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Synthesis and functionalization of a lactam-pyrazole molecular scaffold as a promising anticancer compound

Tesi di laurea sperimentale

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ABSTRACT

The importance of pyrazole and lactam-based molecules in medical and pharmaceutical fields is underlined by the multitude of active ingredients on trade, such as Sildenafil or Apixaban, by Pfizer. In this work, a synthesis of an organic molecule with promising anticancer activity has been developed. This molecular scaffold is characterized by a δ-lactam-fused pyrazolic core, with a well-known biological activity and amenable of further functionalization. The synthetic strategy adopted for the obtainment of the core was based on a 1,3-dipolar cycloaddition of a nitrilimine with an α,β-unsaturated δ-lactam. Secondly, in order to give the final compound an elevated pharmacological activity, a functionalization with a double "side chain", namely molecular fragment able to improve the interaction with particular biological receptors, was achieved. The target compound was thus obtained, with a highly convergent synthesis, and will be tested for antiproliferative activities towards different cellular lines.

RIASSUNTO

L'importanza di molecole contenenti eterocicli pirazolici e δ-lattami in ambito farmaceutico è evidenziata dalla grande quantità di principi attivi presenti sul mercato, quali il Sildenafil o l'Apixaban, commercializzate da Pfizer. Nel mio lavoro di tesi magistrale è stata sviluppata la sintesi di una molecola organica a potenziale attività farmacologica, caratterizzata da un pirazolo biciclico fuso con un δ- lattame, predisposto per successive funzionalizzazioni. La strategia sintetica adottata per l'ottenimento dello scheletro pirazolico si è basata su una cicloaddizione 1,3-dipolare di una nitril-immina con un δ-lattame α,β-insaturo. In secondo luogo si è proceduto, al fine di impartire al composto finale elevata attività farmacologica, ad un'ulteriore funzionalizzazione dello scheletro molecolare in precedenza ottenuto con una doppia "catena laterale", ovvero un frammento molecolare in grado di interagire efficacemente con particolari recettori biologici. Si è infine ottenuto il prodotto "target", che sarà sperimentato per attività antiproliferative nei confronti di svariate linee cellulari cancerose, in collaborazione con gruppi di ricerca nel campo medico-biologico.

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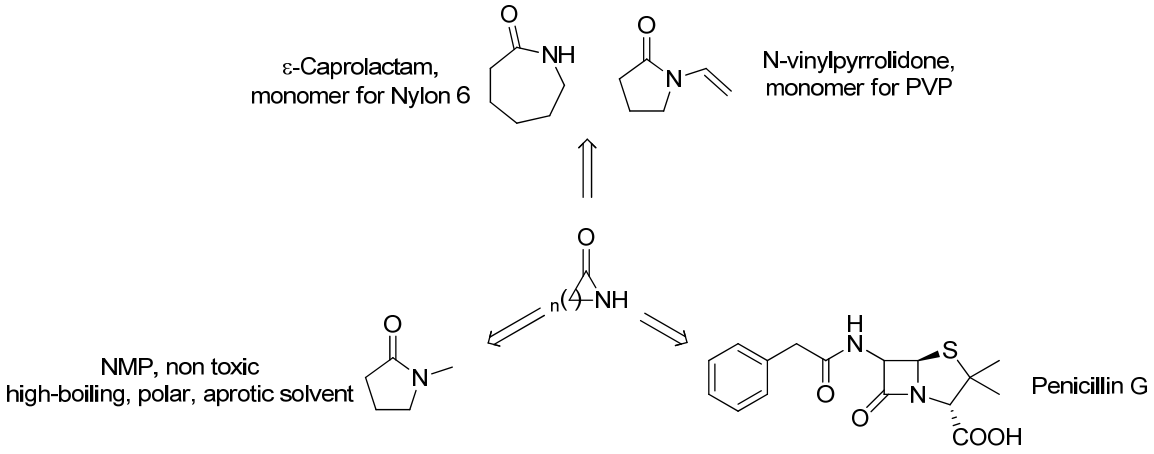
1. Introduction

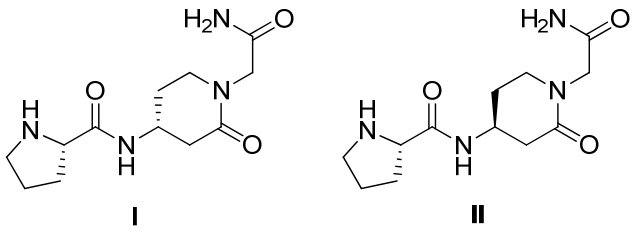
1.1. Lactams in medicinal chemistry

Lactams are cyclic amidic compounds, whose importance arises from the broad spectrum of applicability shown in modern industry. For example, ε-caprolactam and N-vinylpyrrolidone find application in polymer industries as monomers for the synthesis of Nylon 6 and PVP (polyvinylpyrrolidone). N-methylpyrrolidone is used in petrochemical and plastics industries as a solvent, exploiting its non-volatility and capability to dissolve a wide range of materials (a less toxic alternative to typical high-boiling polar aprotic solvents such as HMPA, hexamethylphosphoramide or DMA, dimethylacetamide). Finally, β-lactams are well-known for their antibiotic properties, since the discovery of penicillin G by Fleming in 1928, and are present in a huge number of important drugs.



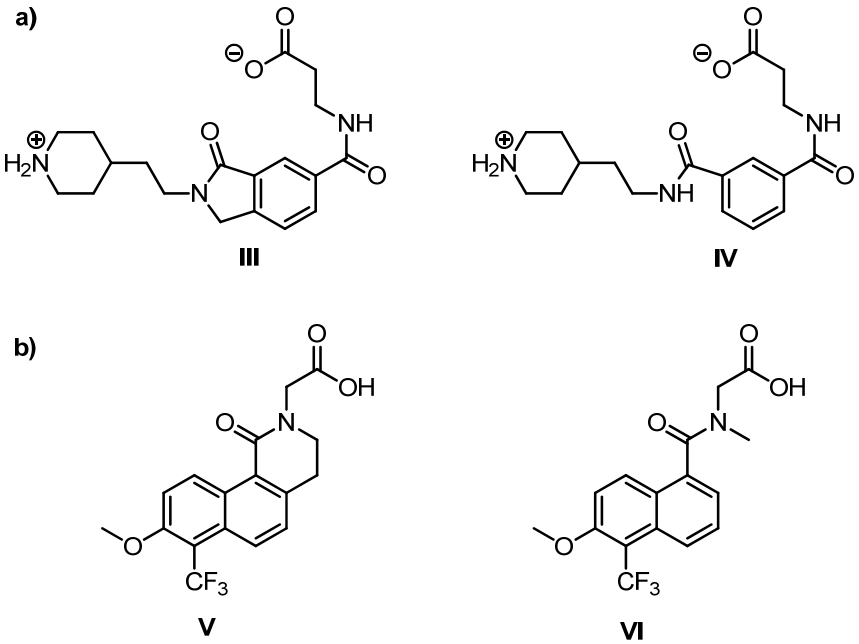
Scheme 1. Some applications of lactams

Nowadays, complex lactamic systems can be found predominantly in pharmaceutic and biologically active compounds, and take part in new libraries of molecules designed for drug discovery. For example, diastereoisomers **I** and **II** were found to be modulating agents for dopamine receptors (increasing antagonist compounds affinity) and are of potential interest for the treatment of neurologic and psychiatric disorders.[1]



Scheme 2. δ-Lactams as modulating agents for dopamine receptors

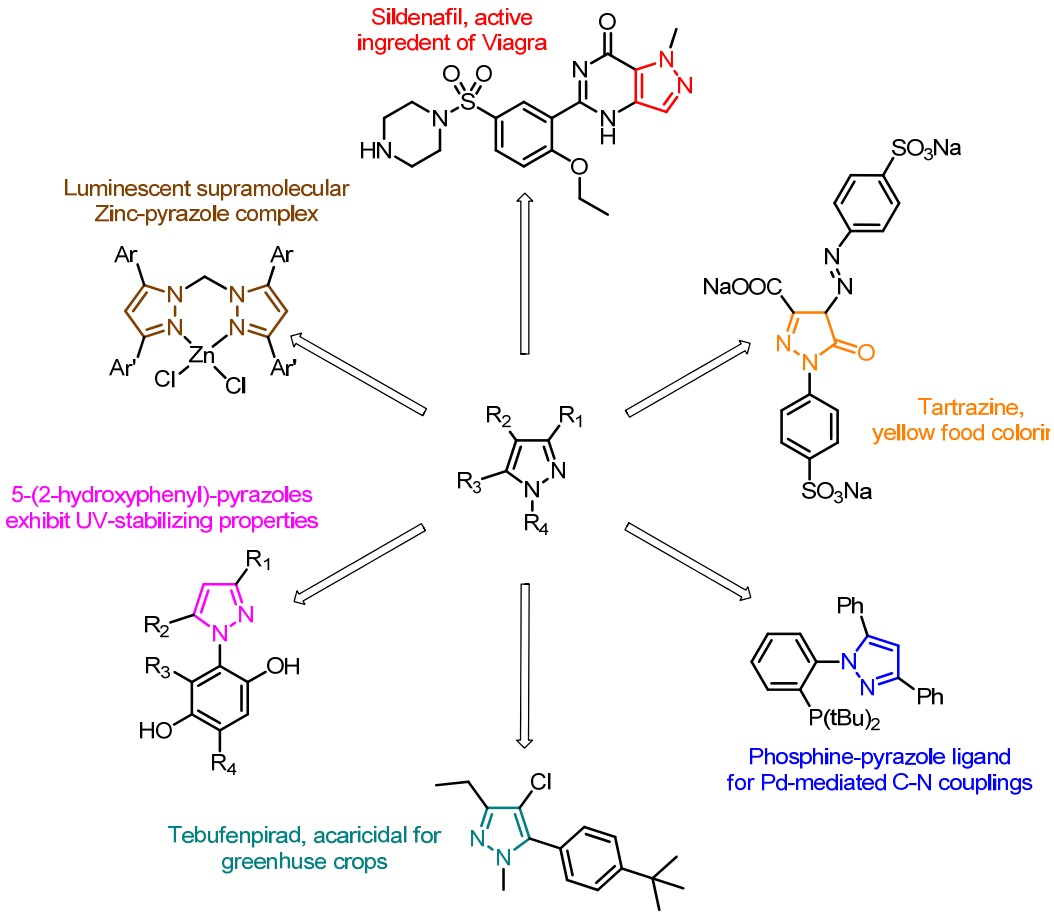
One typical feature of lactams in medicinal chemistry is their better pharmacologic activity shown, compared to very similar non-cyclic amidic compounds. This fact underlines that the lactam itself plays a fundamental role in the interactions of the active core of the molecule with the biologic environment. In view of this, two literature examples may be reported. The first (**Scheme 3a**) shows two fibrinogen-receptors antagonists (**III** and **IV**), studied and used to prevent platelets aggregation in antithrombic therapy. Through computational studies and *in vitro* tests, it has been noted that, while compound **III**, bearing an aromatic-fused lactam, shows an IC_{50} of 25 mM, the corresponding non-cyclic amide **IV** is much worse performing, with a value of 530 mM.[2] Similarly, compound **V**, a naphthalene-fused δ-valerolactam, resulted much more effective against diabetes complicacies, than the corresponding naphtamide **VI** (**Scheme 3b**).[3]



Scheme 3. Lactams are often more active than non-cyclic amides

1.2. Pyrazoles in medicinal chemistry

Pyrazole, a five membered aromatic heterocycle containing two adjacent nitrogen atoms, is a motif found in a number of small molecules that possess a wide range of pharmaceutical and agricultural[4] activities.[5] Moreover, some pyrazoles are used in the food industry, in supramolecular and polymer chemistry, as colorings or UV stabilizers,[6] in the chemistry of complexes with phosphorescent properties[7] and as ligands for transition metal-catalyzed reactions.[8]

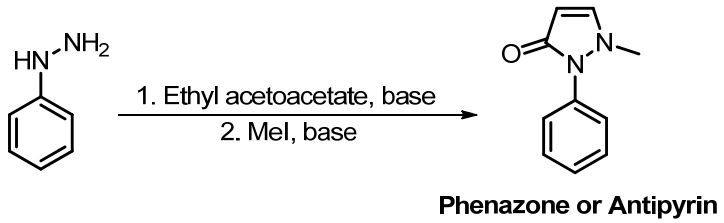


Scheme 4. Some applications of pyrazole derivatives

1.2.1. Simple pyrazoles

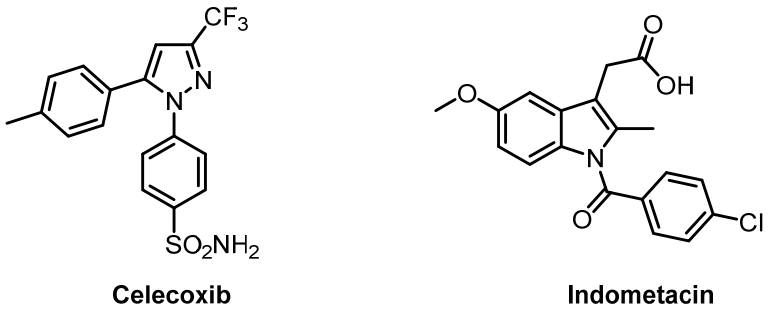
More in detail, a large number of top selling drugs containing pyrazoles can be found, exhibiting antispasmodic, anti-inflammatory, antibacterial, antihyperglicemic, antidepressive and, more recently, antitumor activities.[9] Even if it is true that quite recently the interest in pyrazole pharmaceutical activities has significantly increased, it is

also true that their biological properties are known since a very long time. The discovery of the antipyretic activity of pyrazole-family compounds is, in fact, attributed to Ludwig Knorr, who synthesized Phenazone (or Antipyrin), a simple pyrazolone, in 1883.



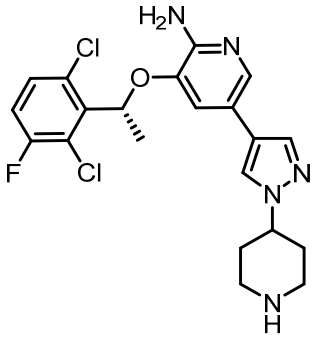
Scheme 5. Phenazone and its synthesis by Ludwig Knorr

In order to underline how many simple substituted pyrazoles are present on the market of pharmaceuticals, two much more recent examples may be reported. First of all, Celecoxib, a simple trisubstituted pyrazole, sold by Pfizer under the trade name of Celebrex, is used as a COX-2 (cyclooxygenase-2) selective nonsteroidal anti-inflammatory drug (FANS), more or less equal to ibuprofen for postoperative pain and similar to paracetamol for pain relief. Moreover, experimental results showed its enhanced activity, compared to Indometacin (characterized by an indolic scaffold), against chronic pathologies, such as arthritis or ankylosing spondylitis.[10]



Scheme 6. Celecoxib and Indometacin, two non-steroidal anti-inflammatory drugs

Secondly, Crizotinib, sold by Pfizer under the trade name of Xalkori, is a disubstituted simple pyrazole with antitumor properties. It acts as inhibitor of a particular enzyme, codified by ROS-1 gene (proto-oncogene-1) and has obtained excellent results in clinical trials against anaplastic large cell lymphoma, neuroblastoma and other advanced state solid tumors.[11]

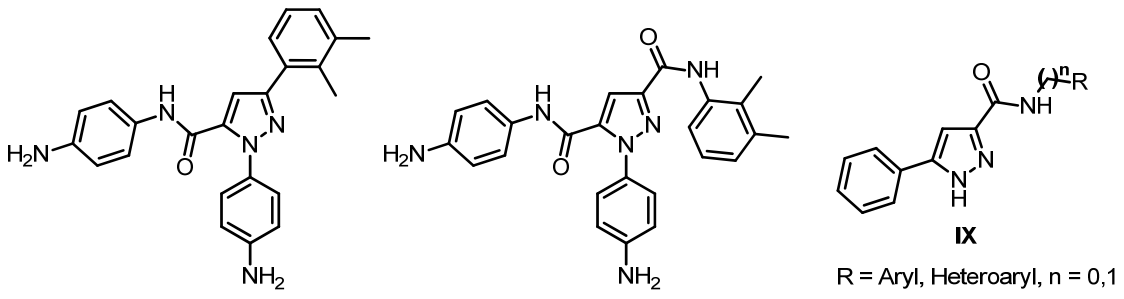


Crizotinib

Scheme 7. Crizotinib, a simple pyrazole exhibiting anticancer activity

In the last decades, discovery and development of new drugs for the treatment of the above-mentioned type of tumors (and many others) has been a challenging feature in medicinal chemistry and drug delivery research. For this purpose, pyrazolic systems are very often found as a recurrent molecular scaffold, synthesized in order to construct libraries of similar, same-core containing compounds. One of the most appealing characteristics of this type of anticancer molecules is the relatively low cost, compared to monoclonal antibodies (other large family of antitumor drugs), obtained through recombining DNA techniques.[12] For example, recent molecular docking studies, followed by *in vitro* tests, have shown compounds **VII** and **VIII** as potential inhibitors of multikinase enzymes, responsible for the growth and the proliferation of HCC (Hepatocellular carcinoma) cells, a liver malignant tumor.[13]

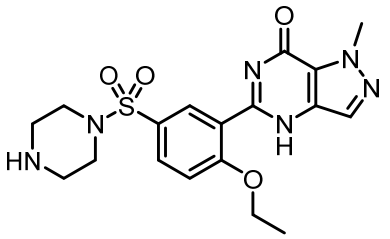
As another example, a new class of 3-carboxamido pyrazoles (**IX**), exhibiting various moieties attached via the amide linker, has been recently synthesized and tested both *in vitro* and *in vivo* as potent inhibitors of c-Jun N-terminal kinases, enzymes playing a key role in arthritis, metabolic diseases and cancer. These compounds have shown micromolar values of IC_{50} (half maximal inhibitory concentration) and significant anti-inflammatory activity.in carrageenan induced rat paw edema.[14]



Scheme 8. Carboxamido-pyrazoles showing anticancer properties

1.2.2. **Ring-fused pyrazoles**

Ring-fused pyrazoles are, if possible, even more important than simple rings in the above mentioned applicative categories. An outstanding example is represented by Viagra (Sildenafil citrate), a very famous drug commercialized by Pfizer that reached a very high commercial success. Its central core is constituted by a pyrimidinone-fused pyrazole.



Sildenafil

Scheme 9. Sildenafil, active ingredient of Viagra

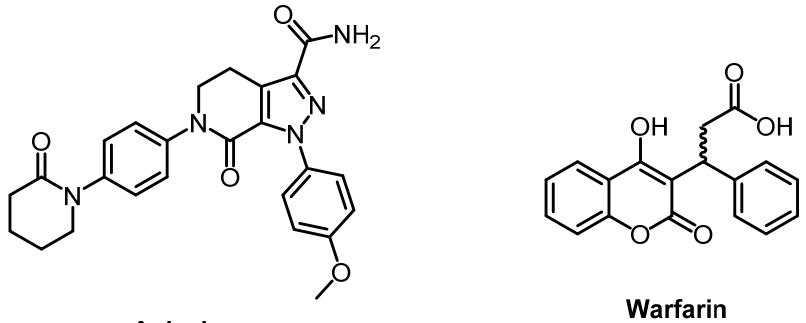
A similar molecular motif is found in Allopurinol, a generic drug included in the World Health Organization's List of Essential Medicine. It is used as an inhibitor of the xanthine-oxidase enzyme for the treatment of hyperuricemia, i.e. an excess of uric acid in blood. This molecule is very similar to xanthine (the substrate oxidized by the enzyme into uric acid) but differs for the five membered ring heterocycle. Thanks to the pyrazolic ring, instead of the imidazole of xanthine, Allopurinol is able to interact with the catalytic center of the enzyme, but is inert towards its oxidative cycle, behaving indeed as a catalyst poison.[15]

Allopurinol **Xanthine**

Scheme 10. Allopurinol and Xanthine

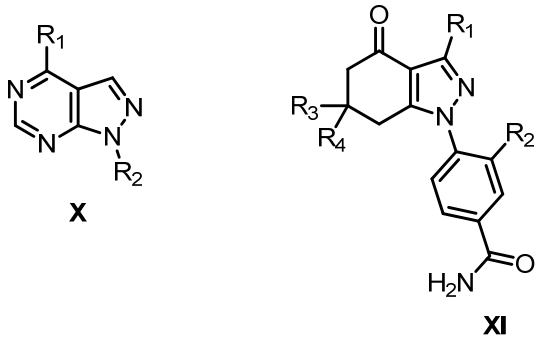
With the target molecule of this work in mind, citation of another ring-fused pyrazole is mandatory. Apixaban, developed by Bristol-Myers Squibb in collaboration with Pfizer, actually in phase III of clinical trials for the excellent anticoagulant properties, contains both the above-mentioned δ-lactamic and the present pyrazolic functionalities fused together. This drug is often prescribed in case of venous thromboembolism, acting as an inhibitor of the Xa factor, a serine protease that plays an important role in the coagulation cascade.[16] Despite some side effects, compared to Warfarin (known under the trade name

of Coumadin), currently the most common drug for the treatment of this disease, Apixaban has been found to be "not inferior" in ictus prevention and superior in secondary hemorrhagings prevention.[17]



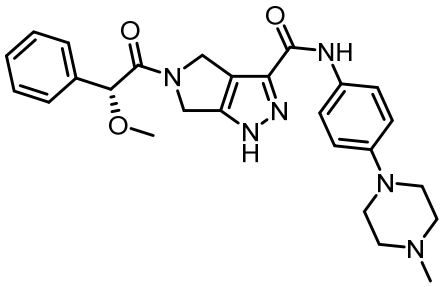
Scheme 11. Apixaban and Warfarin, two anticoagulant drugs

Moreover, condensed pyrazole derivatives have often shown antiproliferative activities towards a wide number of cellular lines and are often employed in anticancer drug discovery screening. For example, pyrazolo-[3,4-d]-pyrimidines (**X**) have been tested as potential Abelson Kinase inhibitors for the treatment of chronic myelogenous leukemia.[18] Furthermore, these molecules have shown promising antiproliferative properties towards breast and skin cancer cells.[19] Similarly, some indazol-4-ones (**XI**) are at present in phase of clinic trials for the promising cytotoxicity shown, even at nanomolar concentrations.



Scheme 12. Two ring-fused pyrazole derivatives exhibiting anticancer activity

Finally, as another example, pyrrolo-[3,4-c]-pyrazole **XII** (Danusertib), an Aurora kinase inhibitor, has advanced in phase II clinical trials for the treatment of Bcr-Abl positive leukemias , due to the good pharmacokinetic properties as inhibitor of Aurora kinases and Bcr-Abl tyrosine kinase (mitosis regulators aberrantly overexpressed in cancer cells), along with general safety profiles shown in phase I clinical studies. Compared with other hinge binder templates, its double heterocycle core offers efficient hydrogen bonds interactions, improving greatly its physiochemical properties.[20]



Danusertib, XII

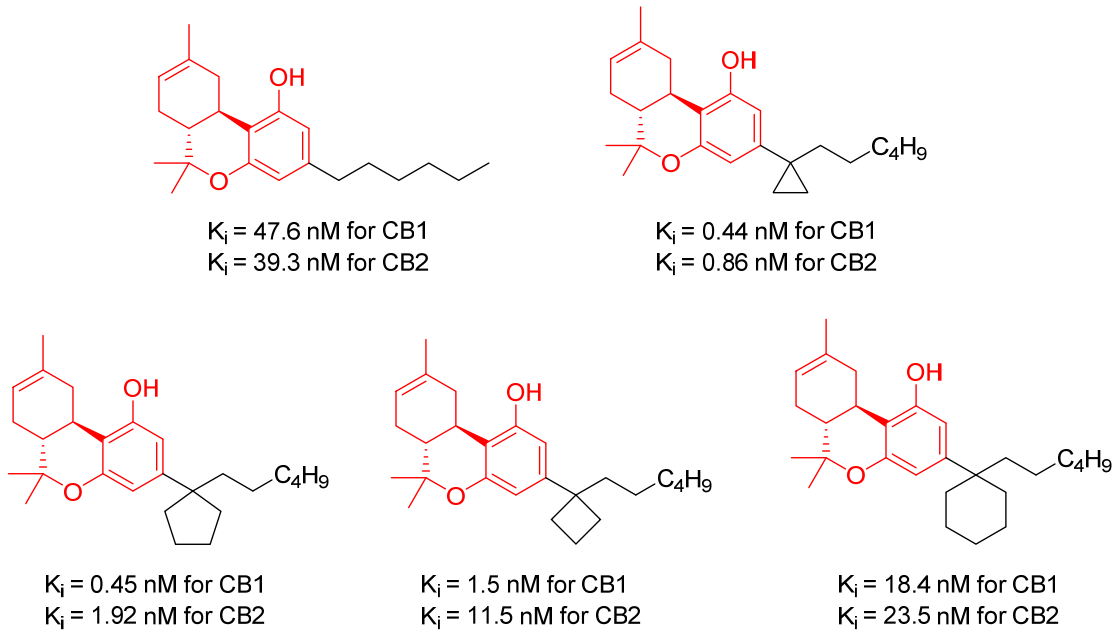
Scheme 13. Danusertib, a ring-fused pyrazole in clinical trials for the treatment of Bcr-Abl positive leukemias.

1.3. "Side chains" in medicinal chemistry

Most often, molecules designed for medicinal chemistry (with either therapeutic or diagnostic purposes) are not constituted by a simple biologically active core, but are provided with one or more "side chains". Typically these "side chains" are made of a "anchor agent", connected through a "spacer" to the central molecular scaffold. The role of the "anchor agent" is to supply the molecule with a number of specific or generic interactions into the biological environment. On the other hand, the spacer serves as chemical linkage, but is also often designed in order to perform extra interactions and give the correct distance between the core and the "anchor", once they are placed in the desired biological site.

More generally, one of the main aims of a "side chain" is to render the active core, which it is attached to, more specific for the desired biological target. Namely, thanks to the interactions of the "anchor agent" with the biological environment near the selected site and the appropriate length of the spacer, the core is brought near to its binding position. The result is not only an enhanced affinity for the substrate but also an improved selectivity.

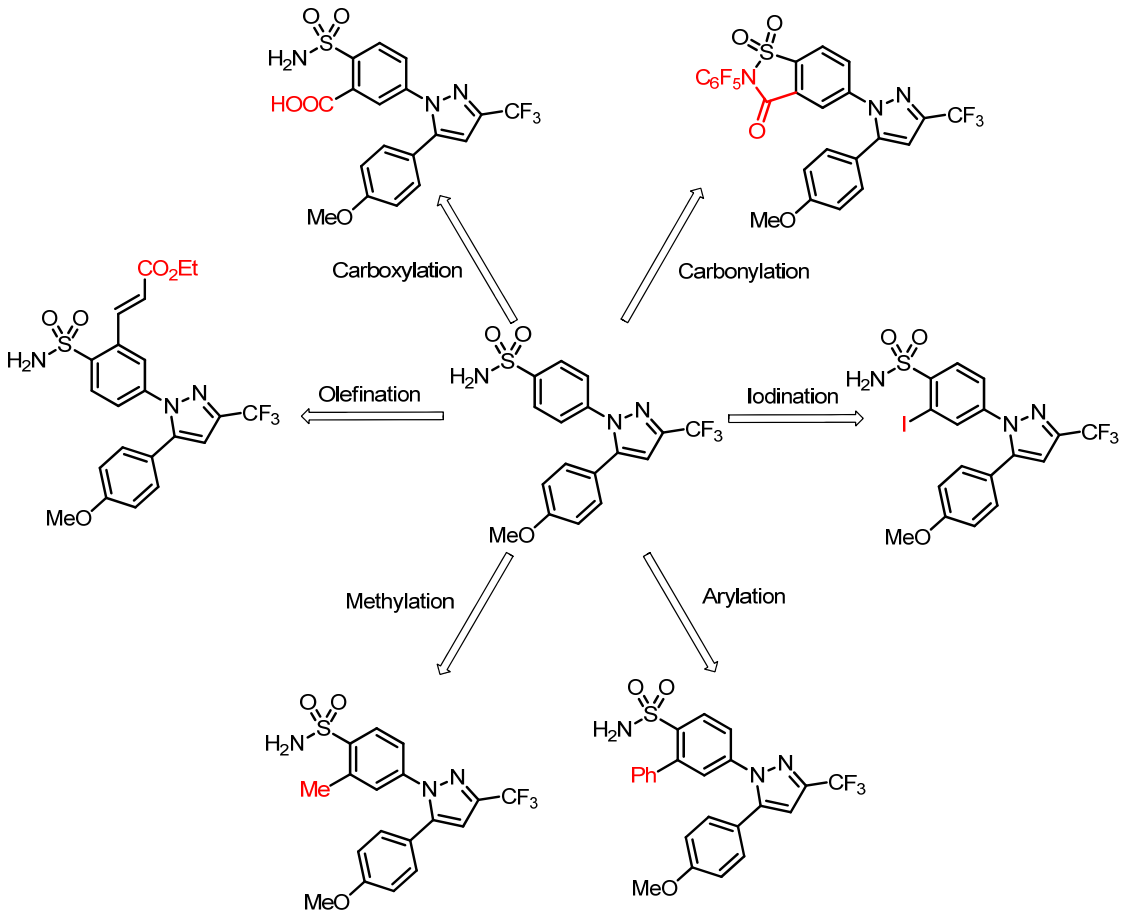
For example, it has been demonstrated that cannabinoid molecules with different side chains (reported in **Scheme 14**) have different binding ability for their biological targets. Namely, cyclopropyl and cyclopentyl groups improve their affinity for CB1 and CB2 cannabinoid receptors, compared to simple aliphatic chains (being therefore efficient pharmacophores), while cyclobutyl group renders them selective for CB1 and cyclohexyl groups quench the binding affinity for both of the receptors.[21]



Scheme 14. Molecules with the same core (red) and different side chains exhibit different binding affinities for their specific receptor. (Affinity is measured by mean of Ki, inhibition constant: namely, the concentration necessary to replace 50 % of a radiolabelled competitor ligand, already attached to the desired receptor).

The other main goal prefixed in side chains design may be schematically called "differentiation" of the core. Modern drug discovery is indeed contingent on identifying lead compounds and rapidly synthesizing analogues.[22] Therefore, in medicinal chemistry and drug design, once a promising central molecular scaffold has been found, it is common procedure to perform an ample screening of different "side chains", in order to find the more active and selective compound. Moreover, upon variation of the side chain, it is possible to address the same active principle towards different biological receptors, expanding the applicability greatly. Finally, a different side chain not influencing the activity and the selectivity of the core may be desirable for pharmaceutical industries when the construction of analogues is concerned and violation of intellectual property may be involved.

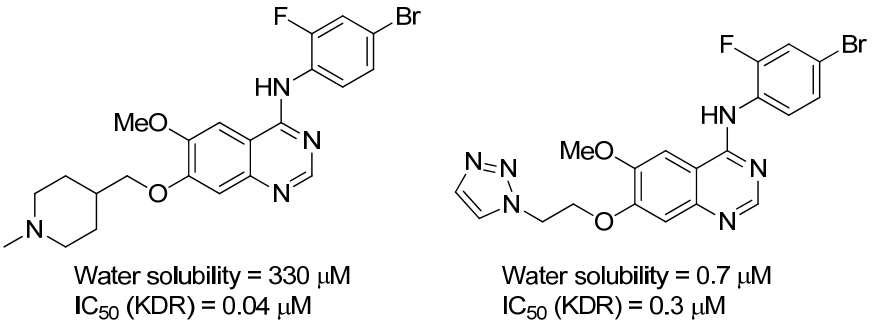
The elegant divergent synthetic approach reported in **Scheme 15** has been thought in order to rapidly functionalize sulfonamide containing drugs, such as Celecoxib. The fascinating feature of the process is not only represented by the broad spectrum of feasible reactions and the atom-economy typical of a direct C-H bond activation, but arises also from the fact that the same pharmacophore (sulfonamide) acts as a regiochemistry director in all the functionalizations performed.[22]



Scheme 15. Divergent functionalization of Celecoxib

In the previously reported examples some "anchor agents" have already been shown; however, additional mention should be given to terminal piperidine containing "side chains", which can be found in a large class of pharmaceutical compounds (see Crizotinib, Sildenafil and Danusertib). With pH tunable hydrolytic properties (acidic when cationic, basic when neutral) these molecular fragments may influence greatly interactions with biological residues such as amino acids and cell membranes, conferring also water solubility at the organic molecule.

For example, introduction of nitrogen containing, basic residues, covering ranges of pKa's from 7 to 9.5, in the side chain (namely piperidines or N-methyl piperidines) originated in a great improvement for 4-anilinoquinazolines in the inhibition of KDR (kinase insert domain-containing receptor), active part of a key factor promoting cancer neovascularization and growth.[23]

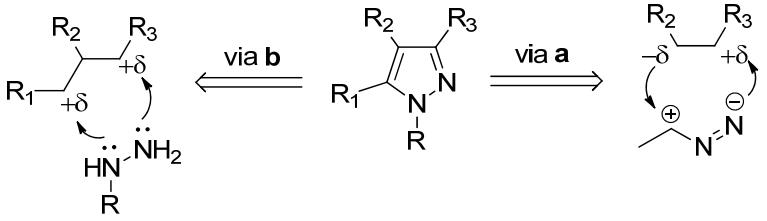


Scheme 16. Effect of a N-methyl-piperidine on solubility and cytotoxicity

Finally some structures of typical "spacers" should be underlined. Aliphatic chains, along with short polymer fragments are very common: the first for lipophilic interactions and great variability in the length and the latter for the ready availability and facile synthesis. However, other more elaborated "spacers" can be found; in particular, chains constituted by two or three p-diaminobenzene fragments are quite appealing for either hydrophilic (NH) and lipophilic (π-stacking) interactions, or the facility of the synthesis and the functionalization.

1.4. Pyrazole synthesis

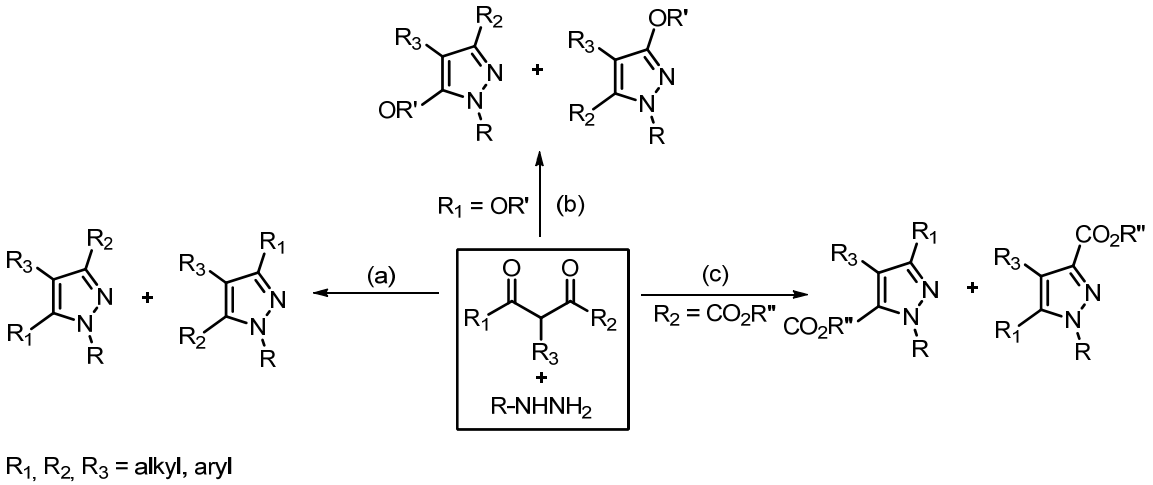
Conventional approaches for the preparation of substituted pyrazoles involve either the construction of two C-N bonds by cyclocondensation of hydrazines with 1,3-dielectrophilic compounds (**Scheme 17**, via **b**) or the generation of one C-N and one C-C bond by 1,3-dipolar [3+2] cycloaddition (**Scheme 17**, via **a**). Each method has its own scope and efficiency limitations, however, general and efficient methodologies have been developed, following these general strategies, with the aim of increasing the yield and the regioselectivity in the preparation of substituted pyrazoles.[5]



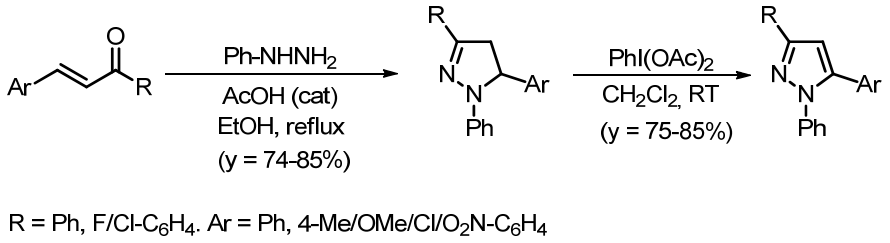
Scheme 17. General approaches for the synthesis of pyrazole

1.4.1. Cyclocondensation of 1,3-dielectrophilic compounds with hydrazines

The most common synthetic method for the preparation of functionalized pyrazoles involves the cyclocondensation (often called Knorr or Knorr-type reaction) of the appropriate hydrazine (mainly arylhydrazines), which acts as a double nucleophile, with a three-carbon unit featuring two electrophilic carbons in a 1,3 relationship, such as 1,3-dicarbonyl or α,β-unsaturated carbonyl compounds. 1,3-Diketones, β-ketoesters and 2,4-diketoesters can be efficiently condensed with hydrazines, affording simple pyrazoles bearing various alkyl or aryl substituents. However, starting from unsymmetrical 1,3-dicarbonyl compounds, mixtures of two regioisomers are often obtained in reactions with substituted hydrazines. On the other hand, condensation of hydrazines with α-enones regioselectively leads to pyrazolines, which must then be oxidized to the corresponding pyrazoles.[5]

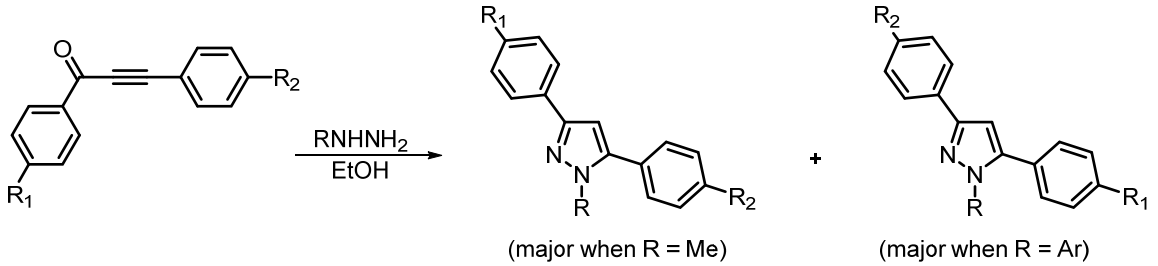


Scheme 18. General cyclocondensation reaction of hydrazines with 1,3-dicarbonyl compounds



Scheme 19. Cyclocondensation of phenylhydrazine with α,β-unsaturated compounds followed by oxidation[24].

The regioselectivity of these cyclocondensations has been widely investigated and has been found to be dependent on a delicate equilibrium between the different reactivity of the groups involved in the condensation, and steric-electronic effects of the substituents on the hydrazine and the 1,3-dielectrophile, with solvent, concentration and temperature effects playing also an important role, both under thermodynamic and kinetic control reaction conditions.[5] For example, in the cyclocondensation of β-aryl, aryl-ynones with hydrazines reported in **Scheme 20**, the observed regioselectivity was explained as a result of an initial 1,4-coniugate addition of the more nucleophilic nitrogen (methyl-substituted in methylhydrazines and unsubstituted in arylhydrazines) to the triple bond of the ynone system.[25]

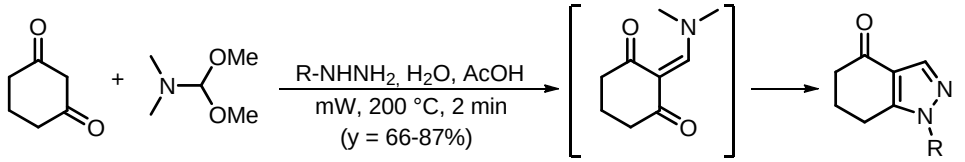


R_1 or R_2 = H, Me, NO_2, OMe, Cl, F

Scheme 20. Example of regiochemistry control in cyclocondensation reactions

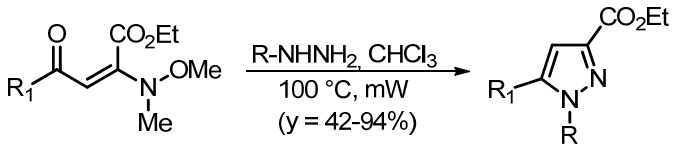
The versatility of this synthetic pathway is underlined not only by the huge number of examples of "classic" reactions that can be found in the past and recent literature, but also by its application in new synthetic methods, such as solventless or solid-phase synthesis. However, these last strategies, despite the high yields, short reaction times and operationally convenient conditions, often lack of selectivity.[5]

Finally, with the target molecule of this work in mind, particular attention must be paid to synthetic strategies aiming for ring-fused pyrazoles and pyrazole-3-carboxylates. While a variety of examples for either the first (**Scheme 21**)[26] or the latter (**Scheme 22,**[27] **Scheme 18** via **c**) is reported in recent literature, to our knowledge, no cyclocondensation method is known to produce, at the same time, a ring-fused pyrazole-3-carboxylate.



R = alkyl, cycloalkyl, aryl, heteroaryl

Scheme 21. Cyclocondensation leading to a ring-fused pyrazole

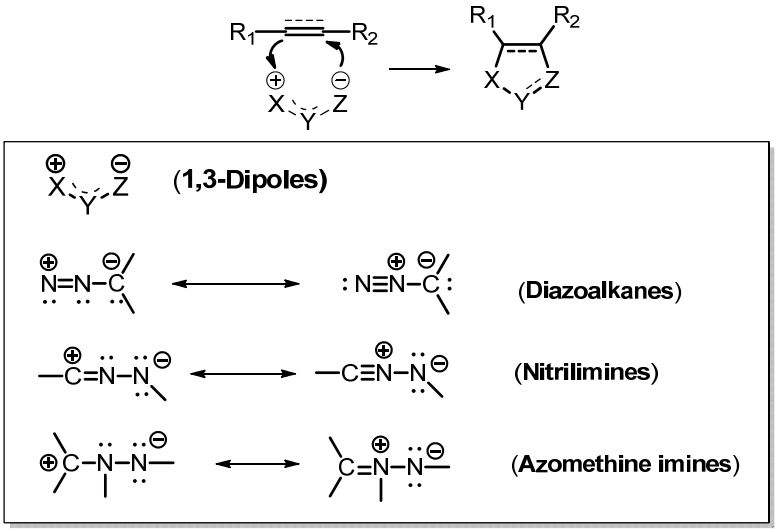


R = Me, Ph. R_1 = alkyl, aryl

Scheme 22. Cyclocondensation leading to a pyrazole-3-carboxylate

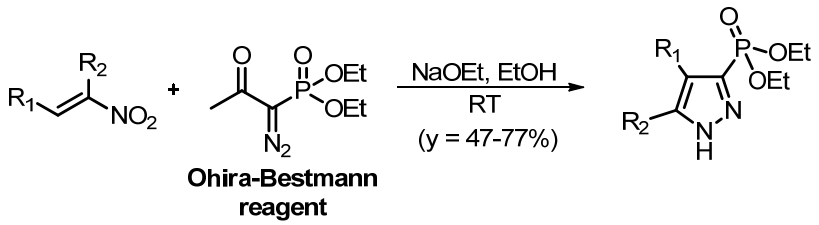
1.4.2. **1,3-Dipolar cycloadditions**

The 1,3-dipolar cycloaddition reaction has been employed as one of the most powerful synthetic tools to provide substituted pyrazoles. Three main classes of 1,3-dipoles have been used as [C,N,N] synthons, namely, diazoalkanes, azomethine imines and nitrilimines; the [C,C] fragment usually comes from activated π-bonds of alkanes and alkynes. Compared to cyclocondensations between hydrazines and 1,3-dielectrophiles, 1,3-dipolar cycloadditions are intrinsically more highly regioselective owing to the significant electronegative difference between the N and the C atom of the substrate.[5]



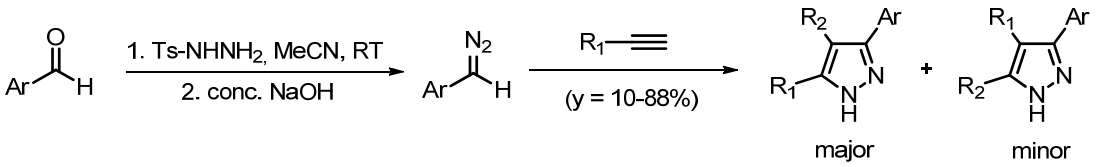
Scheme 23. Most common dipoles for 1,3-dipolar cycloadditions leading to pyrazoles

1,3-Dipolar cycloadditions of diazo compounds with alkynes, leading to N-unsubstituted pyrazoles, can be conducted efficiently under thermal conditions. However, diazo compounds are dangerous to prepare and handle due to their toxicity and potentially explosive nature.[5] Nevertheless, either methods starting from commercially available, quite stable substrates (such as the Ohira-Bestmann reagent[28] shown in **Scheme 24**) or generating the unstable diazo compound in situ[29] (**Scheme 25**) are reported, also in recent literature.



R_1 = Aryl, Heteroaryl. R_2 = H, Br, CO_2Et

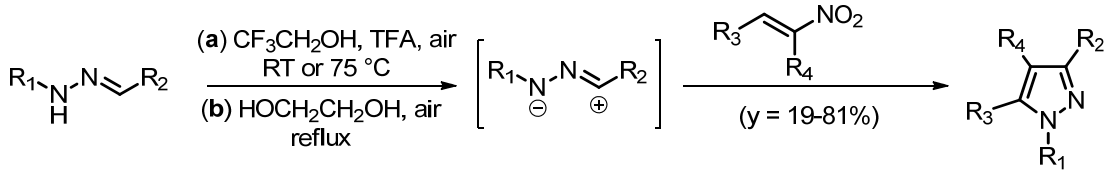
Scheme 24. Cycloaddition of nitroalkenes with Ohira-Bestmann reagent (aromatization occurs by spontaneous HNO_2 elimination)



R_1 = H, Aryl, Heteroaryl, Benzyl. Ts-$NHNH_2$ = Tosylhydrazine
MeCN = Acetonitrile

Scheme 25. In situ formation of the diazo compounds from tosyl-hydrazones and cycloaddition

Azomethine imines are reactive intermediates generated in situ by various methods, generally starting from hydrazones, by thermal decomposition or acid treatment. As a more regioselective direct alternative to the Knorr reaction, the 1,3-dipolar cycloaddition of azomethine-imines with activated π-bonds turned out to be an appealing method for the library synthesis of pharmaceutically relevant pyrazoles for drug discovery efforts. This is shown in **Scheme 26**, following a literature example.[30]

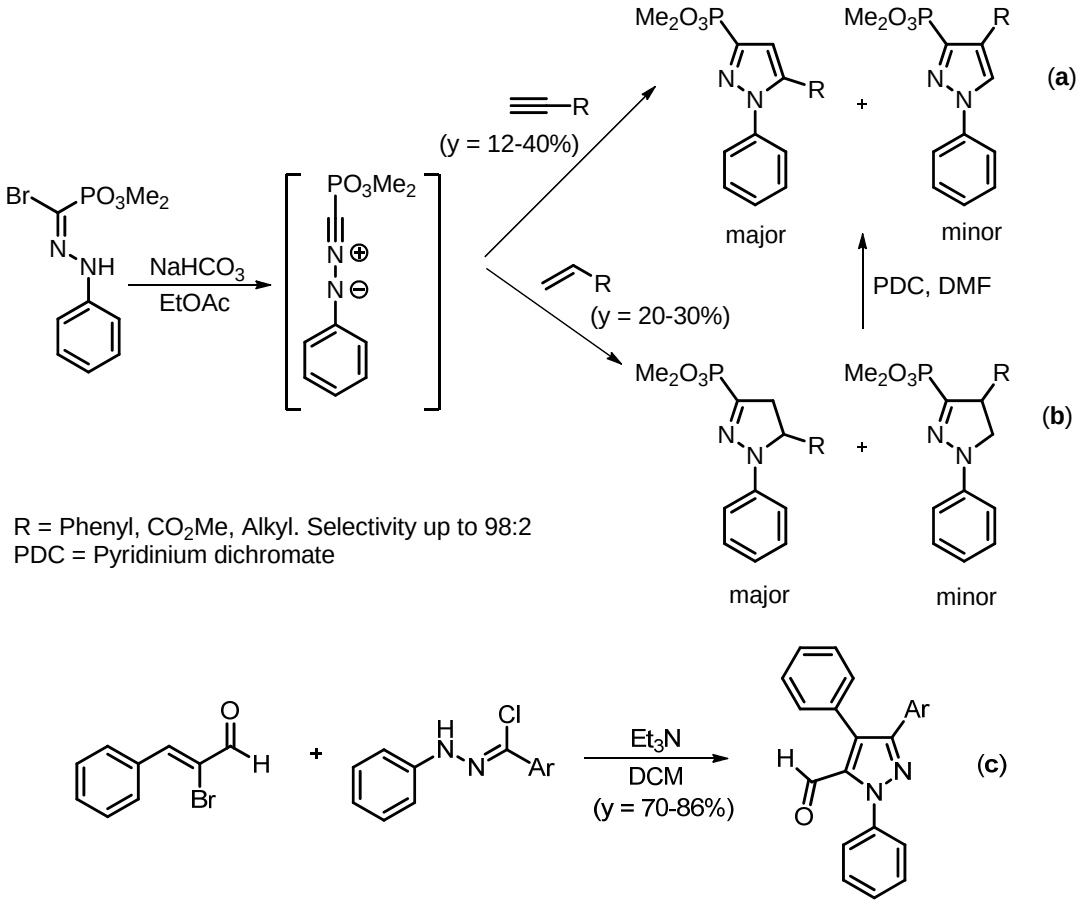


R_1 = Aryl, Methyl. R_2 = Aryl. R_3 = Aryl, Heteroaryl. R_4 = H, Methyl

Scheme 26. Azomethine imine formation and cycloaddition

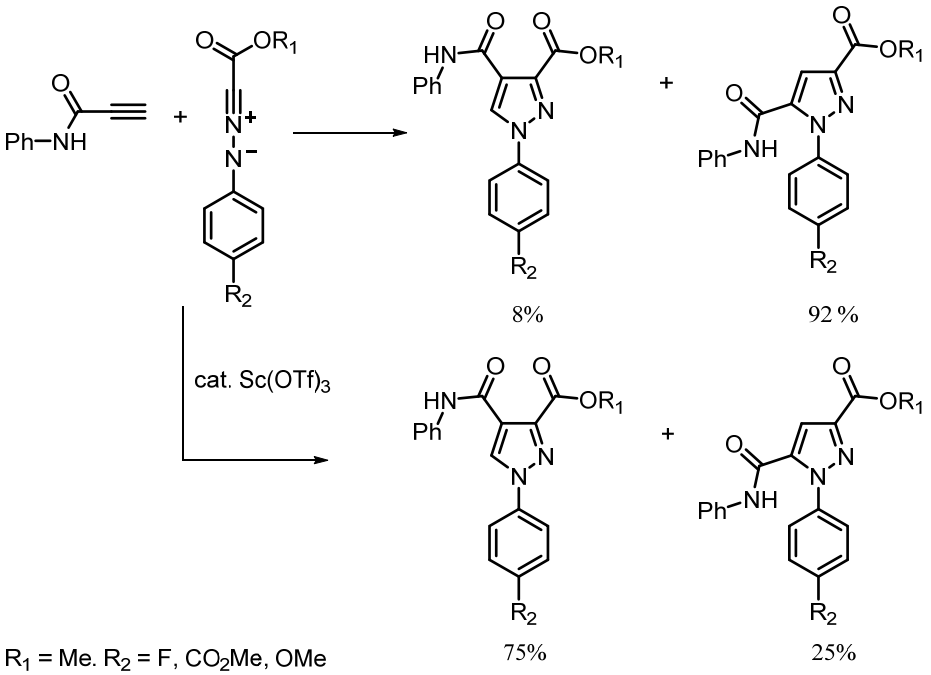
Nitrilimines are generated in situ by treatment of hydrazonoyl halides with a base. Their 1,3-dipolar cycloaddition to alkynes (**Scheme 27,** via **a**[31]) or alkenes bearing a leaving group (such as α-bromo-α,β-unsaturated aldehydes in **Scheme 27,** via **c**[32]) leads directly to pyrazoles, while addition to simple activated double bonds produces pyrazolines

(**Scheme 27,** via **b**[31]) that must be subsequently oxidized to the desired aromatic pyrazole. (Examples in **Scheme 27** are from very recent literature).



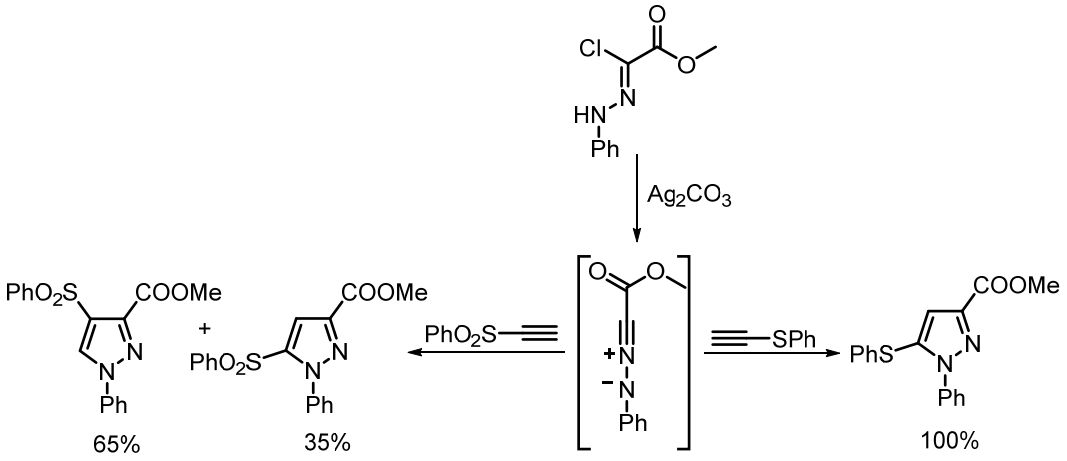
Scheme 27. Nitrilimine formation and cycloaddition with alkynes (**a**), alkenes followed by oxidation (**b**) and alkene bearing a leaving group (**c**)

A very convenient prerogative of this synthetic strategy relies on the facile control and modification of the regiochemistry, as proved in our laboratories. For example, a catalytic amount of scandium triflate, $Sc(OTf)_3$, as Lewis acid, is responsible for the inversion of the regioisomeric ratio in the cycloaddition of N-aryl-C-carboxyalkyl nitrilimine and an activated acetylene, such as N-phenyl-propiolamide.[33]



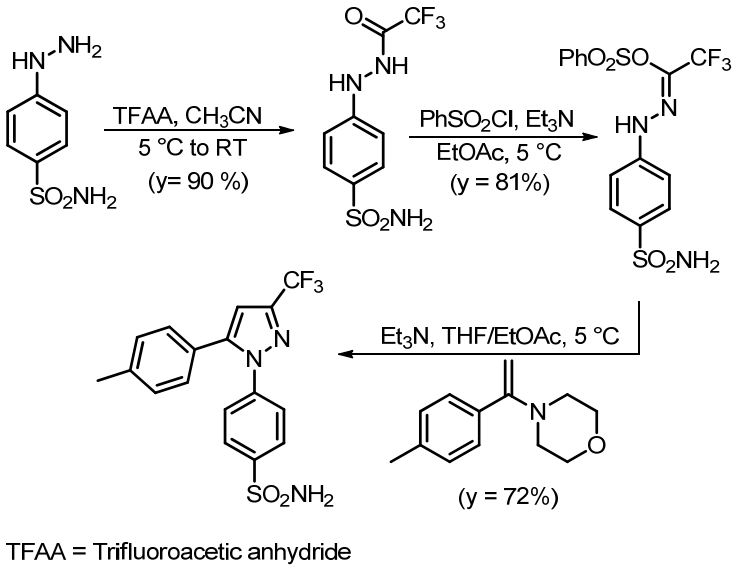
Scheme 28. Regioselectivity reversion with $Sc(OTf)_3$

Moreover, the electronic nature of the activating substituent of the multiple bond acts as a director of regiochemistry. In particular, electron donating groups (EDG), such as sulfide in **Scheme 29**, lead to 5-substitued pyrazoles, while electron-withdrawing groups (EWG) such as sulfone in **Scheme 29**, lead to 4-substitued pyrazoles as major products. These experimental results were broadly investigated by our research group and were confirmed by computational calculations as an interaction between distorted frontier molecular orbitals (HOMO-LUMO) both on the dipolarophile and on the nitrilimine dipole.[34]



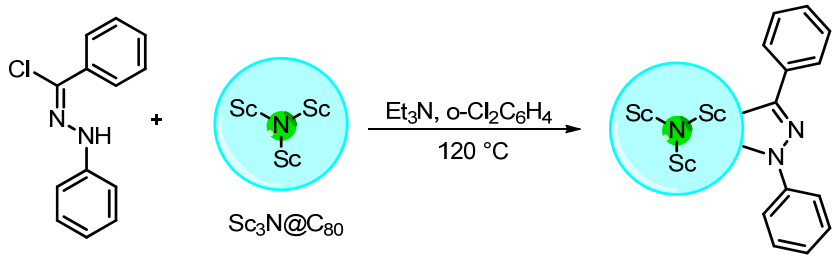
Scheme 29. Regioselectivity directed by acetylene substituents

The 1,3-dipolar cycloaddition of nitrilimines with activated π-bonds has proved to be a very powerful tool in the regioselective synthesis of biologically active pyrazole-based molecules, such as Rimonabant, Apixaban (see **Scheme 11**) and many others. The synthesis of Celecoxib shown in **Scheme 30** may be taken as an example. Cyclocondensation reactions lead to mixtures of regioisomers, requiring the development of a crystallization protocol to obtain a regiopure material, or needed intermediates of complicated synthesis, whose purifications relied on column chromatography. On the other hand, the 1,3-dipolar cycloaddition strategy furnished the desired pyrazole in 52% overall yield from starting material (4-sulfonamide-phenylhydrazine hydrochloride) with a simple, practical and 100% regioselective protocol, employing economical and readily available reagents.[35]



Scheme 30. Celecoxib synthesis through 1,3-dipolar cycloaddition strategy (in the last step, aromatization occurs by morpholine elimination)

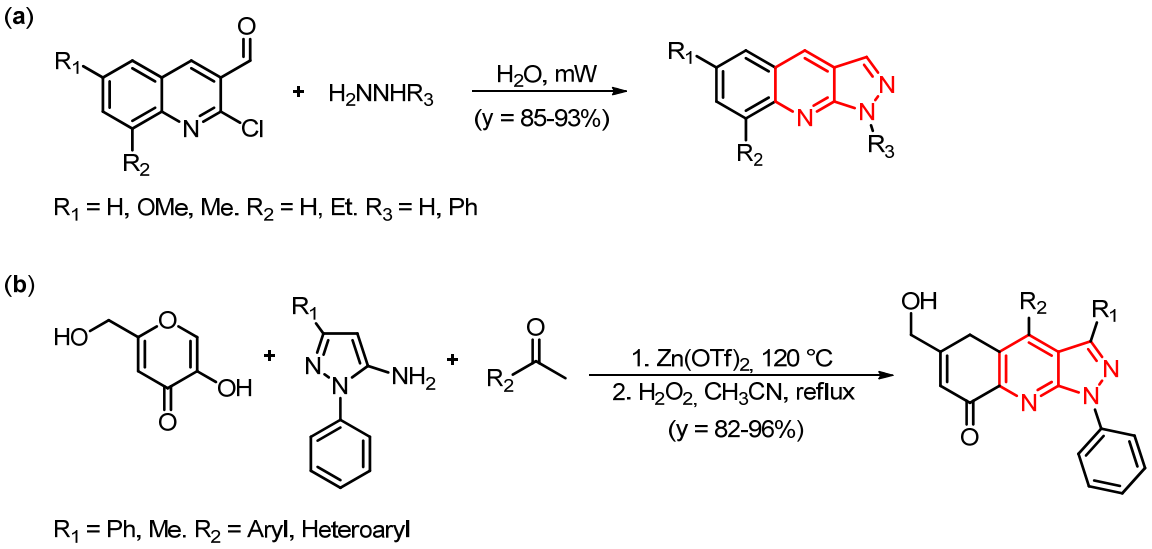
Finally, to underline the versatility and the generality of this strategy, a very recent literature example, employing it successfully on an unusual substrate, is to be cited. Lu et al. reported a stable pyrazole-ring fused derivative of a endohedral metallofullerene, synthesized by regioselective 1,3-dipolar cycloaddition of diphenylnitrilimine to $Sc_3N@C_{80}$. (Scandium nitride encapsulated in a C_{80} fullerene).[36]

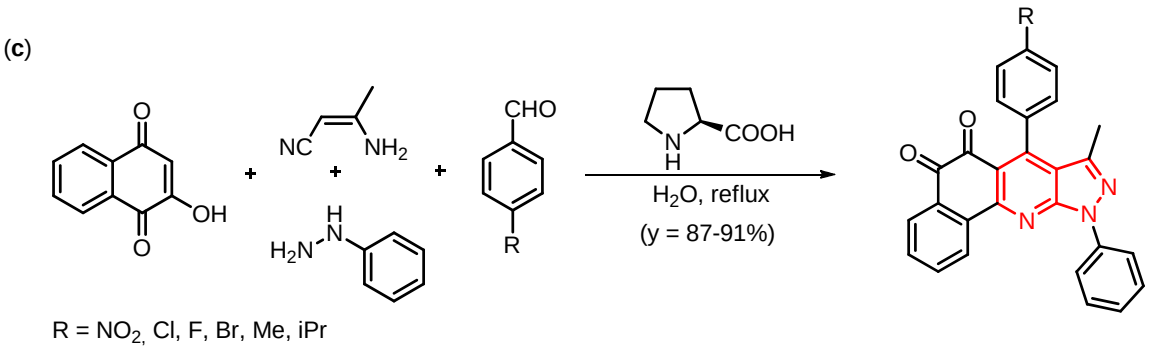


Scheme 31. 1,3-dipolar cycloaddition of diphenylnitrilimine using a fullerene as dipolarophile

1.4.3. **Synthesis of ring-fused pyrazoles**

Because of the excellent bioactivity and the wide range of application, thousands of papers concerning the synthesis of condensed pyrazole derivatives have been published[4]. However, it is possible to summarize all these strategies into three main classes: methods starting from a pre-formed ring to which the pyrazole is subsequently fused, methods starting from a pre-formed pyrazole to which a new ring is subsequently fused and, finally, methods generating both pyrazole and its fused ring at once, following one-pot reaction procedures. In **Scheme 32** the three above-mentioned strategies are exemplified, showing the synthesis of the same pyrazole-pyridinic core in different target molecules.[37,38,39]

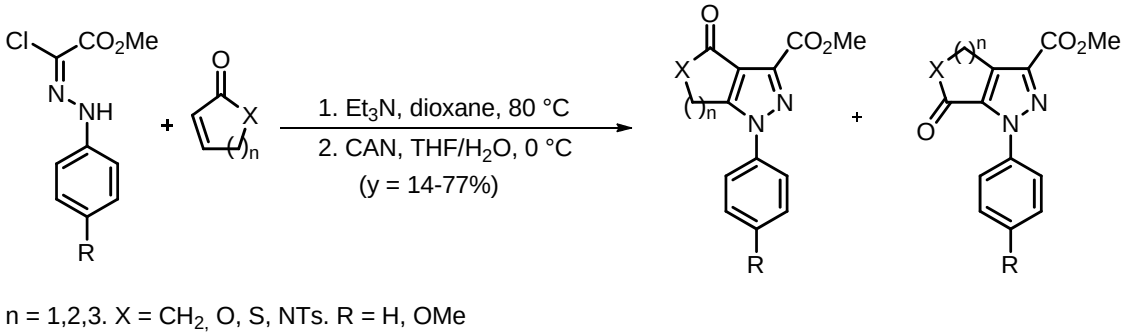




Scheme 32. Three different strategies to create the same bicyclic pyrazole core

Multicomponent one-pot syntheses are well known to produce highly functionalized, complex molecular scaffolds in very convenient, step- and atom-economical procedures. Nevertheless, the outcome of these reactions is very often difficult to predict and control, rendering this strategy quite unappealing for the synthesis of a precise target compound. On the other hand, methods condensing a new cycle on the pyrazole ring suffer from the complexity of the direct functionalization of an heterocycle of hybrid electronic characteristics, not yet completely investigated and understood.[4]

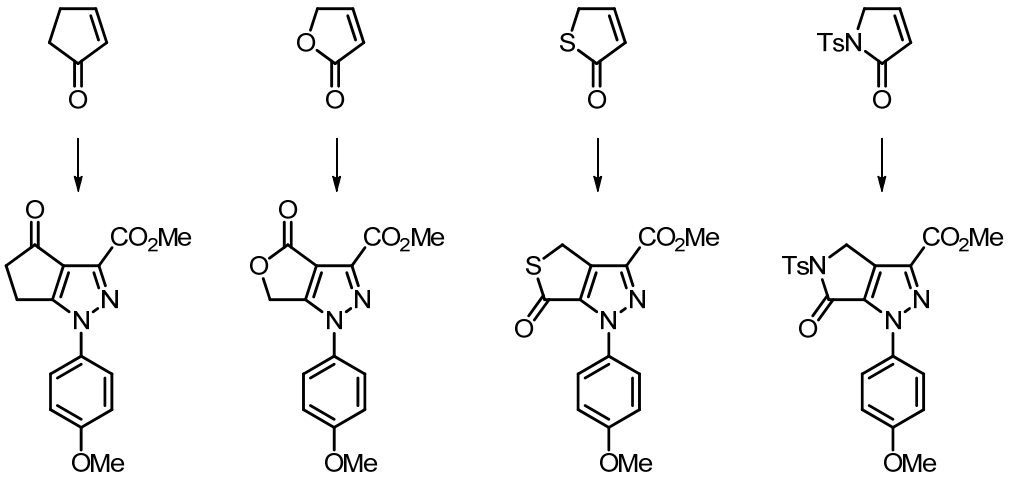
Hence, strategies constructing the pyrazole on pre-existing rings seem to be the more convenient pathway to condensed pyrazole derivatives, due to the wide spectrum of synthetic methods for the obtainment of the heterocycle, also from very different starting materials.[5] For these reasons, our research group has quite recently reported a new synthetic procedure leading to cycloalkenone, lactone, thiolactone and lactam-fused pyrazoles, employing the 1,3-dipolar cycloaddition of various nitrilimines with α,β-unsaturated cyclic systems.[40]



Scheme 33. Cycloaddition of nitrilimines with various α,β-unsaturated cyclic systems

Finally, the regiochemistry of the reaction was broadly investigated and the experimental results were justified through theoretical and computational studies. In most cases, 5-

acyl-pyrazole derivatives were found to be the major products; although, only small and hard dipolarophiles (cyclopentanone and α,β-unsaturated γ-butyrolactone) reacted with electron-rich nitrilimines (such as N-p-methoxyphenyl, C-carboxymethyl nitrilimine) under the mandatory presence of Et_3N as a base, giving an inversion in the selectivity, in favour of 4-acyl-pyrazoles.[40]

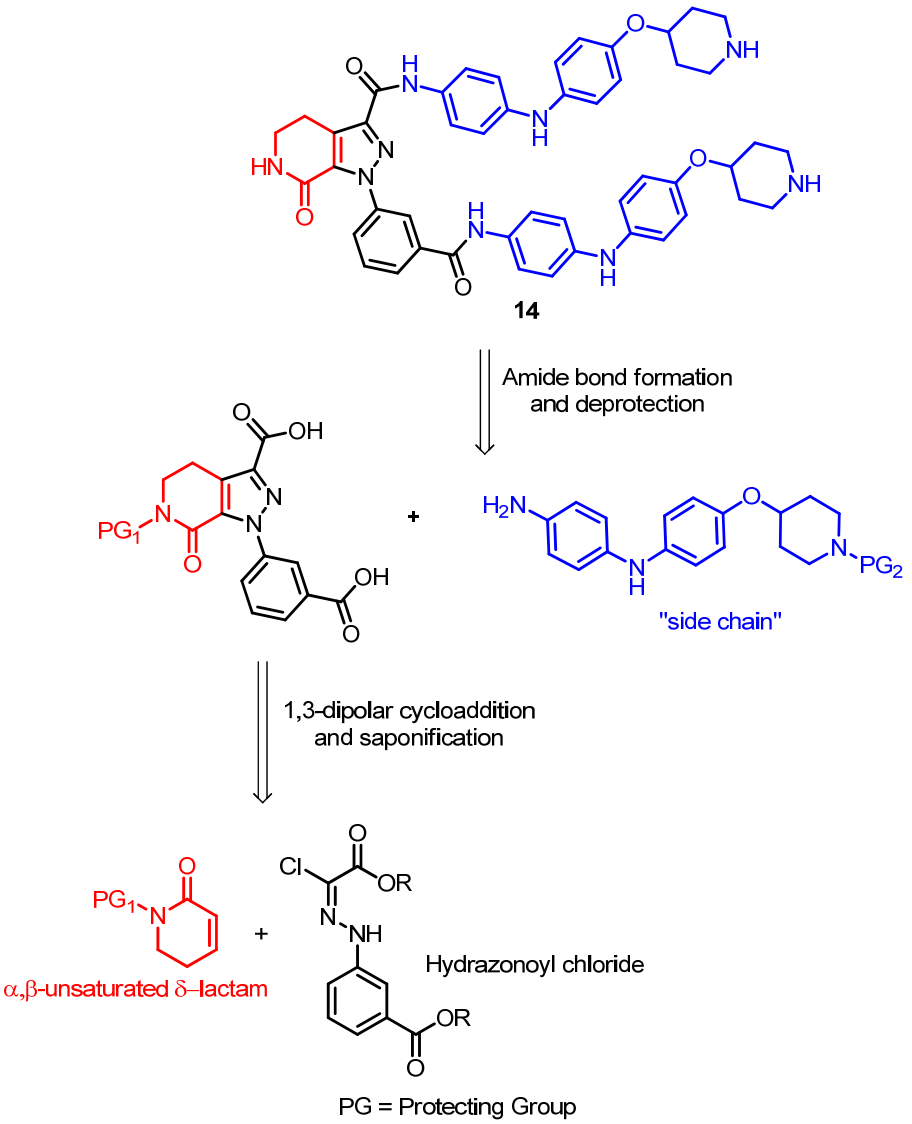


Scheme 34. Major product arising from cycloaddition-oxidation of N-p-methoxyphenyl, C-carboxymethyl- nitrilimine and each of the shown dipolarophiles

2. Aim of the Thesis

In the previous chapter the importance of pyrazoles and lactams derivatives in medicinal chemistry has been underlined, along with a brief discussion about "side chains" in drug design.

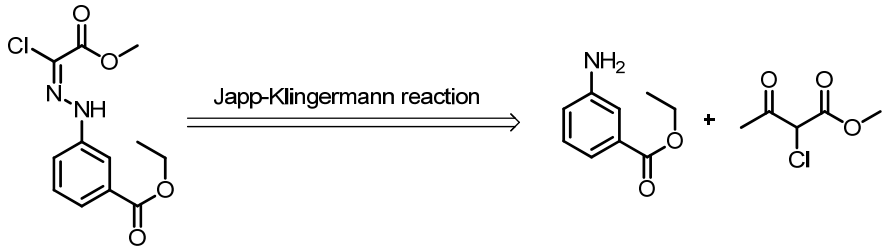
Aim of this thesis is the synthesis of a complex molecular target with potential pharmacologic activity, formed by a lactam-fused pyrazolic central scaffold and a double "side chain". Retrosynthetic analysis, starting from the final product, may help in recognizing the different parts and foreseeing the synthetic strategy adopted.



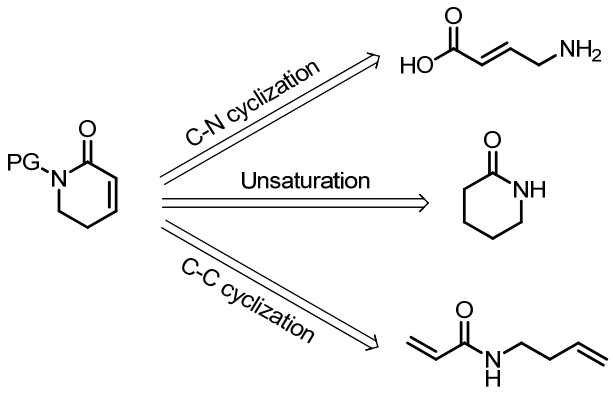
Scheme 35. Retrosynthetic analysis from building blocks to the final target

Following the disconnection approach, it is clear that three building blocks are necessary for the synthesis of the desired product: the α,β-unsaturated δ-lactam, the hydrazonoyl chloride and the "side chain".

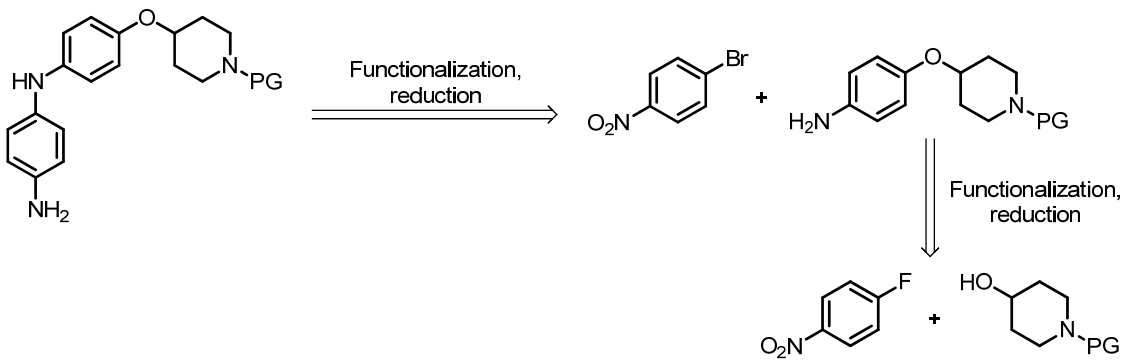
Since none of the three building blocks is commercially available, their synthesis will be the first effort towards the construction of the main intermediates. While the hydrazonoyl chloride can be synthesized with a common literature procedure, the obtainment of the α,β-unsaturated δ-lactam will not prove trivial and many strategies will be envisioned and attempted. Finally, the side chain will be synthesized through a series of aromatic functionalizations and reductions, improving a method already established by our group.



Scheme 36. Retrosynthetic analysis for the hydrazonoyl chloride



Scheme 37. Retrosynthetic analysis for the α,β-unsaturated δ-lactam (three possible ways)



Scheme 38. Retrosynthetic analysis for the "side chain"

As one can see in **Scheme 35**, construction of the central core will be achieved by 1,3-dipolar cycloaddition, followed by oxidation, between the α,β-unsaturated δ-lactam (dipolarophile) and the nitrilimine (dipole) derived from the hydrazonoyl chloride (see chapter 1.4.2 and 1.4.3). This straightforward and regioselective synthetic strategy, for ring-fused pyrazoles, has been firstly reported by our research group (see **Scheme 34**). Optimization of reaction conditions and selectivity will be broadly investigated.

Attachment of the "side chain" will be subsequently attempted via amide bond formation between the two acid moieties on the central core and terminal reactive amine on the "spacer" of the side chain. This is a very common procedure in medicinal chemistry, due to both the high stability of the amide bond and the multitude of mild and functional groups-tolerant synthetic methods for its formation.

As the last step, removal of the protecting groups will afford the final target. In view of this, introduction of suitable protecting groups with the same cleavage method, in the early steps of the synthesis, will be a smart choice to afford, at the end of the process, a step-economical, one-pot multiple deprotection.

At the end of this highly convergent synthesis a double functionalized bicyclic lactam-fused pyrazole system will be obtained, ready to be tested for its cytotoxicity towards various cancerous cell lines.

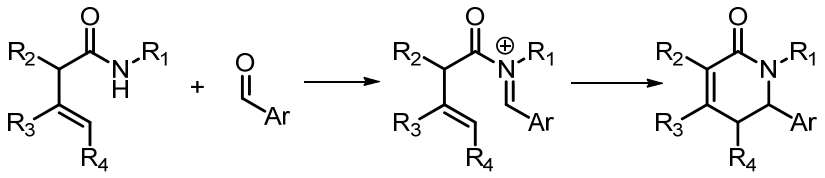
3. Results and Discussion.

3.1. Synthetic methodologies for the dipolarophile and precursor of the 1,3-dipole

Our first efforts in the synthesis of the desired molecular target, were to find practical and scalable synthetic routes to an α,β-unsaturated δ-valerolactam and a hydrazonoyl chloride (precursor of the 1,3-dipole nitril-imine), suitable for the [3+2] dipolar cycloaddition reaction, in order to obtain the central ring-fused pyrazolic scaffold.

3.1.1. Synthetic strategies towards the α,β-unsaturated δ-lactam (dipolarophile)

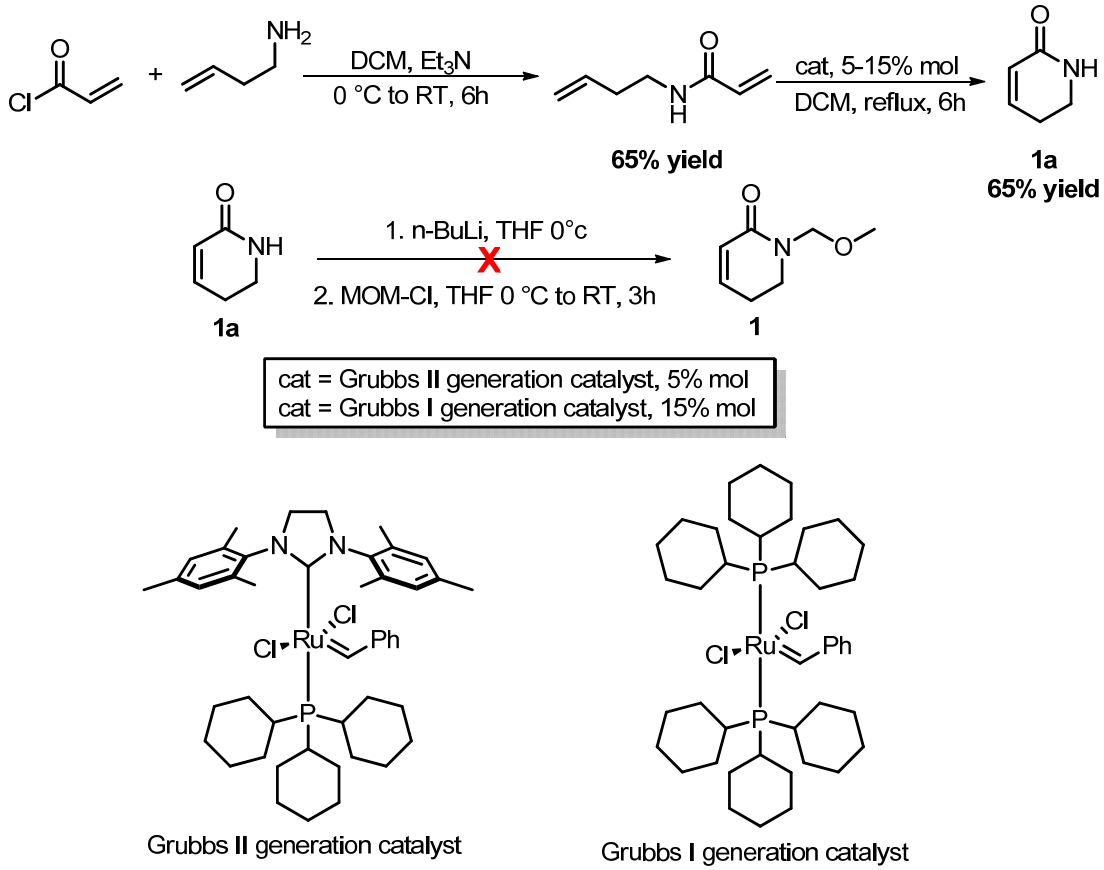
A well-known literature procedure (developed by C. Marson and U. Gabrowska) involves an acid catalyzed condensation of a 3-alkenamide on an aryl-aldehyde followed by a mixed Bischer-Napieralski and Pictet-Spengler-type cyclization, leading to highly functionalized α,β-unsaturated δ-valerolactams with good stereo- and regiochemistry.[41] Despite its simplicity, this method is unsuitable for our purpose, aiming for an unsubstituted cycle.



Scheme 39. Marson and Gabrowska method

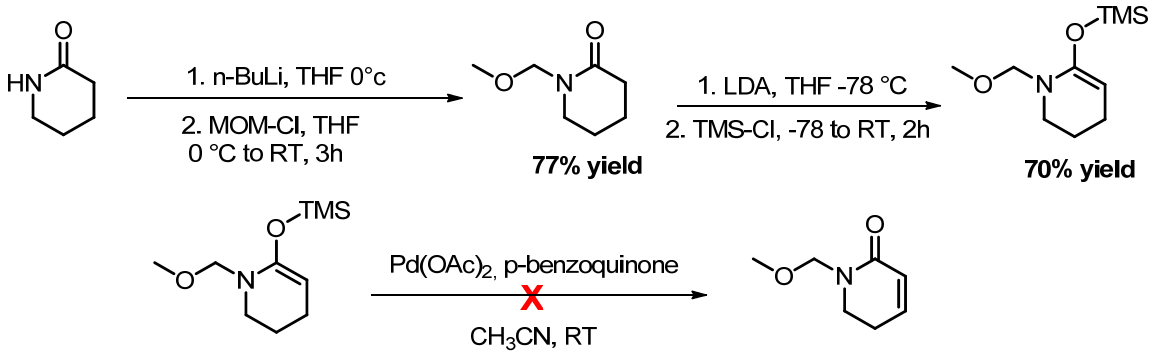
Another very established strategy is the ring closing metathesis, promoted by the Ruthenium-based "Grubbs catalysts".[42] The synthetic sequence leading to the unprotected lactam **1a** was reported in literature[43] and was attempted by our research group. However, besides the high cost of the substrates (homoallylamine in particular), this method proved also to be quite unpractical and very difficult to scale up on a laboratory scale, requiring very high dilution and a slight high amount of the very expensive Grubbs II generation catalyst. While the less expensive I generation one was attempted and promoted successfully the reaction, it required an even higher amount of catalyst, which proved to be inseparable from the product. This one, in the crude form,

failed to undergo the subsequent protection reaction, probably due to traces of impurities and the strategy had to be discarded.



Scheme 40. Ring-closing metathesis method

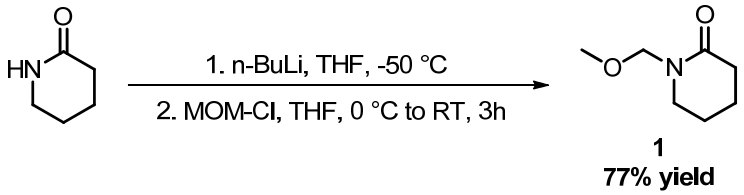
With the most common ring-closing methods set apart, we moved our attention on strategies aiming for α,β-unsaturated cyclic products, starting from the corresponding saturated ones (in this case being the inexpensive and commercially available δ-valerolactam). One common procedure is the Saegusa-Ito oxidation, a Pd(II) promoted β-hydrogen extraction performed on silyl-enol ethers.[44] While it represents a very established procedure for cyclic ketonic substrates, we failed to find any examples in literature about its application on esters or amides. The reaction was attempted anyway but led only to trace-amounts of the desired product.



Scheme 41. Saegusa-Ito oxidation strategy

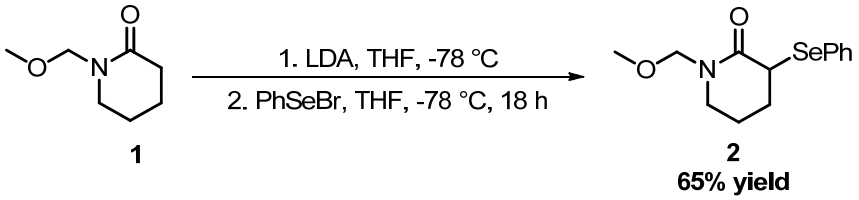
Finally the "selenoxide elimination" method[45] was taken into account as a suitable synthetic strategy. This route proved to be the most efficient and easy to perform on a multigram laboratory scale, with the stoichiometric requirement of the toxic (but quite inexpensive) organoselenium compound (phenylselenyl bromide, PhSeBr) being the only drawback. However, the reagent itself is quite safe to handle and its toxicity should not be of any concern for the final pharmaceutical target, having been used and removed in a very early synthetic step.

For these reasons a three-step procedure was developed and optimized. The process started with the protection of the amide functionality as methoxy-methyl hemiaminal by treatment of δ-valerolactam with *n*-butyllithium and chloromethyl methyl ether (MOM-Cl) in anhydrous THF. Product **1** was thus obtained in 77% yield after purification either by column chromatography on silica gel or by distillation under reduced pressure.



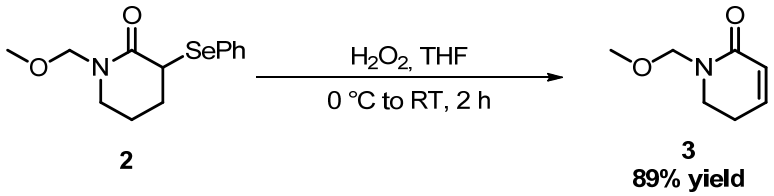
Scheme 42. Selenoxide elimination method, 1st step

In the second step, α-selenylation of the carbonyl was achieved by reacting product **1** with LDA (lithium diisopropylamide) and PhSeBr in anhydrous THF. Product **2** was thus obtained in 65% yield after purification by column chromatography on silica gel.



Scheme 43. Selenoxide elimination method, 2nd step

In the last step the selenoxide was formed by treatment of compound **2** with concentrated H_2O_2 in THF at 0 °C (where it is stable) and thermally decomposed (eliminated) by rising the reaction temperature above 22 °C in a (highly exothermic) one-pot oxidation-reverse cycloaddition reaction. After purification by column chromatography on silica gel, which easily afforded separation from all the selenium-containing coproducts, compound **3** was obtained in 89% yield.

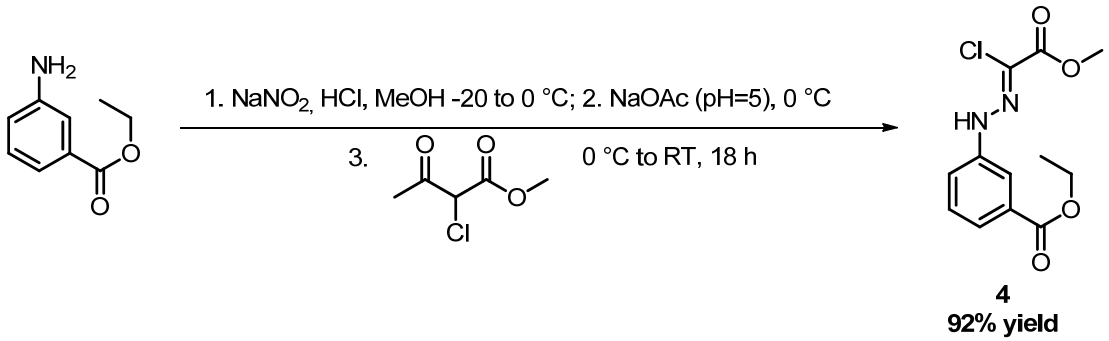


Scheme 44. Selenoxide elimination method, 3rd step

The target α,β-unsaturated δ-lactam, which will be used in the subsequent cycloaddition reaction, was thus obtained in overall (3 steps) 45% yield.

3.1.2. Synthesis of the hydrazonoyl chloride, precursor of the 1,3-dipole

Afterwards, we moved to the synthesis of the precursor of the nitril-imine, ethyl-3-(2-(1-chloro-2-methoxy-2-oxoethyiliden)hydrazinylbenzoate **4**. A well-established method for the obtainment of C-carboxyalkyl-N-aryl hydrazonoyl chlorides is the Japp-Klingermann reaction, involving the nucleophilic attack of the enolate of a 2-chloro-1,3-keto-ester on an aryl diazonium salt, followed by deacylative elimination. So, reaction of ethyl 3-aminobenzoate with $NaNO_2$ and concentrated HCl in cold MeOH formed the diazonium salt that was subsequently reacted with methyl 2-chloro-acetoacetate with a large excess of sodium acetate as the nucleophile for the final elimination. Common aqueous workups provided finally product **4** in 92 % yield. This synthesis is very easy to perform on a very large scale and leads to a solid and stable product that can be stored for months.



Scheme 45 Japp-Klingermann reaction for the synthesis of the hydrazonoyl chloride

3.2. 1,3-Dipolar cycloaddition of the nitril-imine with the α,β-unsaturated δ-lactam followed by oxidation: synthesis of the ring-fused pyrazolic core

With the dipolarophile and the precursor of the 1,3-dipole in hand, the best reaction conditions for the [3+2] dipolar cycloaddition were then investigated.

First of all, taking into account the previous work of our research group[33,34,40,46] underling the requirement of high boiling anhydrous solvents for the reaction to occur, 1,4-dioxane and toluene were tested, either at reflux or at 140 °C (sealed tube), and refluxing toluene proved to be the best choice.

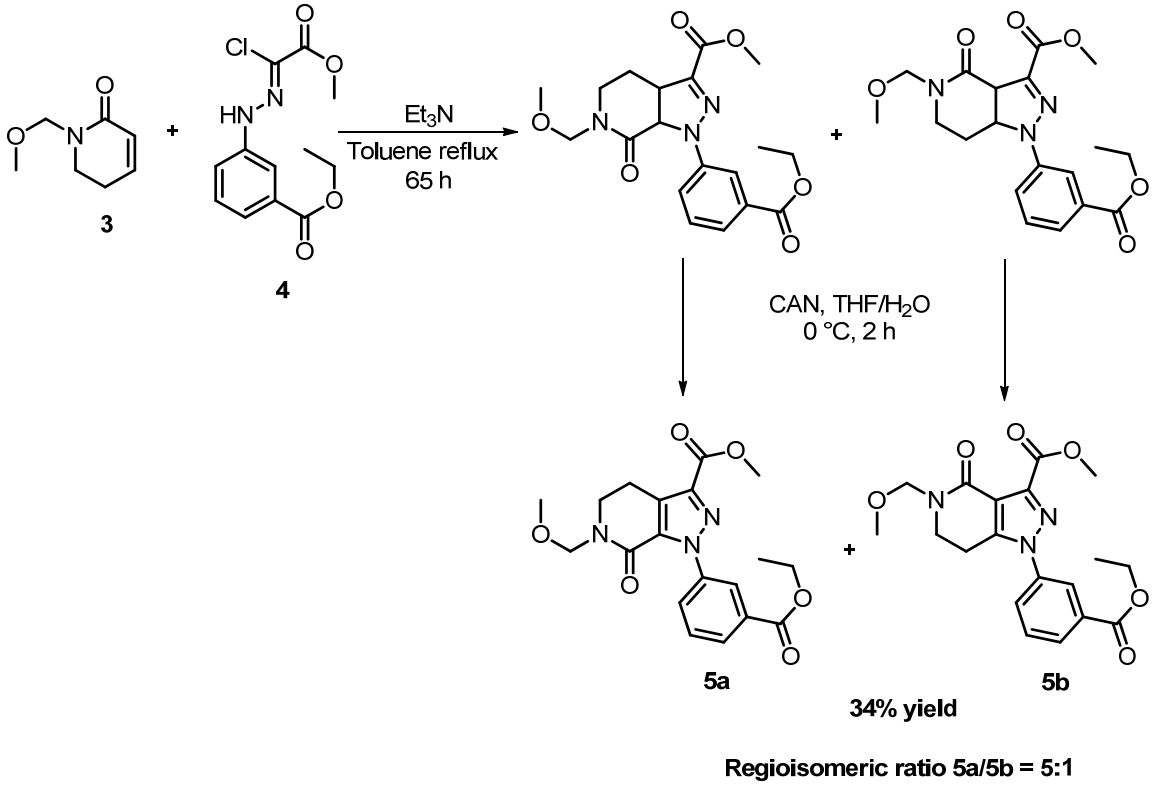
Subsequently, a brief base screening was conducted and the reaction was found to be quite base sensitive: in fact, only aliphatic tertiary amines (such as Et_3N or DIPEA, N,N'-diisopropylethylamine) were able to promote the conversion of the hydrazonoyl chloride into the desired nitrile-imine and perform the addition.

Finally, since the major by-products arose from the decomposition of the nitrile-imine, a reverse in the stoichiometry ratio was attempted, but it resulted in a deterioration of the yield. In summary, the best reaction conditions were found to be Et_3N as a base, 1.5 equivalents of the hydrazonoyl chloride in refluxing toluene.

On the other hand, the subsequent aromatization step was rather trivial: it involved suspension of the cycloaddition crude product in a mixture of THF and water and treatment with cerium ammonium nitrate (CAN). The desired product **5a** was thus obtained in an overall (2 steps) 34% yield after purification by column chromatography on silica gel, which also afforded easy and complete separation from regioisomer **5b**.

All the modification performed affected the yield but not the regioselectivity of the reaction, that was found to be 5:1 in favour of **5a** (from the ^{1}HNMR spectrum of the crude oxidized product).

Table 1. Reaction conditions for the 1,3 dipolar cycloaddition followed by oxidation

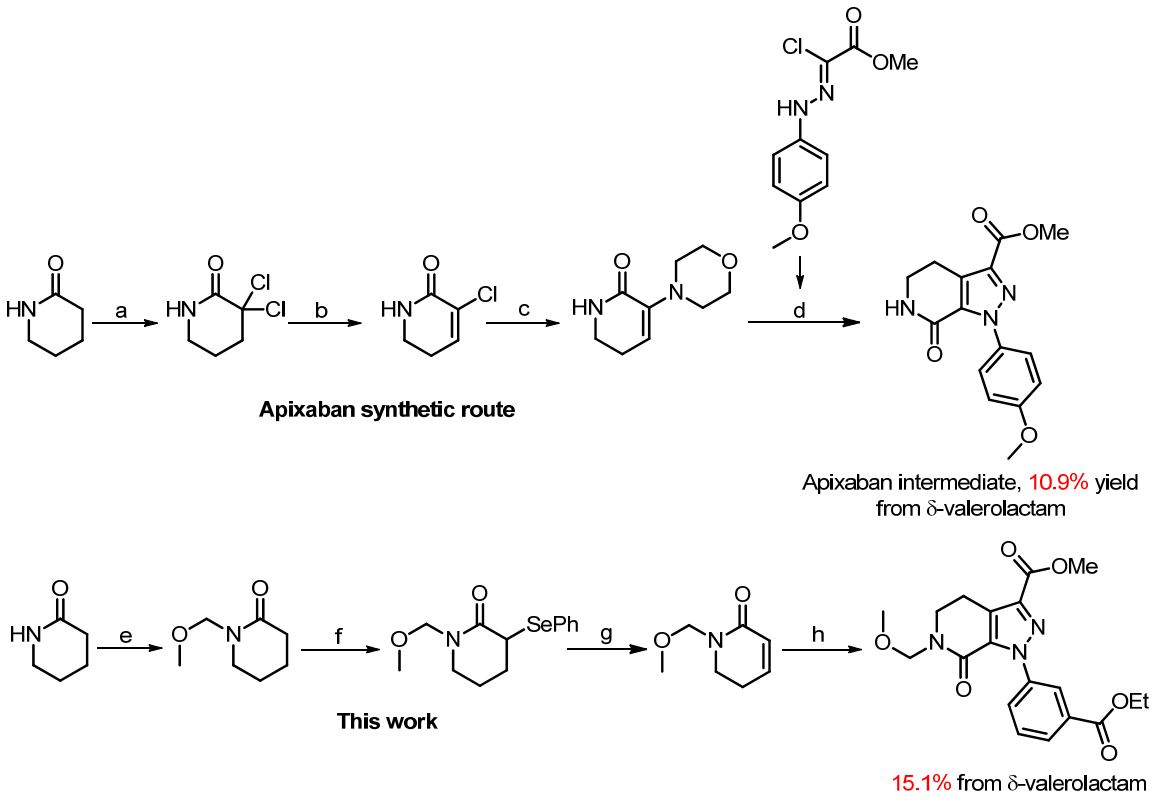


Entry	Solvent	Temp. (°C)	Base	Equiv. of 4	Yield (%)[b]
1	1,4-Dioxane	100	Et_3N	1.5	24
2	1,4-Dioxane	140[c]	Et_3N	1.5	27
3	Toluene	110	Et_3N	1.5	34
4	Toluene	140[c]	Et_3N	1.5	decomp.
5	Toluene	110	DIPEA	1.5	27
6	Toluene	110	Cs_2CO_3	1.5	decomp.
7	Toluene	110	DBU[d]	1.5	0
8[e]	Toluene	110	Et_3N	0.75	27[f]

(**a**) Reaction conditions: 3 (0.25 mmol), 4 (0.375 mmol), base (2.625 mmol, 7 eq. referred to 4), solvent (1.5 mL), 65h; then CAN (0.625 mmol), THF/H_2O 8:6 (3.5 mL), 0 °C, 2 h. (**b**) Yield of 5a isolated after column chromatography, referred to 3. (**c**) Sealed tube. (**d**) 1,5-diazabiciclo(5.4.0)undec-5-ene. (**e**) Reaction conditions: 3 (0.28 mmol), 4 (0.18 mmol), base (1.29 mmol, 7 eq. referred to 4), solvent (1.2 mL), 65h; then CAN (0.7 mmol), THF/H_2O 8:6 (3 mL), 0 °C, 2 h. (**f**) Referred to 4.

Despite the best yield obtained was quite modest, this method still represents, to our knowledge, the only straightforward synthetic strategy to a ring-fused pyrazole. We should also consider that the final values refer always to a two-step procedure cycloaddition plus oxidation. Moreover, it also proved to be better performing, in terms of overall yield, compared to other very similar synthetic sequences.

In particular, one synthetic route to Apixaban[47] shows a much better yielding "cycloaddition"-aromatization step, due to an enamine-driven reaction pathway, in which the dipolarophile double bond is much more nucleophile and reactive towards the electrophilic center of the nitrile-imine. Aromatization is obtained by a subsequent, acid catalyzed, morpholine elimination. This also produced a complete regioselectivity, on account of a stepwise mechanism. However, the insertion of the morpholine "activator" on the lactamic scaffold involves a much more problematic synthesis of the substrate, resulting in a diminished overall yield from δ-valerolactam to the desired ring-fused pyrazole.

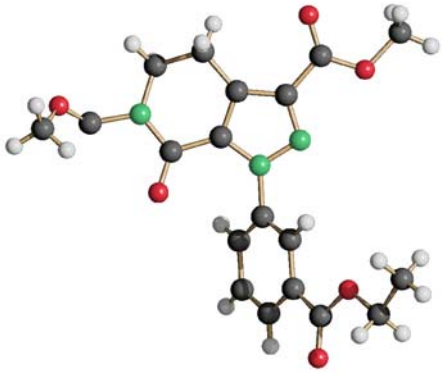


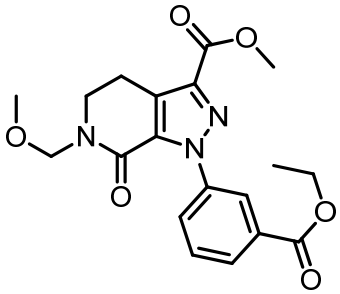
Scheme 46. Comparison between this work and the Apixaban route

a) PCl_5, $CHCl_3$, 66% yield; **b**) Li_2CO_3, DMF, 62% yield; **c**) morpholine, Et_3N, 43% yield; **d**) Et_3N, toluene then TFA, DCM, 62% yield

e) *n*-BuLi, MOMCl, THF, 77% yield; **f**) LDA, PhSeBr, THF, 65% yield; **g**) H_2O_2, THF, 89% yield; **h**) 4, Et_3N, toluene, 34% yield then CAN, THF/H_2O, 34% yield.

Finally, in order to attribute the exact structure of the desired regioisomer, X-Ray diffraction analysis was performed on a single crystal of product **5a**. The structure shown is in complete agreement with the theoretical and experimental studies conducted by our research group and reported in the introduction (*vide supra*).





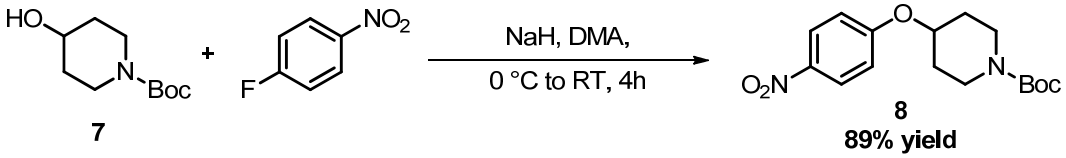
5a

Scheme 47. X-ray structure of the major regioisomer **5a** (*Black = C; White = H; Red= O; Green = N)*

3.3.Synthesis of the "side chain"

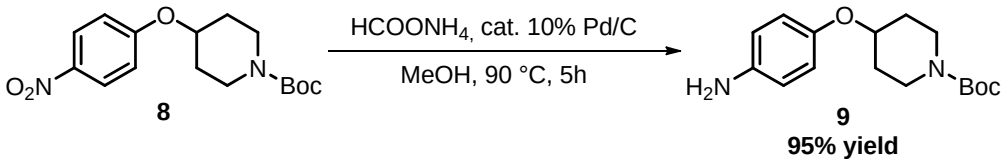
The synthesis of the side chain consisted in a very efficient optimized 4 steps method, starting from commercially available inexpensive starting materials and easily applicable on a multigram laboratory scale.

In the first step nucleophilic aromatic substitution of N-Boc-4-hydroxypiperidine (**7**) on 1-fluoro-4-nitrobenzene was performed in dry N,N'-dimethylacetamide (DMA), using NaH as a base. After purification by column chromatography on silica gel, product **8** was obtained in 89% yield.



Scheme 48. Side chain synthesis, 1st step: nucleophilic aromatic substitution

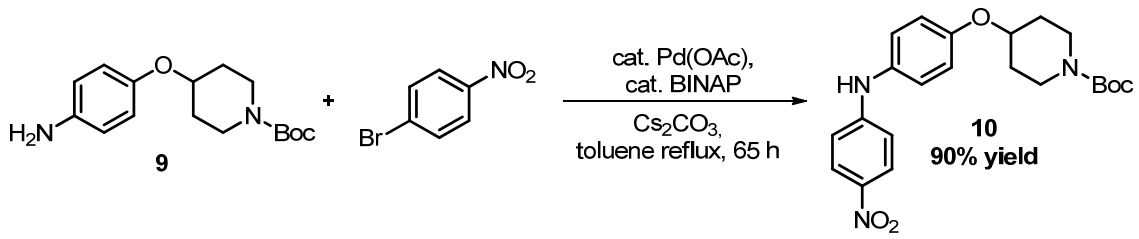
Reduction of the nitro group of **8** into amine **9** was achieved by catalytic hydrogenation using $HCOONH_4$ (ammonium formate) as hydrogen source, 10% Pd on activated carbon as catalyst and degassed MeOH as solvent. Product **9** was thus obtained in 95% yield without any further purification.



Scheme 49. Side chain synthesis, 2nd step: reduction

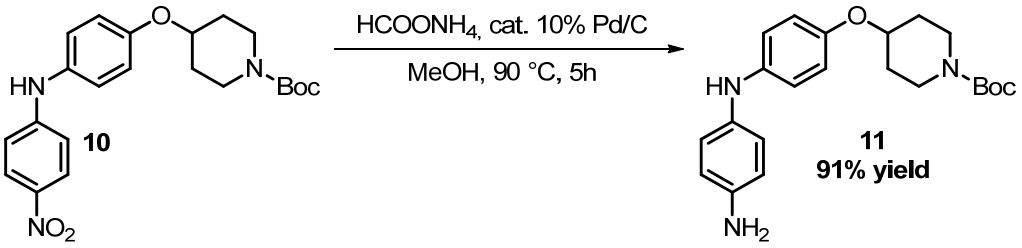
The third step of this strategy involves the attack of the amino group of **9** on the aromatic ring of 4-bromo-1-nitrobenzene for the formation of the desired nitroamine (**10**). However, arylamines synthesis with the formation of a new C-N bond is quite difficult, since classic nucleophilic aromatic substitution requires very often too harsh reaction conditions to take place. Buchwald-Hartwig coupling[48] was therefore taken into account as a feasible synthetic strategy. Despite the use of a microwave or a sealed tube was required by the initial procedure to reach a temperature of 140 °C, treatment of **9** and 4-bromo-1-nitrobenzene with catalytic $Pd(OAc)_2$ (palladium acetate), catalytic BINAP (2,2'-bis(diphenylphosphino)-1,1'-binaftalene) as ligand and Cs_2CO_3 as a base in

refluxing toluene for 65 h resulted in a complete and smooth conversion. After purification by column chromatography on silica gel, product **10** was thus obtained in 90% yield.



Scheme 50. Side chain synthesis, 3rd step: Buchwald-Hartwig coupling

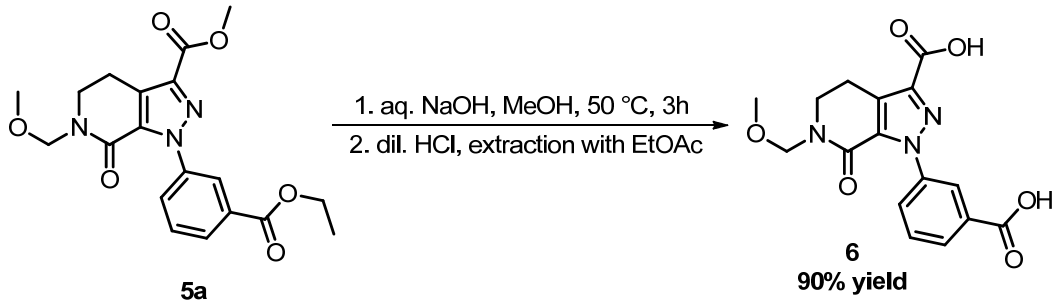
In the final step reduction of **10** to **11** (91% yield) was achieved again by treatment with ammonium formate with Pd/C in refluxing MeOH without any further purifications. The desired "side chain" amine **11** was thus obtained in overall (4 steps) 69% yield.



Scheme 51. Side chain synthesis, 4th step: reduction

3.4. Functionalization and deprotection

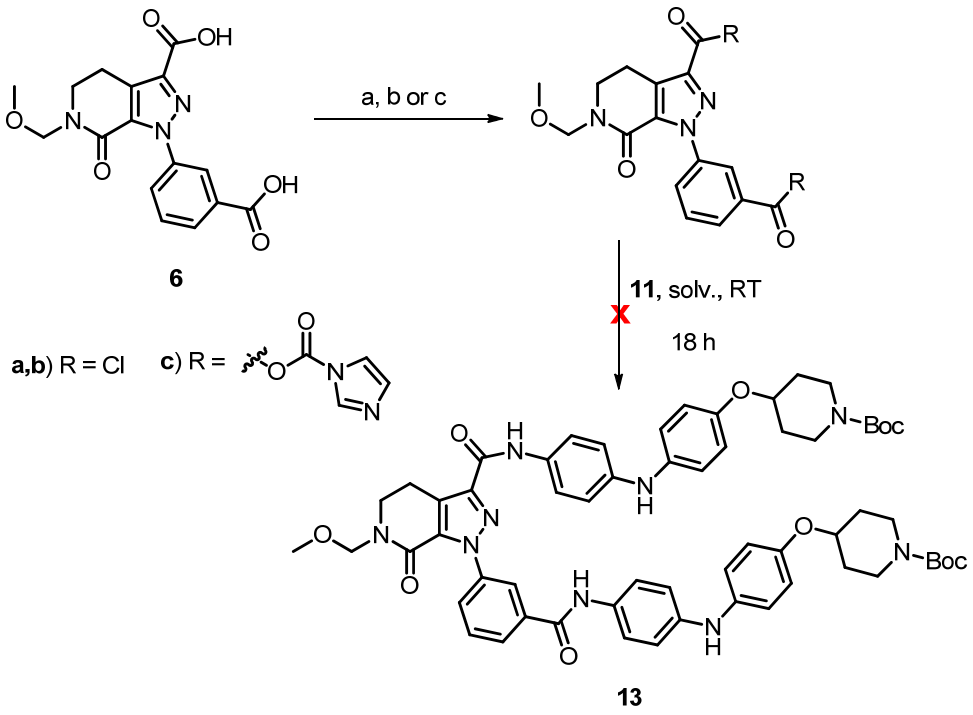
Initially, diacid **6** was easily obtained by saponification of diester **5a** (treatment with excess aqueous NaOH in hot methanol followed by protonation with diluted HCl and solvent extraction) in 90% yield.



Scheme 52. Saponification

Once the diacid core **6** and the amine "side chain" **11** were synthesized on the appropriate scale, our final efforts were focused on the coupling reaction to obtain the protected diamide **13**. Different activation methods were attempted, starting from the acid chloride. This desired reactive intermediate was formed easily both with thionyl chloride ($SOCl_2$) and oxalyl chloride ($C_2O_2Cl_2$). However, this activated intermediate failed to react with amine **11** in the subsequent acylic nucleophilic substitution reaction, leading only to decomposition byproducts.

Activation with CDI (N,N'-carbonyldiimidazole) was tried next and once again the reactive intermediate was formed but, due to its extreme insolubility both in THF and in DMF (N,N'-dimethylformamide), did not react with the amine and decomposed upon work up.

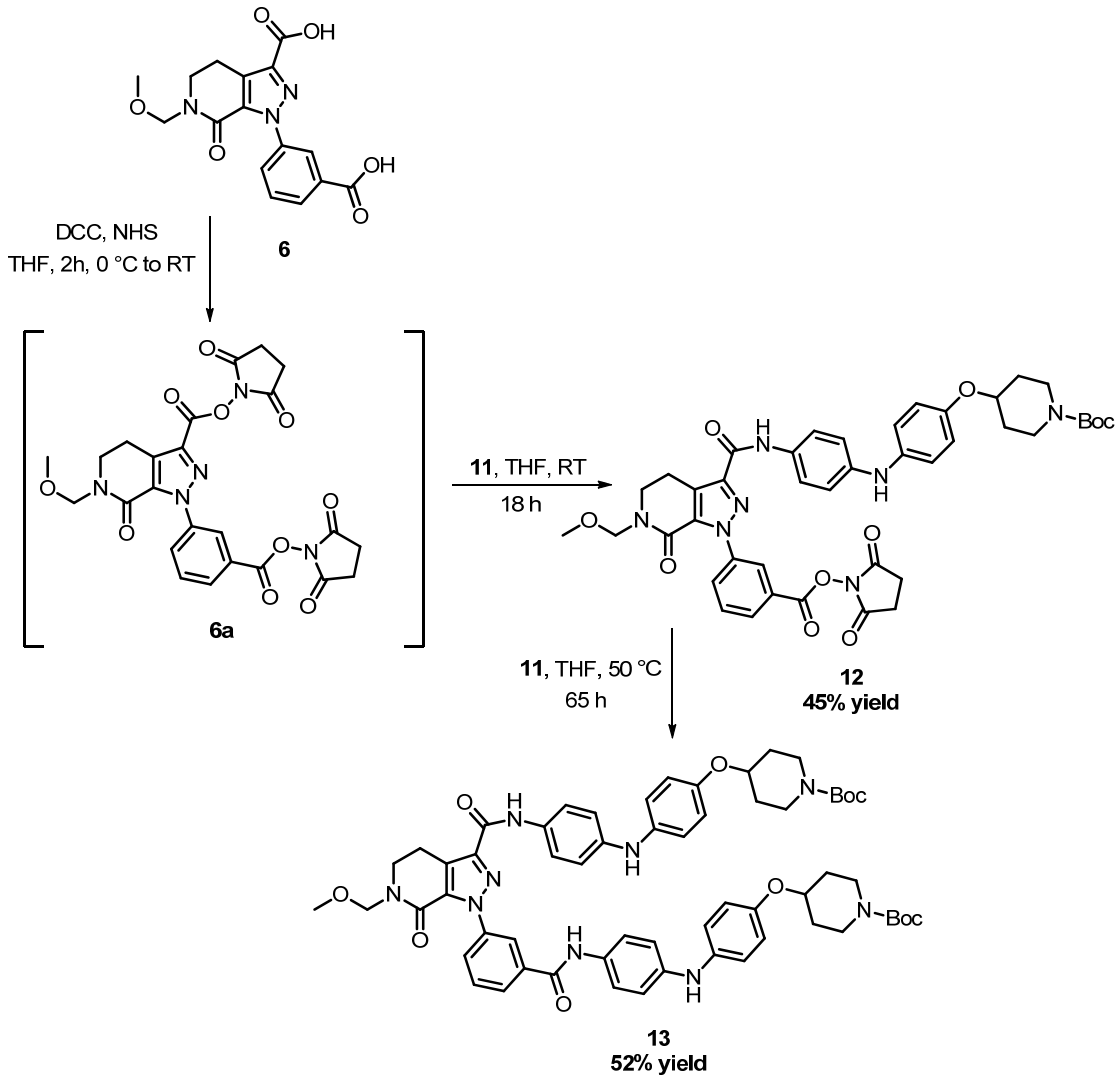


a) $SOCl_2$, neat, 50 °C, 2 h (solv = DCM); b) $(COCl)_2$, cat. DMF, neat, 0°C to RT, 2 h (solv. = DCM); c) CDI (1.5 eq), THF or DMF, RT, 2 h (R = , solv = THF or DMF)

Scheme 53. Failed activation methods

Finally, activation with DCC (N,N'-dicyclohexylcarbodiimide) and NHS (N-hydroxy sucinimide) in anhydrous THF worked well but reaction of **6a** with 2.2 equivalents of **11**, at room temperature, gave unexpectedly intermediate **12** as single product, which proved to be surprisingly stable towards aqueous work up and purification by column chromatography on silica gel (after which it was isolated in 45% yield and completely characterized).

While further treatment of purified **12** with 2 additional equivalents of **11** at 50 °C in THF for 65 h gave product **13** in 52% yield after purification by column chromatography on silica gel, any attempt to synthesize **13** directly from **6** was completely unsuccessful. On account of the enhanced stability of the NHS-ester group of **12**, both an activation with a catalytic amount (10 mol%) of NHS and a direct treatment of the double-activated intermediate **6a** with 5 equivalents of **11,** either at 50 °C or at RT, were attempted, but failed to give any trace of the desired product. For these reasons isolation of intermediate **12** seemed to be necessary for the process to be successful.



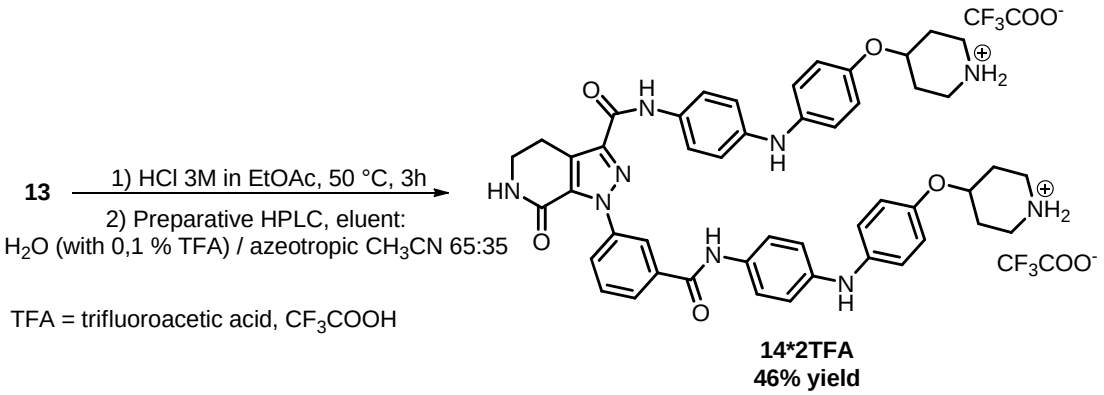
Scheme 54. Diamide formation

This additional purification step is undoubtedly a drawback in the process aiming for the double substituted amide product. However, the unexpected stability of the activated intermediate **12** discloses a very interesting path for further functionalization of the central scaffold with different "side chains", leading to unsymmetrically substituted amidic compounds. This could be a very convenient path to vary the affinity of the core for specific biological receptors and will be investigated by the research group in the next future.

In order to exploit this pathway, the structure of compound **12** must be exactly known. From ^{1}HNMR analysis it resulted that it exists in a regioisomerically pure form but, at present time, we are not completely sure about which of the two activated carboxylic acids of **6a** reacted with **11**. From preliminary ^{1}HNMR studies it is quite probable that structure **12** is correct but bidimensional NMR studies, along with attempts to obtain a

single crystal for X-rays diffraction, are ongoing in our research group, in order to confirm this initial hypothesis.

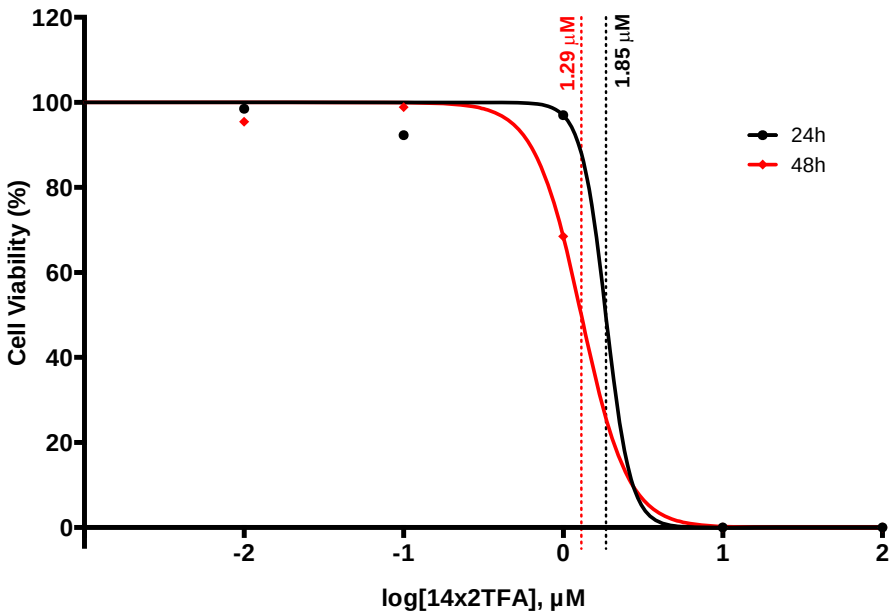
As the last step of the synthetic sequence, deprotection of the three protecting groups was achieved in a single reaction, by treatment of **13** with HCl 3M in EtOAc. This had been possible due to the initial introduction of MOM and Boc groups, both cleavable under acidic conditions. Purification by trituration in MeOH and subsequent semi-preparative HPLC furnished a high purity sample of **14**, suitable for *in vitro* biological tests.



Scheme 55. Deprotection and purification: obtainment of the final molecular target

3.5. Biological test

Molecule **14*2TFA** (from preparative HPLC) has been sent to the Biology Laboratory of Doctor Mario Chiariello, Istituto Toscano Tumori, Siena. This collaborating group performed a first test of cell viability *in vitro* on uterine cervical cancer cells (namely, HeLa cells). As one can see in **Scheme 56**, this showed a very good result of IC_{50} with a concentration of 1.3 μM after 48 hours of incubation and of 1.85 μM after 24 hours, which is comparable with the most common anticancer drugs.[12]



Scheme 56. Plot of the cell viability test. Continue curves represent the trend of the viability or cytotoxicity of HeLa cells treated with different concentrations of **14*2TFA**. Dotted vertical lines represent the value of IC_{50} after 24 or 48 hours of incubation.

4. Conclusions

In conclusion, a double functionalized bicyclic lactam-fused pyrazole system, with promising anticancer activity, has been obtained through a highly convergent synthetic strategy. Every step has been optimized in terms of yield, selectivity and feasibility and every intermediate has been fully characterized, in order to ensure a practical and reproducible pathway from inexpensive starting materials to a highly functionalized molecular target.

More in detail, a wide spectrum of synthetic strategies towards the dipolarophile (α,β-unsaturated δ-lactam **3**) has been investigated and the "selenoxide elimination" has been chosen as the best alternative to obtain the desired product in high yields and on a quite large laboratory scale.

Since the synthesis of the hydrazonoyl chloride **4** and the "side chain" **11** were already established on a small scale, we focused on laboratory scale-up and optimization. These features were completely achieved and very efficient synthetic pathways were found and adopted to obtain suitable amounts of high-purity key intermediates.

The construction of the central bicyclic core has been our main issue. A straightforward procedure, namely 1,3-dipolar cycloaddition followed by oxidative aromatization, established by our research group, has been employed. Screening of reaction conditions and characterization studies about the regioselectivity have been successfully performed.

Subsequently, functionalization *via* amide-bond formation was achieved through a two-steps procedure which serendipitously disclosed a very convenient synthetic pathway to construct a library of compounds on the same central core. This particular feature, along with a deepest characterization of key intermediate **12** will be developed by our research group in the next future.

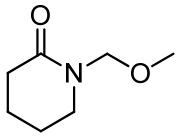
On the other end, a high purity sample of target compound **14** has been obtained. This showed a good result of IC_{50} towards one type of cancer cells in a first test *in vitro* performed by a collaborating biology group. More biological tests and chemical characterizations are ongoing now in our research groups.

5. Experimental section

General Methods. ^{1}H, ^{13}C NMR spectra were recorded on a Varian AS 300, 400 or 600 spectrometer. Chemical shifts (δ) are reported in ppm relative to residual solvent signals for ^{1}H and ^{13}C NMR. Multiplicity is explained in brackets as follow: "s", singlet; "d", doublet; "t", triplet; "q", quadruplet; "sept", septuplet; "m", multiplet; a "b" before the letter means "broad" ^{13}C NMR spectra were acquired with 1H broad band decoupled mode. Chromatographic purifications were performed using 70-230 mesh silica. Mass spectra were recorded on a micromass LCT spectrometer using electrospray (ES) ionisation techniques or using electron impact (EI) ionisation techniques. IR spectra were recorded on a Perkin-Elmer 177 in CCl_4 and on a FT-IR Perkin-Elmer 1600 in KBr. Fusion points were measured on a Buchi SMP-20 apparatus and are uncorrected.

Materials. Analytical grade solvents and commercially available reagents were used as received. Anhydrous THF was obtained by standing overnight on KOH, filtration through a short pad of basic alumina and distilled over Na/benzophenone. All the reactions demanding anhydrous conditions were performed in nitrogen atmosphere, passed through $CaCl_2$ and silica gel with indicator. Anhydrous toluene was obtained by distillation on Na. Dry DMF was obtained by overnight standing on activated 4 Å molecular sieves. Degassed MeOH was obtained by bubbling a nitrogen flux in an ice bath.

Synthesis of 1-(methoxymethyl)piperidin-2-one (1)



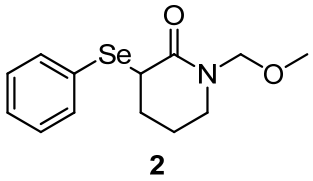
1

In an oven dried round bottom flask equipped with a magnetic stirring bar and under nitrogen atmosphere, 2-piperidinone (5.00 g, 50.4 mmol) was dissolved in 50 mL of anhydrous tetrahydrofuran (THF) and the solution was cooled to -50°C. Then *n*-butyllithium 1.6 M in hexanes (36 mL, 57.6 mmol) was added dropwise, the resulting mixture was placed in an ice bath and allowed to warm up to 0 °C. After 30 min, 4.2 mL of chloromethyl methyl ether (MOM-Cl, 55 mmol) were slowly added and the reaction was stirred at room temperature for 3h.

Hereafter, the reaction was quenched with water (20 mL), diluted with hexane and washed with water (50 mL) and brine (50 mL). The aqueous phases were extracted with dichloromethane (DCM, 3x50 mL), the combined organic extracts were washed again with brine (50 mL), dried over $MgSO_4$ and concentrated *in vacuo*. The crude yellow oil was purified either by column chromatography on silica gel (Ethyl Acetate (EtOAc)/Hexane (Hex) 2:1) or by vacuum distillation (3 mbar, 130 °C, oil bath temperature) to afford 5.50 g (38.5 mmol; 77% yield) of 1-methoxymethyl-piperidin-2-one **1** as a colourless oil.

^{1}HNMR (300 MHz, $CDCl_3$) δ = 4.79 (s, 2H); 3.35-3.32 (m, 2H); 3.28 (s, 3H); 2.42-2.39 (m, 2H); 1.82-1.77 (m, 4H) ppm. **^{13}CNMR** (100 MHz, $CDCl_3$) δ = 171.4; 77.3; 56.2; 46.5; 32.7; 23.3; 21.5 ppm. **GCMS** (EI) m/z = 143 $[M^+]$, 128 $[M - Me]^+$.

Synthesis of 1-(methoxymethyl)-3-(phenylselenyl)piperidin-2-one (2)



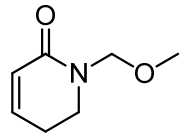
2

In an oven dried round bottom flask equipped with a magnetic stirring bar and under nitrogen atmosphere, diisopropylamine (2.97 mL, 21.2 mmol) was dissolved in 21 mL of anhydrous THF and the solution was cooled to -78°C. Then, n-butyllithium 1.6 M in hexanes (13.2 mL, 21.2 mmol) was added dropwise over a period of 20 min and the resulting mixture was stirred for additional 30 min. A solution of product **1** (2.76 g, 19.3 mmol) in anhydrous THF (14 mL) was then added dropwise and the reaction mixture was left stirring for 1.5 h at -78 °C. In another oven dried round bottom flask equipped with a magnetic stirring bar and under nitrogen atmosphere, the previously formed reaction mixture was added *via syringe*, in portions, to a solution of phenylselenyl bromide (5.00 g, 21.9 mmol) in anhydrous THF (20 mL) cooled to -78 °C. The resulting yellow suspension was stirred at -78 °C for 18 h.

Hereafter, the reaction was quenched with water (45 mL) and the THF was removed *in vacuo*. The mixture was extracted with DCM (3x50 mL), the organic extracts were washed with brine (50 mL), dried over $MgSO_4$ and concentrated *in vacuo*. The crude red oil was purified by column chromatography on silica gel (Hex/EtOAc 1:1) to afford 3.76 g (12.6 mmol, 65% yield) of **2** as an orange oil.

^{1}HNMR (300 MHz, $CDCl_3$) δ = 7.70-7.67 (m, 2H), 7.33-7.27 (m, 3H); 4.86-4.78 (m, 2H); 4.04 (t, J = 5.3 Hz, 1H); 3.41-3.35 (m, 2H); 3.30 (s, 3H); 2.22-2.00 (m, 3H); 1.87-1.75 (m, 1H) ppm. **^{13}CNMR** (100 MHz, $CDCl_3$) δ = 169.5; 134.2; 128.1; 127.2; 76.4; 55.1; 44.8; 41.8; 27.9; 19.9 ppm. **GCMS** (EI) m/z = 295 [M (^{76}Se)] $^+$, 296 [M (^{77}Se)]$^+$, 297 [M (^{78}Se)]$^+$, 299 [M (^{80}Se)]$^+$, 301 [M (^{82}Se)]$^+$, 142 [M - SePh]$^+$.

Synthesis of 1-(methoxymethyl)-5,6-dihydropyridin-2(1H)-one (3)



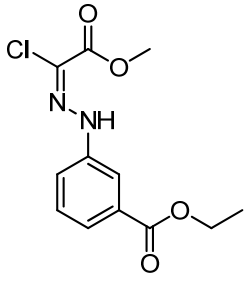
3

In a round bottom flask equipped with a magnetic stirring bar, product **2** (2.75 g, 9.22 mmol) was dissolved in THF (27.5 mL) and cooled to 0 °C. Then, a solution of H_2O_2 35% w/w in H_2O (1.79 g, 18.44 mmol) was slowly added and the reaction mixture was stirred for 30 min. The ice bath was removed, the temperature was allowed to rise slowly up to 25 °C (Caution: highly exothermic reaction!) and the reaction was stirred for 2 h.

Hereafter, water (20 mL) was added and the THF was removed *in vacuo*, The mixture was extracted with DCM (2x50 mL), the organic extracts were washed with a half saturated solution of $NaHCO_3$ (3x50 mL) and brine (50 mL), dried over $MgSO_4$ and evaporated *in vacuo*. The crude orange oil was purified by column chromatography on silica gel (DCM, then DCM/MeOH 20:1) to afford 1.16 g (8.19 mmol, 89% yield) of **3** as a pale brown oil.

^{1}HNMR (300 MHz, $CDCl_3$) δ = 6.63 (dt, J_1 = 9.9 Hz, J_2 = 4.30 Hz, 1H); 5.96 dt (J_1 = 9.8 Hz, J_2 = 1.87 Hz, 1H); 4.84 (s, 2H); 3.48 (t, J = 7.08 Hz, 2H), 3.31 (s, 3H), 2.44-2.38 (m, 2H) ppm. **^{13}CNMR** (100 MHz, $CDCl_3$) δ = 165.1; 140.9; 125.1; 76.8; 55.9; 43.6; 24.2 ppm. **IR** (CCl_4) ν = 2949.0, 2870.3, 1724.6, 1659.8 cm^{-1}. **ESIMS** 164 [M + Na^+]

Synthesis of ethyl 3-(2-(1-chloro-2-methoxy-2-oxoethylidene)hydrazinyl)benzoate (4)



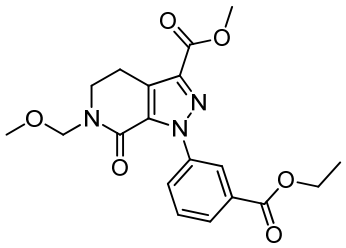
4

In a round bottom flask equipped with a magnetic stirring bar, ethyl 3-aminobenzoate (8.00 g, 48.4 mmol) was dissolved in methanol (MeOH, 50 mL), the solution was cooled to -20 °C and HCl 6N (100 mL, 600 mmol) was carefully added. Then $NaNO_2$ (6.68 g, 96.8 mmol) was added slowly in portions; the resulting mixture was stirred at 0 °C for 15 min and sodium acetate was added until pH = 5 was reached. Finally, a solution of 2-chloro-acetoacetate (7.28 g, 48.4 mmol) in 60 mL of MeOH was added dropwise through a dropping funnel; the ice bath was removed and the dense orange suspension was stirred at room temperature for 18 h.

After removal of all the MeOH *in vacuo*, the mixture was diluted with diethyl ether (Et_2O, 200 mL), washed with a saturated solution of $NaHCO_3$ (5x100 mL) until gas evolution was no longer observed, washed again with brine (2x75 mL), dried over MgSO4 and evaporated *in vacuo* to give 12.55 g (44.12 mmol, 91% yield) of pure **4** as an orange solid (f. p. = 108-110 °C).

^{1}**HNMR** (400 MHz, $CDCl_3$) δ = 8.40 (bs, 1H); 7.80 (m, 1H); 7.75-7.73 (m, 1H); 7.50-7.45 (m, 1H); 7.41-7.39 (m, 1H); 4.38 (q, J = 7.0 Hz, 2H); 3.95 (s, 3H); 1.40 (t, J = 7.2 Hz, 3H) ppm. ^{13}**CNMR** (75 MHz, $CDCl_3$) δ = 165.1; 160.1; 141.8; 131.8; 129.6; 124.1; 118.7; 116.5; 115.4; 61.2; 53.6; 14.3 ppm. **IR** (KBr) ν = 3273.4, 2988.4, 1732.9, 1717.0, 1557.6, 1076.1 cm^{-1}. **ESIMS** 307 [M (^{35}Cl) + Na^+], 309 [M (^{37}Cl) + Na^+]

Synthesis of methyl 1-(3-(ethoxycarbonyl)phenyl)-6-(methoxymethyl)-7-oxo-4,5,6,7-tetrahydro-1H-pyrazolo[3,4-c]pyridine-3-carboxylate (5a)



5a

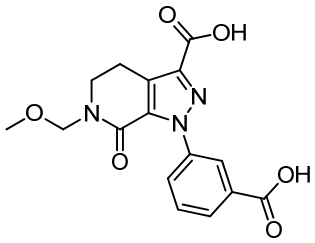
In an oven dried round bottom flask equipped with a magnetic stirring bar and under nitrogen atmosphere, product **3** (1.41 g, 10.0 mmol) and product **4** (4.27 g, 15.0 mmol) were stirred in toluene (60 mL) until a clear solution was obtained. Triethylamine (14.62 mL, 105 mmol) was then added and the reaction was refluxed at 110 °C for 65 h.

The reaction mixture was cooled to room temperature, filtered through a short plug of celite and evaporated *in vacuo*. The crude product was directly suspended in 140 mL of an 8:6 THF/water mixture and cooled to 0 °C. Cerium ammonium nitrate (CAN, 13.7 g, 25.0 mmol) was then added in portions and the reaction mixture was stirred at 0 °C for 2 h.

After removal of all the THF *in vacuo*, the mixture was extracted with EtOAc (3x75 mL), the organic extracts were washed with brine (75 mL), dried over $MgSO_4$ and evaporated *in vacuo*. The crude red sticky oil was purified by column chromatography on silica gel (Hex/EtOAc 1:1) to afford 1.32 g (3.4 mmol, 34% yield) of **5a** as a yellow solid (f. p. = 113-114 °C).

^{1}HNMR (300 MHz, $CDCl_3$) δ = 8.21 (m, 1H); 8.12 (m, 1H); 7.77-7.73 (m, 1H); 7.53 (t, J = 7.8 Hz, 1H); 4.29 (s, 2H); 4.37 (q, J = 7.2 Hz, 2H); 3.97 (s, 3H); 3.77 (t, J = 6.9 Hz, 2H); 3.32 (s, 3H); 3.22 (t, J = 6.9 Hz, 2H); 1.38 (t, J = 7.1, 3H) ppm. **^{13}CNMR** (100 MHz, $CDCl_3$) δ = 165.4; 162.2; 157.8; 139.5; 139.3; 132.8; 131.2; 130.1; 129.8; 128.6; 127.6; 126.8; 76.5; 61.3; 56.2, 52.2; 46.0; 21.0; 14.3 ppm. **IR** (KBr) ν = 2961.0, 1713.2, 1671.6, 1591.7, 1253.8, 1089.5 cm^{-1}. **ESIMS** 410 [M + Na^+]

Synthesis of 1-(3-carboxyphenyl)-6-(methoxymethyl)-7-oxo-4,5,6,7-tetrahydro-1H-pyrazolo[3,4-c]pyridine-3-carboxylic acid (6)



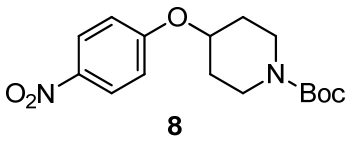
6

In round bottom flask equipped with a magnetic stirring bar, product **5a** (1.32 g, 3.4 mmol) was dissolved in MeOH (20 mL) and heated to 50 °C, then a solution of NaOH (0.54 g, 13.6 mmol) in 7 mL of water was added dropwise and the reaction was stirred for 3h.

Hereafter, water (10 mL) was added, the MeOH was removed *in vacuo* and the aqueous phase was washed with DCM (3x30 mL), acidified with HCl 1 N until pH = 1 was reached and extracted with EtOAc (3x30 mL). The combined organic extracts were dried over $MgSO_4$ and evaporated *in vacuo* to give 1.06 g of product **6** (3.06 mmol, 90% yield) as a red solid (f. p. = 200 °C with decomposition)

^{1}HNMR (400 MHz, CD_3OD) δ = 8.20 (m, 1H); 8.10 (m, 1H); 7.80 (m, 1H); 7.57 (m, 1H); 4.80 (s, 2H); 3.70 (t, J = 6.7 Hz, 2H); 3.25 (s, 3H); 3.10 (t, J = 6.9 Hz, 2H) ppm. **^{13}CNMR** (100 MHz, CD_3OD) δ = 171.1; 167.1; 162.1; 144.0; 143.4, 136.6, 135.2; 133.6, 133.4; 132.4, 132.0, 130.3, 80.3; 50.0; 24.6 ppm. **IR** (KBr) ν = 3011.8 1712.3, 1672.7, 1591.6, 1223.1, 760.1 cm^{-1}. **ESIMS** 368 [$M + Na^+$]

Synthesis of *tert*-butyl 4-(4-nitrophenoxy)piperidine-1-carboxylate (8)



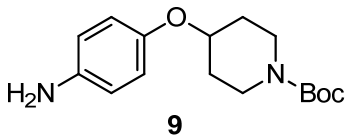
8

In an oven dried round bottom flask equipped with a magnetic stirring bar and under nitrogen atmosphere, NaH 60% in mineral oil (1.76 g, 44.0 mmol) was washed with hexane (3x20 mL) and dried at the high vacuum pump. The flask was filled again with nitrogen and placed in an ice bath; dry N,N'-dimethylacetamide (DMA, 130 mL) and **7** (N-Boc-4-hydroxypiperidine, 8.16 g, 40 mmol) were added in this order and stirred vigorously for 15 min. A solution of 1-fluoro-4-nitrobenzene (5.5 mL, 52.24 mmol) in 20 mL of dry DMA was then added in one portion (a moderate but prolonged heat evolution was observed) and the reaction mixture was stirred at room temperature for 4 h.

Hereafter, the reaction was quenched with a saturated solution of NH_4Cl (100 mL) and diluted with EtOAc (200 mL); the phases were separated and the organic extract was washed with brine (3x100 mL), dried over $MgSO_4$ and evaporated *in vacuo*. The crude brown oil was purified by column chromatography on silica gel (Hex/EtOAc 5:1) to give 11.42 g (35.4 mmol, 89% yield) of **8** as a bright yellow powder (f. p. = 100-102 °C).

^{1}HNMR (400 MHz, $CDCl_3$) δ = 8.21-8.17 (m, 2H); 6.97-6.93 (m, 2H); 4.60 (sept, J = 3.5 Hz, 1H); 3.72-3.66 (m, 2H); 3.41-3.34 (m, 2H); 1.99-1.92 (m, 2H); 1.82-1.74 (m, 2H); 1.47 (s, 9H) ppm. **^{13}CNMR** (100 MHz, $CDCl_3$) δ = 162.8; 155.1; 141.8; 126.4, 115.7, 80.2, 73.3; 40.8 (broad); 30.6; 28.8 ppm. **IR** (KBr) ν = 2954.2, 1698.2, 1590.8, 1509.3, 1412.9, 1340.5, 1262.3 cm^{-1}. **GCMS** (EI) m/z = 322 [M^+], 266 [M - *iso*butylene]$^+$, 249 [M - *i*BuO]$^+$, 222 [M – *iso*butylene - CO_2], 184 [M – $PhNO_2$, - H_2O]$^+$, 128 [M - $PhNO_2$, - H_2O – *iso*butylene]$^+$, 84 [M - $PhNO_2$, - H_2O - *iso*butylene - CO_2]$^+$.

Synthesis of *tert*-butyl 4-(4-aminophenoxy)piperidine-1-carboxylate (9)



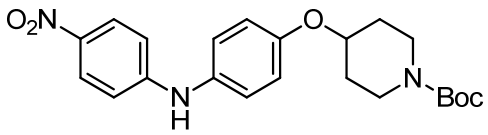
9

In a round bottom flask equipped with a magnetic stirring bar and under nitrogen atmosphere, product **8** (11.42 g, 35.4 mmol) and ammonium formate (21.52 g, 354 mmol) were dissolved in 210 mL of degassed MeOH and the solution was heated to 50 °C. 10% w/w Palladium on activated charcoal (1.10 g) was added and the reaction mixture was stirred at 90 °C for 5 h.

Hereafter, the reaction mixture was filtered through a short plug of celite, washed with DCM (100 mL) and evaporated *in vacuo* to give 9.28 g (32.2 mmol, 95% yield) of pure **9** as a brown solid (f. p. = 80-81 °C).

^{1}HNMR (300 MHz, $CDCl_3$) δ = 6.77-6.71 (m, 2H); 6.63-6.58 (m, 2H); 4.24 (sept, J = 3.6 Hz, 1H); 3.73-3.65 (m, 2H); 3.46 (bs, 2H); 3.29-3.21 (m, 2H); 1.90-1.81 (m, 2H); 1.73-1.62 (m, 2H); 1.45 (s, 9H) ppm. **^{13}CNMR** (100 MHz, $CDCl_3$) δ = 154.9; 150.0; 140.7, 118.3; 116.4; 79.5; 73.7; 40.7 (broad); 30.8; 28.4 ppm. **IR** (KBr) ν = 3460.0, 3361.6, 2955.4, 1685.1, 1509.9, 1419.4, 1229.0, 1049.6 cm^{-1}. **GCMS** (EI) m/z = 291 $[M^+]$, 219 [M - *i*BuO]$^+$, 192 [M – *iso*butylene - CO_2], 184 [M – $PhNH_2$, - H_2O]$^+$, 128 [M - $PhNH_2$, - H_2O - isobutylene]$^+$, 109 [4-aminophenol]$^+$, 84 [M - $PhNH_2$, - H_2O - *iso*butylene - CO_2]$^+$.

Synthesis of *tert*-butyl 4-(4-((4-nitrophenyl)amino)phenoxy)piperidine-1-carboxylate (10)



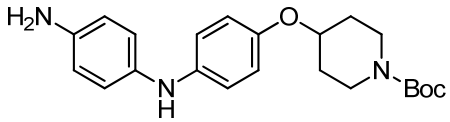
10

In an oven dried round bottom flask equipped with a magnetic stirring bar and under nitrogen atmosphere, product **9** (9.22 g, 32 mmol), 1-bromo-4-nitrobenzene (6.45 g, 32 mmol), palladium acetate (1.10 g, 4.8 mmol), 2,2′-Bis(diphenylphosphino)-1,1′-binaphthalene (BINAP, 3.00 g, 4.8 mmol) and Cs_2CO_3 (13.9 g, 42.7 mmol) were suspended in anhydrous toluene (270 mL) and the resulting mixture was refluxed for 65 h.

Hereafter, the reaction mixture was filtered through a short plug of celite, washed with DCM (150 mL) and evaporated *in vacuo*. The crude brown solid was purified by column chromatography on silica gel (Hex/EtOAc 2:1) to afford 11.93 g (28.9 mmol, 90% yield) of **10** as an intense orange solid (f. p. = 150-152 °C).

^{1}HNMR (400 MHz, $CDCl_3$) δ = 8.10-8.06 (m, 2H); 7.16-7.12 (m, 2H); 6.95-6.91 (m, 2H); 6.80-6.76 (m, 2H); 6.26 (bs, 1H); 4.45 (sept, J = 3.7 Hz, 1H); 3.74-3.68 (m, 2H); 3.37-3.31 (m, 2H); 1.97-1.89 (m, 2H); 1.80-1.72 (m, 2H); 1.47 (s, 9H) ppm. **^{13}CNMR** (100 MHz, $CDCl_3$) δ = 155.23; 155.19; 151.9; 139.5; 132.8; 126.6; 125.6; 117.6; 113.1; 80.0; 73.1; 40.9 (broad); 30.8; 28.8 ppm. **IR** (KBr) ν = 3317.3, 2936.1, 1669.1, 1597.5, 1508.2, 1323.1, 1242.4 cm^{-1}. **ESIMS** 436 [M + Na^+].

Synthesis of *tert*-butyl 4-(4-((4-aminophenyl)amino)phenoxy)piperidine-1-carboxylate (11)



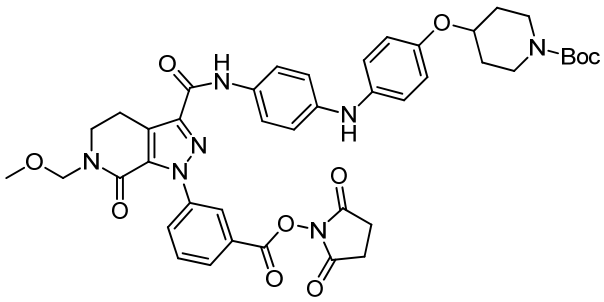
11

In a round bottom flask equipped with a magnetic stirring bar and under nitrogen atmosphere, product **10** (2.57 g, 6.0 mmol) and ammonium formate (3.43 g, 54.5 mmol) were dissolved in 60 mL of degassed MeOH and the solution was heated to 50 °C. 10% w/w Palladium on activated charcoal (0.23 g) was added and the reaction mixture was stirred at 90 °C for 5 h.

Hereafter, the reaction mixture was filtered through a short plug of celite, washed with DCM (40mL) and evaporated *in vacuo*. The crude product was suspended in DCM (50 mL), filtered again on celite and washed with DCM (50 mL) to give 2.10 g (5.5 mmol, 91% yield) of pure **11** as a brown-violet solid (f. p. = 144-145 °C).

^{1}HNMR (300 MHz, $CDCl_3$) δ = 6.89-6.77 (m, 6H); 6.66-6.61 (m, 2H); 4.30 (sept, J = 4.0 Hz); 3.89 (bs, 3H); 3.75-3.67 (m, 2H); 3.32-3.-24 (m, 2H); 1.93-1.84 (m, 2H); 1.76-1.65 (m, 2H); 1.47 (s, 9H) ppm. **^{13}CNMR** (75 MHz, $CDCl_3$) δ = 154.9; 150.6; 141.3; 139.9; 135.4; 121.4; 117.9; 117.8; 116.3; 79.5; 73.4; 40.8 (broad); 30.7; 28.5 ppm. **IR** (KBr) ν = 3462.2, 3367.5, 2960.0, 1684.8, 1 1506.4, 1424.3, 1228.9, 1057.3 cm^{-1}. **ESIMS** 406 [M + Na^+].

Synthesis of Intermediate 12



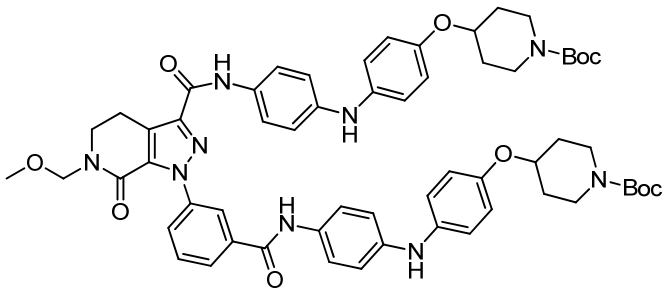
12

In an oven dried Schlenk flask equipped with a magnetic stirring bar and under nitrogen atmosphere, diacid **6** (0.173 g, 0.5 mmol) and N-hydroxysuccinimide (NHS, 0.173 g, 1.5 mmol) were dissolved in anhydrous THF (7.5 mL). The flask was placed in an ice bath, N,N'-dicyclohexylcarbodiimide (0.309 g, 1.5 mmol) was added slowly in portions and the reaction mixture was left stirring for 2 h at room temperature (a white precipitate was readily formed). A solution of amine **11** (0.460 g, 1.2 mmol) in anhydrous THF (3.5 mL) was then added dropwise at 0°C and the resulting mixture was left stirring for 18 h at room temperature.

Hereafter, the reaction mixture was filtered through a short pad of celite, diluted with EtOAc (30 mL) and washed rapidly with water (4x20 mL) and brine (20 mL). The organic phase was dried over $MgSO_4$ and evaporated *in vacuo*. The crude dark solid was purified by column chromatography on silica gel (EtOAc/Hex 5:2) to afford 0.181 g (0.224 mmol, 45% yield) of intermediate **12** as a dark yellow solid (f. p. = 127-130 °C).

^{1}HNMR (600 MHz, $CDCl_3$) δ = 8.65 (bs, 1H); 8.35 (m, 1H); 8.20 (m, 1H); 7.95-7.91 (m, 1H); 7.66 (t, J = 8.2, 1H); 7.56-7.54 (m, 2H); 7.04-7.01 (m, 2H); 6.96-6.94 (m, 2H); 6.88-6.85 (m, 2H); 5.50 (bs, 1H); 4.91 (s, 2H); 4.37 (m, 1H); 3.79 (t, J = 7.1 Hz, 2H); 3.74-3.70 (m, 2H); 3.35-3.29 (m, 4H overlapped with s, 3H); 2.92 (bs, 4H); 1.93-1.89 (m, 2H); 1.77-1.71 (m, 2H); 1.47 (s, 9H) ppm. **^{13}CNMR** (75 MHz, $CDCl_3$) δ = 168.0; 160.0; 158.0; 156.9; 153.9; 151.3; 141.5; 140.5; 138.7; 135.7; 132.3; 130.9; 129.7; 128.9; 128.1; 126.3; 125.8; 124.9; 120.6; 120.0; 116.6; 115.8; 78.6; 76.2; 72.1; 55.2; 45.2; 39.6 (broad); 29.6; 27.4; 24.7; 19.8 ppm. **IR** (KBr) ν = 3497.0, 3350.9, 2929.0, 1773.4, 1741.0, 1674.3, 1595.4, 1504.3, 1423.4, 1229.2 cm^{-1}. **ESIMS** 830 [M + Na^+].

Synthesis of protected diamide 13



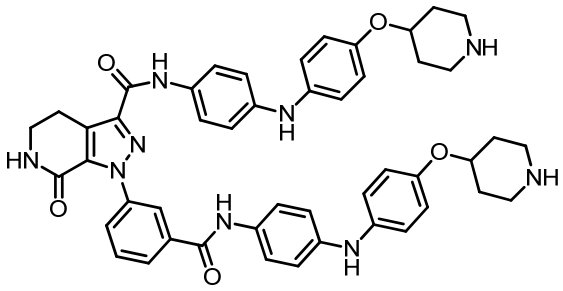
13

In an oven dried Schlenk flask equipped with a magnetic stirring bar and under nitrogen atmosphere, intermediate **12** (0.140 g, 0.173 mmol) and amine **11** were dissolved in anhydrous THF (1.2 mL) and the resulting solution was stirred at 50 °C for 65 h.

Hereafter, the reaction mixture was diluted with EtOAc (15 mL), washed with water (2x15 mL) and brine (15 mL), dried over MgSO4 and evaporated *in vacuo*. The crude product was purified by column chromatography on silica gel (EtOAc/Hex 5:2) to afford 0.095 g (0.09 mmol, 52% yield) of diamide 13 as a dark green solid (f. p. = 200 °C with decomposition).

^{1}**HNMR** (400 MHz, $CDCl_3$) δ = 8.64 (bs, 1H); 8.13 (m, 1H); 8.05 (bs, 1H); 7.91 (m, 1H); 7.76-7.74 (m, 1H); 7.56 (t, J = 7.9 Hz, 1H); 7.53-7.47 (m, 4H); 7.04-7.00 (m, 4H); 6.95-6.91 (m, 4H); 6.88-6.84 (m, 4H); 5.52 (bs, 2H); 4.90 (s, 2H); 4.37 (m, 2H); 3.78-3.69 (m, 6H); 3.34-3.28 (m, 6H overlapped with s, 3H); 1.94-1.87 (m, 4H); 1.78-1.70 (m, 4H); 1.47 (s, 18 H) ppm. ^{13}**CNMR** (100 MHz, $CDCl_3$) δ = 163.4; 158.1; 157.1; 153.9; 151.3; 141.1; 140.7; 140.6; 138.2; 135.6; 134.8; 132.0; 129.4; 128.8; 127.8; 126.9; 125.9; 123.1; 121.0; 120.6; 120.04; 120.02; 116.6; 115.8; 115.7; 78.6; 76.2; 72.1;55.2; 45.3; 39.7 (broad); 29.6; 27.4; 19.9 ppm. **IR** (KBr) ν = 3493.5, 3360.2, 2929.8, 1773.6, 1749.3, 1672.9, 1598.3, 1423.5, 1229.2 cm^{-1}. **ESIMS** 1098 [M + Na^+].

Synthesis of final target 14



14

In a small vial, 1 mL of HCl (12 M) and 3 mL of EtOAc were vigorously shacked until a homogeneous phase is obtained. In a test tube equipped with a magnetic stirring bar compound **13** (0.080 g, 0.075 mmol) was vigorously stirred in 1.5 mL of the previously prepared solution for 3 h at 50 °C (complete dissolution and subsequent precipitation were observed).

Hereafter, the reaction mixture is evaporated *in vacuo* to obtain a crude green solid that can be purified by trituration in MeOH (3 mL), affording 0.031 g (0.034 mmol, 46% yield) of **14*2HCl** as a green solid. A high purity sample may be obtained by purification with semi-preparative HPLC (stationary phase: C_{18} "hydro"; eluent: 65% H_2O with 0.01% TFA/35% azeotropic CH_3CN), giving **14*2TFA** after evaporation of the solvent.

^{1}HNMR (600 MHz, DMSO, d_6) δ = 10.18 (s, 1H); 10.04 (s, 1H); 8.44 (bs, 4H); 8.21 (s, 1H); 8.03 (m, 2H); 7.96-7.81 (m, 3H); 7.66 (s, 1H); 7.60 (m, 4H); 7.09-6.74 (m, 12H); 4.49 (bm, 2H); 3.50 (bm, 2H); 3.25 (bm, 4H); 3.07 (bm, 6H); 2.05 (bm, 4H); 1.80 (bm, 4H) ppm. **ESIMS** 417 [M of **14** + 2]$^{2+}$

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