d, 1H, *J* = 1.8 Hz), 9.50 (s, 1H), 11.72 (bs, 1H); signals imputable to the indole (~ 16%) δ (ppm): 9.40 (s, 1H).

6-(4-Carboxy-1*H*-1,2,3-triazol-1-yl)-1-hydroxy-1*H*-indole-2-carboxylic acid (3.170b)



Methyl ester **3.192b** (24.5 mg, 0.0811 mmol) was dissolved in a 1:1 mixture of THF/methanol (0.8 mL) and treated with 0.2 mL of 2N aqueous solution of LiOH. The reaction was kept under stirring in the dark at RT and it was monitored by TLC. After 48 h, the reaction mixture was concentrated, diluted with water and washed with Et_2O. Then, the aqueous phase was treated with 1N aqueous HCl and extracted several times with EtOAc. The combined ethyl acetate organic phase was dried over anhydrous sodium sulphate and evaporated to afford the desired *N*-hydroxyindol-carboxylic acid product **3.170b** (19.7 mg, 0.0684 mmol, 84% yield) without any further purification. ^{1}H NMR (DMSO-d_6) δ (ppm): 7.13 (s, 1H), 7.71 (dd, 1H, *J* = 8.6, 1.8 Hz), 7.87 (d, 1H, *J* = 8.6 Hz), 8.04 (d, 1H, *J* = 1.4 Hz), 9.48 (s, 1H); signals imputable to the indole (~ 26%) δ (ppm): 7.18-7.21 (m, 1H), 9.38 (s, 1H).

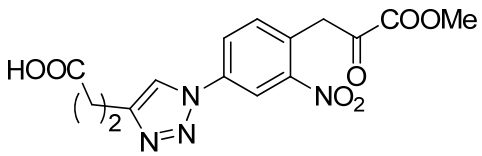
3-(1-(4-Methyl-3-nitrophenyl)-1*H*-1,2,3-triazol-4-yl)propanoic acid (3.190c)



4-Pentynoic acid **3.187** (551 mg, 5.62 mmol) and azide **3.176** (1.00 g, 5.62 mmol) were suspended in a 1:1 mixture of water and tert-butyl alcohol (22.5 mL). A freshly prepared sodium ascorbate aqueous solution (111 mg, 0.562 mmol, in 0.6 mL of water) was added, followed by copper (II) sulfate pentahydrate aqueous solution (14 mg, 0.056 mmol, in 0.2 mL of water). The heterogeneous mixture was stirred vigorously in the dark at RT overnight, then it was heated at 100 °C for 3 days. The reaction mixture was

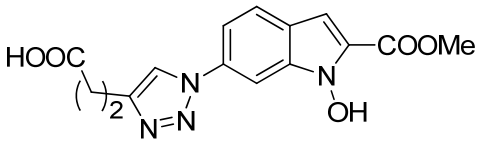
diluted with water, cooled in ice and the precipitate was collected by filtration. After washing the precipitate with cold water, it was dried and then subjected to flash chromatography using *n*-hexane/EtOAc 2:8 as the eluent, to yield the triazole derivative **3.190c** (607 mg, 2.20 mmol, 39% yield). ^{1}H NMR (CD_3OD) δ (ppm): 2.63 (s, 3H), 2.77 (t, 2H, *J* = 7.3 Hz), 3.08 (t, 2H, *J* = 7.4 Hz), 7.66 (d, 1H, *J* = 8.8 Hz), 8.07 (dd, 1H, *J* = 8.2, 2.4 Hz), 8.43 (s, 1H), 8.47 (d, 1H, *J* = 2.4 Hz).

3-(1-(4-(3-Methoxy-2,3-dioxopropyl)-3-nitrophenyl)-1*H*-1,2,3-triazol-4-yl)propanoic acid (3.191c)



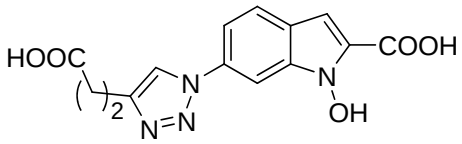
A stirred suspension of sodium hydride (60% dispersion in mineral oil, 504 mg, 12.6 mmol) in 8.2 mL of anhydrous DMF cooled to –15 °C under nitrogen was treated dropwise with a solution containing the nitrotoluene precursor **3.190c** (580 mg, 2.10 mmol) and dimethyl oxalate (1.49 g, 12.6 mmol) in 3.7 mL of anhydrous DMF. The mixture was left under stirring at the same temperature for 10 minutes and then it was slowly warmed to room temperature. The mixture was then left under stirring at RT for 5 h. Once the disappearance of the precursor was verified by TLC, the reaction mixture was slowly poured in an ice-water mixture; the water phase was acidified with 1N aqueous HCl and extracted several times with EtOAc. The combined organic phase was washed with saturated $NaHCO_3$ solution and brine, then dried over anhydrous sodium sulphate. Evaporation under vacuum of the organic solvent afforded a crude product which was purified by column chromatography over silica gel using $CHCl_3$/MeOH 9:1 as the eluent, to yield the nitroaryl-ketoester derivative **3.191c** (403 mg, 1.11 mmol, 53% yield). ^{1}H NMR (DMSO-d_6) δ (ppm): 2.68 (t, 2H, *J* = 7.1 Hz), 2.96 (t, 2H, *J* = 7.1 Hz), 3.86 (s, 3H), 4.68 (s, 2H), 7.74 (d, 1H, *J* = 8.4 Hz), 8.26-8-31 (m, 1H), 8.60 (d, 1H, *J* = 2.0 Hz), 8.80 (s, 1H), 12.20 (bs, 1H); signals imputable to the enol form (~ 72%) δ (ppm): 3.84 (s, 3H), 6.63 (s, 1H), 8.25 (dd, 1H, *J* = 8.8, 2.2 Hz), 8.41 (d, 1H, *J* = 8.6 Hz), 8.48 (d, 1H, *J* = 2.4 Hz), 8.76 (s, 1H).

3-(1-(1-Hydroxy-2-(methoxycarbonyl)-1*H*-indol-6-yl)-1*H*-1,2,3-triazol-4-yl)propanoic acid (3.192c)



Ketoester **3.191c** (380 mg, 1.05 mmol) was dissolved in anhydrous DME (1.2 mL) and the resulting solution was added dropwise to a cooled (0 °C) solution of $SnCl_2 \cdot 2H_2O$ (530 mg, 2.35 mmol) in DME (1.2 mL) containing 4Å molecular sieves, previously activated for 18 hours at 130 °C in oven and cooled to RT in a desiccator over anhydrous calcium chloride. The reaction mixture was stirred under nitrogen at RT for 5 h, then it was diluted with water and repeatedly extracted with EtOAc. The combined organic phase was dried over anhydrous sodium sulphate and evaporated to afford a crude residue that was purified by column chromatography over silica gel using $CHCl_3$/MeOH 9:1 as the eluent, to give a mixture (84.1 mg) of the *N*-hydroxyindol-ester derivative **3.192c** (68%, 57.2 mg, 0.173 mmol, 16% yield) with its indole analogue (32%). ^{1}H NMR (DMSO-d_6) δ (ppm): 2.69 (t, 2H, J = 7.5 Hz), 2.95 (t, 2H, J = 7.4 Hz), 3.88 (s, 3H), 7.18 (d, 1H, J = 0.9 Hz), 7.66 (dd, 1H, J = 8.7, 1.9 Hz), 7.86 (d, 1H, J = 8.8 Hz), 7.92 (d, 1H, J = 1.6 Hz), 8.71 (s, 1H), 11.69 (bs, 1H), 12.28 (bs, 1H); signals imputable to the indole (~ 32%) δ (ppm): 3.90 (s, 3H), 7.24-7.27 (m, 1H), 7.57 (dd, 1H, J = 8.7, 1.9 Hz), 8.59 (s, 1H).

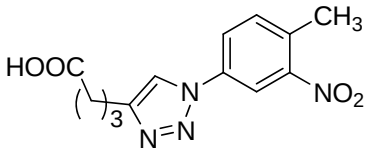
6-(4-(2-Carboxyethyl)-1*H*-1,2,3-triazol-1-yl)-1-hydroxy-1*H*-indole-2-carboxylic acid (3.170c)



Methyl ester **3.192c** (35.0 mg, 0.106 mmol) was dissolved in a 1:1 mixture of THF/methanol (1.1 mL) and treated with 0.3 mL of 2N aqueous solution of LiOH. The reaction was kept under stirring in the dark at RT and it was monitored by TLC. After 5 h, the reaction mixture was concentrated, diluted with water and washed with Et_2O.

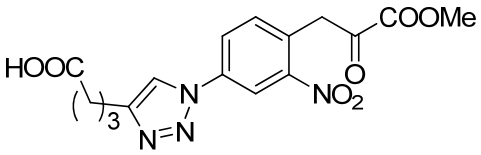
Then, the aqueous phase was treated with 1N aqueous HCl and extracted several times with EtOAc. The combined ethyl acetate organic phase was dried over anhydrous sodium sulphate and evaporated to afford the desired *N*-hydroxyindol-carboxylic acid product **3.170c** (33.2 mg, 0.105 mmol, 99% yield) without any further purification. ^{1}H NMR (DMSO-d_6) δ (ppm): 2.69 (t, 2H, J = 7.4 Hz), 2.95 (t, 2H, J = 7.3 Hz), 7.10 (s, 1H), 7.64 (dd, 1H, J = 8.5, 1.9 Hz), 7.52 (d, 1H, J = 9.0 Hz), 7.90 (d, 1H, J = 2.3 Hz), 8.70 (s, 1H), 12.10 (bs, 1H); signals imputable to the indole (~ 31%) δ (ppm): 7.17-7.19 (m, 1H), 7.54 (dd, 1H, J = 8.4, 2.0 Hz), 8.58 (s, 1H). ^{13}C NMR (DMSO-d_6) δ (ppm): 20.98, 33.17, 98.22, 101.03, 115.86, 115.96 (2C), 120.95, 124.24, 134.26, 135.84, 149.81, 161.08, 173.93.

4-(1-(4-Methyl-3-nitrophenyl)-1*H*-1,2,3-triazol-4-yl)butanoic acid (3.190d)



5-Hexynoic acid **3.188** (0.69 mL, 0.66 g, 5.9 mmol) and azide **3.176** (1.05 g, 5.89 mmol) were suspended in a 1:1 mixture of water and tert-butyl alcohol (23.6 mL). A freshly prepared sodium ascorbate aqueous solution (117 mg, 0.589 mmol, in 0.6 mL of water) was added, followed by copper (II) sulfate pentahydrate aqueous solution (14.7 mg, 0.0589 mmol, in 0.2 mL of water). The heterogeneous mixture was stirred vigorously in the dark for 24 h. The reaction mixture was diluted with water, cooled in ice and the precipitate was collected by filtration. After washing the precipitate with cold water, it was dried and then subjected to flash chromatography using *n*-hexane/EtOAc 3:7 as the eluent, to yield the triazole derivative **3.190d** (1.24 g, 4.27 mmol, 72% yield). ^{1}H NMR (DMSO-d_6) δ (ppm): 1.90 (quint., 2H, J = 7.6 Hz), 2.33 (t, 2H, J = 7.4 Hz), 2.57 (s, 3H), 2.74 (t, 2H, J = 7.6 Hz), 7.73 (d, 1H, J = 8.4 Hz), 8.18 (dd, 1H, J = 8.4, 2.4 Hz), 8.51 (d, 1H, J = 2.4 Hz), 8.77 (s, 1H), 12.11 (bs, 1H).

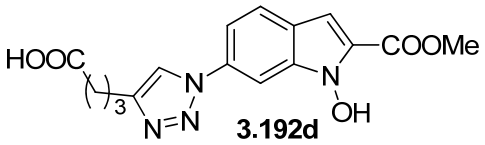
4-(1-(4-(3-Methoxy-2,3-dioxopropyl)-3-nitrophenyl)-1*H*-1,2,3-triazol-4-yl)butanoic acid (3.191d)



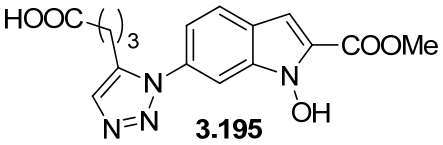
A stirred suspension of sodium hydride (60% dispersion in mineral oil, 385 mg, 9.64 mmol) in 10 mL of anhydrous DMF cooled to −15 °C under nitrogen was treated dropwise with a solution containing the nitrotoluene precursor **3.190d** (700 mg, 2.41 mmol) and dimethyl oxalate (1.42 g, 12.1 mmol) in 7 mL of anhydrous DMF. The mixture was left under stirring at the same temperature for 10 minutes and then it was slowly warmed to room temperature. The mixture was then left under stirring at RT for 4 h. Once the disappearance of the precursor was verified by TLC, the reaction mixture was slowly poured in an ice-water mixture; the water phase was acidified with 1N aqueous HCl and extracted several times with EtOAc. The combined organic phase was washed with saturated $NaHCO_3$ solution and brine, then dried over anhydrous sodium sulphate. Evaporation under vacuum of the organic solvent afforded a crude product which was purified by column chromatography over silica gel using CH_2Cl_2/MeOH 9:1 as the eluent, to yield the nitroaryl-ketoester derivative **3.191d** (960 mg, 2.55 mmol, > 99% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 2.08 (quint., 2H, J = 7.1 Hz), 2.46 (t, 2H, J = 7.2 Hz), 2.88 (t, 2H, J = 7.4 Hz), 3.93 (s, 3H), 4.61 (s, 2H), 7.50 (d, 1H, J = 8.4 Hz), 7.94 (s, 1H), 8.09 (dd, 1H, J = 8.2, 2.4 Hz), 8.51 (d, 1H, J = 2.4 Hz); signals imputable to the enol form (~ 36%) δ (ppm): 3.94 (s, 3H), 6.92 (s, 1H), 7.91 (s, 1H), 8.29 (d, 1H, J = 2.2 Hz), 8.46 (d, 1H, J = 8.8 Hz).

4-(1-(1-Hydroxy-2-(methoxycarbonyl)-1*H*-indol-6-yl)-1*H*-1,2,3-triazol-4-yl)butanoic acid (3.192d)

4-(1-(1-Hydroxy-2-(methoxycarbonyl)-1*H*-indol-6-yl)-1*H*-1,2,3-triazol-5-yl)butanoic acid (3.195)



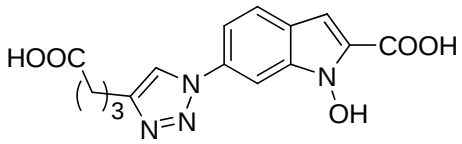
3.192d



3.195

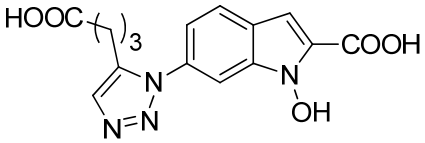
Ketoester **3.191d** (509 mg, 1.35 mmol) was dissolved in anhydrous DME (1.4 mL) and the resulting solution was added dropwise to a cooled (0 °C) solution of $SnCl_2 \cdot 2H_2O$ (684 mg, 3.03 mmol) in DME (1.4 mL) containing 4Å molecular sieves, previously activated for 18 hours at 130 °C in oven and cooled to RT in a desiccator over anhydrous calcium chloride. The reaction mixture was stirred under nitrogen at RT for 3 h, then it was diluted with water and repeatedly extracted with EtOAc. The combined organic phase was dried over anhydrous sodium sulphate and evaporated to afford a crude residue that was purified by column chromatography over silica gel using CH_2Cl_2/MeOH 9:1 as the eluent, to give two different mixtures: *a*) 73.7 mg of the 1,4-regioisomer **3.192d** (83%, 61.2 mg, 0.178 mmol, 13% yield) with its indole analogue (17%); *b*) 17.6 mg of the 1,5-regioisomer **3.195** (79%, 13.9 mg, 0.0404 mmol) with its indole analogue (21%). **3.192d**, ^{1}H NMR ($CDCl_3$) δ (ppm): 2.10 (quint., 2H, *J* = 7.3 Hz), 2.48 (t, 2H, *J* = 7.1 Hz), 2.87 (t, 2H, *J* = 7.4 Hz), 4.01 (s, 3H), 7.09 (s, 1H), 7.50 (dd, 1H, *J* = 8.6, 1.4 Hz), 7.74 (d, 1H, *J* = 8.6 Hz), 7.86 (s, 1H), 7.94-7.96 (m, 1H); signals imputable to the indole (~ 17%) δ (ppm): 3.98 (s, 3H). **3.195**, ^{1}H NMR ($CDCl_3$) δ (ppm): 2.08 (quint., 2H, *J* = 7.5 Hz), 2.43 (t, 2H, *J* = 7.3 Hz), 2.87 (t, 2H, *J* = 7.6 Hz), 4.01 (s, 3H), 7.09 (s, 1H), 7.38-7.45 (m, 1H), 7.72 (d, 1H, *J* = 8.8 Hz), 7.84 (s, 1H), 8.06-8.13 (m, 1H); signals imputable to the indole (~ 21%) δ (ppm): 3.98 (s, 3H).

6-(4-(3-Carboxypropyl)-1*H*-1,2,3-triazol-1-yl)-1-hydroxy-1*H*-indole-2-carboxylic acid (3.170d)



Methyl ester **3.192d** (70.0 mg, 0.203 mmol) was dissolved in a 1:1 mixture of THF/methanol (2 mL) and treated with 0.6 mL of 2N aqueous solution of LiOH. The reaction was kept under stirring in the dark at RT and it was monitored by TLC. After 1 h, the reaction mixture was concentrated, diluted with water and washed with Et_2O. Then, the aqueous phase was treated with 1N aqueous HCl and extracted several times with EtOAc. The combined ethyl acetate organic phase was dried over anhydrous sodium sulphate and evaporated to afford the desired *N*-hydroxyindol-carboxylic acid product **3.170d** (59.8 mg, 0.181 mmol, 89% yield) without any further purification. ^{1}H NMR (DMSO-d_6) δ (ppm): 1.91 (quint., 2H, J = 7.1 Hz), 2.34 (t, 2H, J = 7.3 Hz), 2.74 (t, 2H, J = 7.5 Hz), 7.10 (d, 1H, J = 0.9 Hz), 7.67 (dd, 1H, J = 8.6, 1.8 Hz), 7.83 (d, 1H, J = 8.6 Hz), 7.92 (t, 1H, J = 1.1 Hz), 8.74 (s, 1H), 12.12 (bs, 1H); signals imputable to the indole (~ 30%) δ (ppm): 7.17-7.18 (m, 1H), 7.56 (dd, 1H, J = 8.6, 1.9 Hz), 7.87-7.89 (m, 1H), 8.61 (s, 1H).

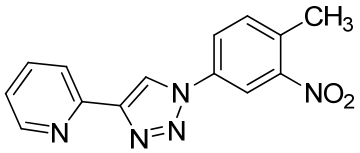
6-(5-(3-Carboxypropyl)-1*H*-1,2,3-triazol-1-yl)-1-hydroxy-1*H*-indole-2-carboxylic acid (3.196)



Methyl ester **3.195** (14.0 mg, 0.0407 mmol) was dissolved in a 1:1 mixture of THF/methanol (0.4 mL) and treated with 0.12 mL of 2N aqueous solution of LiOH. The reaction was kept under stirring in the dark at RT and it was monitored by TLC. After 1 h, the reaction mixture was concentrated, diluted with water and washed with Et_2O. Then, the aqueous phase was treated with 1N aqueous HCl and extracted several times with EtOAc. The combined ethyl acetate organic phase was dried over anhydrous

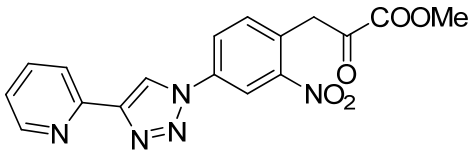
sodium sulphate and evaporated to afford the desired *N*-hydroxyindol-carboxylic acid product **3.196** (13.3 mg, 0.0403 mmol, 99% yield) without any further purification. ^{1}H NMR (acetone-d_6) δ (ppm): 2.00-2.09 (m, 2H), 2.45 (t, 2H, *J* = 7.1 Hz), 2.86 (t, 2H, *J* = 7.4 Hz), 7.20 (d, 1H, *J* = 0.9 Hz), 7.70 (dd, 1H, *J* = 8.6, 2.0 Hz), 7.87 (d, 1H, *J* = 8.6 Hz), 8.03 (t, 1H, *J* = 1.0 Hz), 8.48 (s, 1H), 11.24 (bs, 1H); signals imputable to the indole (~ 10%) δ (ppm): 7.29 (dd, 1H, *J* = 2.2, 0.9 Hz), 7.62 (dd, 1H, *J* = 8.8, 2.0 Hz), 7.89 (d, 1H, *J* = 8.6 Hz), 8.06-8.10 (m, 1H), 8.37 (s, 1H).

2-(1-(4-Methyl-3-nitrophenyl)-1*H*-1,2,3-triazol-4-yl)pyridine (3.190e)



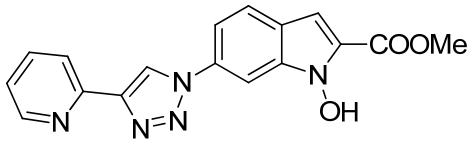
2-Ethynylpyridine **3.189** (0.57 mL, 0.58 g, 5.6 mmol) and azide **3.176** (1.00 g, 5.61 mmol) were suspended in a 1:1 mixture of water and tert-butyl alcohol (22.5 mL). A freshly prepared sodium ascorbate aqueous solution (111 mg, 0.561 mmol, in 0.6 mL of water) was added, followed by copper (II) sulfate pentahydrate aqueous solution (15 mg, 0.056 mmol, in 0.2 mL of water). The heterogeneous mixture was stirred vigorously in the dark at RT for 4 h, then it was heated to 45 °C overnight. The reaction mixture was diluted with water, cooled in ice and the precipitate was collected by filtration. After washing the precipitate with cold water, it was dried and then subjected to flash chromatography using *n*-hexane/EtOAc 6:4 as the eluent, to yield the triazole derivative **3.190e** (1.44 g, 5.12 mmol, 91% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 2.70 (s, 3H), 7.32 (ddd, 1H, *J* = 7.7, 4.9, 1.1 Hz), 7.55 (d, 1H, *J* = 8.4 Hz), 7.85 (td, 1H, *J* = 7.9, 1.7 Hz), 8.03 (dd, 1H, *J* = 8.4, 2.4 Hz), 8.23 (d, 1H, *J* = 7.9 Hz), 8.47 (d, 1H, *J* = 2.4 Hz), 8.63 (d, 1H, *J* = 4.6 Hz), 8.66 (s, 1H).

Methyl 3-(2-nitro-4-(4-(pyridin-2-yl)-1*H*-1,2,3-triazol-1-yl)phenyl)-2-oxopropanoate (3.191e)



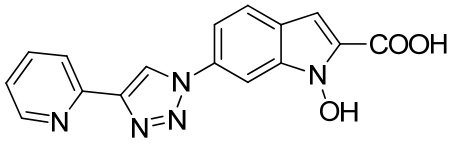
A stirred suspension of sodium hydride (60% dispersion in mineral oil, 400 mg, 10.0 mmol) in 6.5 mL of anhydrous DMF cooled to –15 °C under nitrogen was treated dropwise with a solution containing the nitrotoluene precursor **3.190e** (700 mg, 2.49 mmol) and dimethyl oxalate (1.48 g, 12.5 mmol) in 4.5 mL of anhydrous DMF. The mixture was left under stirring at the same temperature for 10 minutes and then it was slowly warmed to room temperature. The mixture was then left under stirring at RT overnight, then it was heated to 60 °C for 24 h. Once the disappearance of the precursor was verified by TLC, the reaction mixture was slowly poured in an ice-water mixture; the water phase was acidified with 1N aqueous HCl and extracted several times with EtOAc. The combined organic phase was washed with saturated $NaHCO_3$ solution and brine, then dried over anhydrous sodium sulphate. Evaporation under vacuum of the organic solvent afforded a crude product which was purified by column chromatography over silica gel using *n*-hexane/EtOAc 4:6 as the eluent, to yield the nitroaryl-ketoester derivative **3.191e** (469 mg, 1.28 mmol, 51% yield). ^{1}H NMR (DMSO-d_6) signals imputable to the ketonic form δ (ppm): 3.87 (s, 3H), 4.70 (s, 2H), 8.77 (d, 1H, J = 2.2 Hz); signals imputable to the enol form (~ 71%) δ (ppm): 3.85 (s, 3H), 6.64 (s, 1H), 7.42 (ddd, 1H, J = 6.8, 4.8, 1.1 Hz), 7.77 (d, 1H, J = 8.1 Hz), 7.96 (td, 1H, J = 7.7, 1.3 Hz), 8.14 (d, 1H, J = 8.1 Hz), 8.40-8.46 (m, 2H), 8.64-8.70 (m, 2H).

Methyl 1-hydroxy-6-(4-(pyridin-2-yl)-1*H*-1,2,3-triazol-1-yl)-1*H*-indole-2-carboxylate (3.192e)



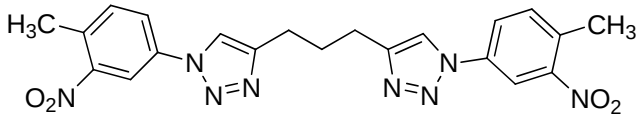
Ketoester **3.191e** (466 mg, 1.27 mmol) was dissolved in anhydrous DME (1.5 mL) and the resulting solution was added dropwise to a cooled (0 °C) solution of $SnCl_2 \cdot 2H_2O$ (641 mg, 2.84 mmol) in DME (2 mL) containing 4Å molecular sieves, previously activated for 18 hours at 130 °C in oven and cooled to RT in a desiccator over anhydrous calcium chloride. The reaction mixture was stirred under nitrogen at RT for 5 h, then it was diluted with water and repeatedly extracted with EtOAc. The combined organic phase was dried over anhydrous sodium sulphate and evaporated to afford a crude residue that was purified by column chromatography over silica gel using *n*-hexane/EtOAc 3:7 as the eluent, to give the *N*-hydroxyindol-ester derivative **3.192e** (23.3 mg, 0.0695 mmol, 5% yield). ^{1}H NMR (acetone-d_6) δ (ppm): 3.93 (s, 3H), 7.31 (d, 1H, J = 0.6 Hz), 7.35 (ddd, 1H, J = 7.5, 4.9, 1.2 Hz). 7.78 (dd, 1H, J = 8.6, 2.0 Hz), 7.88-7.97 (m, 2H), 8.17-8.23 (m, 2H), 8.64 (dq, 1H, J = 4.9, 1.8 Hz), 8.96 (s, 1H), 11.35 (bs, 1H).

1-Hydroxy-6-(4-(pyridin-2-yl)-1*H*-1,2,3-triazol-1-yl)-1*H*-indole-2-carboxylic acid (3.170e)



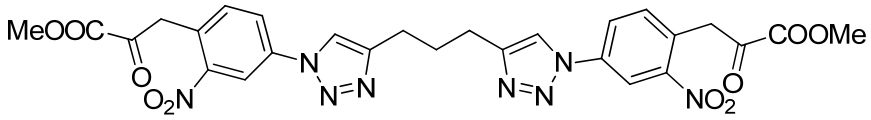
Methyl ester **3.192e** (20.0 mg, 0.0596 mmol) was dissolved in a 1:1 mixture of THF/methanol (0.6 mL) and treated with 0.2 mL of 2N aqueous solution of LiOH. The reaction was kept under stirring in the dark at RT and it was monitored by TLC. After 5 h, the reaction mixture was concentrated, diluted with water and washed with Et_2O. Then, the aqueous phase was treated with 1N aqueous HCl and extracted several times with EtOAc. The combined ethyl acetate organic phase was dried over anhydrous sodium sulphate and evaporated to afford the desired *N*-hydroxyindol-carboxylic acid product **3.170e** (19.0 mg, 0.0591 mmol, 99% yield) without any further purification. ^{1}H NMR (DMSO-d_6) δ (ppm): 7.20 (d, 1H, J = 1.8 Hz), 7.38-7.43 (m, 1H), 7.71 (dd, 1H, J = 8.6, 2.0 Hz), 7.88 (d, 1H, J = 8.8 Hz), 7.96 (t, 1H, J = 8.1 Hz), 8.02 (s, 1H), 8.11-8.16 (m, 1H), 8.63-8.69 (m, 1H), 9.31 (s, 1H), 12.17 (bs, 1H). MS *m/z* 322 ($M+H^+$ 100%), 295 (M^+ –HCN, 60%).

1,3-Bis(1-(4-methyl-3-nitrophenyl)-1*H*-1,2,3-triazol-4-yl)propane (3.198)



1,6-Heptadiyne **3.197** (1.3 mL, 1.0 g, 11 mmol) and azide **3.176** (4.00 g, 22.5 mmol) were suspended in a 1:1 mixture of water and tert-butyl alcohol (89.8 mL). A freshly prepared sodium ascorbate aqueous solution (445 mg, 2.25 mmol, in 2.2 mL of water) was added, followed by copper (II) sulfate pentahydrate aqueous solution (56.0 mg, 0.225 mmol, in 0.7 mL of water). The heterogeneous mixture was stirred vigorously in the dark for 48 h. The reaction mixture was diluted with water, cooled in ice and the precipitate was collected by filtration. After washing the precipitate with cold water, it was dried and then subjected to flash chromatography using *n*-hexane/EtOAc 4:6 as the eluent, to yield the dimer **3.198** (3.68 g, 8.21 mmol, 73% yield). ^{1}H NMR (DMSO-d_6) δ (ppm): 2.10 (t, 2H, J = 7.3 Hz), 2.57 (s, 6H), 2.84 (t, 4H, J = 7.4 Hz), 7.73 (d, 2H, J = 8.8 Hz), 8.17 (dd, 2H, J = 8.2, 2.4 Hz), 8.49 (d, 2H, J = 2.4 Hz), 8.77 (s, 2H).

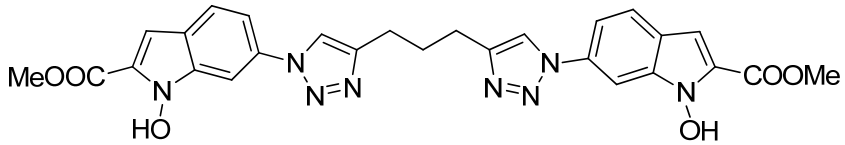
Dimethyl 3,3'-((4,4'-(propane-1,3-diyl)bis(1*H*-1,2,3-triazole-4,1-diyl))bis(2-nitro-4,1-phenylene))bis(2-oxopropanoate) (3.199)



A stirred suspension of sodium hydride (60% dispersion in mineral oil, 2.36 g, 58.9 mmol) in 77 mL of anhydrous DMF cooled to –15 °C under nitrogen was treated dropwise with a solution containing the nitrotoluene precursor **3.198** (3.30 g, 7.36 mmol) and dimethyl oxalate (8.69 g, 73.6 mmol) in 26 mL of anhydrous DMF. The mixture was left under stirring at the same temperature for 10 minutes and then it was slowly warmed to room temperature. The mixture was then left under stirring at RT overnight. Once the disappearance of the precursor was verified by TLC, the reaction mixture was slowly poured in an ice-water mixture; the water phase was acidified with 1N aqueous HCl and extracted several times with EtOAc. The combined organic phase was washed with saturated $NaHCO_3$ solution and brine, then dried over anhydrous

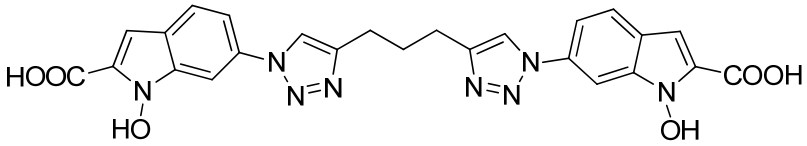
sodium sulphate. Evaporation under vacuum of the organic solvent afforded a crude product which was purified by column chromatography over silica gel using EtOAc/MeOH 9:1 as the eluent, to yield the nitroaryl-ketoester derivative **3.199** (3.08 g, 4.96 mmol, 67% yield). ^{1}H NMR (DMSO-d_6) δ (ppm): 2.05-2.16 (m, 2H), 2.79-2.92 (m, 4H), 3.86 (s, 6H), 4.67 (s, 4H), 7.73 (d, 2H, J = 7.9 Hz), 8.22-8.32 (m, 2H), 8.62 (d, 2H, J = 1.6 Hz), 8.84 (s, 2H); signals imputable to the enol form (~ 66%) δ (ppm): 3.84 (s, 6H), 6.62 (s, 2H), 8.41 (d, 2H, J = 9.0 Hz), 8.49 (d, 2H, J = 2.0 Hz), 8.80 (s, 2H).

Dimethyl 6,6'-(4,4'-(propane-1,3-diyl)bis(1*H*-1,2,3-triazole-4,1-diyl))bis(1-hydroxy-1*H*-indole-2-carboxylate) (3.200)



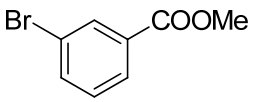
Ketoester **3.199** (800 mg, 1.29 mmol) was dissolved in THF (2.2 mL) and the resulting solution was added to an aqueous solution (2.2 mL) of sodium hypophosphite monohydrate (424 mg, 4.00 mmol). Then 13 mg of 10% palladium over charcoal was added and the resulting suspension was stirred ar RT overnight. Then it was monitored by TLC and $H_2PO_2Na{\cdot}H_2O$ (424 mg, 4.00 mmol) and Pd/C (13 mg) were added and the reaction was heated to 40 °C. After an additional hour, the disappearance of the precursor is verified by TLC and the mixture was concentrated, diluted with water and repeatedly extracted with EtOAc. The combined organic phase was dried over anhydrous sodium sulphate and evaporated to afford a crude residue that was purified by column chromatography over silica gel using CH_2Cl_2/MeOH 95:5 as the eluent, to give the *N*-hydroxyindole **3.200** (158 mg, 0.284 mmol, 22% yield). ^{1}H NMR (DMSO-d_6) δ (ppm): 2.13 (t, 2H, J = 6.9 Hz), 2.84 (t, 4H, J = 7.3 Hz), 3.88 (s, 6H), 7.17 (d, 2H, J = 0.7 Hz), 7.68 (dd, 2H, J = 8.8, 1.8 Hz), 7.85 (d, 2H, J = 8.6 Hz), 7.93 (t, 2H, J = 0.9 Hz), 8.76 (s, 2H), 11.73 (bs, 2H).

6,6'-(4,4'-(Propane-1,3-diyl)bis(1H-1,2,3-triazole-4,1-diyl))bis(1-hydroxy-1H-indole-2-carboxylic acid) (3.170f)



Methyl ester **3.200** (100 mg, 0.180 mmol) was dissolved in a 1:1 mixture of THF/methanol (1.8 mL) and treated with 0.54 mL of 2N aqueous solution of LiOH. The reaction was kept under stirring in the dark at RT and it was monitored by TLC. After 12 h, the reaction mixture was concentrated, diluted with water and washed with Et_2O. Then, the aqueous phase was treated with 1N aqueous HCl and extracted several times with EtOAc. The combined ethyl acetate organic phase was dried over anhydrous sodium sulphate and evaporated to afford the desired *N*-hydroxyindol-carboxylic acid product **3.170f** (85.1 mg, 0.161 mmol, 89% yield) without any further purification. ^{1}H NMR (DMSO-d_6) δ (ppm): 2.13 (t, 2H, J = 7.4 Hz), 2.85 (t, 4H, J = 7.1 Hz), 7.11 (s, 2H), 7.67 (dd, 2H, J = 8.8, 1.6 Hz), 7.84 (d, 2H, J = 8.6 Hz), 7.93 (s, 2H), 8.77 (s, 2H). ^{13}C NMR (DMSO-d_6) δ (ppm): 24.64 (2C), 28.45 (1C), 100.52 (2C), 104.87 (2C), 113.17 (2C), 120.49 (4C), 123.68 (2C), 128.39 (2C), 134.00 (2C), 135.53 (2C), 147.64 (2C), 160.80 (2C). MS *m/z* 546 (M +NH_4^+, 5%), 256 ((M +NH_4^+)/2 –OH, 40%), 239 ((M +NH_4^+)/2 –2OH, 100%).

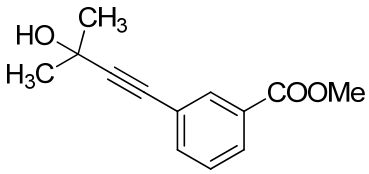
Methyl 3-bromobenzoate (3.207a)



Commercially available 3-bromobenzoic acid **3.206a** (1.00 g, 4.97 mmol) was dissolved in MeOH in a sealed vial, catalytic conc. H_2SO_4 (2 drops) was added and the resulting solution was refluxed for 16 h. After being cooled to RT, the mixture was concentrated, diluted with EtOAc, washed with saturated $NaHCO_3$ aqueous solution and then dried over anhydrous sodium sulphate. The evaporation of the solvent afforded the methyl ester **3.207a** (1.04 g, 4.84 mmol, 97% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 3.92 (s,

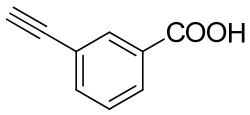
3H), 7.32 (t, 1H, *J* = 7.9 Hz), 7.69 (dt, 1H, *J* = 8.0, 1.6 Hz), 7.97 (dt, 1H, *J* = 7.8, 1.2 Hz), 8.18 (t, 1H, *J* = 1.8 Hz).

Methyl 3-(3-hydroxy-3-methylbut-1-yn-1-yl)benzoate (3.208a)



A solution of $Pd(OAc)_2$ (43 mg, 0.19 mmol) and triphenylphosphine (244 mg, 0.930 mmol) in dry DMF (2.1 mL) was stirred at RT under nitrogen for 10 min. After that period, aryl-bromide **3.207a** (800 mg, 3.72 mmol), copper iodide (36 mg, 0.19 mmol), 2-methyl-3-butyn-2-ol (0.44 mL, 0.38 g, 4.5 mmol) and Et_3N (11.2 mL) were sequentially added under N_2. The mixture was stirred under reflux for 60 h. After being cooled to RT, the mixture was diluted with water and extracted several times with EtOAc. The combined organic phase was washed with saturated $NaHCO_3$ aqueous solution and water, dried over anhydrous sodium sulphate and concentrated. The crude product was purified by flash chromatography (*n*-hexane/ EtOAc 8:2) to yield **3.208a** (717 mg, 3.29 mmol, 88% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 1.62 (s, 6H), 3.91 (s, 3H), 7.38 (t, 1H, *J* = 7.8 Hz), 7.59 (dt, 1H, *J* = 7.8, 1.5 Hz), 7.97 (dt, 1H, *J* = 7.8, 1.5 Hz), 8.09 (t, 1H, *J* = 1.6 Hz).

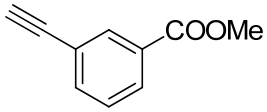
3-Ethynylbenzoic acid (3.209a)



Sodium hydroxide (525 mg, 13.1 mmol) was dissolved in refluxing l-butanol (20.7 mL), **3.208a** (717 mg, 3.28 mmol) was added and the mixture was refluxed for 10 min. The reaction mixture was cooled in an ice bath, diluted with water, acidified with 1N aqueous HCl and extracted several times with EtOAc. The combined organic phase was dried over anhydrous sodium sulphate. Evaporation under vacuum of the organic solvent afforded compound **3.209a** (504 mg, 3.45 mmol, >99% yield). ^{1}H NMR

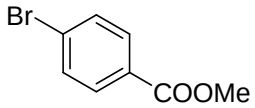
($CDCl_3$) δ (ppm): 3.14 (s, 1H), 7.45 (t, 1H, *J* = 7.7 Hz), 7.72 (d, 1H, *J* = 7.7 Hz), 8.09 (d, 1H, *J* = 7.5 Hz), 8.24 (s, 1H).

Methyl 3-ethynylbenzoate (3.210a)



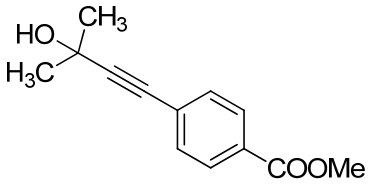
Carboxylic acid **3.209a** (495 mg, 3.39 mmol) was dissolved in MeOH in a sealed vial, catalytic conc. H_2SO_4 (2 drops) was added and the resulting solution was refluxed for 3 h. After being cooled to RT, the mixture was concentrated, diluted with EtOAc, washed with saturated $NaHCO_3$ aqueous solution and then dried over anhydrous sodium sulphate. The evaporation of the solvent afforded a crude residue, which was subjected to flash chromatography using *n*-hexane/EtOAc 95:5 as the eluent, to yield the methyl ester **3.210a** (315 mg, 1.97 mmol, 58% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 3.12 (s, 1H), 3.92 (s, 1H), 7.41 (t, 1H, *J* = 7.8 Hz), 7.67 (dt, 1H, *J* = 7.7, 1.4 Hz), 8.02 (dt, 1H, *J* = 7.8, 1.4 Hz), 8.17 (t, 1H, *J* = 1.3 Hz).

Methyl 4-bromobenzoate (3.207b)



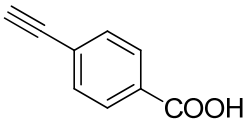
Commercially available 4-bromobenzoic acid **3.206b** (1.00 g, 4.97 mmol) was dissolved in MeOH in a sealed vial, catalytic conc. H_2SO_4 (2 drops) was added and the resulting solution was refluxed for 20 h. After being cooled to RT, the mixture was concentrated, diluted with EtOAc, washed with saturated $NaHCO_3$ aqueous solution and then dried over anhydrous sodium sulphate. The evaporation of the solvent afforded the methyl ester **3.207b** (1.00 g, 4.65 mmol, 94% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 3.92 (s, 3H), 7.58 (AA'XX', 2H, J_{AX} = 8.5 Hz, $J_{AA'/XX'}$ = 2.1 Hz), 7.90 (AA'XX', 2H, J_{AX} = 8.8 Hz, $J_{AA'/XX'}$ = 2.1 Hz).

Methyl 4-(3-hydroxy-3-methylbut-1-yn-1-yl)benzoate (3.208b)



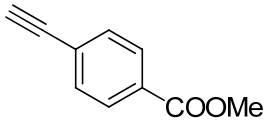
A solution of $Pd(OAc)_2$ (11 mg, 0.050 mmol) and triphenylphosphine (61 mg, 0.23 mmol) in dry DMF (0.5 mL) was stirred at RT under nitrogen for 10 min. After that period, aryl-bromide **3.207b** (200 mg, 0.930 mmol), copper iodide (9.5 mg, 0.050 mmol), 2-methyl-3-butyn-2-ol (0.11 mL, 94 mg, 1.1 mmol) and Et_3N (2.8 mL) were sequentially added under N_2. The mixture was stirred under reflux for 72 h. After being cooled to RT, the mixture was diluted with water and extracted several times with EtOAc. The combined organic phase was washed with saturated $NaHCO_3$ aqueous solution and water, dried over anhydrous sodium sulphate and concentrated. The crude product was purified by flash chromatography (*n*-hexane/ EtOAc 8:2) to yield **3.208b** (169 mg, 0.774 mmol, 83% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 1.63 (s, 6H), 3.91 (s, 3H), 7.47 (AA'XX', 2H, J_{AX} = 8.4 Hz, $J_{AA'XX'}$ = 1.7 Hz), 7.97 (AA'XX', 2H, J_{AX} = 8.3 Hz, $J_{AA'XX'}$ = 1.7 Hz).

4-Ethynylbenzoic acid (3.209b)



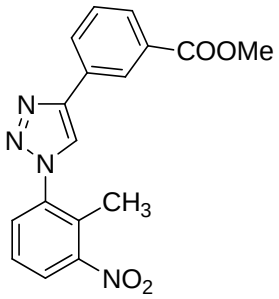
Sodium hydroxide (106 mg, 2.64 mmol) was dissolved in refluxing l-butanol (4.2 mL), **3.208b** (145 mg, 0.664 mmol) was added and the mixture was refluxed for 15 min. The reaction mixture was cooled in an ice bath, diluted with water, acidified with 1N aqueous HCl and extracted several times with EtOAc. The combined organic phase was dried over anhydrous sodium sulphate. Evaporation under vacuum of the organic solvent afforded compound **3.209b** (62.6 mg, 0.428 mmol, 65% yield). ^{1}H NMR (CD_3OD) δ (ppm): 3.72 (s, 1H), 7.56 (d, 2H, J = 7.9 Hz), 7.99 (d, 2H, J = 7.9 Hz).

Methyl 4-ethynylbenzoate (3.210b)



Carboxylic acid **3.209b** (60.0 mg, 0.411 mmol) was dissolved in MeOH in a sealed vial, catalytic conc. H_2SO_4 (2 drops) was added and the resulting solution was refluxed for 4 h. After being cooled to RT, the mixture was concentrated, diluted with EtOAc, washed with saturated $NaHCO_3$ aqueous solution and then dried over anhydrous sodium sulphate. The evaporation of the solvent afforded the methyl ester the methyl ester **3.210b** (59.0 mg, 0.368 mmol, 90% yield). ^{1}H NMR (CD_3OD) δ (ppm): 3.75 (s, 1H), 3.91 (s, 3H), 7.57 (AA'XX', 2H, J_{AX} = 8.6 Hz, $J_{AA'XX'}$ = 1.8 Hz), 7.99 (AA'XX', 2H, J_{AX} = 8.6 Hz, $J_{AA'XX'}$ = 1.7 Hz).

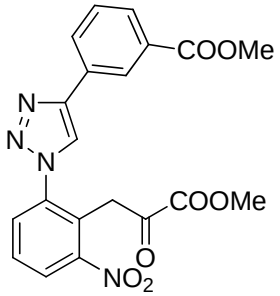
Methyl 3-(1-(2-methyl-3-nitrophenyl)-1*H*-1,2,3-triazol-4-yl)benzoate (3.211)



Alkyne **3.210a** (298 mg, 1.86 mmol) and azide **3.174** (331 mg, 1.86 mmol) were suspended in a 1:1 mixture of water and tert-butyl alcohol (7.4 mL). A freshly prepared sodium ascorbate aqueous solution (36.8 mg, 0.186 mmol, in 0.2 mL of water) was added, followed by copper (II) sulfate pentahydrate aqueous solution (4.6 mg, 0.019 mmol, in 0.06 mL of water). The heterogeneous mixture was stirred vigorously at 200 °C in a microwave reactor for 15 min. The reaction mixture was diluted with water, cooled in ice and the precipitate was collected by filtration. After washing the precipitate with cold water, it was dried and then subjected to flash chromatography using *n*-hexane/EtOAc 7:3 as the eluent, to yield the triazole derivative **3.211** (325 mg, 0.961 mmol, 52% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 2.37 (s, 3H), 3.96 (s, 3H), 7.56 (t,

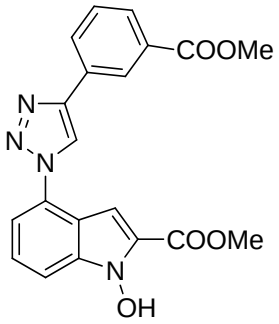
1H, *J* = 8.0 Hz), 7.58 (t, 1H, *J* = 7.8 Hz), 7.69 (dd, 1H, *J* = 8.0, 1.6 Hz), 8.05-8.09 (m, 2H), 8.10 (s, 1H), 8.21 (dt, 1H, *J* = 7.7, 1.5 Hz), 8.51 (t, 1H, *J* = 1.7 Hz).

Methyl 3-(1-(2-(3-methoxy-2,3-dioxopropyl)-3-nitrophenyl)-1*H*-1,2,3-triazol-4-yl)benzoate (3.212)



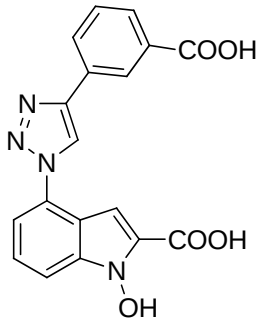
A stirred suspension of sodium hydride (60% dispersion in mineral oil, 152 mg, 3.80 mmol) in 2.6 mL of anhydrous DMF cooled to –15 °C under nitrogen was treated dropwise with a solution containing the nitrotoluene precursor **3.211** (320 mg, 0.946 mmol) and dimethyl oxalate (561 mg, 4.75 mmol) in 2.7 mL of anhydrous DMF. The mixture was left under stirring at the same temperature for 10 minutes and then it was slowly warmed to room temperature. The mixture was then left under stirring at RT for 24 h. Once the disappearance of the precursor was verified by TLC, the reaction mixture was slowly poured in an ice-water mixture; the water phase was acidified with 1N aqueous HCl and extracted several times with EtOAc. The combined organic phase was washed with saturated $NaHCO_3$ solution and brine, then dried over anhydrous sodium sulphate. Evaporation under vacuum of the organic solvent afforded a crude product which was purified by column chromatography over silica gel using *n*-hexane/EtOAc 6:4 as the eluent, to yield the nitroaryl-ketoester derivative **3.212** (202 mg, 0.476 mmol, 50% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 3.90 (s, 3H), 3.95 (s, 3H), 4.37 (s, 2H), 7.56 (t, 1H, *J* = 7.8 Hz), 7.69-7.81 (m, 2H), 8.04-8.16 (m, 2H), 8.12 (s, 1H), 8.32 (dd, 1H, *J* = 7.4, 2.1 Hz), 8.47 (t, 1H, *J* = 1.6 Hz). ^{13}C NMR ($CDCl_3$) δ (ppm): 38.70, 52.43, 53.67, 122.33, 126.45, 127.03 (2C), 129.29, 129.47, 129.89, 129.95, 130.27, 131.06, 131.77, 138.90, 147.66, 149.85, 160.08, 166.65, 188.34.

Methyl 1-hydroxy-4-(4-(3-(methoxycarbonyl)phenyl)-1*H*-1,2,3-triazol-1-yl)-1*H*-indole-2-carboxylate (3.213)



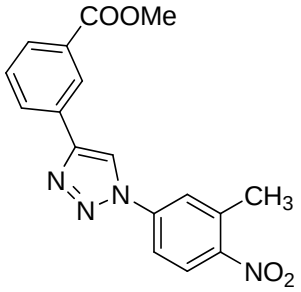
Ketoester **3.212** (150 mg, 0.353 mmol) was dissolved in THF (0.6 mL) and the resulting solution was added to an aqueous solution (0.6 mL) of sodium hypophosphite monohydrate (114 mg, 1.08 mmol). Then 3.5 mg of 10% palladium over charcoal was added and the resulting suspension was stirred ar RT. After 18 h the disappearance of the precursor is verified by TLC and the mixture was concentrated, diluted with water and repeatedly extracted with EtOAc. The combined organic phase was dried over anhydrous sodium sulphate and evaporated to afford a crude residue that was purified by column chromatography over silica gel using *n*-hexane/EtOAc 6:4 as the eluent, to give the *N*-hydroxyindole **3.213** (62.8 mg, 0.160 mmol, 45% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 3.97 (s, 3H), 4.01 (s, 3H), 7.38-7.51 (m, 2H), 7.47 (s, 1H), 7.58 (t, 1H, J = 7.8 Hz), 7.65-7.69 (m, 1H), 8.06 (dt, 1H, J = 7.7, 1.4 Hz), 8.25 (dt, 1H, J = 1.5 Hz), 8.38 (s, 1H), 8.53 (t, 1H, J = 1.7 Hz), 10.53 (bs, 1H).

4-(4-(3-Carboxyphenyl)-1*H*-1,2,3-triazol-1-yl)-1-hydroxy-1*H*-indole-2-carboxylic acid (3.201)



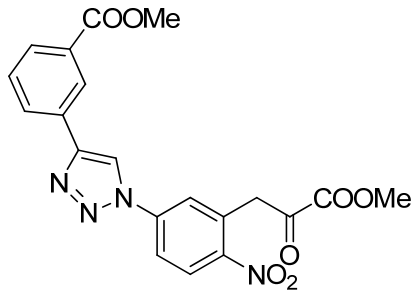
Methyl ester **3.213** (52.0 mg, 0.133 mmol) was dissolved in a 1:1 mixture of THF/methanol (1.3 mL) and treated with 0.4 mL of 2N aqueous solution of LiOH. The reaction was kept under stirring in the dark at RT and it was monitored by TLC. After 27 h, the reaction mixture was concentrated, diluted with water and washed with Et_2O. Then, the aqueous phase was treated with 1N aqueous HCl and extracted several times with EtOAc. The combined ethyl acetate organic phase was dried over anhydrous sodium sulphate and evaporated to afford the desired *N*-hydroxyindol-carboxylic acid product **3.201** (39.5 mg, 0.108 mmol, 82% yield) without any further purification. ^{1}H NMR (DMSO-d_6) δ (ppm): 7.41 (s, 1H), 7.48-7.69 (m, 4H), 7.96 (dt, 1H, *J* = 8.0, 1.4 Hz), 8.27 (dt, 1H, *J* = 7.6, 1.5 Hz), 8.61 (t, 1H, *J* = 1.6 Hz), 9.50 (s, 1H). ^{13}C NMR (DMSO-d_6) δ (ppm): 103.27, 110.53, 112.86, 113.35, 121.33, 124.81, 126.14, 127.88, 128.89, 129.27, 129.60, 129.69, 130.60, 131.51, 136.97, 145.95, 160.73, 167.00. MS *m/z* 365 ($M+H^+$).

Methyl 3-(1-(3-methyl-4-nitrophenyl)-1*H*-1,2,3-triazol-4-yl)benzoate (3.214a)



Alkyne **3.210a** (350 mg, 2.18 mmol) and azide **3.175** (388 mg, 2.18 mmol) were suspended in a 1:1 mixture of water and tert-butyl alcohol (8.7 mL). A freshly prepared sodium ascorbate aqueous solution (43.2 mg, 0.218 mmol, in 0.2 mL of water) was added, followed by copper (II) sulfate pentahydrate aqueous solution (5.4 mg, 0.022 mmol, in 0.07 mL of water). The heterogeneous mixture was stirred 200 °C in a microwave reactor for 15 min. The reaction mixture was diluted with water, cooled in ice and the precipitate was collected by filtration. After washing the precipitate with cold water, it was dried and then subjected to flash chromatography using *n*-hexane/EtOAc 7:3 as the eluent, to yield the triazole derivative **3.214a** (306 mg, 0.904 mmol, 41% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 2.76 (s, 3H), 3.97 (s, 3H), 7.57 (t, 1H, *J* = 7.7 Hz), 7.81 (dd, 1H, *J* = 8.9, 2.4 Hz), 7.89 (d, 1H, *J* = 2.4 Hz), 8.06 (dt, 1H, *J* = 8.0, 1.4 Hz), 8.18-8.26 (m, 2H), 8.37 (s, 1H), 8.50 (t, 1H, *J* = 1.4 Hz).

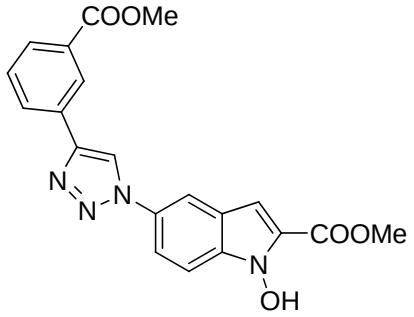
Methyl 3-(1-(3-(3-methoxy-2,3-dioxopropyl)-4-nitrophenyl)-1*H*-1,2,3-triazol-4-yl)benzoate (3.215a)



A stirred suspension of sodium hydride (60% dispersion in mineral oil, 142 mg, 3.56 mmol) in 2.5 mL of anhydrous DMF cooled to −15 °C under nitrogen was treated dropwise with a solution containing the nitrotoluene precursor **3.214a** (300 mg, 0.887 mmol) and dimethyl oxalate (526 mg, 4.45 mmol) in 2.5 mL of anhydrous DMF. The mixture was left under stirring at the same temperature for 10 minutes and then it was slowly warmed to room temperature. The mixture was then left under stirring at RT for4 h. Once the disappearance of the precursor was verified by TLC, the reaction mixture was slowly poured in an ice-water mixture; the water phase was acidified with 1N aqueous HCl and extracted several times with EtOAc. The combined organic phase was

washed with saturated $NaHCO_3$ solution and brine, then dried over anhydrous sodium sulphate. Evaporation under vacuum of the organic solvent afforded a crude product which was purified by column chromatography over silica gel using *n*-hexane/EtOAc 6:4 as the eluent, to yield the nitroaryl-ketoester derivative **3.215a** (109 mg, 0.257 mmol, 29% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 3.97 (s, 3H), 3.98 (s, 3H), 4.69 (s, 2H), 7.58 (t, 1H, *J* = 7.7 Hz), 7.93 (dd, 1H, *J* = 8.9, 2.4 Hz), 7.97 (d, 1H, *J* = 2.4 Hz), 8.07 (dt, 1H, *J* = 7.8, 1.3 Hz), 8.18-8.24 (m, 2H), 8.40 (s, 1H), 8.50 (t, 1H, *J* = 1.4 Hz); signals imputable to the enol form (~ 20%) δ (ppm): 4.00 (s, 3H), 7.05 (s, 1H), 8.38 (s, 1H).

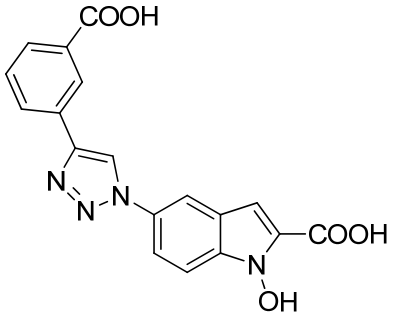
Methyl 1-hydroxy-5-(4-(3-(methoxycarbonyl)phenyl)-1*H*-1,2,3-triazol-1-yl)-1*H*-indole-2-carboxylate (3.216a)



Triethylamine (0.17 mL, 0.12 g, 1.2 mmol) was added dropwise to a stirred solution of $SnCl_2 \cdot 2H_2O$ (81.3 mg, 0.360 mmol) and PhSH (0.12 mL, 0.12 g, 1.1 mmol) in acetonitrile (5 mL) at room temperature to generate a yellow precipitate over a period of 5 min. Then, **3.215a** (100 mg, 0.236 mmol) was transferred to the suspension and stirred at room temperature for 5 more min. After complete consumption of the starting material, the mixture was diluted with water and repeatedly extracted with EtOAc. The combined organic phase was dried over anhydrous sodium sulphate, diluted with toluene and a catalytic amount of *p*-toluensulfonic acid was added. Then it was evaporated to afford a crude residue that was purified by column chromatography over silica gel using $CHCl_3$/MeOH 98:2 as the eluent, to give the *N*-hydroxyindole **3.216a** (43.0 mg, 0.110 mmol, 46% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 3.96 (s, 3H), 4.03 (s, 3H), 7.14 (s, 1H), 7.56 (t, 1H, *J* = 7.8 Hz), 7.68 (d, 1H, *J* = 8.7 Hz), 7.77 (dd, 1H, *J* =

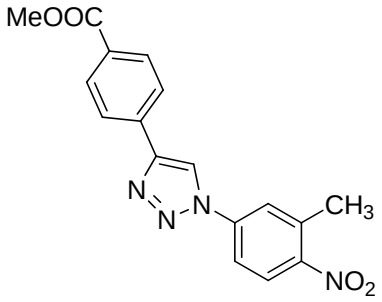
8.6, 1.8 Hz), 8.01-8.08 (m, 2H), 8.20 (dt, 1H, *J* = 7.8, 1.6 Hz), 8.29 (s, 1H), 8.50 (t, 1H, *J* = 1.6 Hz), 10.76 (bs, 1H).

5-(4-(3-Carboxyphenyl)-1*H*-1,2,3-triazol-1-yl)-1-hydroxy-1*H*-indole-2-carboxylic acid (3.202)



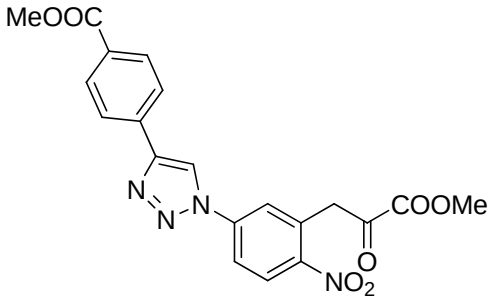
Methyl ester **3.216a** (40.0 mg, 0.102 mmol) was dissolved in a 1:1 mixture of THF/methanol (1.0 mL) and treated with 0.62 mL of 2N aqueous solution of LiOH. The reaction was kept under stirring in the dark at RT and it was monitored by TLC. After 12 h, the reaction mixture was concentrated, diluted with water and washed with Et_2O. Then, the aqueous phase was treated with 1N aqueous HCl and extracted several times with EtOAc. The combined ethyl acetate organic phase was dried over anhydrous sodium sulphate and evaporated to afford the desired *N*-hydroxyindol-carboxylic acid product **3.202** (37.0 mg, 0.102 mmol, 99% yield) without any further purification. ^{1}H NMR (DMSO-d_6) δ (ppm): 7.13 (s, 1H), 7.64 (t, 1H, *J* = 7.7 Hz), 7.67 (dd, 1H, *J* = 8.6, 1.8 Hz), 7.87 (d, 1H, *J* = 2.0 Hz), 7.94 (dt, 1H, *J* = 8.2, 1.4 Hz), 8.18-8.24 (m, 2H), 8.55 (t, 1H, *J* = 1.6 Hz), 9.45 (s, 1H). ^{13}C NMR (DMSO-d_6) δ (ppm): 104.83, 110.73, 113.59, 117.82, 120.22, 120.60, 125.84, 128.58, 129.07, 129.21 (2C), 130.31, 130.69, 131.45, 134.91, 146.11, 160.62, 166.80.

Methyl 4-(1-(3-methyl-4-nitrophenyl)-1*H*-1,2,3-triazol-4-yl)benzoate (3.214b)



Alkyne **3.210b** (500 mg, 3.12 mmol) and azide **3.175** (556 mg, 3.12 mmol) were suspended in a 1:1 mixture of water and tert-butyl alcohol (12.5 mL). A freshly prepared sodium ascorbate aqueous solution (61.8 mg, 0.312 mmol, in 0.3 mL of water) was added, followed by copper (II) sulfate pentahydrate aqueous solution (7.8 mg, 0.031 mmol, in 0.1 mL of water). The heterogeneous mixture was stirred vigorously at 200 °C in a microwave reactor for 15 min. The reaction mixture was diluted with water, cooled in ice and the precipitate was collected by filtration. After washing the precipitate with cold water, it was dried and then subjected to flash chromatography using *n*-hexane/EtOAc 7:3 as the eluent, to yield the triazole derivative **3.214b** (784 mg, 2.32 mmol, 74% yield). ^{1}H NMR (DMSO-d_6) δ (ppm): 2.66 (s, 3H), 3.89 (s, 3H), 8.00-8.15 (m, 5H), 8.18 (d, 1H, J = 2.0 Hz), 8.29 (d, 1H, J = 8.2 Hz), 9.63 (s, 1H).

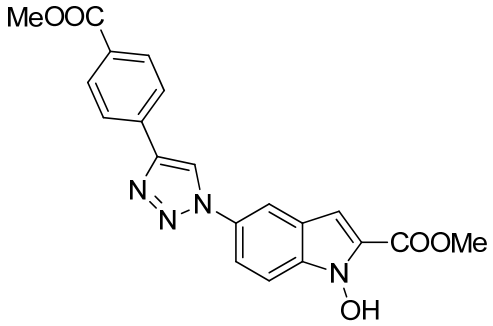
Methyl 4-(1-(3-(3-methoxy-2,3-dioxopropyl)-4-nitrophenyl)-1*H*-1,2,3-triazol-4-yl)benzoate (3.215b)



A stirred suspension of sodium hydride (60% dispersion in mineral oil, 237 mg, 5.92 mmol) in 4.1 mL of anhydrous DMF cooled to –15 °C under nitrogen was treated

dropwise with a solution containing the nitrotoluene precursor **3.214b** (500 mg, 1.48 mmol) and dimethyl oxalate (874 mg, 7.40 mmol) in 4.2 mL of anhydrous DMF. The mixture was left under stirring at the same temperature for 10 minutes and then it was slowly warmed to room temperature. The mixture was then left under stirring at RT for 4 h. Once the disappearance of the precursor was verified by TLC, the reaction mixture was slowly poured in an ice-water mixture; the water phase was acidified with 1N aqueous HCl and extracted several times with EtOAc. The combined organic phase was washed with saturated $NaHCO_3$ solution and brine, then dried over anhydrous sodium sulphate. Evaporation under vacuum of the organic solvent afforded a crude product which was purified by column chromatography over silica gel using *n*-hexane/EtOAc 6:4 as the eluent, to yield the nitroaryl-ketoester derivative **3.215b** (234 mg, 0.551 mmol, 37% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 3.96 (s, 3H), 3.98 (s, 3H), 4.69 (s, 2H), 7.92-8.02 (m, 5H), 8.13-8.18 (m, 2H), 8.38 (s, 1H).

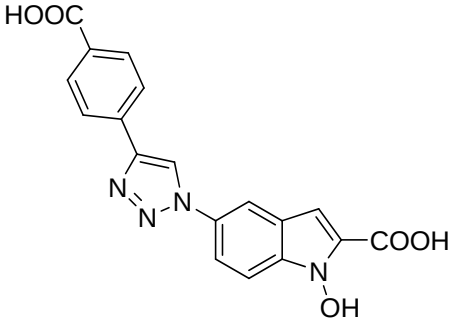
Methyl 1-hydroxy-5-(4-(4-(methoxycarbonyl)phenyl)-1*H*-1,2,3-triazol-1-yl)-1*H*-indole-2-carboxylate (3.216b)



Triethylamine (0.17 mL, 0.12 g, 1.2 mmol) was added dropwise to a stirred solution of $SnCl_2 \cdot 2H_2O$ (81.3 mg, 0.360 mmol) and PhSH (0.12 mL, 0.12 g, 1.1 mmol) in acetonitrile (5 mL) at room temperature to generate a yellow precipitate over a period of 5 min. Then, **3.215b** (100 mg, 0.236 mmol) was transferred to the suspension and stirred at room temperature for 5 more min. After complete consumption of the starting material, the mixture was diluted with water and repeatedly extracted with EtOAc. The combined organic phase was dried over anhydrous sodium sulphate, diluted with toluene and a catalytic amount of *p*-toluensulfonic acid was added. Then it was

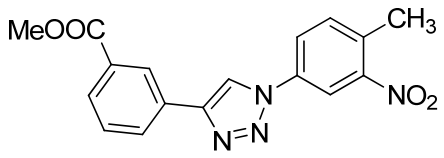
evaporated to afford a crude residue that was purified by column chromatography over silica gel using $CHCl_3$/MeOH 98:2 as the eluent, to give the *N*-hydroxyindole **3.216b** (31.1 mg, 0.0793 mmol, 34% yield). ^{1}H NMR (DMSO-d_6) δ (ppm): 3.89 (s, 3H), 3.90 (s, 3H), 7.26 (s, 1H), 7.71 (d, 1H, J = 8.7 Hz), 7.92 (dd, 1H, J = 8.9, 2.0 Hz), 8.10 (s, 4H), 8.25 (d, 1H, J = 2.0 Hz), 9.48 (s, 1H), 11.77 (bs, 1H).

5-(4-(4-Carboxyphenyl)-1*H*-1,2,3-triazol-1-yl)-1-hydroxy-1*H*-indole-2-carboxylic acid (3.204)



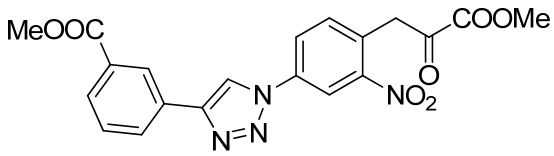
Methyl ester **3.216b** (12.0 mg, 0.0306 mmol) was dissolved in a 1:1 mixture of THF/methanol (0.3 mL) and treated with 0.2 mL of 2N aqueous solution of LiOH. The reaction was kept under stirring in the dark at RT. After 24 h it was monitored by TLC and another portion of LiOH (0.2 mL) was added. After further 72 h, the reaction mixture was concentrated, diluted with water and washed with Et_2O. Then, the aqueous phase was treated with 1N aqueous HCl and extracted several times with EtOAc. The combined ethyl acetate organic phase was dried over anhydrous sodium sulphate and evaporated to afford the desired *N*-hydroxyindol-carboxylic acid product **3.204** (11.0 mg, 0.0302 mmol, 99% yield) without any further purification. ^{1}H NMR (DMSO-d_6) δ (ppm): 6.74 (s, 1H), 7.52-7.72 (m, 3H), 8.07 (s, 4H), 9.39 (s, 1H).

Methyl 3-(1-(4-methyl-3-nitrophenyl)-1*H*-1,2,3-triazol-4-yl)benzoate (3.217a)



Alkyne **3.210a** (418 mg, 2.61 mmol) and azide **3.176** (465 mg, 2.61 mmol) were suspended in a 1:1 mixture of water and tert-butyl alcohol (3.5 mL). A freshly prepared sodium ascorbate aqueous solution (51.7 mg, 0.261 mmol, in 0.2 mL of water) was added, followed by copper (II) sulfate pentahydrate aqueous solution (6.5 mg, 0.026 mmol, in 0.09 mL of water). The heterogeneous mixture was stirred at 200 °C in a microwave reactor for 15 min. The reaction mixture was diluted with water, cooled in ice and the precipitate was collected by filtration. After washing the precipitate with cold water, it was dried and then subjected to flash chromatography using *n*-hexane/EtOAc 7:3 as the eluent, to yield the triazole derivative **3.217a** (729 mg, 2.15 mmol, 83% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 2.71 (s, 3H), 3.97 (s, 3H), 7.57 (t, 1H, *J* = 7.7 Hz), 7.58 (d, 1H, *J* = 8.2 Hz), 8.02-8.10 (m, 2H), 8.20 (dt, 1H, *J* = 7.8, 1.3 Hz), 8.36 (s, 1H), 8.43 (d, 1H, *J* = 2.4 Hz), 8.50 (t, 1H, *J* = 1.7 Hz).

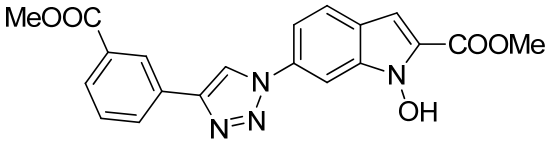
Methyl 3-(1-(4-(3-methoxy-2,3-dioxopropyl)-3-nitrophenyl)-1*H*-1,2,3-triazol-4-yl)benzoate (3.218a)



A stirred suspension of sodium hydride (60% dispersion in mineral oil, 237 mg, 5.92 mmol) in 4.1 mL of anhydrous DMF cooled to –15 °C under nitrogen was treated dropwise with a solution containing the nitrotoluene precursor **3.217a** (500 mg, 1.48 mmol) and dimethyl oxalate (874 mg, 7.40 mmol) in 4.2 mL of anhydrous DMF. The mixture was left under stirring at the same temperature for 10 minutes and then it was slowly warmed to room temperature. The mixture was then left under stirring at RT for 20 h. Once the disappearance of the precursor was verified by TLC, the reaction mixture was slowly poured in an ice-water mixture; the water phase was acidified with 1N aqueous HCl and extracted several times with EtOAc. The combined organic phase was washed with saturated $NaHCO_3$ solution and brine, then dried over anhydrous sodium sulphate. Evaporation under vacuum of the organic solvent afforded a crude product which was purified by column chromatography over silica gel using *n*-hexane/EtOAc

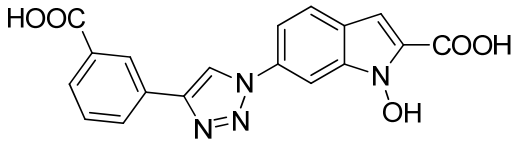
4:6 as the eluent, to yield the nitroaryl-ketoester derivative **3.218a** (385 mg, 0.907 mmol, 61% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 3.93 (s, 3H), 3.97 (s, 3H), 4.65 (s, 2H), 7.57 (t, 1H, *J* = 7.7 Hz), 7.58 (d, 1H, *J* = 8.2 Hz), 8.02-8.10 (m, 2H), 8.20 (dt, 1H, *J* = 7.8, 1.3 Hz), 8.41 (s, 1H), 8.43 (d, 1H, *J* = 2.4 Hz), 8.63 (t, 1H, *J* = 1.7 Hz); signals imputable to the enol form (~ 20%) δ (ppm): 3.98 (s, 3H), 6.98 (s, 1H), 8.45 (d, 1H, *J* = 8.2 Hz).

Methyl 1-hydroxy-6-(4-(3-(methoxycarbonyl)phenyl)-1*H*-1,2,3-triazol-1-yl)-1*H*-indole-2-carboxylate (3.219a)



To a solution containing the ketoester **3.218a** (100 mg, 0.236 mmol) in 0.8 mL of MeOH, was added lead powder (213 mg, 1.03 mmol) and 0.5 mL of HCO_2HNEt_3. The mixture was stirred at 55 °C for 18 h, cooled to RT, and filtered over a pad of Celite. The solvent was removed under reduced pressure and the residue purified by column chromatography over silica gel using $CHCl_3$/MeOH 98:2 as the eluent, to give the *N*-hydroxyindole **3.219a** (27.1 mg, 0.0691 mmol, 29% yield). ^{1}H NMR (acetone-d_6) δ (ppm): 3.85 (s, 3H), 3.92 (s, 3H), 7.20 (d, 1H, *J* = 0.9 Hz), 7.64 (t, 1H, *J* = 7.8 Hz), 7.81 (dd, 1H, *J* = 8.6, 1.8 Hz), 7.91 (d, 1H, *J* = 8.7 Hz), 7.98-8.04 (m, 2H), 8.13 (t, 1H, *J* = 1.4 Hz), 8.28 (dt, 1H, *J* = 7.7, 1.2 Hz), 8.63 (dt, 1H, *J* = 7.2, 1.3 Hz), 10.76 (bs, 1H).

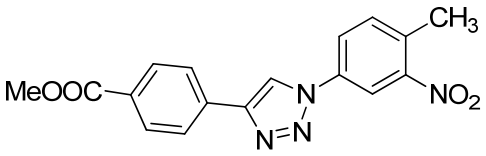
6-(4-(3-Carboxyphenyl)-1*H*-1,2,3-triazol-1-yl)-1-hydroxy-1*H*-indole-2-carboxylic acid (3.203)



Methyl ester **3.219a** (25.0 mg, 0.0637 mmol) was dissolved in a 1:1 mixture of THF/methanol (0.6 mL) and treated with 0.2 mL of 2N aqueous solution of LiOH. The reaction was kept under stirring in the dark at RT and it was monitored by TLC. After

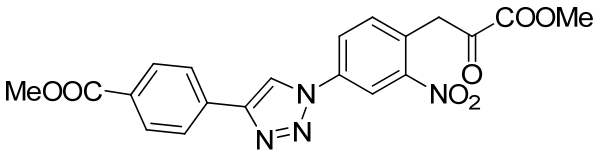
24 h, the reaction mixture was concentrated, diluted with water and washed with Et_2O. Then, the aqueous phase was treated with 1N aqueous HCl and extracted several times with EtOAc. The combined ethyl acetate organic phase was dried over anhydrous sodium sulphate and evaporated to afford the desired *N*-hydroxyindol-carboxylic acid product **3.203** (22.0 mg, 0.0604 mmol, 95% yield) without any further purification. ^{1}H NMR (acetone-d_6) δ (ppm): 7.24 (d, 1H, J = 0.9 Hz), 7.64 (t, 1H, J = 7.7 Hz), 7.82 (dd, 1H, J = 8.6, 1.8 Hz), 7.93 (d, 1H, J = 8.8 Hz), 8.05 (dt, 1H, J = 7.8, 1.4 Hz), 8.15 (t, 1H, J = 1.0 Hz), 8.30 (dt, 1H, J = 7.8, 1.6 Hz), 8.69 (t, 1H, J = 1.6 Hz), 9.30 (s, 1H).

Methyl 4-(1-(4-methyl-3-nitrophenyl)-1*H*-1,2,3-triazol-4-yl)benzoate (3.217b)



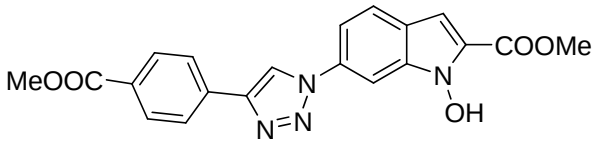
Alkyne **3.210b** (230 mg, 1.44 mmol) and azide **3.176** (256 mg, 1.44 mmol) were suspended in a 1:1 mixture of water and tert-butyl alcohol (5.8 mL). A freshly prepared sodium ascorbate aqueous solution (28.5 mg, 0.144 mmol, in 0.12 mL of water) was added, followed by copper (II) sulfate pentahydrate aqueous solution (3.6 mg, 0.014 mmol, in 0.5 mL of water). The heterogeneous mixture was stirred vigorously at 200 °C in a microwave reactor for 15 min. The reaction mixture was diluted with water, cooled in ice and the precipitate was collected by filtration. After washing the precipitate with cold water, it was dried and then subjected to flash chromatography using *n*-hexane/EtOAc 7:3 as the eluent, to yield the triazole derivative **3.217b** (346 mg, 1.02 mmol, 71% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 2.70 (s, 3H), 3.95 (s, 3H), 7.58 (d, 1H, J = 8.4 Hz), 8.00 (AA'XX', 2H, J_{AX} = 8.4 Hz, $J_{AA'/XX'}$= 1.8 Hz), 8.05 (dd, 1H, J = 8.4, 2.4 Hz), 8.15 (AA'XX', 2H, J_{AX} = 8.2 Hz, $J_{AA'/XX'}$= 1.8 Hz), 8.34 (s, 1H), 8.41 (d, 1H, J = 2.4 Hz).

Methyl 4-(1-(4-(3-methoxy-2,3-dioxopropyl)-3-nitrophenyl)-1*H*-1,2,3-triazol-4-yl)benzoate (3.218b)



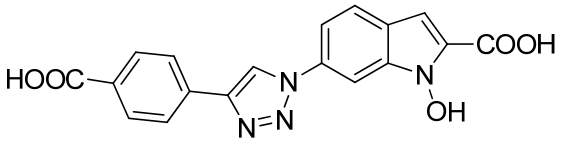
A stirred suspension of sodium hydride (60% dispersion in mineral oil, 133 mg, 3.32 mmol) in 2.3 mL of anhydrous DMF cooled to –15 °C under nitrogen was treated dropwise with a solution containing the nitrotoluene precursor **3.217b** (281 mg, 0.831 mmol) and dimethyl oxalate (490 mg, 4.15 mmol) in 2.4 mL of anhydrous DMF. The mixture was left under stirring at the same temperature for 10 minutes and then it was slowly warmed to room temperature. The mixture was then left under stirring at RT for 19 h. Once the disappearance of the precursor was verified by TLC, the reaction mixture was slowly poured in an ice-water mixture; the water phase was acidified with 1N aqueous HCl and extracted several times with EtOAc. The combined organic phase was washed with saturated $NaHCO_3$ solution and brine, then dried over anhydrous sodium sulphate. Evaporation under vacuum of the organic solvent afforded a crude product which was purified by column chromatography over silica gel using *n*-hexane/EtOAc 1:1 as the eluent, to yield the nitroaryl-ketoester derivative **3.218b** (114 mg, 0.269 mmol, 32% yield). ^{1}H NMR (DMSO-d_6) δ (ppm): 3.85 (s, 3H), 3.87 (s, 3H), 4.71 (s, 2H), 7.80 (d, 1H, J = 8.6 Hz), 8.11 (s, 4H), 8.37 (dd, 1H, J = 8.8, 1.8 Hz), 8.70 (d, 1H, J = 2.4 Hz), 9.67 (s, 1H); signals imputable to the enol form (~ 70%) δ (ppm): 3.89 (s, 3H), 6.67 (s, 1H), 8.10 (m, 4H), 8.33 (dd, 1H, J = 8.8, 1.8 Hz), 8.47 (d, 1H, J = 8.8 Hz), 8.57 (d, 1H, J = 2.2 Hz), 9.63 (s, 1H).

Methyl 1-hydroxy-6-(4-(4-(methoxycarbonyl)phenyl)-1*H*-1,2,3-triazol-1-yl)-1*H*-indole-2-carboxylate (3.219b)



Ketoester **3.218b** (45.0 mg, 0.106 mmol) was dissolved in anhydrous DME (0.11 mL) and the resulting solution was added dropwise to a cooled (0 °C) solution of $SnCl_2 \cdot 2H_2O$ (53.6 mg, 0.238 mmol) in DME (0.11 mL) containing 4Å molecular sieves, previously activated for 18 hours at 130 °C in oven and cooled to RT in a desiccator over anhydrous calcium chloride. The reaction mixture was stirred under nitrogen at RT for 48 h, then it was diluted with water and repeatedly extracted with EtOAc. The combined organic phase was dried over anhydrous sodium sulphate and evaporated to afford a crude residue that was purified by column chromatography over silica gel using $CHCl_3$/MeOH 98:2 as the eluent, to give a mixture (12.9 mg) of the *N*-hydroxyindol-ester derivative **3.219b** (85%, 11.0 mg, 0.0280 mmol, 26% yield) with its indole analogue (15%). ^{1}H NMR (DMSO-d_6) δ (ppm): 3.89 (s, 6H), 7.21 (s, 1H), 7.69 (d, 1H, J = 2.4 Hz), 7.77 (dd, 1H, J = 8.6, 1.8 Hz), 7.92 (d, 1H, J = 8.7 Hz), 8.12 (AB, 4H, J_{AB} = 8.6 Hz), 9.63 (s, 1H), 11.77 (bs, 1H); signals imputable to the indole (~ 15%) δ (ppm): 7.28-7.30 (m, 1H).

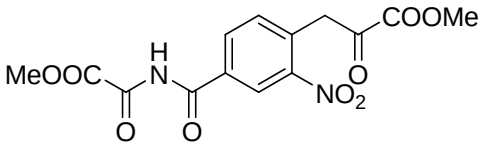
6-(4-(4-Carboxyphenyl)-1*H*-1,2,3-triazol-1-yl)-1-hydroxy-1*H*-indole-2-carboxylic acid (3.205)



Methyl ester **3.219b** (9.0 mg, 0.023 mmol) was dissolved in a 1:1 mixture of THF/methanol (0.23 mL) and treated with 0.1 mL of 2N aqueous solution of LiOH. The reaction was kept under stirring in the dark at RT and it was monitored by TLC. After 24 h, the reaction mixture was concentrated, diluted with water and washed with Et_2O. Then, the aqueous phase was treated with 1N aqueous HCl and extracted several times with EtOAc. The combined ethyl acetate organic phase was dried over anhydrous sodium sulphate and evaporated to afford the desired *N*-hydroxyindol-carboxylic acid product **3.205** (8.3 mg, 0.023 mmol, 99% yield) without any further purification. ^{1}H NMR (DMSO-d_6) δ (ppm): 7.11 (s, 1H), 7.74 (dd, 1H, J = 8.6, 1.5 Hz), 7.89 (d, 1H, J =

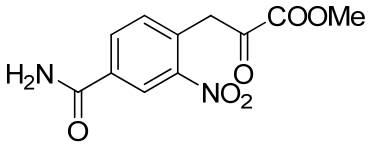
8.8 Hz), 8.02-8.10 (m, 5H), 9.60 (s, 1H); signals imputable to the indole (~ 26%) δ (ppm): 7.18-7.22 (m, 1H).

Methyl 3-(4-(2-methoxy-2-oxoacetylcarbamoyl)-2-nitrophenyl)-2-oxopropanoate (3.224)



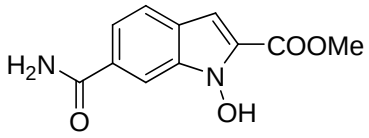
A stirred suspension of sodium hydride (60% dispersion in mineral oil, 932 mg, 23.3 mmol) in 16 mL of anhydrous DMF cooled to –15 °C under nitrogen was treated dropwise with a solution containing commercially available 4-methyl-3-nitrobenzamide **3.222** (700 mg, 3.89 mmol) and dimethyl oxalate (2.75 g, 23.3 mmol) in 11 mL of anhydrous DMF. The mixture was left under stirring at the same temperature for 10 minutes and then it was slowly warmed to room temperature. The mixture was then left under stirring at RT for 2 h. Once the disappearance of the precursor was verified by TLC, the reaction mixture was slowly poured in an ice-water mixture; the water phase was acidified with 1N aqueous HCl and extracted several times with EtOAc. The combined organic phase was washed with saturated $NaHCO_3$ solution and brine, then dried over anhydrous sodium sulphate. Evaporation under vacuum of the organic solvent afforded a crude product which was purified by column chromatography over silica gel using *n*-hexane/EtOAc 3:7 as the eluent, to yield the dialkylated nitroaryl-ketoester derivative **3.224** (860 mg, 2.44 mmol). ^{1}H NMR ($CDCl_3$), signals imputable to the formation of the dialkylated product **3.224** δ (ppm): 3.95 (s, 3H), 4.00 (s, 3H); signals imputable to the enol form (~ 26%) δ (ppm): 3.96 (s, 3H).

Methyl 3-(4-carbamoyl-2-nitrophenyl)-2-oxopropanoate (3.226a)



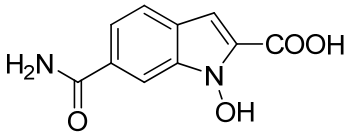
The dialkylated product **3.224** was dissolved in MeOH (8 mL) and silica gel was added (1.6 g). The mixture was stirred at RT for 1.5 h. Then, evaporation under vacuum of the organic solvent afforded a crude product which was purified by column chromatography over silica gel using *n*-hexane/EtOAc 3:7 as the eluent, to yield the amidic nitroaryl-ketoester derivative **3.226a** (484 mg, 1.82 mmol, 2 steps: 47% yield). ^{1}H NMR (DMSO-d_6) δ (ppm): 3.85 (s, 3H), 4.66 (s, 2H), 7.70 (d, 1H, J = 7.7 Hz), 8.19 (dd, 1H, J = 7.7, 1.8 Hz), 8.56 (d, 1H, J = 1.8 Hz), 10.51 (bs, 1H); signals imputable to the enol form (~ 67%) δ (ppm): 3.83 (s, 3H), 6.64 (s, 1H),7.61 (d, 1H, J = 8.1 Hz), 8.15 (dd, 1H, J = 8.1, 1.7 Hz), 8.39 (d, 1H, J = 1.7 Hz).

Methyl 6-carbamoyl-1-hydroxy-1*H*-indole-2-carboxylate (3.227a)



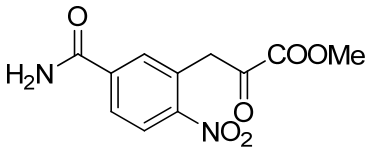
Ketoester **3.226a** (480 mg, 1.80 mmol) was dissolved in anhydrous DME (1.8 mL) and the resulting solution was added dropwise to a cooled (0 °C) solution of $SnCl_2 \cdot 2H_2O$ (911 mg, 4.04 mmol) in DME (1.8 mL) containing 4Å molecular sieves, previously activated for 18 hours at 130 °C in oven and cooled to RT in a desiccator over anhydrous calcium chloride. The reaction mixture was stirred under nitrogen at 0 °C for 6.5 h, then it was diluted with water and repeatedly extracted with EtOAc. The combined organic phase was dried over anhydrous sodium sulphate and evaporated to afford a crude residue that was purified by column chromatography over silica gel using EtOAc as the eluent, to give a mixture (50.0 mg) of the *N*-hydroxyindol-ester derivative **3.227a** (50%, 25.0 mg, 0.107 mmol, 6% yield) with its indole analogue (50%) ^{1}H NMR (DMSO-d_6) δ (ppm): 3.87 (s, 3H), 7.11 (d, 1H, J = 0.9 Hz), 7.58 (dd, 1H, J = 8.8, 1.6 Hz), 7.69 (d, 1H, J = 8.4 Hz), 8.00 (t, 1H, J = 0.8 Hz), 11.60 (bs, 1H); signals imputable to the indole (~ 50%) δ (ppm): 3.89 (s, 3H), 7.19 (dd, 1H, J = 1.9, 0.5 Hz),7.63 (dd, 1H, J = 8.8, 1.6 Hz), 12.25 (bs, 1H).

6-Carbamoyl-1-hydroxy-1*H*-indole-2-carboxylic acid (3.220a)



Methyl ester **3.227a** (43.0 mg, 0.184 mmol) was dissolved in a 1:1 mixture of THF/methanol (1.8 mL) and treated with 0.6 mL of 2N aqueous solution of LiOH. The reaction was kept under stirring in the dark at RT and it was monitored by TLC. After 2 h, the reaction mixture was concentrated, diluted with water and washed with Et_2O. Then, the aqueous phase was treated with 1N aqueous HCl and extracted several times with EtOAc. The combined ethyl acetate organic phase was dried over anhydrous sodium sulphate and evaporated to afford the desired *N*-hydroxyindol-carboxylic acid product **3.220a** (27.5 mg, 0.125 mmol, 68% yield) without any further purification. ^{1}H NMR (DMSO-d_6) δ (ppm): 7.02 (d, 1H, J = 0.7 Hz), 7.56 (dd, 1H, J = 8.6, 1.5 Hz), 7.67 (d, 1H, J = 8.4 Hz), 7.99 (s, 1H); signals imputable to the indole (~ 50%) δ (ppm): 7.11 (dd, 1H, J = 2.0, 0.9 Hz), 7.56 (dd, 1H, J = 7.1, 1.5 Hz).

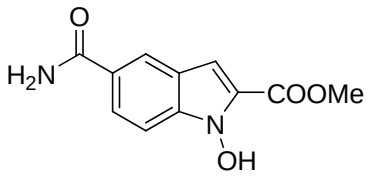
Methyl 3-(5-carbamoyl-2-nitrophenyl)-2-oxopropanoate (3.226b)



A stirred suspension of sodium hydride (60% dispersion in mineral oil, 932 mg, 23.3 mmol) in 16 mL of anhydrous DMF cooled to –15 °C under nitrogen was treated dropwise with a solution containing commercially available 3-methyl-4-nitrobenzamide **3.223** (700 mg, 3.89 mmol) and dimethyl oxalate (2.75 g, 23.3 mmol) in 11 mL of anhydrous DMF. The mixture was left under stirring at the same temperature for 10 minutes and then it was slowly warmed to room temperature. The mixture was then left under stirring at RT for 5 h. Once the disappearance of the precursor was verified by TLC, the reaction mixture was slowly poured in an ice-water mixture; the water phase was acidified with 1N aqueous HCl and extracted several times with EtOAc. The combined organic phase was washed with saturated $NaHCO_3$ solution and brine, then

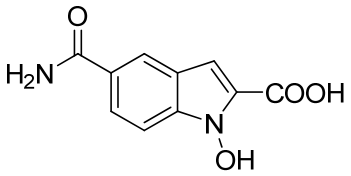
dried over anhydrous sodium sulphate. Evaporation under vacuum of the organic solvent afforded a crude, likely corresponding to the imidic derivative **3.225** (3.5 g). Hence, the crude residue was dissolved in MeOH (7 mL) and silica gel was added (2.8 g). The mixture was stirred at RT overnight. Then, evaporation under vacuum of the organic solvent afforded a crude product which was purified by column chromatography over silica gel using *n*-hexane/EtOAc 3:7 as the eluent, to yield the amidic nitroaryl-ketoester derivative **3.226b** (364 mg, 1.37 mmol, 2 steps: 35% yield). ^{1}H NMR (DMSO-d_6) δ (ppm): 3.85 (s, 3H), 4.64 (s, 2H), 7.68-7.74 (m, 2H), 7.88 (dd, 1H, J = 8.4, 1.8 Hz), 7.96 (d, 1H, J = 1.8 Hz), 8.25 (bs, 1H); signals imputable to the enol form (~ 62%) δ (ppm): 3.83 (s, 3H), 6.59 (s, 1H), 8.00 (dd, 1H, *J* = 8.3, 2.5 Hz), 8.19 (d, 1H, *J* = 8.4 Hz), 8.64 (d, 1H *J* = 1.8 Hz).

Methyl 5-carbamoyl-1-hydroxy-1*H*-indole-2-carboxylate (3.227b)



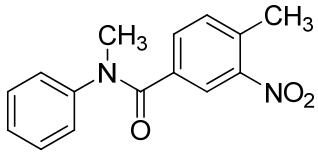
Ketoester **3.226b** (350 mg, 1.31 mmol) was dissolved in anhydrous DME (1.5 mL) and the resulting solution was added dropwise to a cooled (0 °C) solution of $SnCl_2 \cdot 2H_2O$ (782 mg, 3.47 mmol) in DME (1.5 mL) containing 4Å molecular sieves, previously activated for 18 hours at 130 °C in oven and cooled to RT in a desiccator over anhydrous calcium chloride. The reaction mixture was stirred under nitrogen at 0 °C for 2 h, then it was diluted with water and repeatedly extracted with EtOAc. The combined organic phase was dried over anhydrous sodium sulphate and evaporated to afford a crude residue that was purified by column chromatography over silica gel using EtOAc as the eluent, to give the *N*-hydroxyindol-ester derivative **3.227b** (87.0 mg, 0.371 mmol, 28% yield). ^{1}H NMR (DMSO-d_6) δ (ppm): 3.87 (s, 3H), 7.18 (d, 1H, *J* = 0.9 Hz), 7.25 (bs, 1H), 7.48 (d, 1H, *J* = 8.8 Hz), 7.86 (dd, 1H, *J* = 8.8, 1.6 Hz), 7.95 (bs, 1H), 8.26 (d, 1H, *J* = 0.9 Hz), 11.59 (bs, 1H).

5-Carbamoyl-1-hydroxy-1*H*-indole-2-carboxylic acid (3.221a)



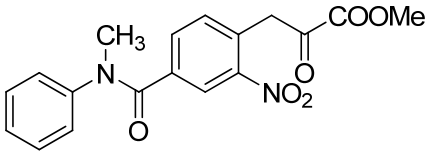
Methyl ester **3.227b** (75.0 mg, 0.320 mmol) was dissolved in a 1:1 mixture of THF/methanol (3.2 mL) and treated with 1.0 mL of 2N aqueous solution of LiOH. The reaction was kept under stirring in the dark at RT and it was monitored by TLC. After 3 h, the reaction mixture was concentrated, diluted with water and washed with Et_2O. Then, the aqueous phase was treated with 1N aqueous HCl and extracted several times with EtOAc. The combined ethyl acetate organic phase was dried over anhydrous sodium sulphate and evaporated to afford the desired *N*-hydroxyindol-carboxylic acid product **3.221a** (45.6 mg, 0.207 mmol, 65% yield) without any further purification. ^{1}H NMR (DMSO-d_6) δ (ppm): 7.11 (d, 1H, J = 0.5 Hz), 7.23 (bs, 1H), 7.45 (d, 1H, J = 8.6 Hz), 7.84 (dd, 1H, J = 8.8, 1.5 Hz), 7.93 (bs, 1H), 8.24 (s, 1H).

***N*,4-Dimethyl-3-nitro-*N*-phenylbenzamide (3.230)**



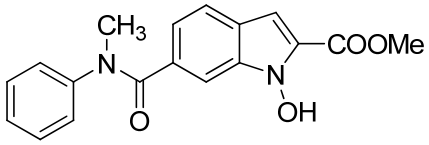
A solution of commercially available 4-methyl-3-nitrobenzoylchloride **3.228** (0.55 mL, 0.75 mg, 3.8 mmol) in CH_2Cl_2 (6.0 mL) was added dropwise to *N*-methylaniline (1.25 mL, 1.20 g, 11.3 mmol) and the reaction was kept under stirring at RT for 2 h. The reaction mixture was acidified with 1N aqueous HCl, extracted several times with CH_2Cl_2 and the combined organic phase was dried over anhydrous sodium sulphate. Evaporation under vacuum of the organic solvent afforded a crude product which was purified by column chromatography over silica gel using *n*-hexane/EtOAc 7:3 as the eluent, to yield the amidic derivative **3.230** (904 mg, 3.34 mmol, 89% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 2.51 (s, 3H), 3.51 (s, 3H), 7.03-7.33 (m, 6H), 7.41 (dd, 1H, J = 8.1, 1.8 Hz), 7.92 (d, 1H, J = 1.8 Hz).

Methyl 3-(4-(methyl(phenyl)carbamoyl)-2-nitrophenyl)-2-oxopropanoate (3.232a)



A stirred suspension of sodium hydride (60% dispersion in mineral oil, 512 mg, 12.8 mmol) in 13 mL of anhydrous DMF cooled to –15 °C under nitrogen was treated dropwise with a solution containing the nitrotoluene precursor **3.230** (865 mg, 3.20 mmol) and dimethyl oxalate (1.89 g, 16.0 mmol) in 10 mL of anhydrous DMF. The mixture was left under stirring at the same temperature for 10 minutes and then it was slowly warmed to room temperature. The mixture was then left under stirring at RT for 3.5 h. Once the disappearance of the precursor was verified by TLC, the reaction mixture was slowly poured in an ice-water mixture; the water phase was acidified with 1N aqueous HCl and extracted several times with EtOAc. The combined organic phase was washed with saturated $NaHCO_3$ solution and brine, then dried over anhydrous sodium sulphate. Evaporation under vacuum of the organic solvent afforded a crude product which was purified by column chromatography over silica gel using *n*-hexane/EtOAc 1:1 as the eluent, to yield the nitroaryl-ketoester derivative **3.232a** (976 mg, 2.74 mmol, 86% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 3.52 (s, 3H), 3.91 (s, 3H), 4.44 (s, 2H), 7.04-7.34 (m, 6H), 7.53 (dd, 1H, *J* = 8.1, 1.6 Hz), 7.84 (d, 1H, *J* = 1.8 Hz); signals imputable to the enol form (~ 69%) δ (ppm): 3.51 (s, 3H), 3.92 (s, 3H), 6.73 (d, 1H, exchangeable, *J* = 1.5 Hz), 6.83 (d, 1H, *J* = 1.1 Hz), 7.48 (dd, 1H, *J* = 8.4, 1.6 Hz), 8.08 (d, 1H, *J* = 8.2 Hz).

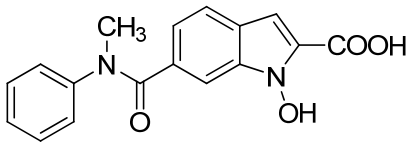
Methyl 1-hydroxy-6-(methyl(phenyl)carbamoyl)-1*H*-indole-2-carboxylate (3.233a)



Ketoester **3.232a** (950 mg, 2.67 mmol) was dissolved in anhydrous DME (2.7 mL) and the resulting solution was added dropwise to a cooled (0 °C) solution of $SnCl_2{\cdot}2H_2O$ (1.13 g, 5.97 mmol) in DME (2.7 mL) containing 4Å molecular sieves,

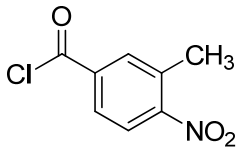
previously activated for 18 hours at 130 °C in oven and cooled to RT in a desiccator over anhydrous calcium chloride. The reaction mixture was stirred under nitrogen at at 0 °C for 6 h, then it was diluted with water and repeatedly extracted with EtOAc. The combined organic phase was dried over anhydrous sodium sulphate and evaporated to afford a crude residue that was purified by column chromatography over silica gel using CH_2Cl_2/MeOH 98:2 as the eluent, to give a mixture (134 mg) of the *N*-hydroxyindol-ester derivative **3.233a** (90%, 121 mg, 0.372 mmol, 14% yield) with its indole analogue (10%) ^{1}H NMR ($CDCl_3$) δ (ppm): 3.54 (s, 3H), 3.96 (s, 3H), 6.86 (d, 1H, *J* = 0.9 Hz), 6.91 (dd, 1H, *J* = 8.4, 1.3 Hz), 7.02-7.31 (m, 6H), 7.52 (s, 1H), 10.27 (bs, 1H); signals imputable to the indole (~ 10%) δ (ppm): 3.49 (s, 3H), 3.92 (s, 3H), 8.87 (bs, 1H).

1-Hydroxy-6-(methyl(phenyl)carbamoyl)-1*H*-indole-2-carboxylic acid (3.220b)



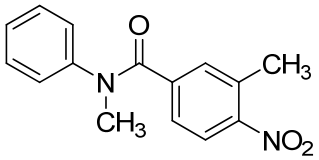
Methyl ester **3.233a** (100 mg, 0.308 mmol) was dissolved in a 1:1 mixture of THF/methanol (3.0 mL) and treated with 0.92 mL of 2N aqueous solution of LiOH. The reaction was kept under stirring in the dark at RT and it was monitored by TLC. After 2 h, the reaction mixture was concentrated, diluted with water and washed with Et_2O. Then, the aqueous phase was treated with 1N aqueous HCl and extracted several times with EtOAc. The combined ethyl acetate organic phase was dried over anhydrous sodium sulphate and evaporated to afford the desired *N*-hydroxyindol-carboxylic acid product **3.220b** (87.0 mg, 0.280 mmol, 91% yield) without any further purification. ^{1}H NMR (DMSO-d_6) δ (ppm): 3.40 (s, 3H), 6.91 (d, 1H, *J* = 0.7 Hz), 6.94 (dd, 1H, *J* = 8.2, 1.5 Hz), 7.12-7.28 (m, 5H), 7.36-7.38 (m, 1H), 7.42 (d, 1H, *J* = 8.4 Hz); signals imputable to the indole (~ 10%) δ (ppm): 3.43 (s, 3H), 6.80-7.00 (m, 1H).

3-Methyl-4-nitrobenzoyl chloride (3.229)



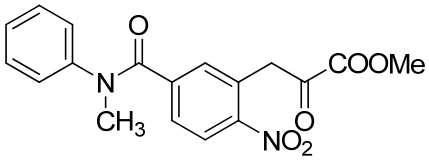
Oxalyl chloride (0.59 mL, 0.84 g, 6.6 mmol) and catalytic DMF (0.2 mL) were added to a suspension of commercially available 3-methyl-4-nitrobenzoic acid **3.90g** (1.00 g, 5.52 mmol) in CH_2Cl_2 (20.0 mL) and the reaction was kept under stirring at RT for 4 h. Evaporation under vacuum of the organic solvent afforded a crude product **3.229** (1.10 g) which was immediately used for the next reaction without any purification.

***N*,3-dimethyl-4-nitro-*N*-phenylbenzamide (3.231)**



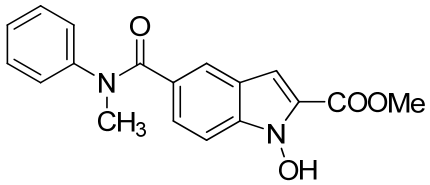
A solution of **3.229** (1.10 g, 5.52 mmol) in CH_2Cl_2 (12.0 mL) was added dropwise to *N*-methylaniline (1.80 mL, 1.77 g, 16.6 mmol) and the reaction was kept under stirring at RT for 2 h. The reaction mixture was acidified with 1N aqueous HCl, extracted several times with CH_2Cl_2 and the combined organic phase was dried over anhydrous sodium sulphate. Evaporation under vacuum of the organic solvent afforded a crude product which was purified by column chromatography over silica gel using *n*-hexane/EtOAc 7:3 as the eluent, to yield the amidic derivative **3.231** (1.35 g, 4.99 mmol, 90% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 2.46 (s, 3H), 3.50 (s, 3H), 7.01-7.31 (m, 6H), 7.34-7.36 (m, 1H), 7.70 (d, 1H, J = 8.4 Hz).

Methyl 3-(5-(methyl(phenyl)carbamoyl)-2-nitrophenyl)-2-oxopropanoate (3.232b)



A stirred suspension of sodium hydride (60% dispersion in mineral oil, 414 mg, 10.4 mmol) in 10 mL of anhydrous DMF cooled to –15 °C under nitrogen was treated dropwise with a solution containing the nitrotoluene precursor **3.231** (700 mg, 2.59 mmol) and dimethyl oxalate (1.53 g, 13.0 mmol) in 7 mL of anhydrous DMF. The mixture was left under stirring at the same temperature for 10 minutes and then it was slowly warmed to room temperature. The mixture was then left under stirring at RT for 21.5 h. Once the disappearance of the precursor was verified by TLC, the reaction mixture was slowly poured in an ice-water mixture; the water phase was acidified with 1N aqueous HCl and extracted several times with EtOAc. The combined organic phase was washed with saturated $NaHCO_3$ solution and brine, then dried over anhydrous sodium sulphate. Evaporation under vacuum of the organic solvent afforded a crude product which was purified by column chromatography over silica gel using *n*-hexane/EtOAc 7:3 as the eluent, to yield the nitroaryl-ketoester derivative **3.232b** (240 mg, 0.674 mmol, 26% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 3.48 (s, 3H), 3.88 (s, 3H), 4.36 (s, 2H), 6.96-7.06 (m, 2H), 7.12-7.32 (m, 5H), 7.89 (d, 1H, J = 8.1 Hz); signals imputable to the enol form (~ 26%) δ (ppm): 3.87 (s,3H), 6.67 (bs, 1H, exchangeable), 6.74 (s, 1H), 7.65 (d, 1H, J = 8.4 Hz), 8.19 (d, 1H, J = 1.6 Hz).

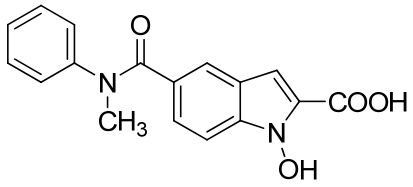
Methyl 1-hydroxy-5-(methyl(phenyl)carbamoyl)-1*H*-indole-2-carboxylate (3.233b)



Ketoester **3.232b** (230 mg, 0.645 mmol) was dissolved in anhydrous DME (0.65 mL) and the resulting solution was added dropwise to a cooled (0 °C) solution of $SnCl_2 \cdot 2H_2O$ (274 mg, 1.45 mmol) in DME (0.65 mL) containing 4Å molecular sieves, previously activated for 18 hours at 130 °C in oven and cooled to RT in a desiccator over anhydrous calcium chloride. The reaction mixture was stirred under nitrogen at at 0 °C for 6.5 h, then it was diluted with water and repeatedly extracted with EtOAc. The combined organic phase was dried over anhydrous sodium sulphate and evaporated to afford a crude residue that was purified by column chromatography over silica gel using

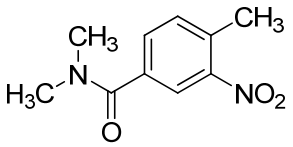
CH_2Cl_2/MeOH 98:2 as the eluent, to give a mixture (55.4 mg) of the *N*-hydroxyindol-ester derivative **3.233b** (50%, 27.7 mg, 0.854 mmol, 13% yield) with its indole analogue (50%) ^{1}H NMR ($CDCl_3$) δ (ppm): 3.52 (s, 3H), 3.91 (s, 3H), 6.85 (s, 1H), 6.98-7.28 (m, 8H), 10.51 (bs, 1H); signals imputable to the indole (~ 50%) δ (ppm): 3.53 (s, 3H), 3.96 (s, 3H), 7.50 (t, 1H, *J* = 1.2 Hz), 7.71-7.73 (m, 1H), 8.92 (bs, 1H).

1-Hydroxy-5-(methyl(phenyl)carbamoyl)-1*H*-indole-2-carboxylic acid (3.221b)



Methyl ester **3.233b** (53.0 mg, 0.163 mmol) was dissolved in a 1:1 mixture of THF/methanol (1.6 mL) and treated with 0.49 mL of 2N aqueous solution of LiOH. The reaction was kept under stirring in the dark at RT and it was monitored by TLC. After 7 h, the reaction mixture was concentrated, diluted with water and washed with Et_2O. Then, the aqueous phase was treated with 1N aqueous HCl and extracted several times with EtOAc. The combined ethyl acetate organic phase was dried over anhydrous sodium sulphate and evaporated to afford the desired *N*-hydroxyindol-carboxylic acid product **3.221b** (50.0 mg, 0.161 mmol, 99% yield) without any further purification. ^{1}H NMR (DMSO-d_6) δ (ppm): 1.91 (s, 3H), 6.93 (s, 1H), 7.07-7.27 (m, 8H); signals imputable to the indole (~ 50%) δ (ppm): 7.50 (d, 1H, *J* = *1*.6 Hz), 7.60-7.63 (m, 1H).

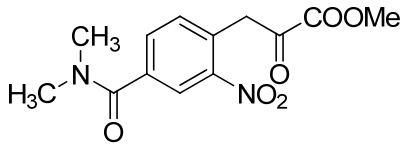
***N,N*,4-Trimethyl-3-nitrobenzamide (3.234)**



A solution of commercially available 4-methyl-3-nitrobenzoylchloride **3.228** (0.50 mL, 0.68 g, 3.4 mmol) in CH_2Cl_2 (6.0 mL) was added dropwise to *N,N*-dimethylaniline (2.0 mL, 0.71 g, 16 mmol) and the reaction was kept under stirring at RT for 30 min. The reaction mixture was acidified with 1N aqueous HCl, extracted several times with

CH_2Cl_2 and the combined organic phase was dried over anhydrous sodium sulphate. Evaporation under vacuum of the organic solvent afforded a crude product which was purified by column chromatography over silica gel using CH_2Cl_2/MeOH 9:1 as the eluent, to yield the amidic derivative **3.234** (642 mg, 3.08 mmol, 90% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 2.63 (s, 3H), 3.02 (s, 3H), 3.12 (s, 3H), 7.40 (d, 1H, *J* = 7.9 Hz), 7.59 (dd, 1H, *J* = 7.7, 1.6 Hz), 8.05 (d, 1H, *J* = 1.6 Hz).

Methyl 3-(4-(dimethylcarbamoyl)-2-nitrophenyl)-2-oxopropanoate (3.236a)

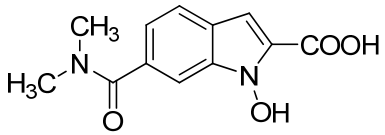


A stirred suspension of sodium hydride (60% dispersion in mineral oil, 469 mg, 11.76 mmol) in 11 mL of anhydrous DMF cooled to –15 °C under nitrogen was treated dropwise with a solution containing the nitrotoluene precursor **3.234** (610 mg, 2.93 mmol) and dimethyl oxalate (1.73 g, 14.7 mmol) in 9 mL of anhydrous DMF. The mixture was left under stirring at the same temperature for 10 minutes and then it was slowly warmed to room temperature. The mixture was then left under stirring at RT for 1.5 h. Once the disappearance of the precursor was verified by TLC, the reaction mixture was slowly poured in an ice-water mixture; the water phase was acidified with 1N aqueous HCl and extracted several times with EtOAc. The combined organic phase was washed with saturated $NaHCO_3$ solution and brine, then dried over anhydrous sodium sulphate. Evaporation under vacuum of the organic solvent afforded a crude product which was purified by column chromatography over silica gel using *n*-hexane/EtOAc 3:7 as the eluent, to yield the nitroaryl-ketoester derivative **3.236a** (821 mg, 2.79 mmol, 95% yield). ^{1}H NMR (DMSO) δ (ppm): 3.03 (s, 3H), 3.13 (s, 3H), 3.94 (s, 3H), 4.58 (s, 2H), 7.21 (d, 1H, *J* = 7.9 Hz), 7.70 (dd, 1H, *J* = 7.9, 1.6 Hz), 8.24 (d, 1H, *J* = 1.6); signals imputable to the enol form (~ 83%) δ (ppm): 3.95 (s, 3H), 6.93 (s, 1H), 7.65 (dd, 1H, *J* = 8.2, 1.8 Hz), 7.98 (d, 1H, *J* = 1.6 Hz), 8.31 (d, 1H, *J* = 8.2 Hz).

Methyl 6-(dimethylcarbamoyl)-1-hydroxy-1*H*-indole-2-carboxylate (3.237a)

Ketoester **3.236a** (507 mg, 1.72 mmol) was dissolved in anhydrous DME (1.7 mL) and the resulting solution was added dropwise to a cooled (0 °C) solution of $SnCl_2 \cdot 2H_2O$ (732 mg, 3.86 mmol) in DME (1.7 mL) containing 4Å molecular sieves, previously activated for 18 hours at 130 °C in oven and cooled to RT in a desiccator over anhydrous calcium chloride. The reaction mixture was stirred under nitrogen at at 0 °C for 5 h, then it was diluted with water and repeatedly extracted with EtOAc. The combined organic phase was dried over anhydrous sodium sulphate and evaporated to afford a crude residue that was purified by column chromatography over silica gel using CH_2Cl_2/MeOH 95:5 as the eluent, to give a mixture (150 mg) of the *N*-hydroxyindol-ester derivative **3.237a** (60%, 90.0 mg, 0.343 mmol, 20% yield) with its indole analogue (40%) ^{1}H NMR ($CDCl_3$) δ (ppm): 2.94-3.16 (m, 6H), 3.97 (s, 3H), 6.85 (d, 1H, *J* = 0.9 Hz), 7.17 (dd, 1H, *J* = 8.2, 1.3 Hz), 7.21 (dd, 1H, *J* = 2.0, 0.8 Hz), 7.34 (d, 1H, *J* = 8.4 Hz); signals imputable to the indole (~ 40%) δ (ppm): 3.96 (s, 3H), 6.84 (dd, 1H, *J* = 8.2, 1.5 Hz), 7.68 (d, 1H, *J* = 8.4 Hz).

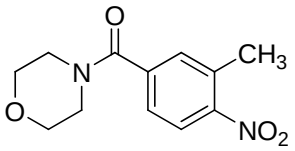
6-(Dimethylcarbamoyl)-1-hydroxy-1*H*-indole-2-carboxylic acid (3.220c)



Methyl ester **3.237a** (130 mg, 0.496 mmol) was dissolved in a 1:1 mixture of THF/methanol (5 mL) and treated with 1.5 mL of 2N aqueous solution of LiOH. The reaction was kept under stirring in the dark at RT and it was monitored by TLC. After 2.5 h, the reaction mixture was concentrated, diluted with water and washed with Et_2O. Then, the aqueous phase was treated with 1N aqueous HCl and extracted several times with EtOAc. The combined ethyl acetate organic phase was dried over anhydrous sodium sulphate and evaporated to afford the desired *N*-hydroxyindol-carboxylic acid

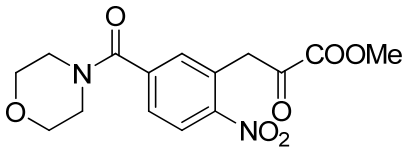
product **3.220c** (122 mg, 0.491 mmol, 99% yield) without any further purification. ^{1}H NMR (DMSO-d_6) δ (ppm): 2.97 (s, 6H), 7.04 (d, 1H, J = 0.7 Hz); 7.10-7.13 (m, 1H), 7.42-7.44 (m, 2H); signals imputable to the indole (~ 40%) δ (ppm): 7.08 (dd, 1H, J = 4.6, 1.5 Hz), 7.68 (d, 1H, J = 8.4 Hz).

(3-Methyl-4-nitrophenyl)(morpholino)methanone (3.235)



A solution of **3.229** (918 mg, 4.60 mmol) in CH_2Cl_2 (10.0 mL) was added dropwise to morpholine (1.21 mL, 1.20 g, 13.8 mmol) and the reaction was kept under stirring at RT overnight. The reaction mixture was acidified with 1N aqueous HCl, extracted several times with CH_2Cl_2 and the combined organic phase was dried over anhydrous sodium sulphate. Evaporation under vacuum of the organic solvent afforded a crude product which was purified by column chromatography over silica gel using EtOAc as the eluent, to yield the amidic derivative **3.235** (1.01 g, 4.04 mmol, 88% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 2.62 (s, 3H), 3.40-3.78 (m, 8H), 7.32-7.39 (m, 2H), 8.00 (d, 1H, J = 8.2 Hz).

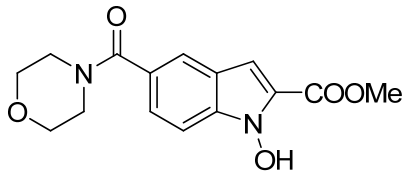
Methyl 3-(5-(morpholine-4-carbonyl)-2-nitrophenyl)-2-oxopropanoate (3.236b)



A stirred suspension of sodium hydride (60% dispersion in mineral oil, 579 mg, 14.5 mmol) in 14 mL of anhydrous DMF cooled to –15 °C under nitrogen was treated dropwise with a solution containing the nitrotoluene precursor **3.235** (906 mg, 3.62 mmol) and dimethyl oxalate (2.14 g, 18.1 mmol) in 11 mL of anhydrous DMF. The mixture was left under stirring at the same temperature for 10 minutes and then it was slowly warmed to room temperature. The mixture was then left under stirring at RT for 4 h. Once the disappearance of the precursor was verified by TLC, the reaction mixture

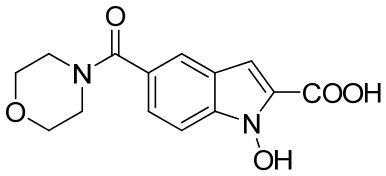
was slowly poured in an ice-water mixture; the water phase was acidified with 1N aqueous HCl and extracted several times with EtOAc. The combined organic phase was washed with saturated $NaHCO_3$ solution and brine, then dried over anhydrous sodium sulphate. Evaporation under vacuum of the organic solvent afforded a crude product which was purified by column chromatography over silica gel using *n*-hexane/EtOAc 1:9 as the eluent, to yield the nitroaryl-ketoester derivative **3.236b** (1.17 g, 3.48 mmol, 96% yield). 50% di forma enolic. ^{1}H NMR ($CDCl_3$) δ (ppm): 3.46-3.80 (m, 8H), 3.94 (s, 3H), 4.57 (s, 2H), 7.38 (d, 1H, *J* = 1.8 Hz), 7.46 (dd, 1H, *J* = 8.4, 1.8 Hz),), 8.21 (d, 1H, *J* = 8.4 Hz); signals imputable to the enol form (~ 48%) δ (ppm): 3.95 (s, 3H), 6.91 (s, 1H), 7.52 (dd, 1H, *J* = 8.4, 1.8 Hz), 7.96 (d, 1H, *J* = 8.4 Hz), 8.28 (d, 1H, *J* = 1.8 Hz).

Methyl 1-hydroxy-5-(morpholine-4-carbonyl)-1*H*-indole-2-carboxylate (3.237b)



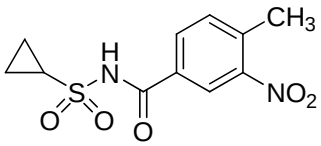
Ketoester **3.236b** (200 mg, 0.595 mmol) was dissolved in anhydrous DME (0.6 mL) and the resulting solution was added dropwise to a cooled (0 °C) solution of $SnCl_2 \cdot 2H_2O$ (253 mg, 1.33 mmol) in DME (0.6 mL) containing 4Å molecular sieves, previously activated for 18 hours at 130 °C in oven and cooled to RT in a desiccator over anhydrous calcium chloride. The reaction mixture was stirred under nitrogen at 0 °C for 5 h, then it was diluted with water and repeatedly extracted with EtOAc. The combined organic phase was dried over anhydrous sodium sulphate and evaporated to afford a crude residue that was purified by column chromatography over silica gel using CH_2Cl_2/MeOH 95:5 as the eluent, to give a mixture (33.0 mg) of the *N*-hydroxyindol-ester derivative **3.237b** (85%, 28.1 mg, 0.0922 mmol, 15% yield) with its indole analogue (15%) ^{1}H NMR ($CDCl_3$) δ (ppm): 3.40-3.90 (m, 8H), 3.99 (s, 3H), 6.93 (d, 1H, *J* = 0.7 Hz), 6.96 (dd, 1H, *J* = 9.0, 1.3), 7.06 (d, 1H, *J* = 8.6 Hz), 7.46 (s, 1H); signals imputable to the indole (~ 15%) δ (ppm): 3.96 (s, 3H), 7.78 (s, 1H).

1-Hydroxy-5-(morpholine-4-carbonyl)-1*H*-indole-2-carboxylic acid (3.221c)



Methyl ester **3.237b** (32.0 mg, 0.105 mmol) was dissolved in a 1:1 mixture of THF/methanol (0.6 mL) and treated with 0.3 mL of 2N aqueous solution of LiOH. The reaction was kept under stirring in the dark at RT and it was monitored by TLC. After 2 h, the reaction mixture was concentrated, diluted with water and washed with Et_2O. Then, the aqueous phase was treated with 1N aqueous HCl and extracted several times with EtOAc. The combined ethyl acetate organic phase was dried over anhydrous sodium sulphate and evaporated to afford the desired *N*-hydroxyindol-carboxylic acid product **3.221c** (30.2 mg, 0.104 mmol, 99% yield) without any further purification. ^{1}H NMR (DMSO-d_6) δ (ppm): 3.53-3.59 (m, 8H), 7.08 (d, 1H, *J* = 0.7 Hz), 7.35 (dd, 1H, *J* = 8.6, 1.5 Hz), 7.47 (d, 1H, *J* = 8.6 Hz), 7.74 (d, 1H, *J* = 1.6 Hz); signals imputable to the indole (~ 15%) δ (ppm): 7.14-7.16 (m, 1H), 7.28 (dd, 1H, *J* = 8.4, 1.5 Hz).

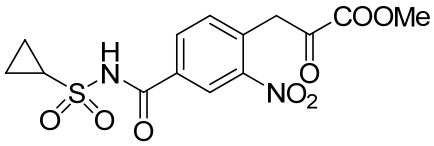
***N*-(Cyclopropylsulfonyl)-4-methyl-3-nitrobenzamide (3.238)**



Cyclopropanesulfonamide (300 mg, 2.48 mmol) was dissolved in 45 mL of CH_2Cl_2 and the resulting solution was cooled to 0 °C, then commercially available 4-methyl-3-nitrobenzoylchloride **3.228** (0.76 mL, 1.04 g, 5.20 mmol) and triethylamine (0.41 mL, 0.30 g, 3.0 mmol) were added dropwise and the reaction was kept under stirring at RT for 72 h. The reaction mixture was diluted with CH_2Cl_2 and washed with saturated aqueous sodium $NaHCO_3$ and water. The organic phase was dried over anhydrous sodium sulphate. Evaporation under vacuum of the organic solvent afforded a crude product which was purified by column chromatography over silica gel using *n*-hexane/EtOAc 7:3 as the eluent, to yield the derivative **3.238** (442 mg, 1.55 mmol, 63%

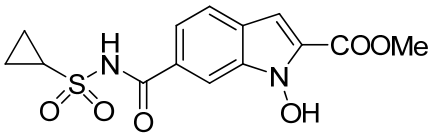
yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 1.22-1.34 (m, 4H), 2.68 (s, 3H), 3.51-3.64 (m, 1H), 7.48 (d, 1H, *J* = 8.2 Hz), 8.00 (dd, 1H, *J* = 8.1, 2.0 Hz), 8.45 (d, 1H, *J* = 1.6 Hz).

Methyl 3-(4-(cyclopropylsulfonylcarbamoyl)-2-nitrophenyl)-2-oxopropanoate (3.239)



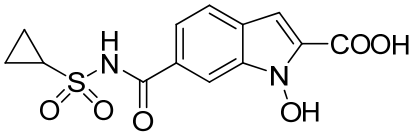
A stirred suspension of sodium hydride (60% dispersion in mineral oil, 432 mg, 10.8 mmol) in 7.5 mL of anhydrous DMF cooled to –15 °C under nitrogen was treated dropwise with a solution containing the nitrotoluene precursor **3.238** (384 mg, 1.35 mmol) and dimethyl oxalate (1.59 g, 13.5 mmol) in 3.8 mL of anhydrous DMF. The mixture was left under stirring at the same temperature for 10 minutes and then it was slowly warmed to room temperature. The mixture was then left under stirring at RT for 18 h. Once the disappearance of the precursor was verified by TLC, the reaction mixture was slowly poured in an ice-water mixture; the water phase was acidified with 1N aqueous HCl and extracted several times with EtOAc. The combined organic phase was washed with saturated $NaHCO_3$ solution and brine, then dried over anhydrous sodium sulphate. Evaporation under vacuum of the organic solvent afforded a crude product which was purified by column chromatography over silica gel using $CHCl_3$/MeOH 95:5 as the eluent, to yield the nitroaryl-ketoester derivative **3.239** (287 mg, 0.775 mmol, 57% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 1.14-1.25 (m, 2H), 1.45-1.54 (m, 2H), 3.07-3.20 (m, 1H), 3.96 (s, 3H), 4.64 (s, 2H), 7.50 (d, 1H, *J* = 8.1 Hz), 8.16 (dd, 1H, *J* = 8.2, 1.8 Hz), 8.68 (d, 1H, *J* = 1.7 Hz); signals imputable to the enol form (~ 3%) δ (ppm): 3.97 (s, 3H), 6.94 (s, 1H), 8.85 (s, 1H).

Methyl 6-(cyclopropylsulfonylcarbamoyl)-1-hydroxy-1*H*-indole-2-carboxylate (3.241a)



Ketoester **3.239** (270 mg, 0.729 mmol) was dissolved in THF (1.2 mL) and the resulting solution was added to an aqueous solution (1.2 mL) of sodium hypophosphite monohydrate (240 mg, 2.26 mmol). Then 7.3 mg of 10% palladium over charcoal was added and the resulting suspension was stirred at RT for 24 h. Then it was monitored by TLC and $H_2PO_2Na{\cdot}H_2O$ (240 mg, 2.26 mmol) and Pd/C (7.3 mg) were added. After further 5 h the disappearance of the precursor is verified by TLC and the mixture was concentrated, diluted with water and repeatedly extracted with EtOAc. The combined organic phase was dried over anhydrous sodium sulphate and evaporated to afford a crude residue that was purified by column chromatography over silica gel using *n*-hexane/EtOAc 5:95 as the eluent, to give the *N*-hydroxyindole **3.241a** (108 mg, 0.319 mmol, 44% yield). ^{1}H NMR (CD_3OD) δ (ppm): 1.08-1.22 (m, 2H), 1.24-1.35 (m, 2H), 3.10-3.23 (m, 1H), 3.94 (s, 3H), 7.13 (d, 1H, J = 0.9 Hz), 7.64 (dd, 1H, J = 8.4, 1.6 Hz), 7.74 (dd, 1H, J = 8.4, 1.0 Hz), 8.14 (t, 1H, J = 1.1 Hz).

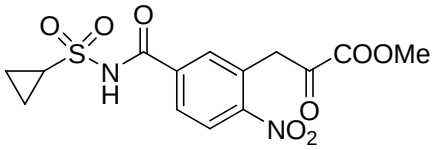
6-(Cyclopropylsulfonylcarbamoyl)-1-hydroxy-1*H*-indole-2-carboxylic acid (3.220d)



Methyl ester **3.241a** (100 mg, 0.296 mmol) was dissolved in a 1:1 mixture of THF/methanol (3 mL) and treated with 0.9 mL of 2N aqueous solution of LiOH. The reaction was kept under stirring in the dark at RT and it was monitored by TLC. After 5 h, the reaction mixture was concentrated, diluted with water and washed with Et_2O. Then, the aqueous phase was treated with 1N aqueous HCl and extracted several times with EtOAc. The combined ethyl acetate organic phase was dried over anhydrous sodium sulphate and evaporated to afford the desired *N*-hydroxyindol-carboxylic acid

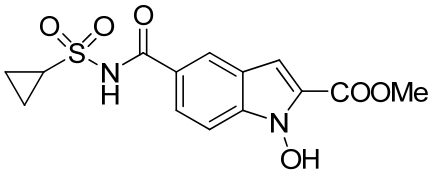
product **3.220d** (92.0 mg, 0.284 mmol, 96% yield) without any further purification. ^{1}H NMR (CD_3OD) δ (ppm): 1.12-1.19 (m, 2H), 1.29-1.34 (m, 2H), 3.14-3.25 (m, 1H), 7.12 (t, 1H, *J* = 0.7 Hz), 7.62 (ddd, 1H, *J* = 8.4, 1.6, 0.7 Hz), 7.74 (d, 1H, *J* = 8.4 Hz), 8.13 (dd, 1H, *J* = 1.6, 0.8 Hz). ^{13}C NMR (CD_3OD) δ (ppm): 6.36 (2C), 32.03, 105.94, 111.93 (2C), 120.80, 123.51 (2C), 129.33, 136.58, 163.20, 168.83.

Methyl 3-(5-(cyclopropylsulfonylcarbamoyl)-2-nitrophenyl)-2-oxopropanoate (3.240)



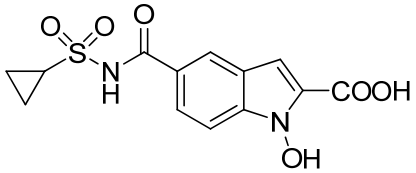
The ketoester precursor **3.91g** (200 mg, 0.748 mmol) and CDI (281 mg, 1.73 mmol) were dissolved in anhydrous THF (5 mL) and the resulting solution was stirred for 1 h at RT. Cyclopropanesulfonamide (182 mg, 1.50 mmol) in 1.9 mL of anhydrous THF and DBU (0.56 mL, 0.57 g, 3.8 mmol) were sequentially added to the reaction mixture, then the reaction was kept under stirring at RT overnight. Then, the mixture was concentrated, acidified with 1N aqueous HCl (pH=1) and repeatedly extracted with EtOAc. The organic phase was dried over anhydrous sodium sulphate. Evaporation under vacuum of the organic solvent afforded a crude product which was purified by column chromatography over silica gel using *n*-hexane/EtOAc 1:0 with 0.01% acetic acid as the eluent, to yield the derivative **3.240** (226 mg, 0.610 mmol, 82% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 0.87-1.08 (m, 4H), 3.04-3.16 (m, 1H), 3.85 (s, 3H), 4.65 (s, 2H), 7.94 (dd, 1H, *J* = 8.4, 2.0 Hz), 8.19 (d, 1H, *J* = 8.1 Hz), 8.84 (bs, 1H); signals imputable to the enol form (~ 73%) δ (ppm): 3.83 (s, 3H), 6.57 (s, 1H), 8.72 (d, 1H, *J* = 1.3 Hz).

Methyl 5-(cyclopropylsulfonylcarbamoyl)-1-hydroxy-1*H*-indole-2-carboxylate (3.241b)



Ketoester **3.240** (100 mg, 0.270 mmol) was dissolved in THF (0.4 mL) and the resulting solution was added to an aqueous solution (0.4 mL) of sodium hypophosphite monohydrate (89.0 mg, 0.840 mmol). Then 2.7 mg of 10% palladium over charcoal was added and the resulting suspension was stirred ar RT. After 72 h the disappearance of the precursor is verified by TLC and the mixture was concentrated, diluted with water and repeatedly extracted with EtOAc. The combined organic phase was dried over anhydrous sodium sulphate and evaporated to afford a crude residue that was purified by column chromatography over silica gel using CH_2Cl_2/MeOH 9:1 as the eluent, to give the *N*-hydroxyindole **3.241b** (24.2 mg, 0.0715 mmol, 26% yield). ^{1}H NMR (CD_3OD) δ (ppm): 1.11-1.35 (m, 4H), 3.11-3.23 (m, 1H), 3.93 (s, 3H), 7.24 (d, 1H, *J* = 0.9 Hz), 7.58 (dd, 1H, *J* = 8.8, 0.7 Hz), 7.89 (dd, 1H, *J* = 8.8, 1.6 Hz), 8.29 (dd, 1H, *J* = 1.6, 0.7 Hz).

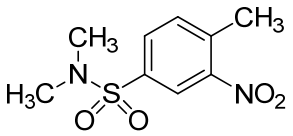
5-(Cyclopropylsulfonylcarbamoyl)-1-hydroxy-1*H*-indole-2-carboxylic acid (3.221d)



Methyl ester **3.241b** (18.0 mg, 0.0532 mmol) was dissolved in a 1:1 mixture of THF/methanol (0.5 mL) and treated with 0.15 mL of 2N aqueous solution of LiOH. The reaction was kept under stirring in the dark at RT and it was monitored by TLC. After 72 h, the reaction mixture was concentrated, diluted with water and washed with Et_2O. Then, the aqueous phase was treated with 1N aqueous HCl and extracted several times with EtOAc. The combined ethyl acetate organic phase was dried over anhydrous sodium sulphate and evaporated to afford the desired *N*-hydroxyindol-carboxylic acid

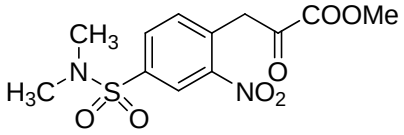
product **3.221d** (15.1 mg, 0.0466 mmol, 88% yield) without any further purification. ^{1}H NMR (CD_3OD) δ (ppm). 1.12-1.33 (m, 4H), 3.11-3.24 (m, 1H), 7.23 (s, 1H), 7.58 (dd, 1H, *J* = 8.8, 0.7 Hz), 7.87 (dd, 1H, *J* = 8.8, 1.2 Hz), 8.28 (dd, 1H, *J* = 1.4, 0.7 Hz). ^{13}C NMR (CD_3OD) δ (ppm): 6.38 (2C), 32.09, 107.83, 110.80 (2C), 122.12, 125.38, 125.60 (2C), 139.31, 163.15, 168.90. MS *m/z* 324 (M^+ 5%), 322 (M^+ –H_2, 100%), 279 (M^+ – COOH, 18%).

_N,N_,4-Trimethyl-3-nitrobenzenesulfonamide (3.246a)



A solution of commercially available 4-methyl-3-nitrobenzenesulfonylchloride **3.245** (750 mg, 3.18 mmol) in CH_2Cl_2 (6.0 mL) was added dropwise to *N,N*-dimethylaniline (2.00 mL, 712 mg, 15.8 mmol) and the reaction was kept under stirring at RT for 30 min. The reaction mixture was acidified with 1N aqueous HCl, extracted several times with CH_2Cl_2 and the combined organic phase was dried over anhydrous sodium sulphate. Evaporation under vacuum of the organic solvent afforded a crude product which was purified by column chromatography over silica gel using CH_2Cl_2 as the eluent, to yield the sulfonamidic derivative **3.246a** (681 mg, 2.79 mmol, 88% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 2.63 (s, 3H), 2.76 (s, 6H), 7.55 (d, 1H, *J* = 8.1 Hz), 7.89 (dd, 1H, *J* = 7.9, 1.8 Hz), 8.35 (d, 1H, *J* = 1.8 Hz).

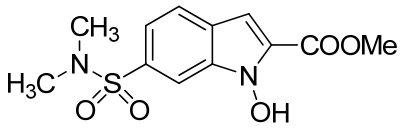
Methyl 3-(4-(*N*,*N*-dimethylsulfamoyl)-2-nitrophenyl)-2-oxopropanoate (3.247a)



A stirred suspension of sodium hydride (60% dispersion in mineral oil, 274 mg, 11.4 mmol) in 11 mL of anhydrous DMF cooled to –15 °C under nitrogen was treated dropwise with a solution containing the nitrotoluene precursor **3.246a** (650 mg, 2.66 mmol) and dimethyl oxalate (1.68 g, 14.3 mmol) in 9 mL of anhydrous DMF. The mixture was left under stirring at the same temperature for 10 minutes and then it was

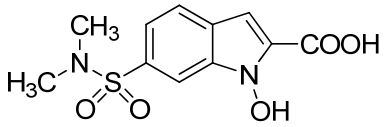
slowly warmed to room temperature. The mixture was then left under stirring at RT for 21 h. Once the disappearance of the precursor was verified by TLC, the reaction mixture was slowly poured in an ice-water mixture; the water phase was acidified with 1N aqueous HCl and extracted several times with EtOAc. The combined organic phase was washed with saturated $NaHCO_3$ solution and brine, then dried over anhydrous sodium sulphate. Evaporation under vacuum of the organic solvent afforded a crude product which was purified by column chromatography over silica gel using *n*-hexane/EtOAc 1:1 as the eluent, to yield the nitroaryl-ketoester derivative **3.247a** (489 mg, 1.48 mmol, 56% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 2.81 (s, 6H), 3.96 (s, 3H), 4.64 (s, 2H), 7.53 (d, 1H, *J* = 7.9 Hz), 8.02 (dd, 1H, *J* = 8.1, 2.0 Hz), 8.54 (d, 1H, *J* = 1.8 Hz); signals imputable to the enol form (~ 25%) δ (ppm): 2.79 (s, 6H), 3.98 (s, 3H), 6.93 (s, 1H), 7.95 (dd, 1H, *J* = 7.7, 1.3 Hz), 8.26 (d, 1H, *J* = 1.8 Hz), 8.47 (d, 1H, *J* = 8.6 Hz).

Methyl 6-(*N*,*N*-dimethylsulfamoyl)-1-hydroxy-1*H*-indole-2-carboxylate (3.248a)



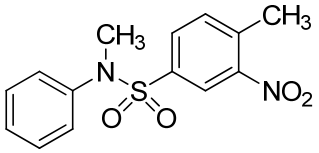
Ketoester **3.247a** (250 mg, 0.757 mmol) was dissolved in anhydrous DME (0.7 mL) and the resulting solution was added dropwise to a cooled (0 °C) solution of $SnCl_2 \cdot 2H_2O$ (382 mg, 1.70 mmol) in DME (0.7 mL) containing 4Å molecular sieves, previously activated for 18 hours at 130 °C in oven and cooled to RT in a desiccator over anhydrous calcium chloride. The reaction mixture was stirred under nitrogen at 0 °C for 3 h, then it was diluted with water and repeatedly extracted with EtOAc. The combined organic phase was dried over anhydrous sodium sulphate and evaporated to afford a crude residue that was purified by column chromatography over silica gel using *n*-hexane/EtOAc 3:7 as the eluent, to give a mixture (149 mg) of the *N*-hydroxyindol-ester derivative **3.248a** (95%, 142 mg, 0.475 mmol, 63% yield) with its indole analogue (5%). ^{1}H NMR ($CDCl_3$) δ (ppm): 2.74 (s, 6H), 4.04 (s, 3H), 7.09 (d, 1H, *J* = 0.9 Hz), 7.47 (dd, 1H, *J* = 8.6, 1.6 Hz), 7.76 (dd, 1H, *J* = 8.4, 0.7 Hz), 8.05 (dt, 1H, *J* = 1.6, 0.8 Hz); signals imputable to the indole (~ 5%) δ (ppm): 2.80 (s, 6H), 4.03 (s, 3H), 7.61 (dd, 1H, *J* = 8.0, 1.6 Hz), 8.25 (d, 1H, *J* = 8.0 Hz).

6-(*N*,*N*-Dimethylsulfamoyl)-1-hydroxy-1*H*-indole-2-carboxylic acid (3.242)



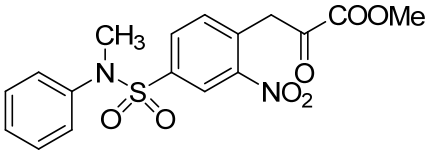
Methyl ester **3.248a** (140 mg, 0.469 mmol) was dissolved in a 1:1 mixture of THF/methanol (4.8 mL) and treated with 1.4 mL of 2N aqueous solution of LiOH. The reaction was kept under stirring in the dark at RT and it was monitored by TLC. After 1 h, the reaction mixture was concentrated, diluted with water and washed with Et_2O. Then, the aqueous phase was treated with 1N aqueous HCl and extracted several times with EtOAc. The combined ethyl acetate organic phase was dried over anhydrous sodium sulphate and evaporated to afford the desired *N*-hydroxyindol-carboxylic acid product **3.242** (132 mg, 0.464 mmol, 99% yield) without any further purification. ^{1}H NMR (DMSO-d_6) δ (ppm): 2.62 (s, 6H), 7.15 (d, 1H, J = 0.7 Hz), 7.41 (dd, 1H, J = 8.4, 1.6 Hz), 7.77 (d, 1H, J = 1.1 Hz), 7.89 (d, 1H, J = 8.6 Hz); signals imputable to the indole (~ 5%) δ (ppm): 2.67 (s, 6H), 7.58 (dd, 1H, J = 8.2, 1.6 Hz), 8.26 (d, 1H, J = 8.4 Hz), 8.99 (d, 1H, J = 1.8 Hz). MS *m/z* 284 (M^+, 34%), 282 ($M^+ - H_2$, 100%).

***N*,4-dimethyl-3-nitro-*N*-phenylbenzenesulfonamide (3.246b)**



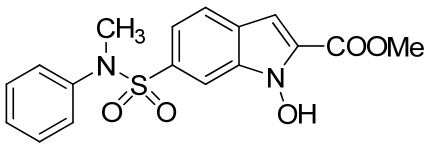
A solution of commercially available 4-methyl-3-nitrobenzenesulfonylchloride **3.245** (1.10 g, 4.67 mmol) in CH_2Cl_2 (8 mL) was added dropwise to *N*-methylaniline (1.55 mL, 1.50 g, 14.0 mmol) and the reaction was kept under stirring at RT for 2 h. The reaction mixture was acidified with 1N aqueous HCl, extracted several times with CH_2Cl_2 and the combined organic phase was dried over anhydrous sodium sulphate. Evaporation under vacuum of the organic solvent afforded the desired sulfonamidic derivative **3.246b** (1.43 g, 4.67 mmol, >99% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 2.67 (s, 3H), 3.22 (s, 3H), 7.07-7.12 (m, 2H), 7.31-7.35 (m, 3H), 7.44 (d, 1H, J = 8.1 Hz), 7.59 (dd, 1H, J = 8.1, 1.8 Hz), 8.15 (d, 1H, J = 1.6 Hz).

Methyl 3-(4-(*N*-methyl-*N*-phenylsulfamoyl)-2-nitrophenyl)-2-oxopropanoate (3.247b)



A stirred suspension of sodium hydride (60% dispersion in mineral oil, 522 mg, 13.0 mmol) in 9.0 mL of anhydrous DMF cooled to –15 °C under nitrogen was treated dropwise with a solution containing the nitrotoluene precursor **3.246b** (1.00 g, 3.26 mmol) and dimethyl oxalate (1.93 g, 16.3 mmol) in 9.3 mL of anhydrous DMF. The mixture was left under stirring at the same temperature for 10 minutes and then it was slowly warmed to room temperature. The mixture was then left under stirring at RT for 21 h. Once the disappearance of the precursor was verified by TLC, the reaction mixture was slowly poured in an ice-water mixture; the water phase was acidified with 1N aqueous HCl and extracted several times with EtOAc. The combined organic phase was washed with saturated $NaHCO_3$ solution and brine, then dried over anhydrous sodium sulphate. Evaporation under vacuum of the organic solvent afforded a crude product which was purified by column chromatography over silica gel using *n*-hexane/EtOAc 1:1 as the eluent, to yield the nitroaryl-ketoester derivative **3.247b** (910 mg, 2.32 mmol, 71% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 3.25 (s, 3H), 3.95 (s, 3H), 4.61 (s, 2H), 7.08-7.13 (m, 2H), 7.32-7.35 (m, 3H), 7.42 (d, 1H, J = 8.1 Hz), 7.72 (dd, 1H, J = 8.1, 1.8 Hz), 8.32 (d, 1H, J = 1.8 Hz).

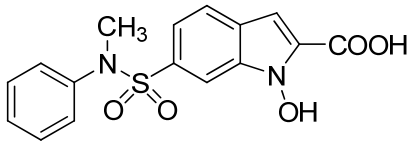
Methyl 1-hydroxy-6-(*N*-methyl-*N*-phenylsulfamoyl)-1*H*-indole-2-carboxylate (3.248b)



Ketoester **3.247b** (450 mg, 1.15 mmol) was dissolved in THF (1.9 mL) and the resulting solution was added to an aqueous solution (1.9 mL) of sodium hypophosphite monohydrate (378 mg, 3.57 mmol). Then 11.6 mg of 10% palladium over charcoal was

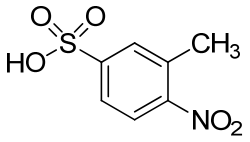
added and the resulting suspension was stirred ar RT. After 2 h the disappearance of the precursor is verified by TLC and the mixture was concentrated, diluted with water and repeatedly extracted with EtOAc. The combined organic phase was dried over anhydrous sodium sulphate and evaporated to afford a crude residue that was purified by column chromatography over silica gel using $CHCl_3$/MeOH 98:2 as the eluent, to give the *N*-hydroxyindole **3.248b** (96.1 mg, 0.267 mmol, 23% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 3.22 (s, 3H), 4.03 (s, 3H), 7.07 (s, 1H), 7.08-7-13 (m, 3H), 7.18 (dd, 1H, *J* = 8.6, 1.6 Hz), 7.27-7.32 (m, 2H), 7.65 (d, 1H, *J* = 8.4 Hz), 7.90 (d, 1H, *J* = 0.7 Hz), 10.32 (bs, 1H).

1-Hydroxy-6-(*N*-methyl-*N*-phenylsulfamoyl)-1*H*-indole-2-carboxylic acid (3.243)



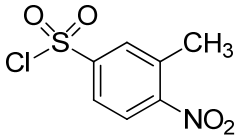
Methyl ester **3.248b** (90.0 mg, 0.250 mmol) was dissolved in a 1:1 mixture of THF/methanol (2.5 mL) and treated with 0.8 mL of 2N aqueous solution of LiOH. The reaction was kept under stirring in the dark at RT and it was monitored by TLC. After 24 h, the reaction mixture was concentrated, diluted with water and washed with Et_2O. Then, the aqueous phase was treated with 1N aqueous HCl and extracted several times with EtOAc. The combined ethyl acetate organic phase was dried over anhydrous sodium sulphate and evaporated to afford the desired *N*-hydroxyindol-carboxylic acid product **3.243** (75.4 mg, 0.218 mmol, 87% yield) without any further purification. ^{1}H NMR (DMSO-d_6) δ (ppm): 3.15 (s, 3H), 7.08-7.15 (m, 4H), 7.27-7.34 (m, 3H), 7.56-7.57 (m, 1H), 7.79 (d, 1H, *J* = 8.2 Hz). ^{13}C NMR (DMSO-d_6) δ (ppm): 30.69, 104.21, 109.97, 118.31, 122.89, 123.53, 126.12 (2C), 127.08, 128.78 (2C), 130.01, 131.67, 133.87, 141.14, 160.62. MS *m/z* 346 (M^+, 17%), 330 (M^+ –O, 14%), 240 (M^+ –PhNMe, 10%), 224 (M^+ –O –PhNMe, 18%), 177 (M^+ –PhNMe –SO_2 +H, 51%), 106 ($PhNMe^{\cdot+}$, 100%).

3-Methyl-4-nitrobenzenesulfonic acid (3.250)



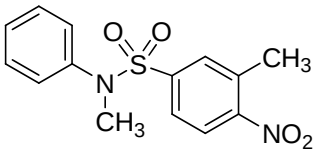
Commercially available 4-amino-3-methylbenzenesulfonic acid **3.249** (1.00 g, 5.94 mmol), acetic acid (24.3 mL), and 35% hydrogen peroxide (6.8 mL, 7.5 g, 77 mmol) were heated for 2 h with stirring at 75 °C. The sulfonic acid gradually dissolved to give a clear solution. Then, the reaction mxture was cooled to RT, sodium hydrogen carbonate was added and the solution was evaporated to a thick syrup. The crude product was purified by column chromatography over silica gel using CH_2Cl_2/MeOH 8:2 with 0.01% acetic acid as the eluent, to yield the nitro-derivative **3.250** (836 mg, 3.85 mmol, 72% yield). ^{1}H NMR (DMSO-d_6) δ (ppm): 2.52 (s, 3H), 7.59-7.67 (m, 2H), 7.95 (d, 1H, J = 8.2 Hz).

3-Methyl-4-nitrobenzene-1-sulfonyl chloride (3.251)



Thionyl chloride (2.7 mL, .4.4 g, 37 mmol) and catalytic DMF (0.8 mL) were added to the sulfonic acid derivative **3.250** (710 mg, 3.27 mmol) and the reaction was kept under stirring at 100 °C for 3 h. Evaporation under vacuum of the organic solvent afforded a crude product **3.251** (5.10 g) which was immediately used for the next reaction without any purification.

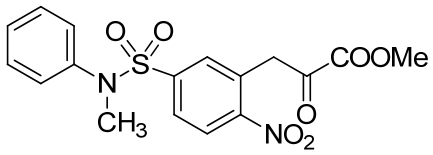
***N*,3-Dimethyl-4-nitro-*N*-phenylbenzenesulfonamide (3.252)**



A solution of **3.251** (5.10 g) in CH_2Cl_2 (5.2 mL) was added dropwise to *N*-methylaniline (0.98 mL, 0.97 g, 9.0 mmol) and the reaction was kept under stirring at

RT for 1 h. The reaction mixture was acidified with 1N aqueous HCl, extracted several times with CH_2Cl_2 and the combined organic phase was dried over anhydrous sodium sulphate. Evaporation under vacuum of the organic solvent afforded a crude product which was purified by column chromatography over silica gel using *n*-hexane/EtOAc 85:15 as the eluent, to yield the sulfonamidic derivative **3.252** (565 mg, 1.85 mmol, 2 steps: 56% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 2.57 (s, 3H), 3.22 (s, 3H), 7.08-7.14 (m, 2H), 7.30-7.37 (m, 3H), 7.45-7.53 (m, 2H), 7.97 (d, 1H, *J* = 8.2 Hz).

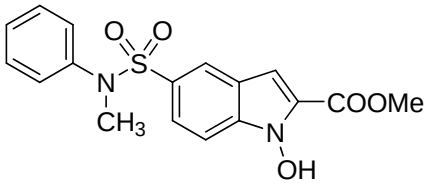
Methyl 3-(5-(*N*-methyl-*N*-phenylsulfamoyl)-2-nitrophenyl)-2-oxopropanoate (3.253)



A stirred suspension of sodium hydride (60% dispersion in mineral oil, 277 mg, 6.92 mmol) in 4.9 mL of anhydrous DMF cooled to −15 °C under nitrogen was treated dropwise with a solution containing the nitrotoluene precursor **3.252** (530 mg, 1.73 mmol) and dimethyl oxalate (1.02 g, 8.65 mmol) in 3.2 mL of anhydrous DMF. The mixture was left under stirring at the same temperature for 10 minutes and then it was slowly warmed to room temperature. The mixture was then left under stirring at RT for 6 h. Once the disappearance of the precursor was verified by TLC, the reaction mixture was slowly poured in an ice-water mixture; the water phase was acidified with 1N aqueous HCl and extracted several times with EtOAc. The combined organic phase was washed with saturated $NaHCO_3$ solution and brine, then dried over anhydrous sodium sulphate. Evaporation under vacuum of the organic solvent afforded a crude product which was purified by column chromatography over silica gel using *n*-hexane/EtOAc 7:3 as the eluent, to yield the nitroaryl-ketoester derivative **3.253** (378 mg, 0.964 mmol, 56% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 3.22 (s, 3H), 3.96 (s, 3H), 4.50 (s, 2H), 7.06-7.15 (m, 2H), 7.29-7.39 (m, 3H), 7.44 (d, 1H, *J* = 2.0 Hz), 7.68 (dd, 1H, *J* = 8.6, 2.0 Hz), 8.21 (d, 1H, *J* = 8.4 Hz); signals imputable to the enol form (~ 16%) δ (ppm): 3.26

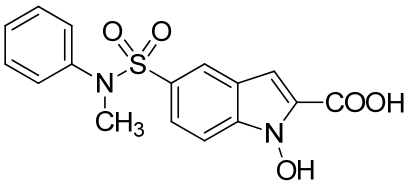
(s, 3H), 6.72 (d, 1H, exchangeable, J = 1.6 Hz), 6.83 (d, 1H, J = 1.3 Hz), 7.44-7.50 (m, 1H), 7.89 (d, 1H, J = 8.4 Hz), 8.53 (d, 1H, J = 2.2 Hz).

Methyl 1-hydroxy-5-(*N*-methyl-*N*-phenylsulfamoyl)-1*H*-indole-2-carboxylate (3.254)



Triethylamine (0.20 mL, 0.13 g, 1.3 mmol) was added dropwise to a stirred solution of $SnCl_2 \cdot 2H_2O$ (86.3 mg, 0.382 mmol) and PhSH (0.10 mL, 0.13 g, 1.2 mmol) in acetonitrile (2 mL) at room temperature to generate a yellow precipitate over a period of 5 min. Then, **3.253** (100 mg, 0.255 mmol) was transferred to the suspension and stirred at room temperature for 5 more min. After complete consumption of the starting material, the mixture was diluted with water and repeatedly extracted with EtOAc. The combined organic phase was dried over anhydrous sodium sulphate, diluted with toluene and a catalytic amount of *p*-toluensulfonic acid was added. Then it was evaporated to afford a crude residue that was purified by column chromatography over silica gel using *n*-hexane/EtOAc 1:1 as the eluent, to give the *N*-hydroxyindole **3.254** (45.4 mg, 0.126 mmol, 49% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 3.18 (s, 3H), 4.03 (s, 3H), 7.05-7.14 (m, 4H), 7.23-7.31 (m, 2H), 7.42 (dd, 1H, J = 8.9, 1.6 Hz), 7.55 (dd, 1H, J = 8.9, 0.8 Hz), 7.95 (t, 1H, J = 0.8 Hz), 10.51 (bs, 1H).

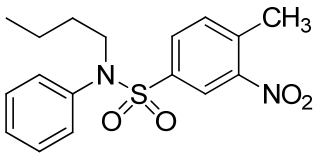
1-Hydroxy-5-(*N*-methyl-*N*-phenylsulfamoyl)-1*H*-indole-2-carboxylic acid (3.244)



Methyl ester **3.254** (35.0 mg, 0.0971 mmol) was dissolved in a 1:1 mixture of THF/methanol (0.9 mL) and treated with 0.3 mL of 2N aqueous solution of LiOH. The

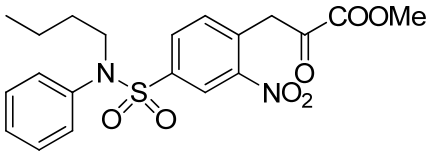
reaction was kept under stirring in the dark at RT and it was monitored by TLC. After 1 h, the reaction mixture was concentrated, diluted with water and washed with Et_2O. Then, the aqueous phase was treated with 1N aqueous HCl and extracted several times with EtOAc. The combined ethyl acetate organic phase was dried over anhydrous sodium sulphate and evaporated to afford the desired *N*-hydroxyindol-carboxylic acid product **3.244** (33.3 mg, 0.0961 mmol, 99% yield) without any further purification. ^{1}H NMR (acetone-d_6) δ (ppm): 3.20 (s, 3H), 7.10-7.15 (m, 2H), 7.26-7.33 (m, 4H), 7.42 (dd, 1H, J = 8.9, 1.7 Hz), 7.63 (dt, 1H, J = 9.0, 0.8 Hz), 7.99 (dd, 1H, J = 1.6, 0.7 Hz). ^{13}C NMR (acetone-d_6) δ (ppm): 38.47, 107.39, 110.63, 121.26, 124.52, 124.74, 127.25 (2C), 127.73, 128.64, 129.46 (2C), 129.93, 137.65, 142.91, 161.54. HPLC, t_R = 8.9 min.

_N_-Butyl-4-methyl-3-nitro-_N_-phenylbenzenesulfonamide (3.262)



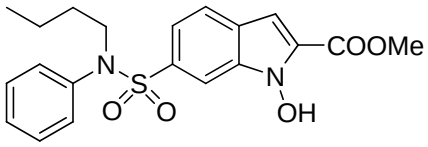
A solution of commercially available 4-methyl-3-nitrobenzenesulfonylchloride **3.245** (600 mg, 2.55 mmol) in CH_2Cl_2 (4.4 mL) was added dropwise to *N*-methylaniline (1.22 mL, 1.14 g, 7.64 mmol) and the reaction was kept under stirring at RT for 16 h. The reaction mixture was acidified with 1N aqueous HCl, extracted several times with CH_2Cl_2 and the combined organic phase was dried over anhydrous sodium sulphate. Evaporation under vacuum of the organic solvent afforded a crude product corresponding to the sulfonamidic derivative **3.262** (748 mg, 2.15 mmol, 84% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 0.87 (t, 3H, J = 6.8 Hz), 1.25-1.42 (m, 4H), 2.67 (s, 3H), 3.56 (t, 2H, J = 6.6 Hz), 7.02-7.07 (m, 2H), 7.30-7.35 (m, 3H), 7.43 (d, 1H, J = 8.1 Hz), 7.63 (dd, 1H, J = 8.1, 1.8 Hz), 8.17 (d, 1H, J = 1.8 Hz).

Methyl 3-(4-(_N_-butyl-_N_-phenylsulfamoyl)-2-nitrophenyl)-2-oxopropanoate (3.263)



A stirred suspension of sodium hydride (60% dispersion in mineral oil, 230 mg, 5.75 mmol) in 3.4 mL of anhydrous DMF cooled to –15 °C under nitrogen was treated dropwise with a solution containing the nitrotoluene precursor **3.262** (500 mg, 1.44 mmol) and dimethyl oxalate (848 mg, 7.18 mmol) in 2.5 mL of anhydrous DMF. The mixture was left under stirring at the same temperature for 10 minutes and then it was slowly warmed to room temperature. The mixture was then left under stirring at RT for 24 h. Once the disappearance of the precursor was verified by TLC, the reaction mixture was slowly poured in an ice-water mixture; the water phase was acidified with 1N aqueous HCl and extracted several times with EtOAc. The combined organic phase was washed with saturated $NaHCO_3$ solution and brine, then dried over anhydrous sodium sulphate. Evaporation under vacuum of the organic solvent afforded a crude product which was purified by column chromatography over silica gel using *n*-hexane/EtOAc 7:3 as the eluent, to yield the nitroaryl-ketoester derivative **3.263** (476 mg, 1.10 mmol, 76% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 0.87 (t, 3H, *J* = 6.8 Hz), 1.29-1.39 (m, 4H), 3.57 (t, 2H, *J* = 6.6 Hz), 3.97 (s, 3H), 4.62 (s, 2H), 7.03-7.09 (m, 2H), 7.31-7.36 (m, 3H), 7.41 (d, 1H, *J* = 8.6 Hz), 7.72 (dd, 1H, *J* = 8.6, 1.8 Hz), 8.36 (s, 1H); signals imputable to the enol form (~ 48%) δ (ppm): 3.96 (s, 3H), 6.91 (s, 1H), 7.76 (dd, 1H, *J* = 8.1, 1.8 Hz), 8.05 (d, 1H, *J* = 2.0 Hz), 8.38 (d, 1H, *J* = 8.4 Hz).

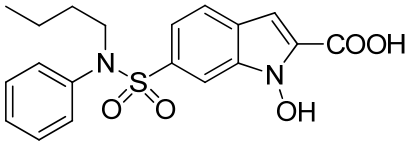
Methyl 6-(*N*-butyl-*N*-phenylsulfamoyl)-1-hydroxy-1*H*-indole-2-carboxylate (3.264)



Triethylamine (0.32 mL, 0.23 g, 2.3 mmol) was added dropwise to a stirred solution of $SnCl_2 \cdot 2H_2O$ (103 mg, 0.457 mmol) and PhSH (0.21 mL, 0.23 g, 2.1 mmol) in acetonitrile (3.6 mL) at room temperature to generate a yellow precipitate over a period of 5 min. Then, **3.263** (200 mg, 0.457 mmol) was transferred to the suspension and stirred at room temperature for 5 more min. After complete consumption of the starting material, the mixture was diluted with water and repeatedly extracted with EtOAc. The combined organic phase was dried over anhydrous sodium sulphate, diluted with

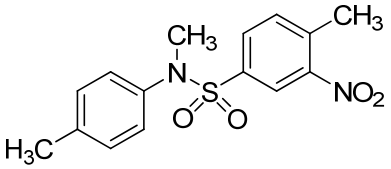
toluene and a catalytic amount of *p*-toluensulfonic acid was added. Then it was evaporated to afford a crude residue that was purified by column chromatography over silica gel using *n*-hexane/EtOAc 6:4 as the eluent, to give the *N*-hydroxyindole **3.264** (45.4 mg, 0.113 mmol, 25% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 0.85 (t, 3H, J = 7.0 Hz), 1.36-1.39 (m, 4H), 3.57 (t, 2H, J = 6.7 Hz), 4.02 (s, 3H), 7.02-7.07 (m, 3H), 7.22 (d, 1H, J = 1.1 Hz), 7.27-7.31 (m, 3H), 7.66 (d, 1H, J = 8.6 Hz), 7.90 (d, 1H, J = 0.8 Hz), 10.30 (bs, 1H).

6-(*N*-Butyl-*N*-phenylsulfamoyl)-1-hydroxy-1*H*-indole-2-carboxylic acid (3.255)



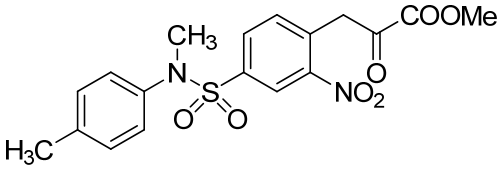
Methyl ester **3.264** (26.0 mg, 0.0647 mmol) was dissolved in a 1:1 mixture of THF/methanol (0.7 mL) and treated with 0.2 mL of 2N aqueous solution of LiOH. The reaction was kept under stirring in the dark at RT and it was monitored by TLC. After 16 h, the reaction mixture was concentrated, diluted with water and washed with Et_2O. Then, the aqueous phase was treated with 1N aqueous HCl and extracted several times with EtOAc. The combined ethyl acetate organic phase was dried over anhydrous sodium sulphate and evaporated to afford the desired *N*-hydroxyindol-carboxylic acid product **3.255** (21.6 mg, 0.0556 mmol, 86% yield) without any further purification. ^{1}H NMR (acetone-d_6) δ (ppm): 0.86 (t, 3H, J = 7.0 Hz), 1.35-1.42 (m, 4H), 3.66 (t, 2H, J = 6.4 Hz), 7.08-7.13 (m, 2H), 7.22 (d, 1H, J = 0.7 Hz), 7.28-7.37 (m, 4H), 7.77 (s, 1H), 7.83 (d, 1H, J = 8.6 Hz). ^{13}C NMR (acetone-d_6) δ (ppm): 13.84, 20.15, 50.71, 105.91, 110.99, 119.82, 123.74, 124.80, 128.44, 129.62, 129.86, 135.30, 135.78, 140.24, 161.28. HPLC, t_R = 10.1 min.

***N*,4-Dimethyl-3-nitro-*N*-*p*-tolylbenzenesulfonamide (3.265a)**



A solution of commercially available 4-methyl-3-nitrobenzenesulfonylchloride **3.245** (1.12 g, 4.76 mmol) in CH_2Cl_2 (8 mL) was added dropwise to *N*-methyl-*p*-toluidine (1.78 mL, 1.70 g, 14.0 mmol) and the reaction was kept under stirring at RT for 2 h. The reaction mixture was acidified with 1N aqueous HCl, extracted several times with CH_2Cl_2 and the combined organic phase was dried over anhydrous sodium sulphate. Evaporation under vacuum of the organic solvent afforded a crude product which was purified by column chromatography over silica gel using *n*-hexane/EtOAc 7:3 as the eluent, to yield the sulfonamidic derivative **3.265a** (1.52 g, 4.74 mmol, 99% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 2.35 (s, 3H), 2.67 (s, 3H), 3.19 (s, 3H), 6.96 (AA'XX', 2H, J_{AX} = 8.4 Hz, $J_{AA'XX'}$ = 2.2 Hz), 7.13 (d, 2H, J = 8.1 Hz), 7.44 (d, 1H, J = 8.2 Hz), 7.60 (dd, 1H, J = 9.0, 1.8 Hz), 8.15 (d, 1H, J = 1.8 Hz).

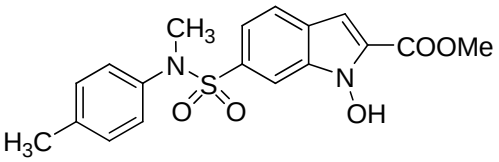
Methyl 3-(4-(*N*-methyl-*N*-*p*-tolylsulfamoyl)-2-nitrophenyl)-2-oxopropanoate (3.266a)



A stirred suspension of sodium hydride (60% dispersion in mineral oil, 320 mg, 8.00 mmol) in 6 mL of anhydrous DMF cooled to –15 °C under nitrogen was treated dropwise with a solution containing the nitrotoluene precursor **3.265a** (640 mg, 2.00 mmol) and dimethyl oxalate (1.18 g, 10.0 mmol) in 4 mL of anhydrous DMF. The mixture was left under stirring at the same temperature for 10 minutes and then it was slowly warmed to room temperature. The mixture was then left under stirring at RT for 24 h. Once the disappearance of the precursor was verified by TLC, the reaction mixture was slowly poured in an ice-water mixture; the water phase was acidified with 1N aqueous HCl and extracted several times with EtOAc. The combined organic phase was washed with saturated $NaHCO_3$ solution and brine, then dried over anhydrous sodium sulphate. Evaporation under vacuum of the organic solvent afforded a crude product which was purified by column chromatography over silica gel using *n*-hexane/EtOAc 7:3 as the eluent, to yield the nitroaryl-ketoester derivative **3.266a** (406 mg, 0.999

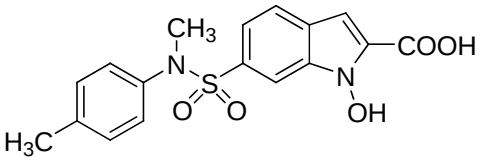
mmol, 50% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 2.35 (s, 3H), 3.21 (s, 3H), 3.97 (s, 3H), 4 62 (s, 2H), 6.98 (d, 2H, *J* = 8.4 Hz), 7.13 (d, 2H, *J* = 8.4 Hz), 7.69 (dd, 1H, *J* = 8.4, 1.8 Hz), 8.04 (d, 1H, *J* = 1.8 Hz), 8.38 (d, 1H, *J* = 8.4 Hz).

Methyl 1-hydroxy-6-(*N*-methyl-*N*-*p*-tolylsulfamoyl)-1*H*-indole-2-carboxylate (3.267a)



To a solution containing the ketoester **3.266a** (102 mg, 0.251 mmol) in 15 mL of MeOH, was added lead powder (448 mg, 2.16 mmol) and 0.5 mL of HCO_2HNEt_3. The mixture was stirred at 55 °C for 6 h, cooled to RT, and filtered over a pad of Celite. The solvent was removed under reduced pressure and the residue purified by column chromatography over silica gel using $CHCl_3$/MeOH 98:2 as the eluent, to give the *N*-hydroxyindole **3.267a** (75.2 mg, 0.201 mmol, 80% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 2.32 (s, 3H), 3.17 (s, 3H), 4.01 (s, 3H), 6.96 (AA'XX', 2H, J_{AX} = 8.6 Hz, $J_{AA'XX'}$ = 2.2 Hz), 7.05 (d, 1H, *J* = 0.7 Hz), 7.07 (d, 2H, *J* = 8.6 Hz), 7.17 (dd, 1H, *J* = 8.6, 1.6 Hz), 7.63 (d, 1H, *J* = 8.6 Hz), 7.89 (t, 1H, *J* = 0.7 Hz), 10.13 (bs, 1H).

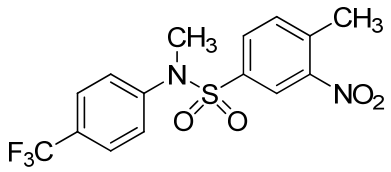
1-Hydroxy-6-(*N*-methyl-*N*-*p*-tolylsulfamoyl)-1*H*-indole-2-carboxylic acid (3.256)



Methyl ester **3.267a** (75.0 mg, 0.200 mmol) was dissolved in a 1:1 mixture of THF/methanol (2 mL) and treated with 0.6 mL of 2N aqueous solution of LiOH. The reaction was kept under stirring in the dark at RT and it was monitored by TLC. After the complete consumption of the starting material, the reaction mixture was concentrated, diluted with water and washed with Et_2O. Then, the aqueous phase was treated with 1N aqueous HCl and extracted several times with EtOAc. The combined

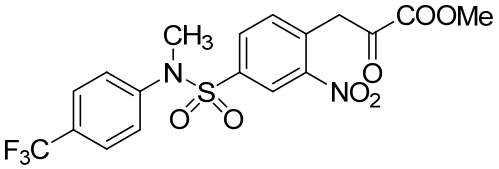
ethyl acetate organic phase was dried over anhydrous sodium sulphate and evaporated to afford the desired *N*-hydroxyindol-carboxylic acid product **3.256** (67.8 mg, 0.188 mmol, 94% yield) without any further purification. ^{1}H NMR ($CDCl_3$) δ (ppm): 2.31 (s, 3H), 3.21 (s, 3H), 7.02 (AA´XX´, 2H, J_{AX} = 8.6 Hz, $J_{AA'XX'}$ = 2.1 Hz), 7.08-7.18 (m, 2H), 7.23 (d, 1H, *J* = 1.0 Hz), 7.24 (dd, 1H, *J* = 8.4, 1.6 Hz), 7.78 (dt, 1H, *J* = 1.8, 0.8 Hz), 7.83 (dd, 1H, *J* = 8.4, 0.8 Hz). ^{13}C NMR ($CDCl_3$) δ (ppm): 20.97, 38.67, 105.88, 111.21, 120.01, 123.648, 124.91, 127.22, 130.06, 134.06, 135.25, 137.67, 140.14, 161.27. MS *m/z* 359 (M^+ –H).

_N_,4-Dimethyl-3-nitro-_N_-(4-(trifluoromethyl)phenyl)benzenesulfonamide (3.265b)



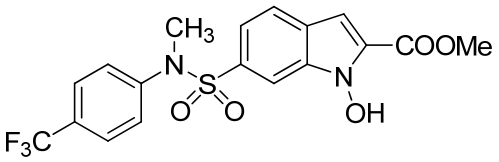
To a solution of 4-trifluoromethyl-*N*-methylaniline (0.40 mL, 0.50 g, 2.9 mmol) in dry CH_2Cl_2 (10 mL), pyridine (0.35 mL, 0.34 g, 4.3 mmol) and catalytic DMAP (15.0 mg) were added, then the resulting mixture was cooled to 0 °C. Subsequently, commercially available 4-methyl-3-nitrobenzenesulfonylchloride **3.245** (807 mg, 3.43 mmol) in CH_2Cl_2 (10 mL) was added dropwise and the reaction was kept under stirring at RT overnight. The reaction mixture was acidified with 1N aqueous HCl, extracted several times with CH_2Cl_2 and the combined organic phase was dried over anhydrous sodium sulphate. Evaporation under vacuum of the organic solvent afforded a crude product which was purified by column chromatography over silica gel using *n*-hexane/EtOAc 93:7 as the eluent, to yield the sulfonamidic derivative **3.265b** (1.19 g, 3.18 mmol, >99% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 2.68 (s, 3H), 3.24 (s, 3H), 7.24-7.30 (m, 2H), 743-7.64 (m, 4H), 8.19 (d, 1H, *J* = 1.5 Hz).

Methyl 3-(4-(*N*-methyl-*N*-(4-(trifluoromethyl)phenyl)sulfamoyl)-2-nitrophenyl)-2-oxopropanoate (3.266b)



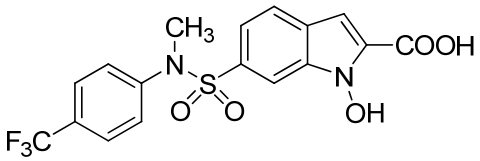
A stirred suspension of sodium hydride (60% dispersion in mineral oil, 320 mg, 8.00 mmol) in 6 mL of anhydrous DMF cooled to –15 °C under nitrogen was treated dropwise with a solution containing the nitrotoluene precursor **3.265b** (748 mg, 2.00 mmol) and dimethyl oxalate (1.18 g, 10.0 mmol) in 4 mL of anhydrous DMF. The mixture was left under stirring at the same temperature for 10 minutes and then it was slowly warmed to room temperature. The mixture was then left under stirring at RT for 24 h. Once the disappearance of the precursor was verified by TLC, the reaction mixture was slowly poured in an ice-water mixture; the water phase was acidified with 1N aqueous HCl and extracted several times with EtOAc. The combined organic phase was washed with saturated $NaHCO_3$ solution and brine, then dried over anhydrous sodium sulphate. Evaporation under vacuum of the organic solvent afforded a crude product which was purified by column chromatography over silica gel using *n*-hexane/EtOAc 7:3 as the eluent, to yield the nitroaryl-ketoester derivative **3.266b** (405 mg, 0.880 mmol, 44% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 3.27 (s, 3H), 3.96 (s, 3H), 4 63 (s, 2H), 7.25-7.32 (m, 2H), 7.44 (d, 1H, J = 8.1 Hz), 7.59-7.65 (m, 2H), 7.69 (dd, 1H, J = 7,9, 1.8 Hz), 8.37 (d, 1H, J = 1.5 Hz); signals imputable to the enol form (~ 49%) δ (ppm): 3.26 (s, 3H), 3.98 (s, 3H), 6.90 (bs, 1H, exchangeable), 6.97 (d, 1H, J = 1.3 Hz), 8.07 (d, 1H, J = 2.0 Hz), 8.40 (d, 1H, J = 8.4 Hz).

Methyl 1-hydroxy-6-(*N*-methyl-*N*-(4-(trifluoromethyl)phenyl)sulfamoyl)-1*H*-indole-2-carboxylate (3.267b)



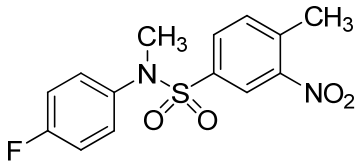
To a solution containing the ketoester **3.266b** (275 mg, 0.598 mmol) in 4.1 mL of MeOH, was added lead powder (1.10 g, 5.31 mmol) and 1.1 mL of HCO_2HNEt_3. The mixture was stirred at 55 °C for 12 h, cooled to RT, and filtered over a pad of Celite. The solvent was removed under reduced pressure and the residue purified by column chromatography over silica gel using *n*-hexane/EtOAc 75:25 as the eluent, to give the *N*-hydroxyindole **3.267b** (182 mg, 0.425 mmol, 71% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 3.23 (s, 3H), 4.03 (s, 3H), 7.07 (d, 1H, *J* = 0.9 Hz), 7.13 (dd, 1H, *J* = 8.6, 1.6 Hz), 7.24-7.29 (m, 2H), 7.53-7.59 (m, 2H), 7.66 (dd, 1H, *J* = 8.6, 0.7 Hz), 7.91 (dt, 1H, *J* = 1.5, 0.8 Hz), 10.36 (bs, 1H).

1-Hydroxy-6-(*N*-methyl-*N*-(4-(trifluoromethyl)phenyl)sulfamoyl)-1*H*-indole-2-carboxylic acid (3.257)



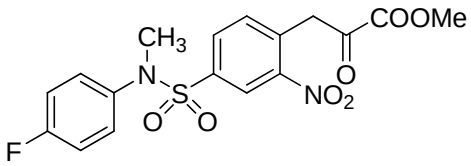
Methyl ester **3.267b** (85.0 mg, 0.198 mmol) was dissolved in a 1:1 mixture of THF/methanol (1 mL) and treated with 0.6 mL of 2N aqueous solution of LiOH. The reaction was kept under stirring in the dark at RT and it was monitored by TLC. After 3 h, the reaction mixture was concentrated, diluted with water and washed with Et_2O. Then, the aqueous phase was treated with 1N aqueous HCl and extracted several times with EtOAc. The combined ethyl acetate organic phase was dried over anhydrous sodium sulphate and evaporated to afford the desired *N*-hydroxyindol-carboxylic acid product **3.257** (75.0 mg, 0.181 mmol, 91% yield) without any further purification. ^{1}H NMR (acetone-d_6) δ (ppm): 3.29 (s, 3H), 7.20 (dd, 1H, *J* = 8.4, 1.8 Hz), 7.21 (d, 1H, *J* = 1.8 Hz), 7.40-7.50 (m, 2H), 7.65-7.74 (m, 2H), 7.78-7.86 (m, 2H). ^{13}C NMR (acetone-d_6) δ (ppm): 38.12, 105.71, 111.18, 119.61, 123.96, 125.16, 126.62 (q, 2C, *J* = 3.7 Hz), 127.13 (2C), 127.75, 128.66 (q, *J* = 33.0 Hz), 130.20, 132.38 (q, *J* = 269.8 Hz), 133.39, 146.30, 161.38. MS *m/z* 415 ($M+H^+$, 5%), 239 ($CF_3PhN(Me)SO_2 + H^+$, 20%), 177 ($M + H^+ - CF_3PhN(Me)SO_2$, 100%).

_N_-(4-Fluorophenyl)-_N_,4-dimethyl-3-nitrobenzenesulfonamide (3.265c)



To a solution of 4-fluoromethyl-*N*-methylaniline (0.32 mL, 0.33 g, 2.7 mmol) in dry CH_2Cl_2 (10 mL), pyridine (0.32 mL, 0.32 g, 4.0 mmol) and catalytic DMAP (15.0 mg) were added, then the resulting mixture was cooled to 0 °C. Subsequently, commercially available 4-methyl-3-nitrobenzenesulfonylchloride **3.245** (695 mg, 2.94 mmol) in CH_2Cl_2 (10 mL) was added dropwise and the reaction was kept under stirring at RT overnight. The reaction mixture was acidified with 1N aqueous HCl, extracted several times with CH_2Cl_2 and the combined organic phase was dried over anhydrous sodium sulphate. Evaporation under vacuum of the organic solvent afforded a crude product which was purified by column chromatography over silica gel using *n*-hexane/EtOAc 7:3 as the eluent, to yield the sulfonamidic derivative **3.265c** (836 mg, 2.58 mmol, 97% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 2.68 (s, 3H), 3.20 (s, 3H), 6.96-7.12 (m, 4H), 7.45 (d, 1H, J = 8.1 Hz), 7.59 (dd, 1H, J = 8.1, 1.8 Hz), 8.17 (d, 1H, J = 1.8 Hz).

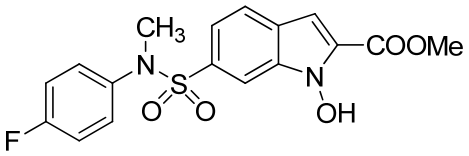
Methyl 3-(4-(_N_-(4-fluorophenyl)-_N_-methylsulfamoyl)-2-nitrophenyl)-2-oxopropanoate (3.266c)



A stirred suspension of sodium hydride (60% dispersion in mineral oil, 320 mg, 8.00 mmol) in 6 mL of anhydrous DMF cooled to −15 °C under nitrogen was treated dropwise with a solution containing the nitrotoluene precursor **3.265c** (648 mg, 2.00 mmol) and dimethyl oxalate (1.18 g, 10.0 mmol) in 4 mL of anhydrous DMF. The mixture was left under stirring at the same temperature for 10 minutes and then it was slowly warmed to room temperature. The mixture was then left under stirring at RT. Once the disappearance of the precursor was verified by TLC, the reaction mixture was

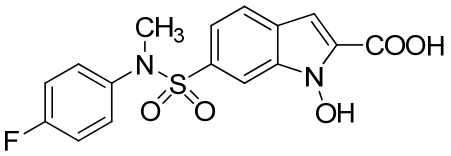
slowly poured in an ice-water mixture; the water phase was acidified with 1N aqueous HCl and extracted several times with EtOAc. The combined organic phase was washed with saturated $NaHCO_3$ solution and brine, then dried over anhydrous sodium sulphate. Evaporation under vacuum of the organic solvent afforded a crude product which was purified by column chromatography over silica gel using *n*-hexane/EtOAc 7:3 as the eluent, to yield the nitroaryl-ketoester derivative **3.266c** (512 mg, 1.25 mmol, 62% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 3.23 (s, 3H), 3.96 (s, 3H), 4.63 (s, 2H), 6.94-7.12 (m, 4H), 7.44 (d, 1H, *J* = 8.1 Hz), 7.72 (dd, 1H, *J* = 8.1, 1.8 Hz), 8.35 (d, 1H, *J* = 1.8 Hz); signals imputable to the enol form (~ 30%) δ (ppm): 3.21 (s, 3H), 3.98 (s, 3H), 6.90 (s, 1H), 8.05 (d, 1H, *J* = 1.8 Hz), 8.40 (d, 1H, *J* = 8.4 Hz).

Methyl 6-(*N*-(4-fluorophenyl)-*N*-methylsulfamoyl)-1-hydroxy-1*H*-indole-2-carboxylate (3.267c)



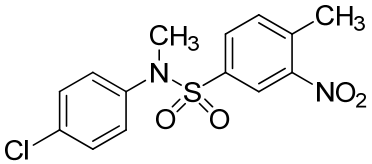
To a solution containing the ketoester **3.266c** (150 mg, 0.366 mmol) in 4 mL of MeOH, was added lead powder (1.00 g, 4.83 mmol) and 1 mL of HCO_2HNEt_3. The mixture was stirred at 55 °C for 12 h, cooled to RT, and filtered over a pad of Celite. The solvent was removed under reduced pressure and the residue purified by column chromatography over silica gel using *n*-hexane/EtOAc 1:1 as the eluent, to give the *N*-hydroxyindole **3.267c** (100 mg, 0.264 mmol, 72% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 3.15 (s, 3H), 3.98 (s, 3H), 6.89-7.87 (m, 5H), 7.13 (dd, 1H, *J* = 8.5, 1.6 Hz), 7.62 (d, 1H, *J* = 8.4 Hz), 7.83 (s, 1H), 10.20 (bs, 1H).

6-(*N*-(4-Fluorophenyl)-*N*-methylsulfamoyl)-1-hydroxy-1*H*-indole-2-carboxylic acid (3.258)



Methyl ester **3.267c** (80.0 mg, 0.211 mmol) was dissolved in a 1:1 mixture of THF/methanol (2.4 mL) and treated with 0.7 mL of 2N aqueous solution of LiOH. The reaction was kept under stirring in the dark at RT and it was monitored by TLC. After 12 h, the reaction mixture was concentrated, diluted with water and washed with Et_2O. Then, the aqueous phase was treated with 1N aqueous HCl and extracted several times with EtOAc. The combined ethyl acetate organic phase was dried over anhydrous sodium sulphate and evaporated to afford the desired *N*-hydroxyindol-carboxylic acid product **3.258** (76.0 mg, 0.209 mmol, 99% yield) without any further purification. ^{1}H NMR (acetone-d_6) δ (ppm): 3.22 (s, 3H), 7.04-7.19 (m, 4H), 7.22 (d, 1H, J = 0.9 Hz), 7.23 (dd, 1H, J = 8.4, 1.6 Hz), 7.75 (dt, 1H, J = 1.6, 0.8 Hz), 7.84 (dd, 1H, J = 8.6, 0.8 Hz). ^{13}C NMR (acetone-d_6) δ (ppm). 38.71, 105.95, 111.25, 116.19 (d, 2C, J = 22.9 Hz), 119.95, 123.78, 125.05, 129.48 (d, 2C, J = 9.2 Hz), 130.08, 133.55, 135.32, 138.89 (d, J = 3.7 Hz), 161.16, 162.13 (d, J = 244.4 Hz).

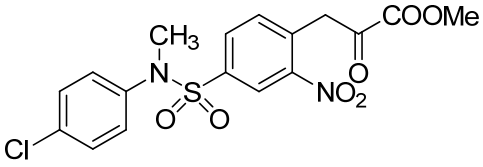
***N*-(4-Chlorophenyl)-*N*,4-dimethyl-3-nitrobenzenesulfonamide (3.265d)**



To a solution of 4-chloro-*N*-methylaniline (0.35 mL, 0.40 g, 2.9 mmol) in dry CH_2Cl_2 (10 mL), pyridine (0.35 mL, 0.34 g, 4.3 mmol) and catalytic DMAP (15.0 mg) were added, then the resulting mixture was cooled to 0 °C. Subsequently, commercially available 4-methyl-3-nitrobenzenesulfonylchloride **3.245** (807 mg, 3.43 mmol) in CH_2Cl_2 (10 mL) was added dropwise and the reaction was kept under stirring at RT overnight. The reaction mixture was acidified with 1N aqueous HCl, extracted several

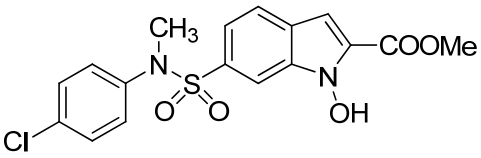
times with CH_2Cl_2 and the combined organic phase was dried over anhydrous sodium sulphate. Evaporation under vacuum of the organic solvent afforded a crude product which was purified by column chromatography over silica gel using *n*-hexane/EtOAc 8:2 as the eluent, to yield the sulfonamidic derivative **3.265d** (1.17 g, 3.43 mmol, >99% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 2.68 (s, 3H), 3.20 (s, 3H), 7.05 (AA´XX´, 2H, J_{AX} = 8.8 Hz, $J_{AA'/XX'}$ = 2.6 Hz), 7.31 (AA´XX´, 2H, J_{AX} = 9.0 Hz, $J_{AA'/XX'}$ = 2.6 Hz), 7.45 (d, 1H, *J* = 8.1 Hz), 7.57 (dd, 1H, *J* = 8.1, 1.8 Hz), 8.19 (d, 1H, *J* = 1.8 Hz).

Methyl 3-(4-(*N*-(4-chlorophenyl)-*N*-methylsulfamoyl)-2-nitrophenyl)-2-oxopropanoate (3.266d)



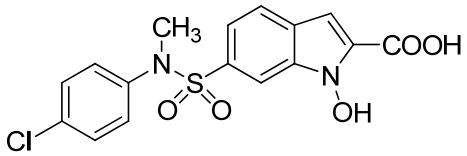
A stirred suspension of sodium hydride (60% dispersion in mineral oil, 320 mg, 8.00 mmol) in 6 mL of anhydrous DMF cooled to −15 °C under nitrogen was treated dropwise with a solution containing the nitrotoluene precursor **3.265d** (682 mg, 2.00 mmol) and dimethyl oxalate (1.18 g, 10.0 mmol) in 4 mL of anhydrous DMF. The mixture was left under stirring at the same temperature for 10 minutes and then it was slowly warmed to room temperature. The mixture was then left under stirring at RT. Once the disappearance of the precursor was verified by TLC, the reaction mixture was slowly poured in an ice-water mixture; the water phase was acidified with 1N aqueous HCl and extracted several times with EtOAc. The combined organic phase was washed with saturated $NaHCO_3$ solution and brine, then dried over anhydrous sodium sulphate. Evaporation under vacuum of the organic solvent afforded a crude product which was purified by column chromatography over silica gel using *n*-hexane/EtOAc 6:4 as the eluent, to yield the nitroaryl-ketoester derivative **3.266d** (126 mg, 0.295 mmol, 15% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 3.21 (s, 3H), 3.95 (s, 3H), 4.62 (s, 2H), 7.02-7.09 (m, 2H), 7.26-7.33 (m, 2H), 7.44 (d, 1H, *J* = 7.9 Hz), 7.68 (dd, 1H, *J* = 8.0, 1.7 Hz), 8.34 (d, 1H, *J* = 1.8 Hz); signals imputable to the enol form (~ 24%) δ (ppm): 3.20 (s, 3H), 3.97 (s, 3H), 6.89 (s, 1H), 8.38 (d, 1H, *J* = 8.6 Hz).

Methyl 6-(*N*-(4-chlorophenyl)-*N*-methylsulfamoyl)-1-hydroxy-1*H*-indole-2-carboxylate (3.267d)



To a solution containing the ketoester **3.266d** (120 mg, 0.281 mmol) in 1.5 mL of MeOH, was added lead powder (300 mg, 1.45 mmol) and 0.3 mL of HCO_2HNEt_3. The mixture was stirred at 55 °C for 12 h, cooled to RT, and filtered over a pad of Celite. The solvent was removed under reduced pressure and the residue purified by column chromatography over silica gel using *n*-hexane/EtOAc 6:4 as the eluent, to give the *N*-hydroxyindole **3.267d** (85.0 mg, 0.215 mmol, 77% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 3.17 (s, 3H), 4.02 (s, 3H), 6.95-7.07 (m, 3H), 7.14 (dd, 1H, J = 8.5, 1.7 Hz), 7.20-7.29 (m, 2H), 7.65 (d, 1H, J = 8.4 Hz), 7.89 (s, 1H), 10.32 (bs, 1H).

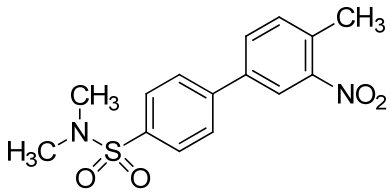
6-(*N*-(4-Chlorophenyl)-*N*-methylsulfamoyl)-1-hydroxy-1*H*-indole-2-carboxylic acid (3.259)



Methyl ester **3.267d** (50.0 mg, 0.127 mmol) was dissolved in a 1:1 mixture of THF/methanol (2 mL) and treated with 0.4 mL of 2N aqueous solution of LiOH. The reaction was kept under stirring in the dark at RT and it was monitored by TLC. After complete consumption of the starting material, the reaction mixture was concentrated, diluted with water and washed with Et_2O. Then, the aqueous phase was treated with 1N aqueous HCl and extracted several times with EtOAc. The combined ethyl acetate organic phase was dried over anhydrous sodium sulphate and evaporated to afford the desired *N*-hydroxyindol-carboxylic acid product **3.259** (44.0 mg, 0.116 mmol, 91% yield) without any further purification. ^{1}H NMR (acetone-d_6) δ (ppm): 3.22 (s, 3H), 7.14-7.25 (m, 4H), 7.36 (AA´XX´, 2H, J_{AX} = 9.0 Hz, $J_{AA'/XX'}$ = 2.4 Hz), 7.77 (pseudo-*t*, 1H, J = 0.8 Hz), 7.83 (dd, 1H, J = 8.4, 0.4 Hz). ^{13}C NMR (acetone-d_6) δ (ppm): 38.69,

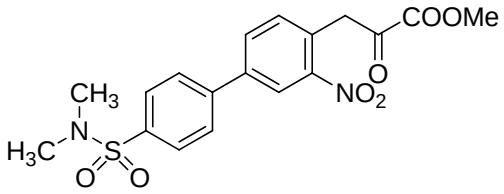
105.99, 111.49, 120.08, 124.09, 125.16, 129.09, 129.82, 132.55, 133.17, 133.66, 135.41, 141.86, 161.56. MS *m/z* 403 ($M+Na^+$, 9%), 370 ($M+Na^+$ –O –OH, 100%).

_N_,_N_,4'-Trimethyl-3'-nitro-[1,1'-biphenyl]-4-sulfonamide (3.269)



4-Bromo-*N*,*N*-dimethylbenzenesulfonamide **3.268** (664 mg, 2.51 mmol) was placed in a vial together with 4-methyl-3-nitrobenzeneboronic acid **3.127** (500 mg, 2.77 mmol), sodium carbonate (798 mg, 7.53 mmol), $Pd(OAc)_2$ (2.3 mg, 0.010 mmol), tetrabutylammonium bromide (809 mg, 2.51 mmol) and water (5 mL). The vial was sealed and heated under stirring at 150 °C in a microwave reactor for 5 min. The reaction mixture was then diluted with water and repeatedly extracted with EtOAc. The combined organic phase was dried over anhydrous sodium sulphate and evaporated to afford a crude residue that was purified by column chromatography over silica gel using *n*-hexane/EtOAc 6:4 as the eluent, to give pure **3.269** (766 mg, 2.39 mmol, 95% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 2.67 (s, 3H), 2.76 (s, 6H), 7.48 (d, 1H, J = 7.7 Hz), 7.74-7.79 (m, 3H), 7.89 (AA'XX', 2H, J_{AX} = 8.4 Hz, $J_{AA'XX'}$ = 1.9 Hz), 8.23 (d, 1H, J = 1.8 Hz).

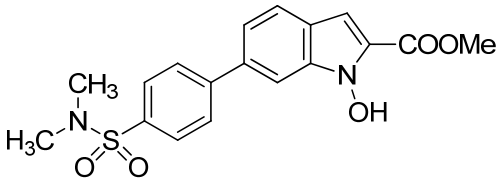
Methyl 3-(4'-(*N*,*N*-dimethylsulfamoyl)-3-nitro-[1,1'-biphenyl]-4-yl)-2-oxopropanoate (3.273a)



A stirred suspension of sodium hydride (60% dispersion in mineral oil, 350 mg, 8.76 mmol) in 6.2 mL of anhydrous DMF cooled to –15 °C under nitrogen was treated dropwise with a solution containing the nitrotoluene precursor **3.269** (700 mg, 2.19

mmol) and dimethyl oxalate (1.29 g, 11.0 mmol) in 4.1 mL of anhydrous DMF. The mixture was left under stirring at the same temperature for 10 minutes and then it was slowly warmed to room temperature. The mixture was then left under stirring at RT for 2 h. Once the disappearance of the precursor was verified by TLC, the reaction mixture was slowly poured in an ice-water mixture; the water phase was acidified with 1N aqueous HCl and extracted several times with EtOAc. The combined organic phase was washed with saturated $NaHCO_3$ solution and brine, then dried over anhydrous sodium sulphate. Evaporation under vacuum of the organic solvent afforded a crude product which was purified by column chromatography over silica gel using *n*-hexane/EtOAc 4:6 as the eluent, to yield the nitroaryl-ketoester derivative **3.273a** (686 mg, 1.69 mmol, 77% yield). ^{1}H NMR $CDCl_3$) δ (ppm): 2.76 (s, 6H), 3.96 (s, 3H), 4.61 (s, 2H), 7.46 (d, 1H, *J* = 7.9 Hz), 7.76-7.93 (m, 5H), 8.42 (d, 1H, *J* = 1.6 Hz); signals imputable to the enol form (~ 36%) δ (ppm): 3.97 (s, 3H), 6.75 (d, 1H, exchangeable, *J* = 1.1 Hz), 6.97 (d, 1H, *J* = 1.3 Hz), 8.16 (d, 1H, *J* = 2.0 Hz), 8.41 (d, 1H, *J* = 8.6 Hz).

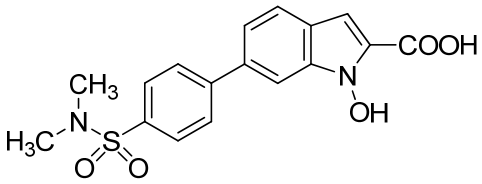
Methyl 6-(4-(*N*,*N*-dimethylsulfamoyl)phenyl)-1-hydroxy-1*H*-indole-2-carboxylate (3.274a)



Triethylamine (0.30 mL, 0.25 g, 2.5 mmol) was added dropwise to a stirred solution of $SnCl_2 \cdot 2H_2O$ (167 mg, 0.738 mmol) and PhSH (0.20 mL, 0.24 g, 2.2 mmol) in acetonitrile (3.8 mL) at room temperature to generate a yellow precipitate over a period of 5 min. Then, **3.273a** (200 mg, 0.492 mmol) was transferred to the suspension and stirred at room temperature for 5 more min. After complete consumption of the starting material, the mixture was diluted with water and repeatedly extracted with EtOAc. The combined organic phase was dried over anhydrous sodium sulphate, diluted with toluene and a catalytic amount of *p*-toluensulfonic acid was added. Then it was evaporated to afford a crude residue that was purified by column chromatography over silica gel using *n*-hexane/EtOAc 4:6 as the eluent, to give the *N*-hydroxyindole **3.274a**

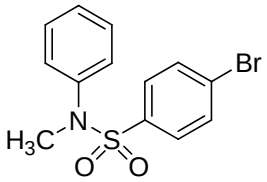
(73.2 mg, 0.196 mmol, 40% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 2.76 (s, 6H), 4.02 (s, 3H), 7.07 (s, 1H), 7.40 (dd, 1H, J = 8.6, 1.5 Hz), 7.74 (d, 1H, J = 8.6 Hz), 7.77-7.79 (m, 1H), 7.85 (s, 4H), 10.38 (bs, 1H).

6-(4-(*N*,*N*-Dimethylsulfamoyl)phenyl)-1-hydroxy-1*H*-indole-2-carboxylic acid (3.260)



Methyl ester **3.274a** (45.0 mg, 0.120 mmol) was dissolved in a 1:1 mixture of THF/methanol (1.2 mL) and treated with 0.36 mL of 2N aqueous solution of LiOH. The reaction was kept under stirring in the dark at RT and it was monitored by TLC. After overnight, the reaction mixture was concentrated, diluted with water and washed with Et_2O. Then, the aqueous phase was treated with 1N aqueous HCl and extracted several times with EtOAc. The combined ethyl acetate organic phase was dried over anhydrous sodium sulphate and evaporated to afford the desired *N*-hydroxyindol-carboxylic acid product **3.260** (41.5 mg, 0.115 .mmol, 96% yield) without any further purification. ^{1}H NMR (DMSO-d_6) δ (ppm): 2.66 (s, 6H), 7.06 (d, 1H, J = 1.2 Hz), 7.51 (dd, 1H, J = 8.6, 1.6 Hz), 7.76-7.85 (m, 4H), 8.02 (AA´XX´, 2H, J_{AX} = 8.8 Hz, $J_{AA'XX'}$ = 1.4 Hz). ^{13}C NMR (DMSO-d_6) δ (ppm): 37.64 (2C), 104.40, 107.87, 119.84, 121.11, 122.95, 127.58 (2C), 127.90, 128.21 (2C), 133.09, 134.88, 136.21, 144.87, 161.04. HPLC, t_R = 8.5 min.

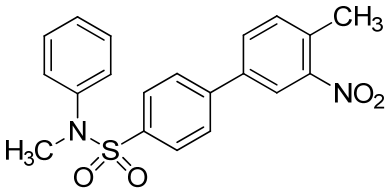
4-Bromo-*N*-methyl-*N*-phenylbenzenesulfonamide (3.271)



A solution of commercially available 4-bromobenzenesulfonylchloride **3.270** (600 mg, 2.35 mmol) in CH_2Cl_2 (4.0 mL) was added dropwise to *N*-methylaniline (0.76 mL,

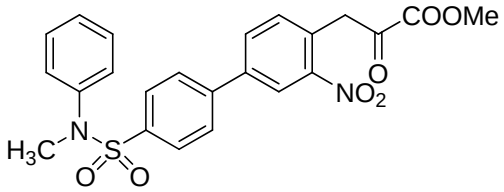
0.76 g, 7.0 mmol) and the reaction was kept under stirring at RT for 4 h. The reaction mixture was acidified with 1N aqueous HCl, extracted several times with CH_2Cl_2 and the combined organic phase was dried over anhydrous sodium sulphate. Evaporation under vacuum of the organic solvent afforded a crude product corresponding to the sulfonamidic derivative **3.271** (696 mg, 2.13 mmol, 91% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 3.18 (s, 3H), 7.07-7.11 (m, 2H), 7.27-7.35 (m, 3H), 7.39 (AA'XX', 2H, J_{AX} = 8.4 Hz, $J_{AA'XX'}$ = 1.8 Hz), 7.60 (AA'XX', 2H, J_{AX} = 8.2 Hz, $J_{AA'XX'}$ = 1.8 Hz).

_N_,4'-Dimethyl-3'-nitro-_N_-phenyl-[1,1'-biphenyl]-4-sulfonamide (3.272)



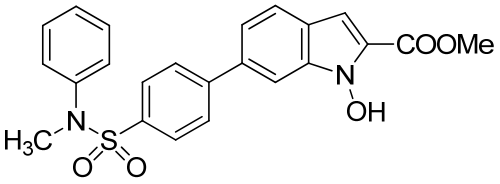
A solution of $Pd(OAc)_2$ (12.1 mg, 0.0540 mmol) and triphenylphosphine (70.8 mg, 0.270 mmol) in absolute ethanol (4.0 mL) and anhydrous toluene (4.0 mL) was stirred at RT under nitrogen for 10 min. After that period, compound **3.271** (586 mg, 1.80 mmol), 4.0 mL of 2M aqueous Na_2CO_3, and 4-methyl-3-nitrobenzeneboronic acid **3.127** (520 mg, 2.88 mmol) were sequentially added. The resulting mixture was heated at 100 °C in a sealed vial under nitrogen overnight. After being cooled to RT, the mixture was diluted with water and extracted with EtOAc. The combined organic phase were dried and concentrated. The crude product was purified by flash chromatography (*n*-hexane/ EtOAc 8:2) to yield **3.272** (442 mg, 1.16 mmol, 64% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 2.66 (s, 3H), 3.22 (s, 3H), 7.11-7.16 (m, 2H), 7.27-7.35 (m, 3H), 7.46 (d, 1H, J = 7.9 Hz), 7.66 (d, 4H, J = 1.6 Hz), 7.74 (dd, 1H, J = 7.9, 1.8 Hz), 8.22 (d, 1H, J = 1.8 Hz).

Methyl 3-(4'-(*N*-methyl-*N*-phenylsulfamoyl)-3-nitro-[1,1'-biphenyl]-4-yl)-2-oxopropanoate (3.273b)



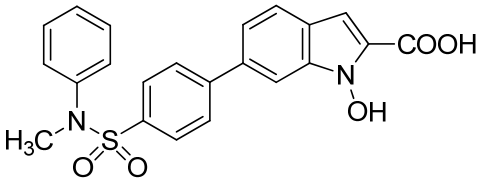
A stirred suspension of sodium hydride (60% dispersion in mineral oil, 141 mg, 3.53 mmol) in 1.9 mL of anhydrous DMF cooled to –15 °C under nitrogen was treated dropwise with a solution containing the nitrotoluene precursor **3.272** (337 mg, 0.883 mmol) and dimethyl oxalate (522 mg, 4.42 mmol) in 1.6 mL of anhydrous DMF. The mixture was left under stirring at the same temperature for 10 minutes and then it was slowly warmed to room temperature. The mixture was then left under stirring at RT for 3 h. Once the disappearance of the precursor was verified by TLC, the reaction mixture was slowly poured in an ice-water mixture; the water phase was acidified with 1N aqueous HCl and extracted several times with EtOAc. The combined organic phase was washed with saturated $NaHCO_3$ solution and brine, then dried over anhydrous sodium sulphate. Evaporation under vacuum of the organic solvent afforded a crude product which was purified by column chromatography over silica gel using *n*-hexane/EtOAc 1:1 as the eluent, to yield the nitroaryl-ketoester derivative **3.273b** (358 mg, 0.764 mmol, 86% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 3.23 (s, 3H), 3.96 (s, 3H), 4.61 (s, 2H), 7.10-7.16 (m, 2H), 7.29-7.34 (m, 3H), 7.45 (d, 1H, J = 7.9 Hz), 7.68 (d, 4H, J = 2.0), 7.86 (dd, 1H, J = 7.9, 2.0 Hz), 8.42 (d, 1H, J = 1.8 Hz); signals imputable to the enol form (~ 29%) δ (ppm): 3.97 (s, 3H), 6.76 (d, 1H, exchangeable, J = 1.5 Hz), 6.97 (s, 1H), 7.84 (dd, 1H, J = 8.4, 2.0 Hz), 8.15 (d, 1H, J = 1.6 Hz).

Methyl 1-hydroxy-6-(4-(*N*-methyl-*N*-phenylsulfamoyl)phenyl)-1*H*-indole-2-carboxylate (3.274b)



Triethylamine (0.23 mL, 0.17 g, 1.7 mmol) was added dropwise to a stirred solution of $SnCl_2 \cdot 2H_2O$ (112 mg, 0.498 mmol) and PhSH (0.15 mL, 0.16 g, 1.5 mmol) in acetonitrile (2.6 mL) at room temperature to generate a yellow precipitate over a period of 5 min. Then, **3.273b** (150 mg, 0.320 mmol) was transferred to the suspension and stirred at room temperature for 5 more min. After complete consumption of the starting material, the mixture was diluted with water and repeatedly extracted with EtOAc. The combined organic phase was dried over anhydrous sodium sulphate, diluted with toluene and a catalytic amount of *p*-toluensulfonic acid was added. Then it was evaporated to afford a crude residue that was purified by column chromatography over silica gel using *n*-hexane/EtOAc 6:4 as the eluent, to give the *N*-hydroxyindole **3.274b** (82.7 mg, 0.189 mmol, 59% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 3.23 (s, 3H), 4.02 (s, 3H), 7.07 (d, 1H, J = 1.1 Hz), 7.15 (AA'XX', 2H, J_{AX} = 8.6 Hz, $J_{AA'/XX'}$ = 1.9 Hz), 7.28-7.33 (m, 3H), 7.39 (dd, 1H, J = 8.6, 1.6 Hz), 7.62 (AA'XX', 2H, J_{AX} = 8.8 Hz, $J_{AA'/XX'}$ = 1.9 Hz), 7.70-7.78 (m, 4H).

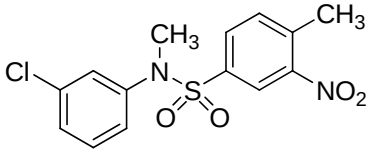
1-Hydroxy-6-(4-(*N*-methyl-*N*-phenylsulfamoyl)phenyl)-1*H*-indole-2-carboxylic acid (3.261)



Methyl ester **3.274b** (72.0 mg, 0.165 mmol) was dissolved in a 1:1 mixture of THF/methanol (1.7 mL) and treated with 0.50 mL of 2N aqueous solution of LiOH. The reaction was kept under stirring in the dark at RT and it was monitored by TLC. After 4

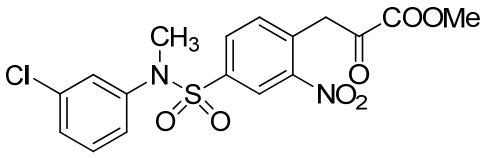
h, the reaction mixture was concentrated, diluted with water and washed with Et_2O. Then, the aqueous phase was treated with 1N aqueous HCl and extracted several times with EtOAc. The combined ethyl acetate organic phase was dried over anhydrous sodium sulphate and evaporated to afford the desired *N*-hydroxyindol-carboxylic acid product **3.261** (61.8 mg, 0.146 mmol, 89% yield) without any further purification. ^{1}H NMR (acetone-d_6) δ (ppm): 3.25 (s, 3H), 7.16-7.22 (m, 3H), 7.30-7.39 (m, 3H), 7.52 (dd, 1H, J = 8.3, 1.6 Hz), 7.64 (AA´XX´, 2H, J_{AX} = 8.4 Hz, $J_{AA'/XX'}$ = 1.9 Hz), 7.81 (d, 1H, J = 8.6 Hz), 7.85-7.87 (m, 1H), 7.95 (AA´XX´, 2H, J_{AX} = 8.4 Hz, $J_{AA'/XX'}$ = 1.8 Hz). ^{13}C NMR (acetone-d_6) δ (ppm): 38.67, 106.02, 109.04, 121.28, 122.72, 124.01, 127.42 (2C), 127.98, 128.35 (2C), 129.26 (2C), 129.68 (2C), 136.67, 137.23, 137.25, 142.89, 146.68, 162.21.

_N_-(3-Chlorophenyl)-_N_,4-dimethyl-3-nitrobenzenesulfonamide (3.277a)



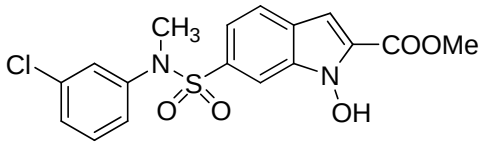
3-Chloro-*N*-methylaniline (0.47 mL; 0.55 g, 3.9 mmol) and anhydrous $NaHCO_3$ (1.94 g) were ground together into fine powder, and 4-methyl-3-nitrobenzenesulfonylchloride **3.245** (915 mg, 3.88 mmol) was added under vigorous stirring at room temperature. The progress of reaction was monitored by TLC until the conversion of amine was completed. After 30 min., the reaction mixture was diluted with water, acidified with 1N aqueous HCl, extracted several times with Et_2O and the combined organic phase was dried over anhydrous sodium sulphate. Evaporation under vacuum of the organic solvent afforded a crude product corresponding to the sulfonamidic derivative **3.277a** (995 mg, 2.92 mmol, 75% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 2.68 (s, 3H), 3.20 (s, 3H), 7.00-7.06 (m, 1H), 7.11-7.13 (m, 1H), 7.26-7.30 (m, 2H), 7.47 (d, 1H, J = 8.1 Hz), 7.60 (dd, 1H, J = 8.1, 1.8 Hz), 8.17 (d, 1H, J = 1.8 Hz).

Methyl 3-(4-(*N*-(3-chlorophenyl)-*N*-methylsulfamoyl)-2-nitrophenyl)-2-oxopropanoate (3.278a)



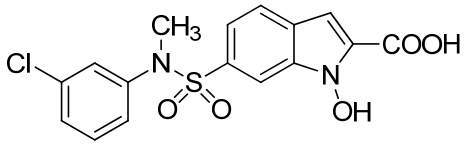
A stirred suspension of sodium hydride (60% dispersion in mineral oil, 282 mg, 7.04 mmol) in 4.2 mL of anhydrous DMF cooled to –15 °C under nitrogen was treated dropwise with a solution containing the nitrotoluene precursor **3.277a** (600 mg, 1.76 mmol) and dimethyl oxalate (1.04 g, 8.80 mmol) in 3.1 mL of anhydrous DMF. The mixture was left under stirring at the same temperature for 10 minutes and then it was slowly warmed to room temperature. The mixture was then left under stirring at RT for 24 h. Once the disappearance of the precursor was verified by TLC, the reaction mixture was slowly poured in an ice-water mixture; the water phase was acidified with 1N aqueous HCl and extracted several times with EtOAc. The combined organic phase was washed with saturated $NaHCO_3$ solution and brine, then dried over anhydrous sodium sulphate. Evaporation under vacuum of the organic solvent afforded a crude product which was purified by column chromatography over silica gel using *n*-hexane/EtOAc 1:1 as the eluent, to yield the nitroaryl-ketoester derivative **3.278a** (321 mg, 0.752 mmol, 43% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 3.23 (s, 3H), 3.96 (s, 3H), 4.63 (s, 2H), 7.14-7.16 (m, 1H), 7.27-7.31 (m, 3H), 7.45 (d, 1H, *J* = 8.1 Hz), 7.72 (dd, 1H, *J* = 7.8, 1.7 Hz), 8.36 (d, 1H, *J* = 1.8 Hz); signals imputable to the enol form (~ 33%) δ (ppm): 3.22 (s, 3H), 3.98 (s, 3H), 6.91 (s, 1H), 7.69 (dd, 1H, *J* = 8.1, 1.9 Hz), 8.05 (d, 1H, *J* = 1.8 Hz), 8.40 (d, 1H, *J* = 8.5 Hz).

Methyl 6-(*N*-(3-chlorophenyl)-*N*-methylsulfamoyl)-1-hydroxy-1*H*-indole-2-carboxylate (3.279a)



Triethylamine (0.28 mL, 0.20 g, 2.0 mmol) was added dropwise to a stirred solution of $SnCl_2 \cdot 2H_2O$ (135 mg, 0.597 mmol) and PhSH (0.18 mL, 0.20 g, 1.8 mmol) in acetonitrile (3.1 mL) at room temperature to generate a yellow precipitate over a period of 5 min. Then, **3.278a** (170 mg, 0.398 mmol) was transferred to the suspension and stirred at room temperature for 5 more min. After complete consumption of the starting material, the mixture was diluted with water and repeatedly extracted with EtOAc. The combined organic phase was dried over anhydrous sodium sulphate, diluted with toluene and a catalytic amount of *p*-toluensulfonic acid was added. Then it was evaporated to afford a crude residue that was purified by column chromatography over silica gel using *n*-hexane/EtOAc 1:1 as the eluent, to give the *N*-hydroxyindole **3.279a** (73.1 mg, 0.185 mmol, 47% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 3.19 (s, 3H), 4.03 (s, 3H), 7.01-7.13 (m, 2H), 7.08 (d, 1H, *J* = 0.9 Hz), 7.17 (dd, 1H, *J* = 8.6, 1.6 Hz), 7.21-7.27 (m, 2H), 7.67 (dd, 1H, *J* = 8.5, 0.6 Hz), 7.92 (t, 1H, *J* = 0.7 Hz), 10.37 (bs, 1H).

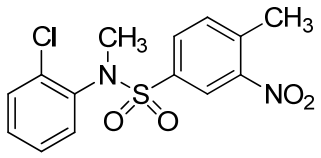
6-(*N*-(3-Chlorophenyl)-*N*-methylsulfamoyl)-1-hydroxy-1*H*-indole-2-carboxylic acid (3.275)



Methyl ester **3.279a** (31.7 mg, 0.0803 mmol) was dissolved in a 1:1 mixture of THF/methanol (0.80 mL) and treated with 2.4 mL of 2N aqueous solution of LiOH. The reaction was kept under stirring in the dark at RT and it was monitored by TLC. After 18 h, the reaction mixture was concentrated, diluted with water and washed with Et_2O. Then, the aqueous phase was treated with 1N aqueous HCl and extracted several times with EtOAc. The combined ethyl acetate organic phase was dried over anhydrous sodium sulphate and evaporated to afford the desired *N*-hydroxyindol-carboxylic acid product **3.275** (22.0 mg, 0.578 mmol, 72% yield) without any further purification. ^{1}H NMR (acetone-d_6) δ (ppm): 3.26 (s, 3H), 7.10-7.16 (m, 1H), 7.22 (dd, 1H, *J* = 8.4, 1.6 Hz), 7.23 (d, 1H, *J* = 0.9 Hz), 7.25-7.28 (m, 1H), 7.32-7.36 (m, 2H), 7.79-7.81 (m, 1H), 7.84 (dd, 1H, *J* = 8.9, 0.6 Hz). ^{13}C NMR (acetone-d_6) δ (ppm): 38.34, 105.20, 111.25,

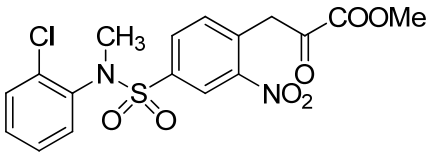
119.61, 123.76, 125.10, 125.34, 127.27, 127.74, 130.22, 130.88, 133.24, 134.37, 134.74, 144.17, 161.85.

_N_-(2-Chlorophenyl)-_N_,4-dimethyl-3-nitrobenzenesulfonamide (3.277b)



To a solution of 4-chloro-*N*-methylaniline (0.43 mL, 0.50 g, 3.5 mmol) in dry CH_2Cl_2 (6.0 mL), pyridine (0.43 mL, 0.42 g, 5.3 mmol) and catalytic DMAP (15.0 mg) were added, then the resulting mixture was cooled to 0 °C. Subsequently, commercially available 4-methyl-3-nitrobenzenesulfonylchloride **3.245** (1.00 g, 4.24 mmol) in CH_2Cl_2 (19 mL) was added dropwise and the reaction was kept under stirring at RT for 72 h. The reaction mixture was acidified with 1N aqueous HCl, extracted several times with CH_2Cl_2 and the combined organic phase was dried over anhydrous sodium sulphate. Evaporation under vacuum of the organic solvent afforded a crude product which was purified by column chromatography over silica gel using *n*-hexane/EtOAc 2:8 as the eluent, to yield the sulfonamidic derivative **3.277b** (528 mg, 1.55 mmol, 37% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 2.69 (s, 3H), 3.27 (s, 3H), 7.27-7.43 (m, 4H), 7.49 (d, 1H, J = 8.4 Hz), 7.85 (dd, 1H, J = 8.1, 1.8 Hz), 8.35 (d, 1H, J = 2.0 Hz).

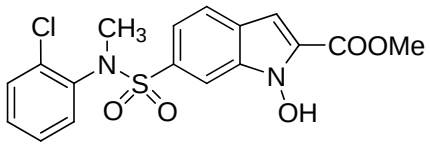
Methyl 3-(4-(_N_-(2-chlorophenyl)-_N_-methylsulfamoyl)-2-nitrophenyl)-2-oxopropanoate (3.278b)



A stirred suspension of sodium hydride (60% dispersion in mineral oil, 245 mg, 6.12 mmol) in 3.3 mL of anhydrous DMF cooled to –15 °C under nitrogen was treated dropwise with a solution containing the nitrotoluene precursor **3.277b** (521 mg, 1.53 mmol) and dimethyl oxalate (903 mg, 7.65 mmol) in 2.7 mL of anhydrous DMF. The mixture was left under stirring at the same temperature for 10 minutes and then it was

slowly warmed to room temperature. The mixture was then left under stirring at RT for 24 h. Once the disappearance of the precursor was verified by TLC, the reaction mixture was slowly poured in an ice-water mixture; the water phase was acidified with 1N aqueous HCl and extracted several times with EtOAc. The combined organic phase was washed with saturated $NaHCO_3$ and solution brine, then dried over anhydrous sodium sulphate. Evaporation under vacuum of the organic solvent afforded a crude product which was purified by column chromatography over silica gel using *n*-hexane/EtOAc 65:35 as the eluent, to yield the nitroaryl-ketoester derivative **3.278b** (281 mg, 0.658 mmol, 43% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 3.29 (s, 3H), 3.96 (s, 3H), 4.64 (s, 2H), 7.27-7.48 (m, 4H), 7.97 (dd, 1H, *J* = 8.1, 1.8 Hz), 8.20 (d, 1H, *J* = 8.8 Hz), 8.53 (d, 1H, *J* = 1.6 Hz); signals imputable to the enol form (~ 30%) δ (ppm): 3.32 (s, 3H), 3.98 (s, 3H), 6.94 (s, 1H), 8.08 (dd, 1H, *J* = 8.1, 1.8 Hz), 8.44 (d, 1H, *J* = 8.4 Hz), 8.60 (d, 1H, *J* = 1.5 Hz).

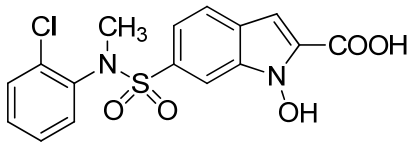
Methyl 6-(*N*-(2-chlorophenyl)-*N*-methylsulfamoyl)-1-hydroxy-1*H*-indole-2-carboxylate (3.279b)



Triethylamine (0.14 mL, 0.10 g, 1.0 mmol) was added dropwise to a stirred solution of $SnCl_2 \cdot 2H_2O$ (45.7 mg, 0.203 mmol) and PhSH (0.09 mL, 0.1 g, 0.9 mmol) in acetonitrile (1.6 mL) at room temperature to generate a yellow precipitate over a period of 5 min. Then, **3.278b** (86.5 mg, 0.203 mmol) was transferred to the suspension and stirred at room temperature for 5 more min. After complete consumption of the starting material, the mixture was diluted with water and repeatedly extracted with EtOAc. The combined organic phase was dried over anhydrous sodium sulphate, diluted with toluene and a catalytic amount of *p*-toluensulfonic acid was added. Then it was evaporated to afford a crude residue that was purified by column chromatography over silica gel using *n*-hexane/EtOAc 6:4 as the eluent, to give the *N*-hydroxyindole **3.279b** (12.1 mg, 0.0306 mmol, 15% yield). ^{1}H NMR ($CDCl_3$) δ (ppm): 3.24 (s, 3H), 4.03 (s,

3H), 7.09 (d, 1H, *J* = 0.9 Hz), 7.19-7.32 (m, 3H), 7.37-7.44 (m, 1H), 7.47 (dd, 1H, *J* = 8.6, 1.6 Hz), 7.73 (dd, 1H, *J* = 8.6, 0.7 Hz), 8.07 (dt, 1H, *J* = 1.8, 0.8 Hz), 10.37 (bs, 1H).

6-(*N*-(2-Chlorophenyl)-*N*-methylsulfamoyl)-1-hydroxy-1*H*-indole-2-carboxylic acid (3.276)



Methyl ester **3.279b** (12.0 mg, 0.0304 mmol) was dissolved in a 1:1 mixture of THF/methanol (0.3 mL) and treated with 0.09 mL of 2N aqueous solution of LiOH. The reaction was kept under stirring in the dark at RT and it was monitored by TLC. After 24 h, the reaction mixture was concentrated, diluted with water and washed with Et_2O. Then, the aqueous phase was treated with 1N aqueous HCl and extracted several times with EtOAc. The combined ethyl acetate organic phase was dried over anhydrous sodium sulphate and evaporated to afford the desired *N*-hydroxyindol-carboxylic acid product **3.276** (10.2 mg, 0.0268 mmol, 88% yield) without any further purification. ^{1}H NMR (acetone-d_6) δ (ppm): 3.24 (s, 3H), 7.11-7.16 (m, 1H), 7.26 (d, 1H, *J* = 0.9 Hz), 7.31 (td, 1H, *J* = 7.4 1.8 Hz), 7.39 (td, 1H, *J* = 7.3, 1.8 Hz), 7.50 (dd, 1H, *J* = 8.4, 1.6 Hz), 7.51-7.55 (m, 1H), 7.91 (dd, 1H, *J* = 8.6, 0.7 Hz), 7.95 (dt, 1H, *J* = 1.6, 0.8 Hz), 10.80 (bs, 1H). ^{13}C NMR (acetone-d_6) δ (ppm): 38.78, 105.99, 111.16, 119.95, 124.05, 125.01, 128.56, 130.55, 131.33 (2C), 135.10, 135.43, 136.16, 138.21, 139.74, 161.19. MS *m/z* 380 (M^+, 20%), 268 (M^+ –C_6H_5Cl), 240 (M^+ –*o*ClPhNMe). HPLC, t_R = 9.4 min.

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