Luísa da Conceição Costa Rainho de Carvalho

Mestre em Química

Development of Novel Benzimidazole-based COX Inhibitors New Synthetic Strategies and Screening of Heterocyclic Structures

Dissertação para obtenção do Grau de Doutor em Química, especialidade Química Orgânica

Orientador: Doutora Maria Manuel Marques, Investigadora Auxiliar, FCT-UNL
Co-orientador: Professora Doutora Eduarda Fernandes, Professora Associada com Agregação, FF-UP

Júri:

Presidente: Prof. Dr.ª Ana Aguiar Ricardo, Prof. Catedrática, FCT-UNL
Arguentes: Prof. Dr. Rui Moreira, Prof. Catedrático, FF-UL
Dr. Pedro Góis, Investigador FCT, FF-UL
Vogais: Prof. Dr. Nuno Maulide, Full Professor, University of Vienna
Prof. Dr. Eurico Cabrita, Professor Auxiliar, FCT-UNL

Maio de 2014

Luísa da Conceição Costa Rainho de Carvalho

Development of Novel Benzimidazole-based COX Inhibitors: New Synthetic Strategies and Screening of Heterocyclic Structures

Lisboa

2014

“Learn from yesterday, live for today, hope for tomorrow.
The important thing is not to stop questioning.”
Albert Einstein

“Nothing in life is to be feared, it is only to be understood.
Now is the time to understand more, so that we may fear less.”
Marie Curie

Acknowledgements

First of all I would like to express my gratitude to both my research advisors, Dr. Maria Manuel Marques and Prof. Dr. Eduarda Fernandes for all the scientific guidance, encouragement and dedication during this fruitful journey.

I want to personally thank to Dr. Maria Manuel Marques for the high quality scientific support that allowed me to grow as a student and researcher. I am sure that the last seven years of collaborative work generated not only two rewarding thesis but also a long-lasting friendship.

I want also to express a special acknowledgement to Prof. Dr. Eduarda Fernandes for receiving me in her laboratory in Faculdade de Farmácia do Porto, and for all the support given to the biological experiments necessary to the present thesis. I am also grateful to her PhD student, Daniela Ribeiro, for all the diligent support and valuable explanations in a foreign area to myself, and to all other Fernandes´s group elements that kindly received me.

I am much obliged to Prof. Dr. Eurico Cabrita and his entire group, especially to Dr. Aldino Viegas, for all the precious help and discussions, and for allowing me to perform time consuming experiments in the NMR machines. I also acknowledge Portuguese Nuclear Magnetic Resonance Network.

I want to acknowledge Prof. Dr. Nuno Maulide, for receiving me in his former laboratory at Max Planck Institute fur Kohlenforshung, for all the support and for giving me the opportunity to contact with nice brilliant people. I would also like to express my acknowledgments to his entire group as well as to Ben List group that gently accepted me. A personal gratitude goes to those with whom I worked more closely: Desislava Petkova, Dr. Daniel O'Donovan, and to Dr. Bo Peng for all the valuable discussions, suggestions and friendship. Many thanks go to Eckhard Wöstefeld and Gerlinde Giessner for being always so friendly and helpful solving the laboratorial and administrative issues. I could also not forget Hélio Faustino with whom I shared this great experience in the MPI and that was a great companion during our stay in Mülheim-am-der-Ruhr.

I am grateful to the analytical departments, namely the NMR and MS technicians, and to Fernanda Alves and Ana Silva, for all the kindness, patience and laboratorial support.

I would also thank to Prof. Dr. Teresa Avilés and to Dr. Vitor Rosa for allowing me to use the lab installations to perform air sensitive reactions.

I acknowledge to Prof. José Graça and to Dr. Sara Santos in Instituto Superior de Agronomia for the compounds isolation in preparative HPLC.

I gratefully acknowledge to Fundação para a Ciência e Tecnologia the funding sources (SFRH/BD/63407/2009 and PTDC/QUI/65187/2006) that made my PhD possible.

I am mostly grateful to Monica Estevão, my PhD fellow and friend, that grow up with me along this path, with whom I shared all the expectations and failures, for all the unspoken understanding, for the good discussions and assistance, for the undoubtedly excellent collaboration that evolved into our friendship.

I would also want to thank to the newbie in the PhD saga, Marina Pires, for all the optimism brought to our group, for the fresh inquisitive spirit and friendship.

I also have to thank to those that crossed my way during the time spent in the laboratory 202, and that influenced me in different and certainly prolific ways. I know that besides the rough moments I will keep fond memories of the Phd time!

I would also express my recognition to Prof. Dr. Maria Manuela Brotas de Carvalho, that inspired me to pursue Chemistry, and that encouraged me along my entire academic path. I would also manifest my true affection to her family for all optimism, support and friendship.

To all my friends I express my deep gratitude for their comprehension and support during this endeavor.

I also want to express a profound acknowledgement to Luís Pinto, that changed my life in ways (that I think) he doesn't realize. For being gentle and concerned about everybody, for being a model of hard work and to truly understand the crazy time spent in an Organic Chemistry lab and how it shapes our perception of life. For being there for me in the good and bad moments, my sincere love.

Um grande obrigado ao meu pai e à minha mãe, que nunca impuseram restrições no caminho que decidi seguir e que sempre acolheram e incentivaram com otimismo todos os meus objetivos.

Resumo

O trabalho descrito nesta dissertação teve como principal objetivo a síntese de novos inibidores selectivos da ciclooxigenase-2 (COX-2) baseados no núcleo de benzimidazole. A biblioteca de compostos foi construída através de uma estratégia racional de *design* de fármacos, recorrendo a diferentes metodologias de avaliação: *docking*, avaliação biológica e estudos de STD-RMN.

Após aprofundados estudos de *docking* que tiveram por base a avaliação de diversos núcleos heterocíclicos, foi escolhida uma biblioteca de compostos cujos resultados foram os mais promissores. Os compostos foram avaliados tendo por base a premissa de que compostos com uma distribuição espacial em y dos substituintes aromáticos, possuem elevada capacidade de inibição seletiva da COX-2. Assim, o padrão de substituição ideal do núcleo de benzimidazole incluiu a substituição das posições *N*-1 e C-2 com anéis aromáticos, sendo que em *N*-1 o anel se encontra substituído em *meta*, enquanto o anel em C-2 contém um grupo sulfonamida em posição *para*. Foi ainda verificado por *docking*, que a presença de uma cadeia alquilíca contendo um grupo carboxílico ou ester na posição 4 do anel de benzimidazole, serviria de "âncora" formando interações relevantes com resíduos específicos no centro activo da proteína. Assim, a biblioteca de compostos foi sintetizada com sucesso utilizando diferentes estratégias sintéticas, sendo que um dos passos chave para formar o núcleo benzimidazólico englobou o acoplamento cruzado C-N catalisado por paládio. Os compostos foram obtidos com rendimentos globais de 27 a 41% para os derivados éster e ácido, ao final de sete e oito passos de síntese, respetivamente.

A biblioteca de compostos foi testada de modo a avaliar a atividade inibitória dos mesmos para a COX-1 e COX-2, bem como determinar a sua seletividade para a COX-2, em sangue humano total. Os resultados demonstram que os derivados ácido (**II.78a-e**) não foram ativos ou tiveram uma fraca atividade, quer para a COX-1 quer para a COX-2, e.g. o composto **II.78d** apresentou percentagens de inibição iguais a 23 ± 8% e 18 ± 2% (50 μM), respetivamente. Ao invés, os derivados éster (**II.77a-e**) revelaram-se inibidores potentes da COX-2, alguns dos quais com percentagens de inibição semelhantes à do celecoxib (72 ± 10 %, a 5 μM), usado como controlo. De facto, o composto **II.77d** foi aquele que demonstrou os melhores resultados, i.e. 60 ± 8%, para uma concentração igual a 0.5 μM. No entanto, apesar da menor (mas significativa) percentagem de inibição, os compostos **II.77a-e** também inibiram a COX-1 demonstrando uma fraca seletividade para a COX-2, e.g. a percentagem de inibição obtida para **II.77d** foi de 41 ± 14% (0.625 μM).

Os estudos de RMN por diferença de transferência de saturação (STD) mostraram que ambos os derivados ácido e éster interagem com a COX-2. No entanto, através de estudos de competição com fármacos conhecidos (ibuprofeno, naproxeno e diclofenac), é sugerido que os derivados ácido (**II.78a-e**) interagem com a COX-2 num terceiro local de ligação. Ao invés, os estudos de competição efetuados com **II.77a**, sugerem que este tipo de compostos compete com o naproxeno pelo mesmo local, não competindo pelo local de ligação do diclofenac. Este resultado indica que os benzimidazoles **II.77a-e** se ligam no centro ativo da COX-2de forma similar ao naproxeno.

Os resultados obtidos com os estudos de STD-NMR corroboram os resultados da avaliação biológica, sendo que os ácidos não se ligam a qualquer centro de decisão na enzima, ao contrário dos ésteres que parecem inibir a COX-2 com igual potência e de forma similar ao naproxeno. Foram também parcialmente validados os resultados obtidos por *docking*, já que este conseguiu prever a elevada potência dos compostos, mas falhou na previsão da seletividade dos mesmos.

Dada a relevância da ligação C-N em compostos heterocíclicos, tais como benzimidazoles, e a necessidade de desenvolver uma síntese mais sustentável para estes compostos, foi desenvolvida uma metodologia para as reações de acoplamento cruzado C-N em fase sólida. Assim foram efetuados estudos de arilaminação catalisada por paládio de halo-arenos covalentemente ligados a um suporte sólido solúvel – o polietilenoglicol (PEG) 2000, atuando este como suporte sólido e como solvente. O acoplamento C-N entre as diferentes anilinas e os (*para*- ou *orto*-) bromo ou (*para*- ou *meta*-) cloro arenos imobilizados em PEG, foi efetuado com sucesso na ausência de qualquer solvente orgânico, usando o sistema catalítico Pd_2dba_3/XPhos. Os rendimentos obtidos variaram entre os 40 a 100%. Tentou-se ainda o mesmo tipo de acoplamento com aminas alquilícas e hidrazinas, apesar da reação para este tipo de substratos não se mostrar adequada.

Nesta dissertação são também descritos os estudos efetuados para a síntese enantioselectiva de estruturas heterocíclicas, aplicando a metodologia desenvolvida no grupo do Prof. Dr. Nuno Maulide: a ativação electrofilica de amidas com concomitante rearranjo de Claisen. Deste modo foram feitos esforços para a síntese enantioselectiva de cromanonas e coumaranonas, bem como dos correspondentes derivados azotados, as indolinonas e dihidroquinolinonas. No entanto, verificou-se que apesar de a metodologia funcionar para alguns tipos de substratos, a hidrólise dos auxiliares quirais usados revelou-se uma tarefa difícil.

Sendo o grupo sulfonamida um importante farmacóforo amplamente utilizado, foram realizados diversos ensaios com o objetivo de obter um reagente de sulfonilação versátil para a preparação de sulfonamidas. Os ensaios realizados consistiram em utilizar metabissulfito de potássio ou trimetilsilil clorosulfonato como fonte de SO_2. Enquanto a utilização do metabisulfito de potássio se revelou infrutífera, no segundo caso foi obtido o correspondente cloreto de sulfonilo ainda que em quantidades vestigiais.

Os resultados descritos nesta dissertação demonstram o sucesso na preparação de novos derivados heterocíclicos com relevante atividade anti-inflamatória. É também reportado o desenvolvimento de importantes metodologias para a síntese deste tipo de compostos. Este trabalho permitiu ainda elucidar alguns dos mecanismos envolvidos na atividade inibitória da COX, através de estudos de interação entre os compostos sintetizados e as proteínas estudadas. Desta forma este trabalho contribuiu para o *design* racional de novas entidades com elevado potencial como fármacos anti-inflamatórios.

Termos chave: síntese, heterociclos, benzimidazole, química medicinal, inibidores da COX.

Abstract

The main objective of this dissertation was the synthesis of novel selective cycloxygenase-2 (COX-2) inhibitors based on the benzimidazole scaffold. The generated library was constructed using a rational design strategy where several screening methodologies were used in a synergic effort: docking studies, biological evaluation and STD-NMR monitoring.

Drawing from initial docking studies that evaluated several heterocyclic scaffolds, benzimidazole structures were chosen as the most promising inhibitors. The compounds were analysed based on the known correlation between the y shape drugs (possessing two aryl substituents in 1,2 relationship) and its high inhibitory activity and selectivity towards COX-2. Thus, the ideal substitution pattern of benzimidazole scaffold included the *N*-1 and C-2 substitution with aromatic rings: a *meta* substituted ring in *N*-1, whereas the C-2 aromatic ring contains a sulfonamide in *para* position. It was also verified by docking, that the presence of an alkylic chain possessing an acid or an ester group in position 4 could act as an anchor, forming relevant interactions with specific residues at the active site of the protein. Thus, the library was successfully synthesized employing several synthetic approaches, where the C-N cross-coupling reaction was one of the key steps to attain the benzimidazole ring. The compounds were achieved with overall yields of 27 to 41% for the ester and acid derivatives, after seven and eight synthetic steps, respectively.

The library of compounds was biologically evaluated by a whole blood assay (WBA) in order to verify the inhibitory activity towards COX-1 and COX-2, as well as to determine its COX-2 selectivity. The results show that the acid derivatives (**II.78a-e**) were not active or exhibit a poor inhibition either for COX-1 and COX-2, e.g. **II.78d** yielded inhibitory percentages of 23 ± 8% and 18 ± 2% (at 50 μM), respectively. In opposition, the ester derivatives (**II.77a-e**) were excellent inhibitors towards COX-2, some of which showing similar inhibition percentages to celecoxib (72 ± 10 %, 5 μM), used as control. In fact, compound **II.77d** obtained the best results, i.e. 60 ± 8%, at 0.5 μM. However, although the lower (but significant) inhibitory percentage, compounds **II.77a-e** also inhibited COX-1 showing a poor selectivity towards COX-2, e.g. the inhibitory percentage obtained for **II.77d** was 41 ± 14% (0.625 μM).

The saturation transfer difference (STD) NMR experiments demonstrated that both ester and acid derivatives interact with COX-2. However, from the several competition studies conducted against known drugs (ibuprofen, naproxen and diclofenac), it is suggested that the acid compounds (**II.78a-e**) can potentially bind to a third binding site. On the other hand, the competitive studies performed with **II.77a**, indicate that the ester derivatives bind in naproxen site and that do not compete for diclofenac binding site. This result suggests that benzimidazoles **II.77a-e** have a similar binding behavior such as naproxen.

The STD-NMR studies corroborate the biological evaluation results, showing that the acids do not bind to any decision centre in the enzyme in opposition to the esters that seem to inhibit COX-2 in a similar way as naproxen. The results obtained by docking were also partially validated, since the method established the high potency of the ester-based benzimidazoles, although failing to predict its unselective character.

Due to the relevance of C-N bond formation in heterocyclic compounds, such as benzimidazoles, and the need of a more sustainable synthesis for these compounds, it was developed a C-N cross-coupling methodology in solid-phase. Thus, several studies were performed on Pd-catalyzed arylamination using several PEG bound aryl halides and aromatic amines, where PEG 2000 acted as solvent and solid-support. The C-N cross-coupling of several substituted anilines and the PEG bound (*para*- or *ortho*-) bromo or (*para*- or *meta*-) cloro arenes was successfully achieved under solventless conditions, using the Pd_2dba_3/XPhos catalytic system. The obtained yields varied from 40 to 100%. The same cross-coupling reaction was attempted using alkyl amines and hydrazines, however without successful results.

In the present dissertation are also described the studies towards the enantioselective synthesis of heterocyclic structures, applying the methodology developed by Maulide's group: the amide electrophilic activation with concomitant Claisen rearragement. Several efforts were conducted to enanteoselectively synthesize chromanones and coumaranones, as well as the nitrogenated counterparts, the indolinones and dihydroquinolinones. However, despite the success of the method for some derivatives, the hydrolysis of the chiral auxiliaries was a difficult endeavor.

Since the sulfonamide group is an important and widely used pharmacophore, several experiments were conducted in order to obtain a versatile sulfonylation reagent to prepare sulfonamides. The experiments used potassium metabisulfite and trimethylsilylchlorosulfonate as SO_2 surrogates. While the approach using potassium metabisulfite was unsuccessful, in the second case it was obtained trace amount of the corresponding sulfonyl chloride.

The results described in this dissertation demonstrate the successful preparation of novel heterocyclic derivatives with relevant anti-inflammatory activity. It is also reported the development of important methodologies to synthesize these compounds. This work also allowed the elucidation some of the mechanisms involved in COX inhibitory activity, *via* interaction studies between the prepared compounds and the studied proteins. Thus, this work has contributed to the rational design of new entities with high potential as anti-inflammatory drugs.

Keywords: synthesis, heterocycles, benzimidazole, medicinal chemistry, COX inhibitors.

Contents

List of Schemes

List of Figures

List of Tables

Abbreviations and Symbols

AA	arachidonic acid
Ac	acetyl
Al	alkyl, aliphatic
Ar	aryl, aromatic
aq.	aqueous
BINAP	2,2'-bis(diphenylphosphino)-1,1'-binaphthyl
Bn	benzyl
Boc	*tert*-butyloxycarbonyl
BOP	benzotriazol-1-yloxytris(dimethyl-amino)-phosphonium hexafluorophosphate
Bu	butyl
calcd	calculated
cat.	catalyst
CDI	*N*, *N*'-carbonyldiimidazole
conv.	conversion
COX	cyclooxygenase
Cy	cyclohexyl
d	doublet
DABCO	1,4-diazabicyclo[2.2.2]octane
DABSO	DABCO-bis(sulfur dioxide)
dba	dibenzylideneacetone
DCC	dicyclohexylcarbodiimide
DCM	dichloromethane
DEAD	diethyl azodicarboxylate
DHP	dihydropyran
DIC	diisopropylcarbodiimide
DMAP	4-(*N*,*N*-dimethylamino)pyridine
DMEDA	*N*,*N*'-dimethylethylenediamine
DMF	*N*,*N*-dimethylformamide
DMSO	dimethylsulfoxide
DPPA	diphenylphosphoryl azide
DTBMP	2,6-di(*tert*-butyl)-4-methylpyridine
dr	diastereomeric ratio
EDG	electron-donating group
EI	electron impact
ee	enantiomeric excess

e.g.	exempli gratia (for example)
EM	exact mass
epi	epimer
equiv	equivalent(s)
er	enantiomeric ratio
Et	ethyl
ESI	electrospray ionization
EWG	electron-withdrawing group
GC (GC-MS)	gas chromatography (gas chromatography coupled with mass detection)
HBTU	O-(benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate
HMDS	hexamethyldisilazane
HOBt	1-hydroxybenzotriazole hydrate
HPLC	high performance liquid chromatography
HRMS	high resolution mass spectrometry
i	iso
i.e.	id est (that is)
IC_{50}	half maximal inhibitory concentration
IR	infrared
J	constant coupling
L	ligand
LDA	lithium diisopropylamide
Lit.	literature
LPS	lipopolysacharide
LUMO	lowest unoccupied molecular orbital
m	*meta*
m	multiplet
M	metal
M	molar (concentration)
CPBA	chloroperbenzoic acid
Me	methyl
m.p.	melting point
MS	mass spectrometry
Ms	methylsulfonyl
MTBE	methyl *tert*-butyl ether
MW	molecular weight
m/z	atomic mass units per charge
N	normal (concentration)

NMR	nuclear magnetic resonance spectroscopy
NSAIDs	non steroidal anti-inflammatory drugs
Nu-H/Nu	nucleophile
o	*ortho*
p	*para*
PG	prostaglandin
Ph	phenyl
Phen	phenantroline
Phth	phthalimide
POX	peroxidase
Pr	propyl
Pyr	pyridine
pTLC	preparative thin layer chromatography
rac.	racemic
Rf	retardation factor
rfx	reflux
rt	room temperature
s	singlet
STD	saturation-transfer difference
t	triplet
t	*tert*
TBAB	tetrabutylammonium bromide
TBAF	tetrabutylammonium fluoride
TBDPS	*tert*-butyl-diphenylsilyl
TBHP	*tert*-butyl hydroperoxide
TBS	tetrabutyldimethylsilyl
TEA	triethylamine
TEMPO	2,2,6,6-tetramethylpiperidine N-oxyl
Tf	triflate (trifluoromethylsulfonyl)
TFA	trifluoroacetic acid
Tf_2O	triflic anhydride
TfOH	triflic acid
THF	tetrahydrofuran
THP	tetrahydropyran-2-yl
TLC	thin layer chromatography
TMS	trimethylsilyl
Tol	tolyl

Ts	*para*-toluenesulfonyl
TX	thromboxane
WBA	whole blood assay
wt	weight
δ	chemical shift

I. Introduction

I.1 Inflammation and COX role

Inflammation is the immune system's response to infection or injury and a body's attempt of self-protection, restoration of tissue structure and physiological function. Inflammation has also been implicated in several pathologies such as arthritis or cancer, as well as in neurodegenerative and cardiovascular diseases.

The acute phase of inflammation is characterized by the rapid influx of several blood granulocytes to the affected tissues causing some external signs such as redness, heat, swelling and pain. In fact, what is happening in physiological terms is the fast overproduction of several inflammation mediators that contributes to the development of the inflammation signs. These mediators include the prostanoids – prostaglandins (PG), prostacyclins and thromboxanes (TX) – which are end products of arachidonic acid (AA) metabolism (Figure I.1).[1,2]

There are several enzymes involved in this metabolites biosynthetic pathway, including the prostaglandin G/H endoperoxide synthase, commonly known as cyclooxygenase (COX).

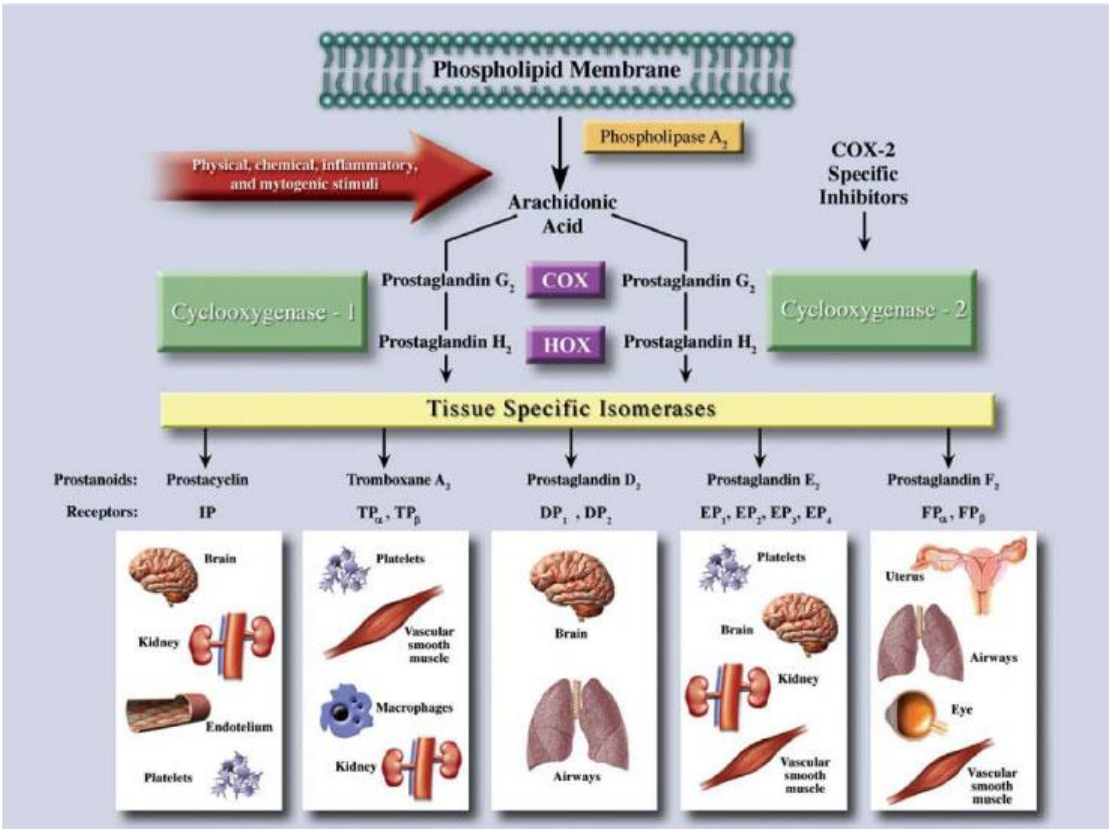


Figure I.1. Arachidonic acid metabolic pathway (adapted from Fitzgerald and Patrono).[2]

In 1971, COX was identified as the enzyme responsible for the formation of prostaglandins during AA metabolism, eliciting a variety of physiological effects such as gastric ephithelial cytoprotection or symptoms associated with the inflammatory response. [3]

Only twenty years later, in 1991, it was discovered that COX exists as two distinct isoforms referred to as COX-1 and COX-2 that are bifunctional enzymes containing both cyclooxygenase and peroxidase (POX) activity.[4] COX-1 is constitutively expressed in most cells, synthesising prostaglandins at low levels to maintain physiological functions, such as cytoprotection in the gastrointestinal tract. On the other hand, COX-2 is usually viewed (in a simplistic way) as the inducible isoform in response to inflammatory stimuli, resulting in a high production and release of prostanoids. However, COX-1 is also inducible under inflammatory conditions in the kidney, and COX-2 is constitutively expressed in several tissues, contributing to the generation of autoregulatory and homeostatic prostanoids.[5]

I.2 COX structure and its isoforms

In order to develop selective and effective anti-inflammatory drugs, it is important to understand the COX structure, the differences on the active site of the different isoenzymes, COX-1 and COX-2 and consequently the binding mode of substrates and its ideal structural features.

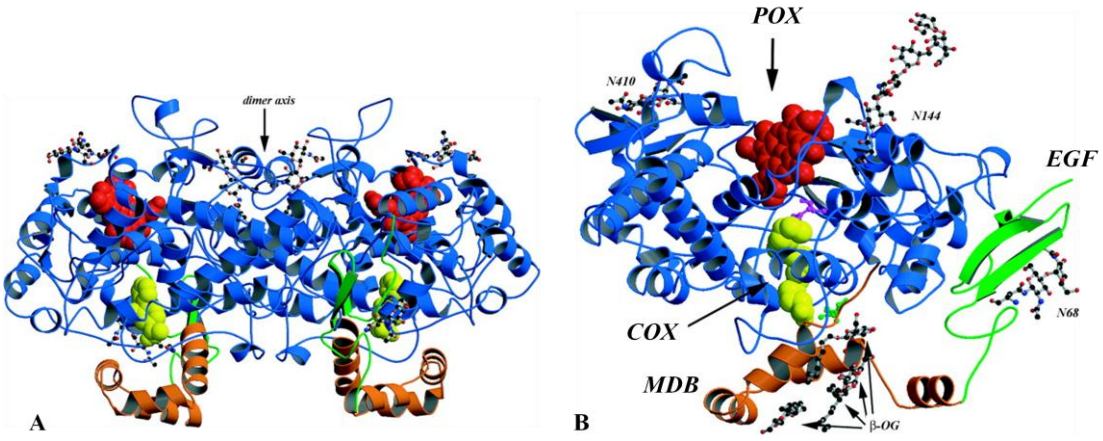
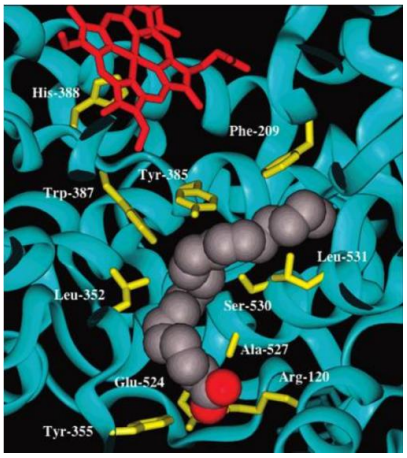


Figure I.2. Ovine COX-1 structure: a) COX-1 homodimer with bound flurbiprofen (yellow); b) COX-1 monomer with bound AA (yellow). EGF (green), MBD (gold), catalytic domain (blue), heme groups (red) and *N*-linked sugars are shown in ball-and-stick rendering (adapted from Garavito *et al.*).[6]

Both COX isoforms are structural homodimers that contains three distinct domains: an extracellular growth factor (EGF) domain, a membrane binding domain (MBD) and a catalytic domain (Figure I.2).[7] The EGF domains occur in membrane proteins that are inserted into a single leaflet of the cytoplasmic membrane and may be involved in the transport and insertion of COX isoforms. The MBD consists of four amphipatic helices that are responsible for anchoring COX to the membrane. The catalytic domain of COX isoforms is divided into peroxidase and cyclooxygenase active sites, which are spatially separated (by the heme prosthetic group) but structurally and functionally interconnected. Thus, COX has altogether two cyclooxygenase and two peroxidase active sites.

The COX active site is the place where two oxygen molecules will oxidize the AA to form PGG_2, beginning the set of reactions of the prostanoids biosynthetic pathway (Figure I.3.a). In the entirely separate peroxidase site, PGG_2 is reduced to PGH_2 by a two electron reduction (Figure I.3.b).[8] The cyclooxygenase active site is buried deep within the protein, and contains a long hydrophobic tunnel that begins at the MBD. The MBD acts like a funnel, guiding the AA and O_2 directly from the membrane into the core of the globular domain for processing.

a)



b)

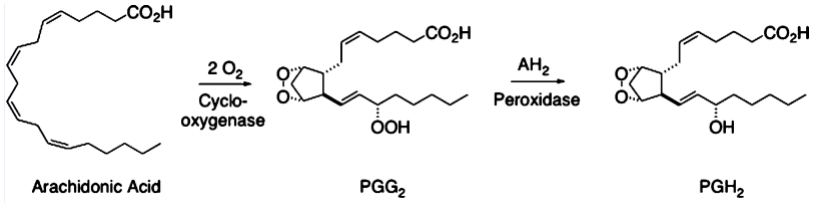


Figure I.3. a) AA bound in the active site of ovine COX-1; b) Prostaglandin biosynthetic pathway (adapted from Marnett *et al.*).[8]

The two COX isoforms – COX-1 and COX-2 – have high sequence identity (about 60% within species), very similar three-dimensional structures and nearly indistinguishable kinetic parameters with AA as substrate.[9] The COX active site is composed of twenty-four residues, though COX-2 is about 20% larger and has a slightly different shape than that of COX-1. This extra size is the structural feature exploited to construct selective COX-2 inhibitors.

The difference relies on the amino acids present three particular positions in COX-1 and COX-2: substitution of Ile523 in COX-1 by a Val523 in COX-2 results in the presence of a small side pocket adjacent to the active site channel, appreciably increasing the volume of the COX-2 active site; the substitution of Ile434 in COX-1 with Val434 in COX-2 in the surrounding second shell further increases the effective size of the active site channel by enhancing the local mobility of side chains within the side pocket. Additionally the combination of these two differences at positions 523 and 434 in COX-2 causes a movement of Phe518 that further increases the size of the side pocket (Figure I.4).

Finally, the substitution of His513 in COX-1 with Arg513 in COX-2 alters the chemical environment of the side pocket. This substitution results in a stable positive charge being placed at the center of this pocket, which can interact with polar moieties entering the pocket.

For example, Arg513 appears to interact with the 4-methysulfonyl or 4-sulfonamoylphenyl substituents of diarylheterocyclic COX-2 inhibitors and give rise to the time-dependent inhibition displayed by this class of inhibitors.

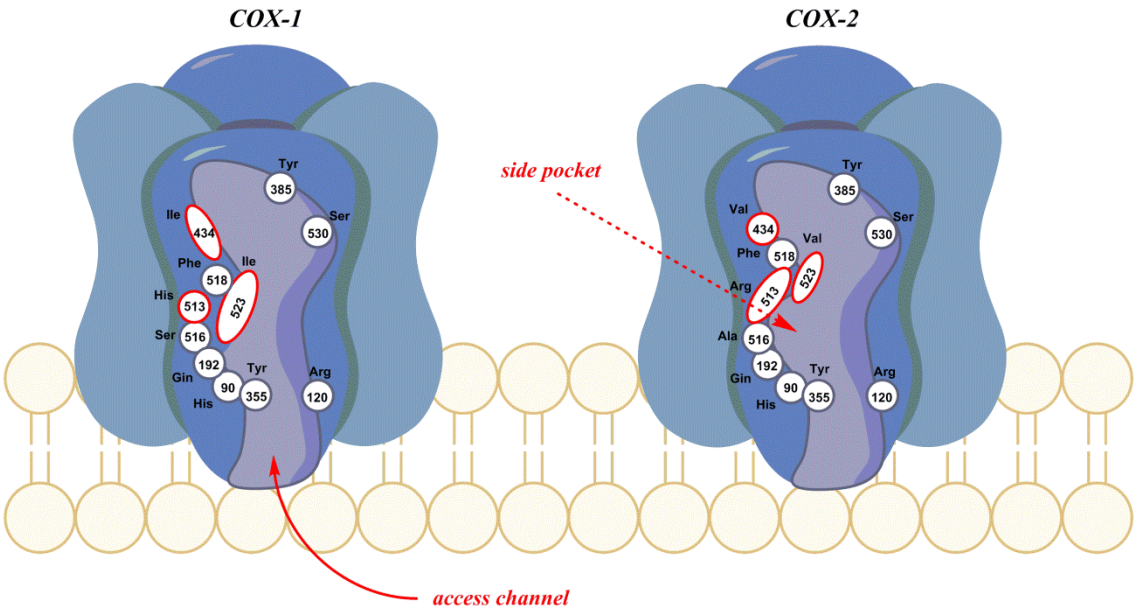


Figure I.4. Differences in the active sites of COX-1 and COX-2.

Other crucial amino acids include Arg120, Tyr355 and Glu524, which together form a narrow constriction in the hydrophobic channel acting as a gate to the bottom of the COX active site. The residue of Tyr385 at the top of the channel is the radical donor for the cyclooxygenase reaction. The anti-inflammatory drugs binding site involves the upper half of this channel from Arg120 to near Tyr385.

Only three of the channel residues are polar – Arg120, Ser353, and Ser530. Ser530 is the site of acetylation by aspirin, and Arg120 binds to the carboxylate groups of fatty acids and many COX inhibitors.

Recently, it was proposed that COX-2 acts as a conformational heterodimer having a catalytic (E_{cat}) and an allosteric monomer (E_{allo}).[10] It is suggested that heme binding – to the peroxidase site of a monomer – defines one monomer as E_{cat} (while the other is defined as E_{allo}). It is suggested that some substrates and certain inhibitors (e.g. celecoxib, diclofenac) bind the cyclooxygenase site of E_{cat}. The nonsubstrate fatty acids and some COX inhibitors (*e.g.* naproxen, flurbiprofen) preferentially bind to the COX site of E_{allo}. Moreover, E_{allo} allosterically influences the catalytic

efficiency of the partner E_{cat} subunit, i.e. E_{cat} is regulated by E_{allo} in a manner-related way the ligand is bound to E_{allo}.

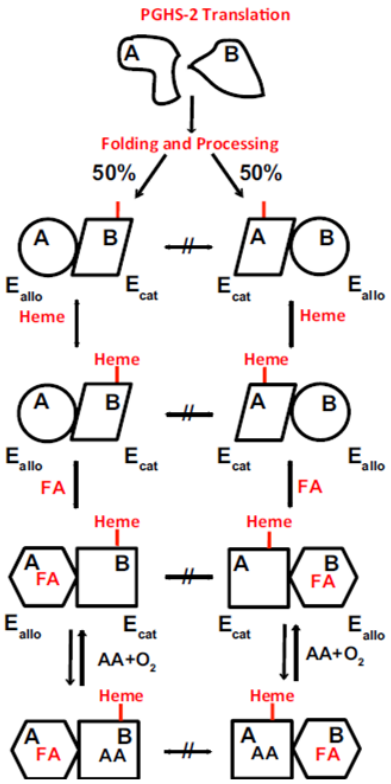


Figure I.5. Model for the folding and processing of native human COX-2 yielding two pre-existent conformational heterodimers that are allosterically regulated by fatty acids (adapted from Smith *et al.*).[10f]

It is suggested that both native COX-2 subunits have identical primary structure folds, but are processed so as to form a pair of stable conformational heterodimers. Once a conformational heterodimer is formed, the monomers comprising the dimer do not flux between E_{allo} and E_{cat} forms (Figure I.5).

I.3 COX inhibitors: is COX-2 selectivity worthy?

Acetylsalicylic acid, mostly known as aspirin, was the first drug used for the treatment of inflammation and since 1899 is the world's most widely used pain reliever. However, the reason *why* it works was not fully understood until 1971.[11] John Vane found that aspirin have influence on the production of certain prostaglandins that are associated with inflammation, elucidating the aspirin's medical efficacy. For this discovery, Vane was awarded the Nobel Prize for Medicine in 1982.

Since then, several Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) were developed and commercialized, such as paracetamol,[12] ibuprofen,[13] indomethacin,[14] diclofenac,[15] naproxen[16] and nimesulide (Figure I.6).[17]

These NSAIDs were found to inhibit prostaglandin synthesis through COX inhibition. However, most of them act as nonselective inhibitors of COX, inhibiting both COX-1 and COX-2 isoenzymes, which conducts to significant side effects specifically at gastrointestinal and renal levels.[18]

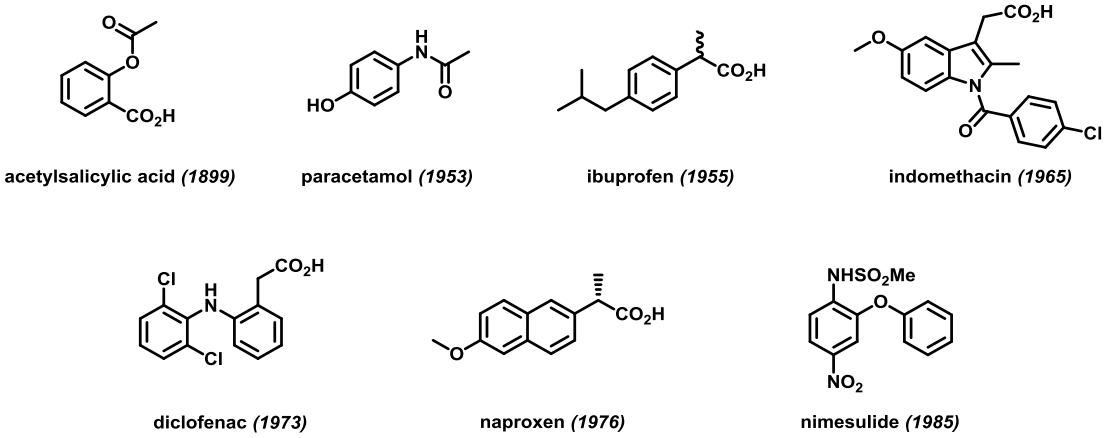


Figure I.6. Examples of early developed NSAIDs and corresponding release date.

In late 20th century, the unraveling of COX structure and the discovery of COX-2 isoform,[7a] aided researchers to understand the selectivity observed with certain compounds and conducted to the development of new potent and high selective inhibitors towards COX-2. In 1990, the compound DuP 697 was reported as the first selective COX-2 inhibitor,[19] followed by the release of NS 398 a couple of years later (Figure I.7).[20] It was rapidly perceived that the shape of DuP 697, composed of a 1,2-diaryl heterocycle template, was an important feature to accommodate the drug inside the COX active site. This observation formed the basis of the early work in the field of selective COX-2 inhibitors.

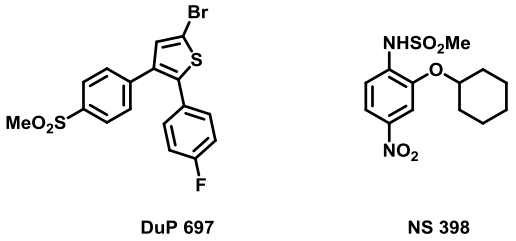


Figure I.7. The first two selective COX-2 inhibitors: DuP 697 and NS 398.

In the late 90s, several highly selective COX-2 inhibitors (usually known as coxibs) were released to the market: celecoxib (Celebrex®),[21] rofecoxib (Vioxx®),[22] valdecoxib (Bextra®)[23] and etoricoxib (Arcoxia®).[24] All these compounds possess the same key structural features: a central 5- or 6-membered heterocylic core with two aryl substituents in a 1,2 relationship. One of the aryl groups has a sulfonyl group in the 4-position whilst the other is either unsubstituted or carries a small hydrophobic group. The central heterocycle is either unsubstituted or bears a small lipophilic

group. Other coxibs possess different structures, such as lumiracoxib that resemble diclofenac structure (Figure I.8).[25]

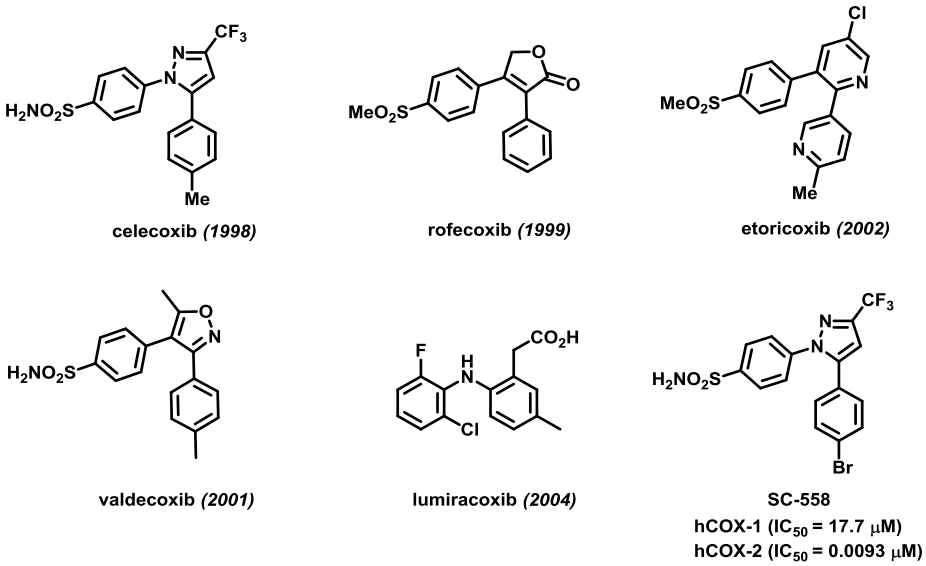


Figure I.8. Commercialized coxibs (and corresponding delivery dates on market) and SC-558 with the corresponding IC_{50} values for COX-1 and COX-2.[7b]

Besides the 1,2-diaryl feature, the coxibs selectivity has been attributed to the sulfonyl functionality placed in *para* position of one of the aromatic rings. It is generally accepted that the methylsulfonyl or sulfonamide groups of the inhibitor can interact with specific key residues within the side pocket of COX-2 (Arg513, His90, Phe518 and Gln192).[7b] This is not allowed in COX-1 due to the steric bulk of Ile-523. In fact, the crystal structure of COX-2 with SC-558 – one of the most selective COX-2 inhibitors – demonstrated how the sulfonamide group of the inhibitor binds to these side pocket residues, establishing the key structural features for the COX-2 selectivity (Figure I.9).

It is verified that the *para*-bromophenyl ring of SC-558 is positioned toward the top of the COX-2 active site, making hydrophobic contacts with Phe381, Leu384, Tyr385, Phe513 and Trp387. The CF_3 group at the position 3 of the pyrazole ring is oriented toward a hydrophobic pocket consisting of Met113, Val116, Val349, Tyr355, Leu359 and Leu531. However, other distinct anchoring sites contribute to the inhibitors binding in the COX-2 active site. A distinctive feature in COX-2 selective inhibitors is generally the lack of a free carboxylate group, which may be related to their low affinity toward COX-1.[26]

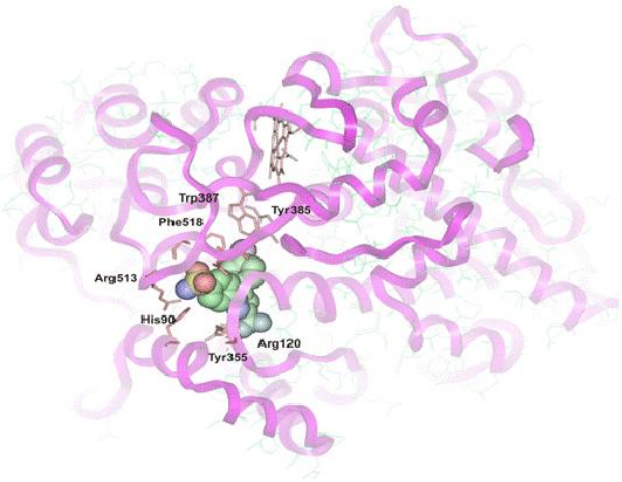


Figure I.9. Crystal structure of SC-558 with COX-2.[7b]

For example, the crystal structure of COX-2 with indomethacin reveals that the carboxyl group can form an ionic interaction with Arg120 and Tyr355 at the constriction site at the base of the active site.[7b] The same was verified for naproxen.[16] Other carboxylic acid-containing inhibitors, such as diclofenac and lumiracoxib, bind to COX-2 in a different orientation, placing their carboxylates at the top of the active site, allowing them to form hydrogen bonds with Ser530 and Tyr385 (Figure I.10).[27]

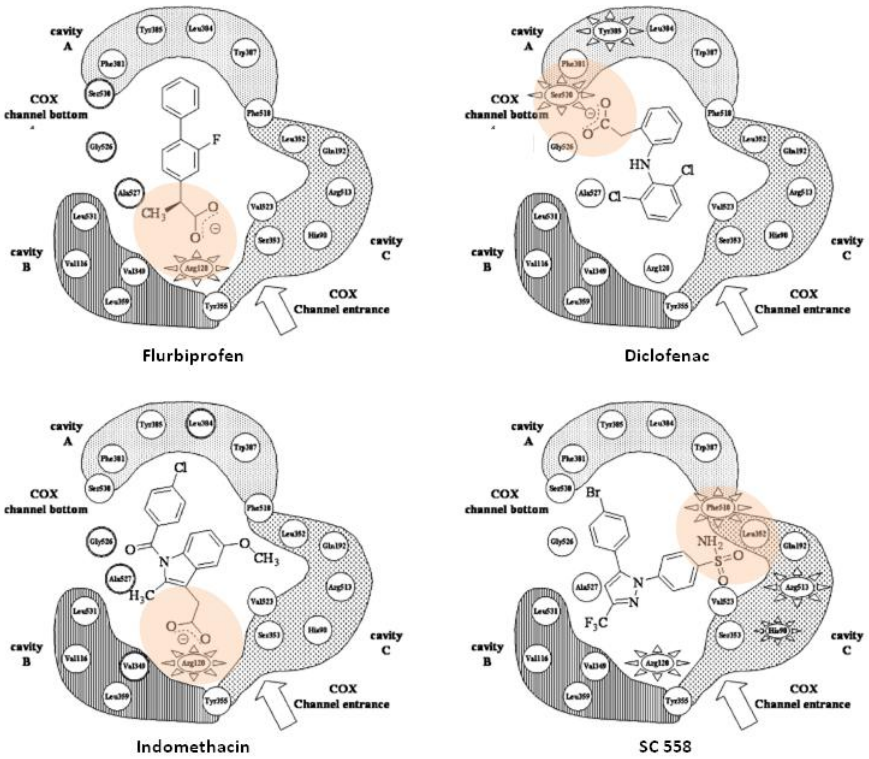


Figure I.10. Representation of several ligands inside COX-2 active site as inferred from X-ray data (adapted from Ermondi *et al*.).[28]

Coxibs were designed based on the hypothesis that selective inhibition of the COX-2 isoform should reduce pain and inflammation without compromising the integrity of the gastric mucosa.
As expected, these inhibitors presented reduced side effects at gastrointestinal level. However, due to acute cardiovascular side effects, Merck withdrew rofecoxib from the market in September 2004, while valdecoxib (Pfizer) was also removed (in 2005) from the United States and Europe.[29]

Some groups support that selective COX-2 inhibition is associated to an increased incidence of thrombotic events that could be reduced by a balanced inhibition of both isoenzymes.[30] In fact, it is proposed that COX-2 inhibitors do not block thromboxane A_2 (TXA_2), which causes platelet aggregation, and selectively inhibit the beneficial vascular effects of prostacyclin (PGI_2).With loss of the antiplatelet and vasodilatory effects of prostacyclin, a relative excess of TXA_2 would promote vasoconstriction, platelet aggregation and thrombosis.[31]

However, these facts are not plain since the risks of myocardial infarction were verified both in coxibs and in certain non-selective NSAIDs. Thus, the selective inhibition of the COX-2 is not the only cause of adverse side effects, and other factors must be considered such as patient medical background, dose- and duration-regimens.[2,32]

As a result, gastrointestinal and cardiovascular risks must be carefully assessed in order to tune the inhibitory activity of the anti-inflammatory drugs: while gastrointestinal effects were less frequently verified for coxibs than NSAIDs, serious cardiovascular events occur at approximately equal rates.[33]

These facts have been encouraging the scientific community to pursue new pharmaceutical entities devoid of adverse side effects.

Heterocyclic compounds play an exclusive role in drug discovery and benzimidazole structures were found to be trendy structures, having numerous biological applications,[34] being also described as highly promising COX inhibitors (Figure I.11).[35]

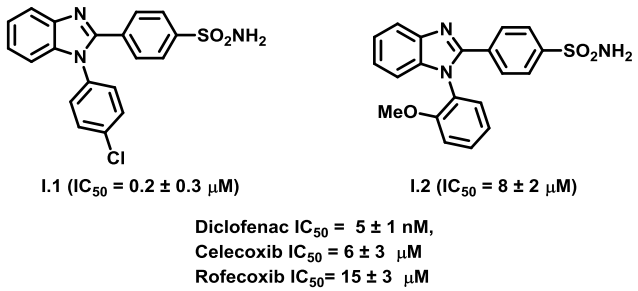


Figure I.11. IC_{50} (COX-2) for benzimidazole structures and comparison with some COX inhibitors **(**obtained using human monocytic cell lines).[35a]

According to the presented facts, it is envisaged that benzimidazoles are an excellent choice to attain new NSAIDs with a balanced COX inhibition, i.e. with less adverse side effects than the presently commercialized anti-inflammatory drugs.

I.4 Main objectives and Thesis structure

The main objective of the work described in the present thesis was to develop highly potent COX inhibitors with a balanced COX-1/COX-2 inhibition, employing a rational drug design which takes advantage of several techniques such as docking, NMR and biological assays. Moreover, it was also proposed to develop a new synthetic methodology to assemble the benzimidazole-based inhibitors, which possess a specific substitution pattern relevant for the inhibitory activity.

Additionally, it was planned to extend the amide electrophilic activation methodology – developed by Maulide's group – to prepare relevant heterocyclic compounds, and to develop a SO_2 surrogate to construct sulfonamides in a broad and versatile way.

This work is divided in four main chapters:

Chapter II. Describes the several synthetic approaches used to assemble the benzimidazole-based inhibitors. It also comprises the evaluation of their inhibitory activity towards COX-1 and COX-2 and the STD-NMR experiments performed with COX-2.

The work described in this chapter is published in:

"Developments towards regioselective synthesis of 1,2 disubstituted benzimidazoles", Luísa C. R. Carvalho, Eduarda Fernandes, M. Manuel B. Marques, *Chem. – Eur. J.* **2011**, 12544.

"1,2-Diaryl benzimidazoles as potent COX inhibitors", Luísa C. R. Carvalho, S. Erhardt, E. Cabrita, Daniela Ribeiro, Raquel S. G. R. Seixas, Artur M. S. Silva, Eduarda Fernandes, M. Manuel B. Marques, **2014**. (Paper under preparation)

Chapter III. Describes a new methodology useful to assemble heterocyclic structures on solid phase: the Pd-catalyzed arylamination using several PEG bound aryl halides and aromatic amines, where PEG 2000 acted as solvent and solid-support.

The work described in this chapter is published in:

"Pd-catalyzed amination on soluble polymer support: arylation of anilines with PEG-supported aryl halides" Luísa C. R. Carvalho, Marina J. Pires, Eduarda Fernandes, M. Manuel B. Marques, *RSC Advances* **2013**, 25711.

Chapter IV. Describes the amide electrophilic activation/Claisen rearrangement methodology developed in Maulide's group, applied to substrates incorporating a phenyl ring within the alkyl tether. Are also described the attempts to expand the procedure to its aza analogs.

The work performed in this section took place in Maulide's former lab at Max Planck Institute für Kohlenforshung from October 2012 until December 2012.

Chapter V. Involves the studies to develop a versatile SO_2 surrogate to prepare sulfonamides.

II. Novel Benzimidazole-based COX inhibitors

My contribution to the work was the synthesis of all compounds, part to the biological evaluation and the STD-NMR studies.

The biological evaluation was performed in collaboration with Prof. Dr. Eduarda Fernandes (FF-UP).

The STD-NMR studies were performed in collaboration with Prof. Dr. Eurico Cabrita (FCT-UNL).

II.1 Backgroud

II.1.1 1,2-Disubstituted benzimidazole assembly

Heterocycles have a very important role in drug discovery since the majority of therapeutic drugs, which includes the NSAIDs, contain a heterocyclic unit. Benzimidazoles are trendy heterocyclic structures employed in several areas such as materials science[36] or in the pharmaceutical industry. A successful example is Nexium (esomeprazole), a proton pump inhibitor used to treat peptic ulcers and gastroesophageal reflux disease, that became one of the most widely prescribed drugs, with sales of about $5 billion in 2009 (Figure II.1). In 1872, when Hobrecker reported the first benzimidazole synthesis, of 2,5- and 2,6-dimethylbenzimidazole, he never suspected that benzimidazole scaffold would become such a preeminent structure.[37]

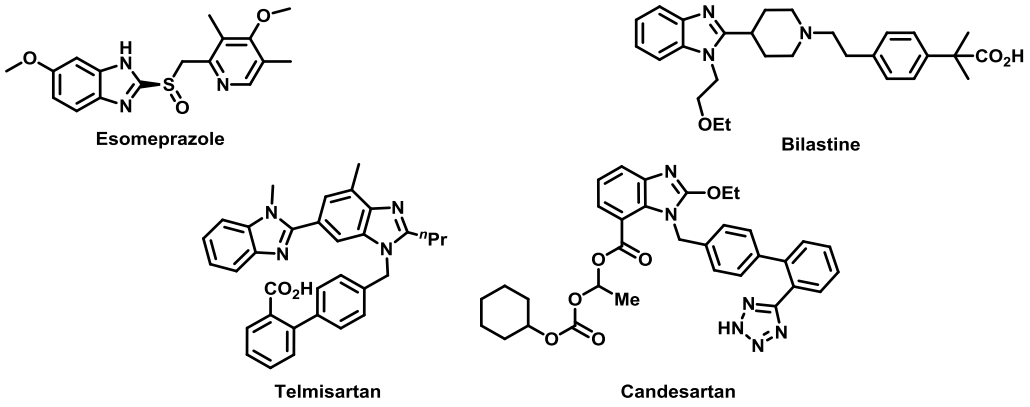


Figure II.1. Examples of some commercially available benzimidazole-based drugs.

1,2-Disubstituted benzimidazoles and their derivatives are a representative branch of this family. Reported as valuable bioactive structures, such as specific angiotensin II receptor type 1 selective antagonists,[38] or hepatitis C virus NS5B polymerase inhibitors,[39] they exhibit several other pharmacological activities including antidiabetic,[40] antihistamine,[41] analgesic,[42] antiviral,[43] chemotherapeutic,[44] antifungal,[45] and antiparasitic[46] applications. The relevance of these compounds can be demonstrated by the profusion of pharmaceutical products in the market, for example, the antihypertensives Micardis® (telmisartan) and Atacand® (candesartan), or the Bilaxten® (bilastine), a histamine H1 receptor antagonist for the oral treatment of allergic rhinitis and chronic idiopathic urticaria. Moreover, 1,2-disubstituted benzimidazoles were also described as intermediates for dyes and polymers,[47] and have frequently been used as ligands. [48] In addition, there have also been reports of their use as possible precursors for aminoboronic acids with an interest as bifunctional organic catalysts.[49]

Undoubtedly, benzimidazoles are important scaffolds and significant efforts have been made to develop new synthetic strategies for their assembling, both in solution[50] and in solid phase.[51] Solid-

phase synthesis (SPS) is extremely useful for combinatorial approaches towards benzimidazole libraries with increased structural complexity.

While methods to prepare 1- or 2-substituted benzimidazoles have highly increased during the last years,[52] the assembly of 1,2-disubstutitued benzimidazoles remains an intricate task.[53]

The classical and most common methods to assemble benzimidazoles involve the condensation of *o*-phenylenediamine with aldehydes, carboxylic acids or their derivatives (nitriles, amidates, orthoesters) (Figure II.2, route a).[54] The *N*-alkylation of benzimidazole derivatives is also a common alternative (Figure II.2, route b).[40]

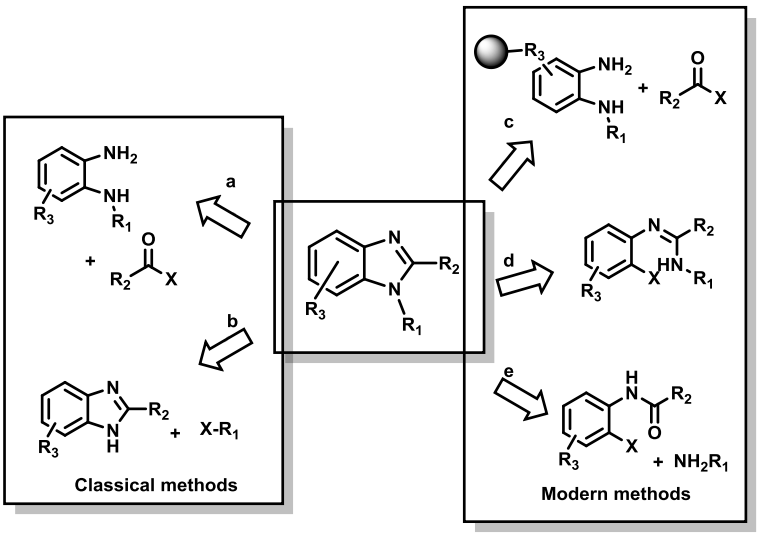


Figure II.2. Available methods to assemble 1,2-disubstituted benzimidazoles.

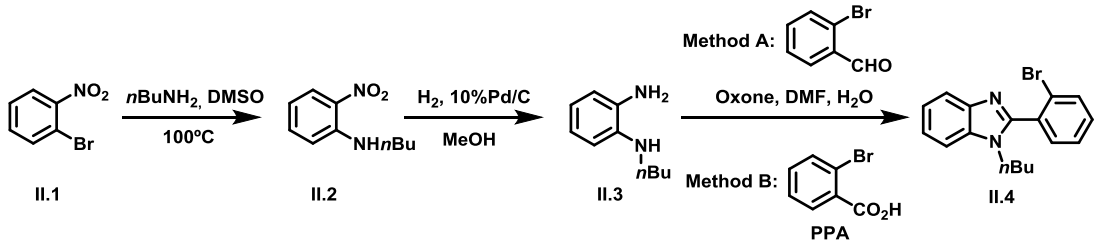
However, these methods are usually not regioselective for the synthesis of the 1,2-disubstitutued benzimidazoles and are limited to the available starting materials. Consequently, improvements were made towards the development of new strategies, such as the metal-catalyzed aryl-amination chemistry (Figure II.2, route d) [50c] and the cascade arylamination/condensation method (Figure II.2, route e).[50a,b] The development of SPS (Figure II.2, route c),[51] allied with the optimization of microwave conditions [55] allowed a more rapid, efficient and sustainable route to achieve this relevant class of benzimidazoles.

II.1.1.1 The classical methods struggle

The classical synthetic methods to assemble the benzimidazole moiety are still widely used due to its inherent simplicity. One of the most used traditional procedures rely on the condensation of aldehydes, carboxylic acids or its derivatives with 1,2-diaminoarenes followed by cyclization.[54] The lack of differentiation between the two nitrogen atoms that usually lead to a mixture of regioisomers, makes this procedure inappropriate to assemble 1,2-disubstituted benzimidazoles. Additionally, these methods are also restricted to the commercially available starting materials, fact that constitutes a main limitation to construct more complex molecules. An example is the

condensation of *o*-phenylenediamine with aldehydes, one of the most studied approach to prepare simple 1,2-disubstituted structures. The employment of several catalysts under mild conditions,[56, 57,58] usually provides the products in good yields.

When the two nitrogen atoms need to be differentiated, the reduction and cyclization from *o*-nitroanilines is commonly used. First the free amine is adequately reacted, followed by the nitro group reduction and subsequent reaction with a suitable substrate to give the benzimidazole scaffold.[39,59] *o*-Halo nitrobenzene structures like *o*-bromonitrobenzene (**II.1**) can also be used in order to achieve the corresponding *N*-substituted *o*-nitroaniline **II.2** (Scheme II.1), which after hydrogenation affords the corresponding amine **II.3** that can undergo subsequent acylation and cyclization to **II.4**.[59]

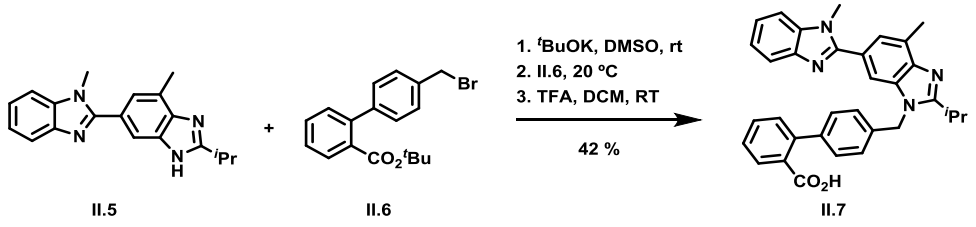


Scheme II.1. Reduction and cyclization from *o*-nitroanilines.[59]

II.1.1.2 Functionalization of the benzimidazole ring

Another current method to attain 1,2-disubstituted benzimidazoles is the *N*-alkylation of 2-substituted benzimidazoles that is often achieved by reaction with either an alkyl halide or an acyl chloride. Although commonly used, this method is usually not regiospecific, tending to elicit substitution on the sterically less hindered nitrogen.

In the first reported synthesis of Telmisartan (**II.7**), the alkylation of **II.5** with the 4-(bromomethyl)-2-biphenylcarboxylic acid *tert*-butyl ester (**II.6**) afforded a mixture of two *bis*-benzimidazoles (Scheme II.2).[60]

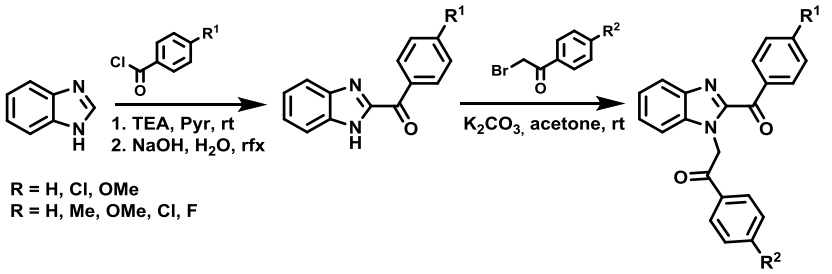


Scheme II.2. First reported Telmisartan synthesis.[60]

N-alkylation of benzimidazole moiety could also be achieved performing an alkylation *via* Mitsunobu reaction or with alkyl bromides as described by Erion and co-workers.[40]

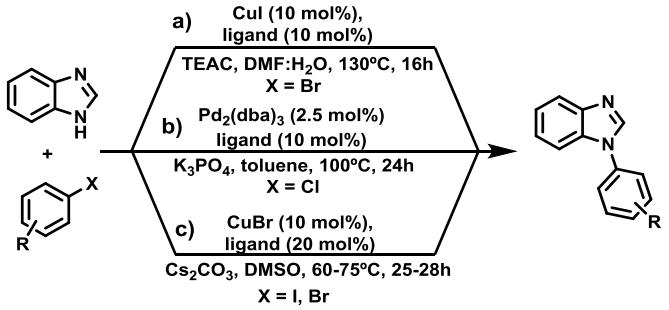
The acylation of position 2 was described by Kayalgil and co-workers that reported the synthesis of 1,3-diarylpyrazino[1,2-a]benzimidazole derivatives.[61] The reaction of benzimidazole

moiety with acyl chlorides gave the corresponding 2-aryloylbenzimidazole derivatives which were reacted with 2-bromoacetophenones to give 1-(2-aryl-2-oxoethyl)-2-aryloylbenzimidazoles intermediates (Scheme II.3).[61] Several other methods for the functionalization of C-2 position were developed to achieve 1,2-disubstituted benzimidazoles.[62]



Scheme II.3. Kayalgil and co-workers approach to 1-(2-aryl-2-oxoethyl)-2-aryloylbenzimidazoles synthesis.[61]

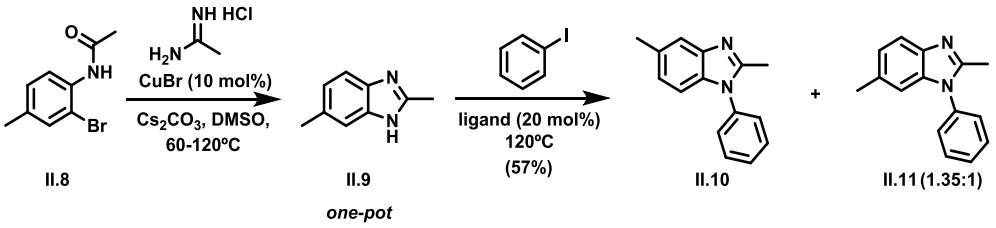
Due to its limitations in scope, metal-catalyzed *N*-arylation of benzimidazoles has been scarcely used, and has only been applied to unsubstituted benzimidazoles (Scheme II.4, a-c).[63] The recent advances on Pd-based catalytic systems for efficient C-N coupling reactions have been highly useful. Buchwald group has described the arylation of simple imidazole or benzimidazole rings with aryl halides (Scheme II.4, b).[64]



Scheme II.4. Metal-catalyzed *N*-arylation of benzimidazoles.

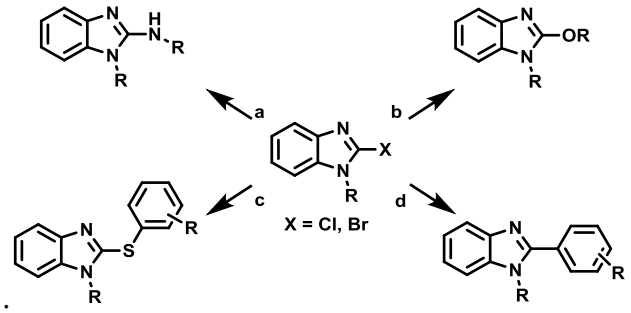
On the other hand, Bao group have developed a copper-catalyzed reaction using ethyl 2-oxocyclohexanecarboxylate as ligand (Scheme II.4, c). The group has demonstrated that under these conditions benzimidazole was less reactive towards aryl halides than imidazole, requiring higher temperatures to give the product in moderate yields.[65]

The *N*-arylation of several 2-substituted 1*H*-benzimidazoles in a one-pot manner was also reported by Zhao and co-workers (Scheme II.5).[66] The method relied on the use of amidine hydrochloride to form the 2-substituted 1*H*-benzimidazoles in the presence of CuBr and Cs_2CO_3, and sequential addition of the aryl iodide and ligand (3,4,7,8-tetramethyl-1,10-phenantroline) to afford the desired products in moderate yields. When 2-bromo 4-methylacetaniline (**II.8**) was employed, a mixture of the two regioisomers (**II.10** and **II.11**) was obtained.



Scheme II.5. *N*-arylation of several 2-substituted 1*H*-benzimidazoles *via* one-pot approach by Zhao and co-workers.[66]

2-Halo substituted benzimidazoles, such as 2-chloro or 2-bromobenzimidazoles, are also important intermediates for functionalization of the benzimidazole scaffold at the position 2 (Scheme II.6). These compounds are versatile intermediates to prepare of 2-hetero-benzimidazoles (Scheme II.6, paths a, b and c) and 2-arylbenzimidazoles (Scheme II.6, path d).



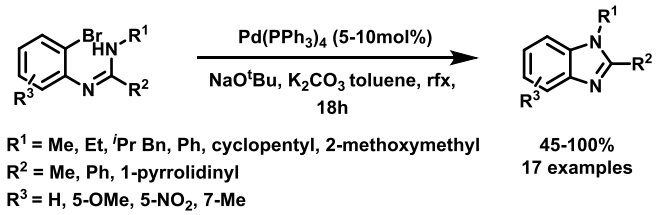
Scheme II.6. Benzimidazole C-2 functionalization *via* 2-halobenzimidazoles.[62b-e]

In order to improve the regioselectivity of the synthesis of *N*-substituted benzimidazoles, novel methods have been developed.

II.1.1.3 Metal-catalyzed intramolecular amination/cyclization

The development of a metal-catalyzed intramolecular aryl-amination/cyclization approach was an elegant way to circumvent the limitations of classical methods, allowing the construction of complex benzimidazoles in a regioselective way.

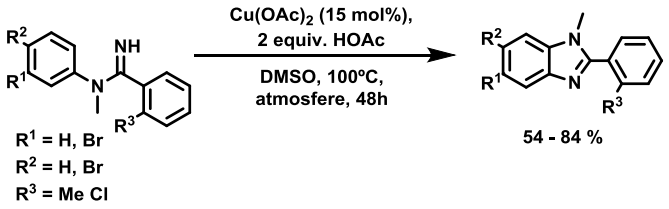
Firstly reported by Brain and co-workers (Scheme II.7),[67] this method was readily adopted with success by several groups that used several catalytic systems including for example, palladium-, copper- or cobalt-based salts.



Scheme II.7. Brain's approach rely on the arylation of amidines.

In fact, the recent advances on metal-mediated coupling chemistry stimulated the development of these new methodologies, allowing the easy assembling of heterocyclic compounds. Palladium catalyzed aryl-amination chemistry has been extensively improved, mainly due to the development of new ligand systems.[68] Concerning copper catalysis, the development of *N*,*N*-, *N*,*O*- and *O*,*O*-bidentate ligands enhanced Ullmann-type coupling reactions.[69]

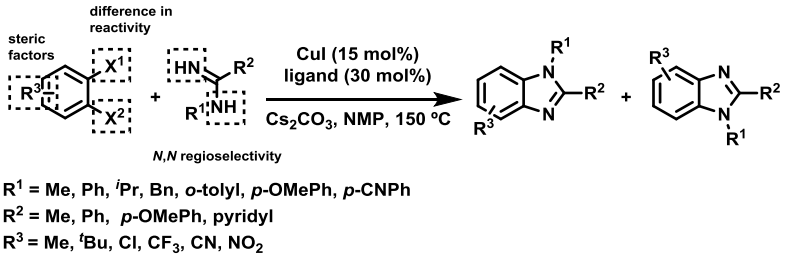
In 2008, Buchwald group developed a copper-catalyzed amination for the synthesis of substituted 2-arylbenzimidazoles in good yields from the corresponding amidines, also demonstrating that the procedure could be extended to the preparation of *N*-methylated benzimidazoles (Scheme II.8).[50c]



Scheme II.8. Buchwald Cu-catalyzed amination.[50c]

Mani and co-workers which previously reported the CuI-catalyzed amination reaction of 1,2-dihaloarenes with guanidines and amidines, to achieve 1-*H*-2-substituted benzimidazoles,[70] developed a comprehensive study on the regioselectivity of the reaction of 1,2-dihaloarenes with *N*-substituted amidines to obtain 1,2-disubstituted benzimidazoles (Scheme II.9).

Several factors and its influence on the regioselectivity were investigated, such as the chemoselectivity of nitrogen atoms on the first amination step; the steric and electronic effect of the substituent on the arene ring; the reactivity control between differentiated halides or even the reaction with different amidines.[71]



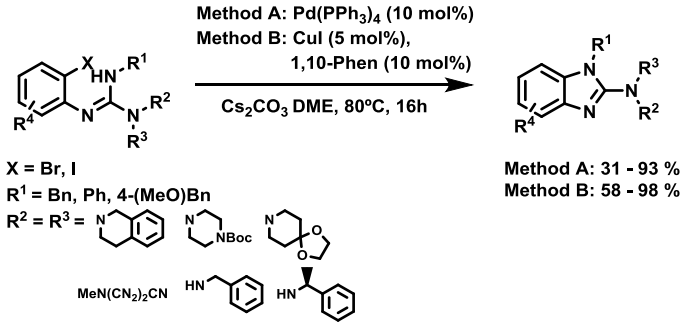
Scheme II.9. Comprehensive study of the regioselectivity described by Mani and Deng.[71]

Other methods using heterogeneous systems were also described for the synthesis of 1,2-disubstituted benzimidazoles. Punniyamurthy group reported the use of copper(II) oxide nanoparticles under ligand-free conditions. The reactions revealed to be efficient and general for the synthesis of 2-alkyl, 2-aryl and 2-amino benzimidazoles and the catalyst could be recovered and recycled without loss of activity and selectivity.[72] The same group described a cobalt-catalyzed intramolecular C–N cross-coupling using *Z*-*N*-(2-halophenyl)-*N*-phenylamidines and *N*-(2-

bromophenyl)benzamides as starting materials to obtain the corresponding substituted benzimidazoles in excellent yields.[73]

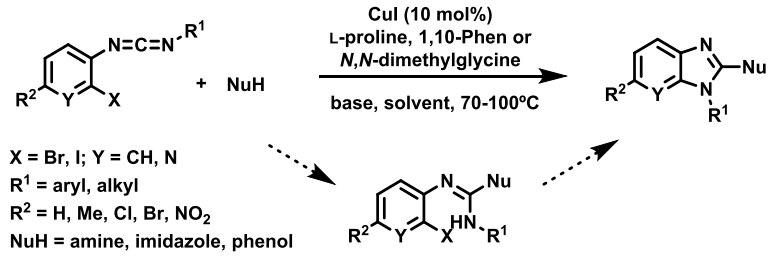
The synthesis of benzimidazoles using a copper-catalyzed intramolecular amination in water was also reported.[74] Although successfully applied for the synthesis of 1*H*-2-substituted benzimidazoles with good yields, it presented a limited scope for 1,2-disubstituted benzimidazoles.

The intramolecular amination method was also successfully applied to the synthesis of *N*-substituted 2-heterobenzimidazoles. Batey and co-workers reported a palladium or copper-catalyzed intramolecular aryl guanidinylation for the formation of 2-aminobenzimidazoles in moderate to high yields (Scheme II.10).[75] Other fused imidazole systems like purine or xanthine nucleus, could also be readily obtained by this method.[76]



Scheme II.10. Synthesis of 2-aminobenzimidazoles by a Cu-catalyzed guanidinylation method.[75]

While by the usual method the precursors *o*-haloguanidines needed to be previously synthesized, Bao and Lv demonstrated that 2-heterobenzimidazoles like 2-amino, 2-imidazyl and 2-phenoxyl benzimidazoles could be easily prepared from *o*-haloarylcarbodiimides.[77] Thus, the group developed a one-pot copper-catalyzed addition/C-N coupling method (Scheme II.11), similar to the previously described metal-catalyzed intramolecular amination. Since diaza compounds have been previously described as ligands in copper catalyzed coupling reactions, Bao group also investigated the same approach in the absence of ligand.[78]

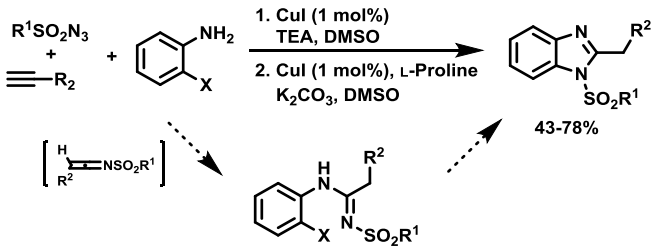


Scheme II.11. Employment of *o*-haloarylcarbodiimides developed by Bao and co-workers.[77]

Bao group also reported the use of $Cu(OAc)_2/O_2$ system to prepare benzimidazole derivatives directly from the reaction of diphenylcarbodiimides with different nucleophiles, *via* addition/C-H

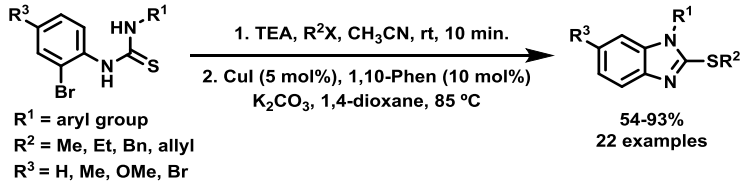
activation in a one-pot cascade procedure.[79] The same approach was adopted by Cai and co-workers that synthesized a variety of 2-aminobenzimidazoles from *o*-haloanilines and carbodiimides *via* copper-catalyzed reaction, in the presence of NaO^{t}Bu using *N*-methyl-2-pyrrolidinone (NMP) as solvent in a "ligand-free" manner.[80]

Wang and co-workers, following a previous work from the group,[81] developed a three-component reaction of sulfonylazides, terminal alkynes and 2-bromoaniline to achieve 2-substituted 1-sulfonylbenzimidazoles. The proposed mechanism involves the copper-catalyzed azide/alkyne addition to form a ketenimine intermediate that is attacked by the amine to generate the corresponding *N*-sulfonylamidine. The copper-catalyzed intramolecular C-N coupling afforded the products in moderate to good yields (Scheme II.12).[82]



Scheme II.12. Wang's three-component reaction. [82]

The intramolecular copper-catalyzed aryl amination can also be applied to the synthesis of *N*-substituted 2-mercapto benzimidazoles. The preparation of these compounds was described by Muzart and co-workers who attained the desired products by the *S*-alkylation of thiourea derivatives, followed by the intramolecular C-N coupling (Scheme II.13).[83]



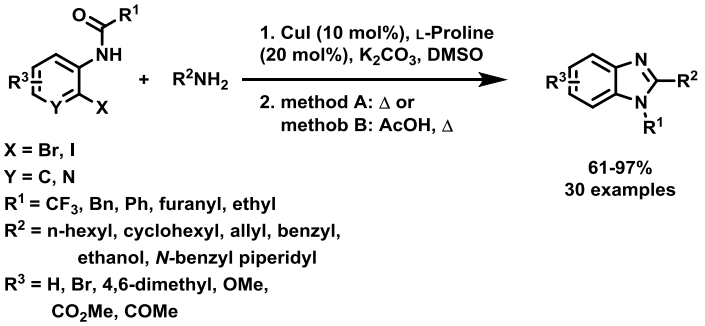
Scheme II.13. Synthesis of N-substituted 2-mercapto benzimidazoles by Muzart and co-workers.[83]

They also demonstrated that benzimidazole thiones could be obtained from 2-mercapto benzimidazoles substituted with a *p*-methoxybenzyl group.

Despite of the advantages of the method, it commonly required a multi-step synthesis to prepare the *o*-halo-aryl or alkylamidine precursors. Thus, cascade arylamination method emerged as a suitable alternative.

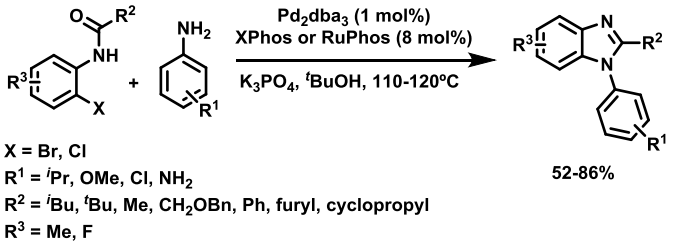
II.1.1.4 Cascade arylamination/condensation method

In 2007, Ma and co-workers developed a mild process to achieve 1,2-disubstituted benzimidazoles.[50b] Encouraged by previous studies, the group investigated the *o*-NHCOR group effect on copper-catalyzed aryl-amination, producing the corresponding *o*-aminoanilides. The 1,2-disubstituted benzimidazoles were achieved in a one-pot manner through an intramolecular condensation/cyclization route (Scheme II.14) in moderate to high yields.



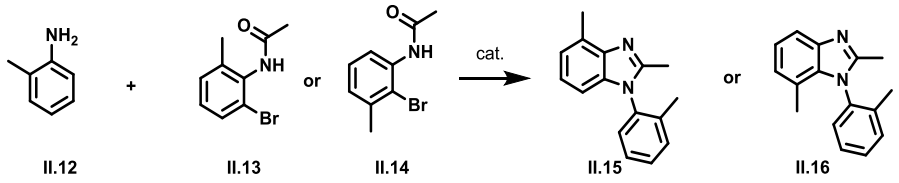
Scheme II.14. Intramolecular condensation/cyclization procedure developed by Ma and co-workers.[50b]

In a related report, Buchwald and co-workers developed a palladium-catalyzed amination of *o*-bromo or *o*-chloro acetanilide followed by cyclization to afford the corresponding *N*-arylbenzimidazoles (Scheme II.15).[50a]



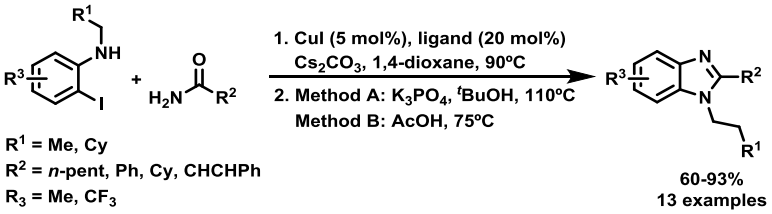
Scheme II.15. Palladium catalyzed arylamination/condensation method.[50a]

When isomeric forms of aryl bromides were applied (**II.13** and **II.14**, respectively), the method provided access to the regioselective synthesis of compounds **II.15** and **II.16** in high yields, proving the advantage of the method (Scheme II.16).



Scheme II.16. Regioselective synthesis of compounds **II.15** and **II.16**.

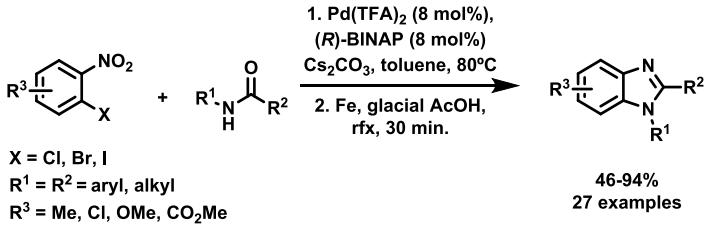
As alternative to amination approach, Buchwald and Zheng reported a copper-catalyzed amidation method for the preparation of *N*-alkylbenzimidazoles. The first amidation step was accomplished using CuI as catalyst and *trans*-*N*,*N*-dimethyl-1,2-cyclohexanediamine as ligand. Further dehydration conditions allowed the preparation of *N*-alkylbenzimidazoles in a regioselective form (Scheme II.17).[84]



Scheme II.17. Copper-catalyzed amidation by Buchwald and Zheng.[84]

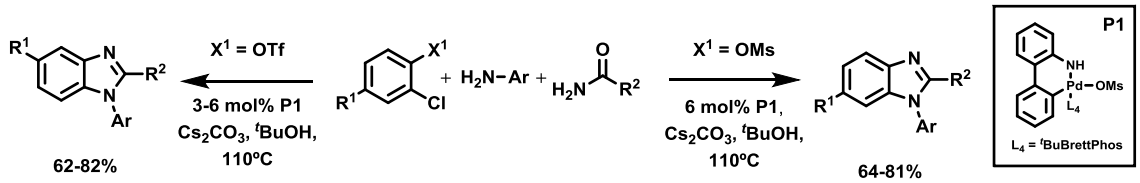
This method was also successfully applied by Legraverend and Ibrahim in the synthesis of substituted purines.[85]

Lindenschimdt and co-workers developed a general palladium-catalyzed amidation of 2-halonitroarenes with secondary amides using a $Pd(TFA)_2$/(*R*)-BINAP system. After reductive aminocyclization with Fe/AcOH the desired 1,2-disubstituted benzimidazoles were achieved with moderate to high yields (Scheme II.18).[86]



Scheme II.18. Amidation of 2-halonitroarenes described by Lindenschimdt.[86]

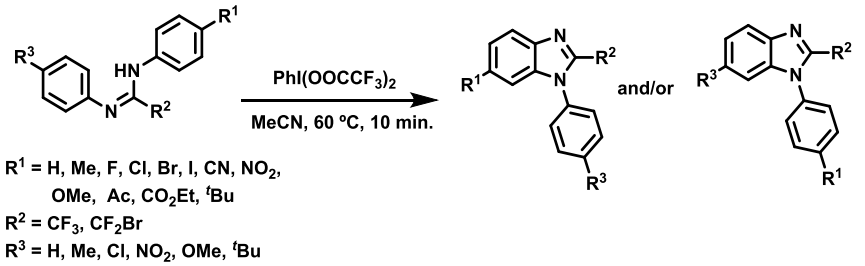
Very recently, Buchwald reported a three component amination/amidation method for the regiocontrolled synthesis of *N*-arylbenzimidazoles (Scheme II.19).[87]



Scheme II.19. Cascade Palladium catalysis.[87]

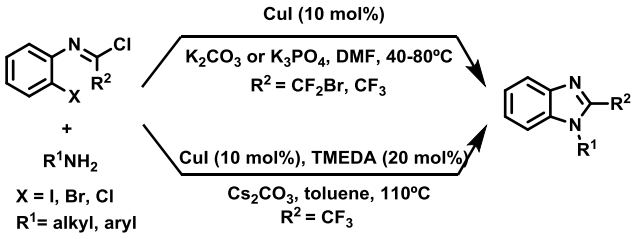
II.1.1.5 Other methods

Fluorinated compounds constitute a significant part of the benzimidazole family. Resembling the metal-catalyzed intramolecular amination process, Wu and co-workers reported the synthesis of *N*-alkyl and *N*-aryl 2-fluoroalkylbenzimidazoles by intramolecular cyclization reaction of several amidines mediated by [bis(trifluoroacetoxy)-iodo]benzene (BTI) (Scheme II.20).[88]



Scheme II.20. Synthesis of *N*-substituted 2-fluoroalkylbenzimidazoles by Wu *et al.*[88]

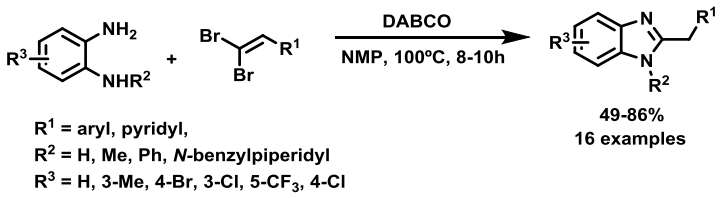
Wu[89] and Zhang[90] groups have independently developed similar procedures to assemble *N*-substituted 2-fluoromethyl benzimidazoles. Both groups reported the double amination of fluorinated acetimidoyl halides with primary amines catalyzed by CuI. While Wu group applied a ligand free approach, Zhang group used a CuI/TMEDA system (Scheme II.21). Wu approach consisted on a Cu-catalyzed coupling of imidoyl chlorides and primary amines under mild conditions.



Scheme II.21. *N*-substituted 2-fluoromethyl benzimidazoles assembly.[89-90]

Zhang group enlarged the scope using different (2-haloaryl)-trifluoroacetimidoyl chlorides with Cl, Br or I in *ortho* position in the presence of a CuI/TMEDA system. It was demonstrated that the presence of trifluoromethyl group is essential for the amination reaction to proceed.

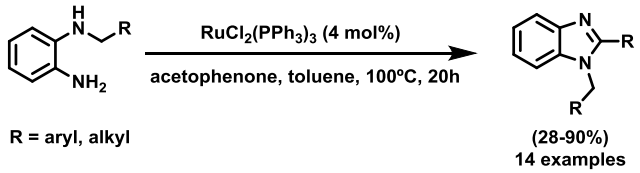
Schmidt and co-workers[91] described the preparation of substituted benzimidazoles from 1,1-dibromoethenes and *o*-diaminobenzenes. The reaction proceeds in the presence of 1,4-diazabiclyco[2.2.2]octane (DABCO), using NMP as solvent at 100ºC (Scheme II.22). The proposed mechanism, based on experimental observations, involves the generation of an alkynyl bromide intermediate upon treatment of dibromide with base. Reaction of the generated intermediate with a corresponding diamine provides an alkynylamine compound, that subsequently cyclises to give the desired substituted benzimidazoles.



Scheme II.22. Synthesis of substituted benzimidazoles from 1,2 dibromoarenes.[91]

More recently Siddapa group[92] used dibromomethylarenes to accesses substituted benzimidazoles in the presence of KO^{t}Bu as base followed by the addition of iodine and catalytic amount of benzoylperoxide under reflux conditions.

Cho and Kim reported the synthesis of 1,2-disubstituted benzimidazoles from *N*-alkyl-1,2-diaminoarenes by means of alkyl group transfer, in the presence of a ruthenium catalyst and acetophenone as hydrogen acceptor (Scheme II.23).[93]



Scheme II.23. Ruthenium catalyzed alkyl group transfer to prepare benzimidazoles.[93]

The development of the novel approaches for benzimidazole synthesis offer alternatives to the restricted classical methods, permitting the access to molecules with increased structural diversity in a straightforward way. However several challenges remain, in particular concerning regioselectivity particularly in solid-phase approaches, for which recently developed solution phase methods might be applied. Moreover, the new advances in metal-mediated C-N couplings could open new possibilities to the successful assembly of heterocyclic compounds.

II.1.2 Evaluation of the inhibitory activity of NSAIDs

The methods applied to evaluate the inhibitory capacity of anti-inflammatory drugs towards COX were developed in the last decades, in an intimate effort to construct new COX inhibitors. Each of these assay systems comprises advantages and drawbacks, and the type of assay must be carefully selected based on the aim of the experiment.

The first *in vitro* testing systems were described in the early 90s and were based on the use of cell lines cultured in the presence of the drugs.[94] The progress made on these assays conducted to systems that use isolated enzymes, receiving exact information about the enzyme-drug interaction while avoiding other cellular influences.[95] However, their major advantages also constitute a drawback since these assays do not mimic the patho-physiological conditions. In fact, several important parameters such as cell-cell interactions, plasma-protein binding of the drug and other blood compounds are neglected.

The use of isolated cell assays has the advantage of being derived from fresh whole blood. However, all these assays are vulnerable to artifacts that take place during cell separation steps, also sharing the disadvantages of isolated enzyme assays since several interactions – besides the drug-cell interplay – are being ignored. Additionally they are time-consuming and therefore inappropriate for routine testing.

Nevertheless, enzyme and cell based assays are useful and powerful tools to determine preliminary drug kinetics and dose-response relationships. Some examples of *in vitro* COX inhibition assay systems use purified/recombinant enzymes, or cell lines obtained from either human or animal sources. [96,97]

Whole blood assays (WBAs) were developed in order to overcome the main drawbacks of isolated enzyme-based or isolated cell assays.[96,98] Unlike in vitro assays, WBAs are carried out in the presence of all blood components, and the compounds activity can be examined in a semi-physiological environment similar to *in vivo* conditions, i.e. 37 °C, homeostasis and cell-cell interactions remain intact.

The WBA for COX inhibition determination has been ameliorated, and recently an improved method was reported by Laufer.[97,99] This WBA is more rapid (stimulation takes only 3-5 h compared to other existing protocols) allowing routine testing.

Thus, human WBA is a simple, fast and reliable method to examine the capacity of NSAIDs at inhibiting COX activity and can be applied for rapid and routine screening purposes. This method can be roughly divided in 3 phases (Figure II.3):

1) Sample preparation (or stimulation of blood);
2) ELISA (enzyme-linked immunosorbent assay) test;
3) Detection and evaluation of results.

In this assay the variability of different donors has to be considered because of metabolism and different enzyme expression. The sample preparation consists on the amplification of PGE_2 content and the stimulation of COX in the presence of the drug (in a fixed concentration) for a predetermined incubation time.

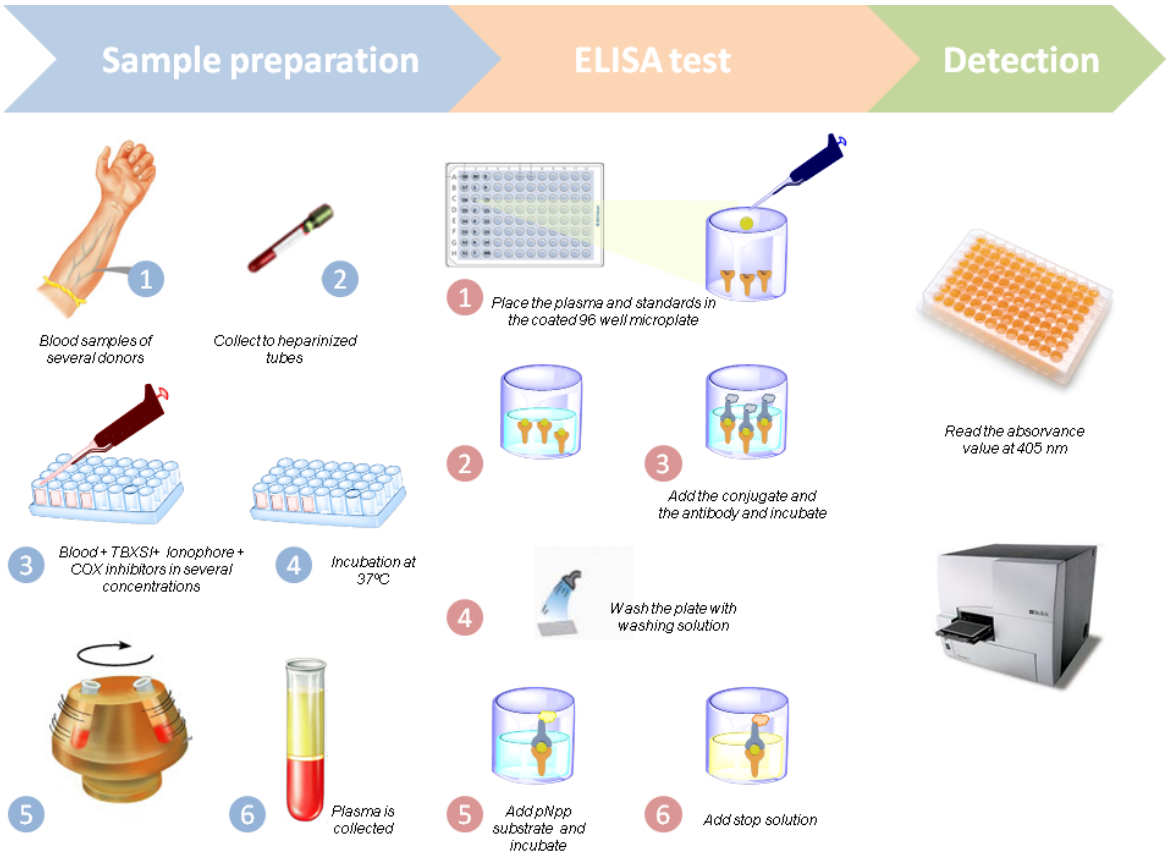


Figure II.3. Whole blood assay stages.

The ELISA test employed in these studies is a commercially available, colorimetric competitive enzyme immunoassay (EIA) kit – the PGE_2 EIA kit. This assay monitors the activity of COX-1 or COX-2 inhibitors by measuring the levels of downstream PGE_2. These levels are inversely related with the added colorimetric reagent which absorvance is read at 405 nm, i.e. the lower the production of PGE_2 (which indicates a higher inhibitory activity), the higher the absorvance.

The *in vitro* evaluation of NSAIDs and selective COX-2 inhibitors can provide the IC_{50} values for both the COX-1 and COX-2 isoforms. This information allows to calculate COX-2 selectivity (ratio of COX-1 IC_{50}/COX-2 IC_{50}), which can be used to compare the different COX inhibitors (Table II.1).

Table II.1 Classification of NSAIDs according to their COX-1/2 inhibitory activities.

Class	Properties	Examples
Group 1	NSAIDs that inhibit both COX-1 and COX-2 completely with little selectivity	Aspirin, ibuprofen, diclofenac, indomethacin, naproxen, piroxicam
Group 2	NSAIDs that inhibit COX-2 with a 5-50 fold	Celecoxib, etodolac, meloxicam, selectivity nimesulide
Group 3	NSAIDs that inhibit COX-2 with a > 50 fold selectivity	Rofecoxib, NS-398
Group 4	NSAIDs that are weak inhibitors of both isoforms	5-Aminosalicylic acid, sodium salicylate, nabumetone, sulfasalazine

Interestingly, Vane and coworkers classified NSAIDs and COX-2-selective compounds based on a careful *in vitro* analysis of COX-1 and 2 inhibition and selectivity data obtained using a human WBA, as shown in Table II.2.[96]

Table II.2. NSAIDs COX-1 and 2 inhibitory activities and COX-2 selectivity index determined using WBAs by Vane *et al.*[96]

Drug	Whole Blood Assay IC_{50} (μM)		Selectivity index
	COX-1	COX-2	
Indomethacin	0.013	1.0	0.013
Fluorbiprofen	0.075	5.5	0.013
Ketoprofen	0.047	2.9	0.016
Aspirin	1.7	> 100	0.017
Naproxen	9.3	28	0.33
Ibuprofen	7.6	7.2	1.05
Diclofenac	0.075	0.038	1.97
Paracetamol	> 100	49	> 2.04
Meloxicam	5.7	2.1	2.7
Nimesulide	10	1.9	5.26
Celecoxib	6.7	0.87	7.7
Valdecoxib	26	0.87	29.8
Rofecoxib	19	0.53	35.8
Etoricoxib	116	1.1	105.4
Lumiracoxib	67	0.13	515

The aim of the study was to present a simple, fast and reliable method to examine the capacity of the studied compounds to inhibit COX-1 and COX-2 and to evaluate their selectivity.

II.1.3 Saturation Transfer Difference (STD) NMR experiments

Reported fifteen years ago by Meyer and Mayer,[100] saturation transfer difference (STD) NMR turned to be a popular ligand-based NMR technique to study molecular interactions of small ligands with biologically relevant macromolecules, such as proteins or nucleic acids.[101,102] It allows the identification of the ligand moieties important for binding, which are important tools for identification of lead structures in the rational drug-discovery process.

The success of this technique is a consequence of its robustness and the fact that it is focused on the signals of the ligand, without any need of processing NMR information about the receptor and only using small quantities of the nonlabeled macromolecule. In fact, this technique, developed over the last decade, offers several advantages over the usual methods to study the binding activities.

The STD-NMR experiment is based on the Nuclear Overhauser Effect (NOE). It starts by irradiating the spectral region containing resonances of the receptor and where no resonances of free ligands are located (such as 0 ppm to -1 ppm), so the protons of the macromolecular receptor are selectively saturated. Due to effective spin diffusion, saturation quickly propagates across the entire receptor. If the ligand contains any residues that bind the receptor, saturation will also spread onto these residues. By chemical exchange, that saturation is carried into solution where is detected. The result will be that intensity of the ligand signals will be attenuated (Figure II.4).

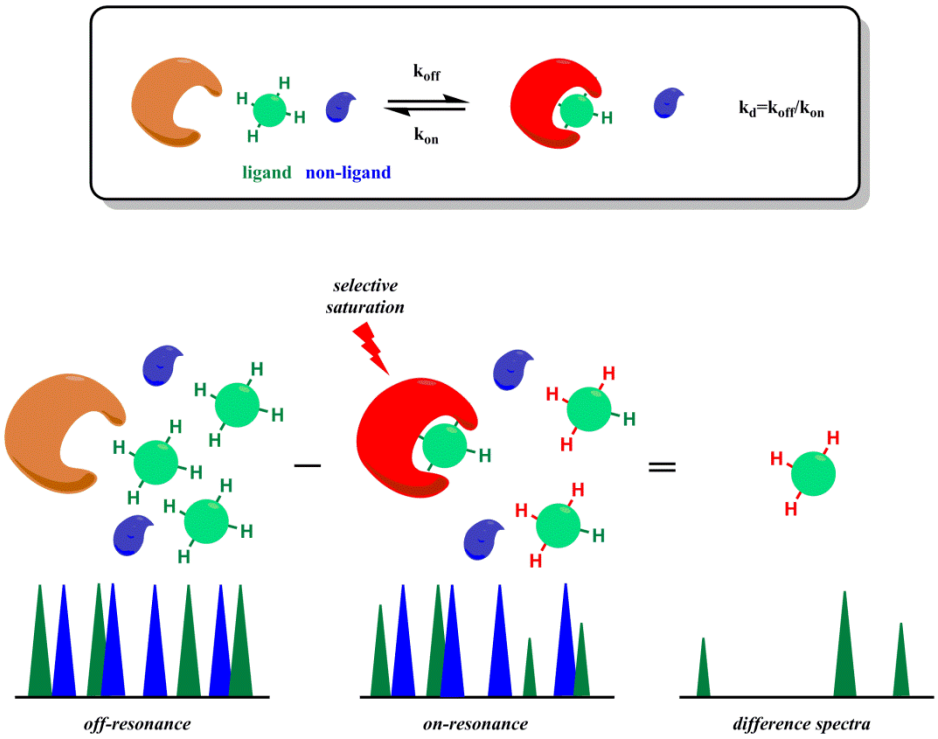


Figure II.4. The STD experiment.

By subtraction of a spectrum, with I_{sat} (on-resonance spectrum), from a spectrum without protein irradiation, with I_0 (off-resonance spectrum), the STD-NMR spectrum is obtained, possessing only signals from the ligand residues that bind to the protein. Other compounds that may be present but do not bind to the receptor will not receive any saturation transfer. Thus, their signals will be of equal intensity on the *on-resonance* and the *off-resonance* spectra and, as a consequence, after subtraction no signals will appear in the difference spectrum from the nonbinding small molecule(s) (Figure II.4).

Thus, the STD spectrum ($I_{STD} = I_0 - I_{SAT}$) contains only the signals of the residues that received saturation transfer from the protein (via spin diffusion, through the nuclear Overhauser effect). The moieties of the ligand having the closest contact to the protein shows the most intense NMR signals, enabling the mapping of the ligand's binding epitope. The term binding epitope is frequently used in the STD-NMR literature to characterize the hydrogens of the ligand that are closer to the protein upon binding.

STD-NMR is a very sensitivity method allowing using as little as 1 nmol of protein with a molecular weight >10 kDa. Moreover, the ligand is normally used in an approximately 50 to 100-fold molar excess over the protein, allowing one to work with low protein and ligand concentrations.

However, the STD-NMR experiment relies on the residence time of the ligand in the protein binding site. The degree of ligand saturation obviously depends on this binding time and on the exchange between the bound and the free ligand state, that allows the screening of binding ligands (with dissociation constants, K_D, ranging from 10^{-3} $molL^{-1}$ to 10^{-8} $molL^{-1}$).[103] The dissociation of the weak binding ligand will transfer this saturation into solution where the free ligand is detected possessing narrow line widths. For those ligands that have strong affinity with protein, a decrease in intensity is observed. In fact, the STD-NMR main drawback is the incapacity to detect the high-affinity ligands which possess a slow chemical exchange on the NMR time-scale. Thus, the detection of these ligands can be made by the competition STD-NMR binding experiments. This approach can be used for screening of ligands over a wider affinity range including high-affinity ligands that would be missed by the STD-NMR method.[103b]

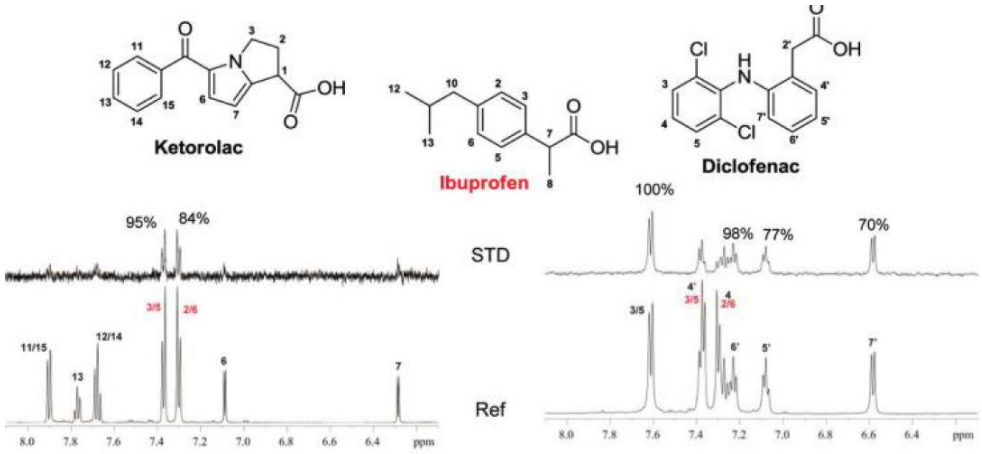


Figure II.5 Competitive experiments between ibuprofen and a) ketorolac, b) diclofenac. [104]

STD-NMR was already used in our group to characterize the binding mode of some anti-inflammatory drugs such as ibuprofen, diclofenac, and ketorolac to COX-1 and COX-2.[104] Based on these studies and in crystallographic structures, the authors proposed that ketorolac should bind to the COX-2 active site in an orientation similar to the already reported for diclofenac. Moreover, the combination of STD-NMR with competition experiments constitutes a valuable tool to address the proposed behavior of COX-2 as functional heterodimer and complements enzyme activity studies in the effort to rationalize COX inhibition mechanisms (Figure II.5).

II.2 Research Objectives

The main objective of this chapter was the development of new benzimidazole-based structures that could act as powerful and selective COX-2 inhibitors. Consequently, it was adopted a rational design strategy comprising docking studies, an original synthetic plan, STD-NMR studies and the biological evaluation of the synthesized compounds. This iterative approach, combining different and complementary tasks allowed an efficient and sustainable construction and monitoring of the new anti-inflammatory candidates (Figure II.6).

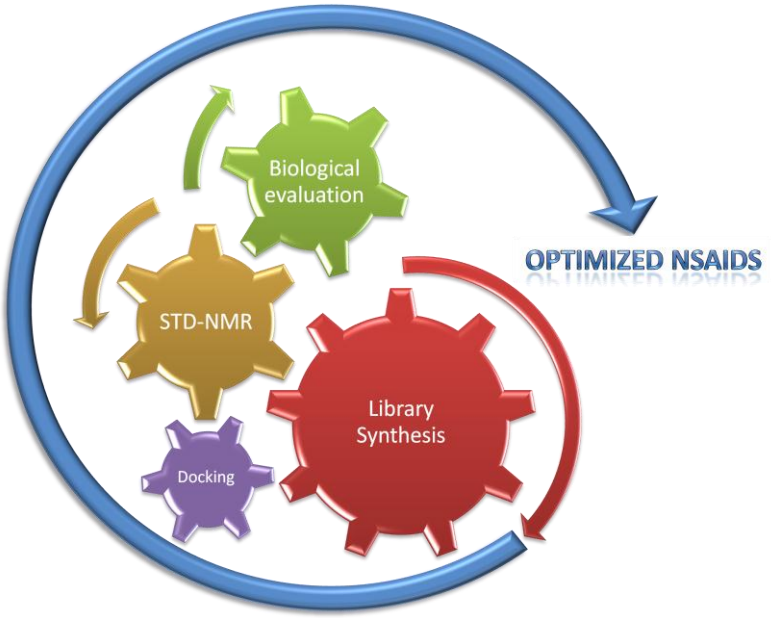


Figure II.6. Rational drug design approach used for the development of novel NSAIDs.

The first step consisted on the screening of all the literature background in order to direct the docking studies and to perceive which structures would have the optimum substitution pattern as potential COX inhibitors. The docking studies narrowed the heterocyclic possibilities to a small group of molecules based on the benzimidazole scaffold. Thus, it was proposed to synthesise this library employing an optimized and straightforward synthetic methodology.

In order to validate the anti-inflammatory activity, the generated benzimidazole library was further evaluated using two different screening methodologies; the biological assays in order to confirm the inhibitory activity towards COX, and the STD-NMR experiments which can unravel the binding mechanism (whitin COX-2) of the studied compounds.

II.3 Results and Discussion

II.3.1 Docking studies and proposed structures

The docking studies performed by Dr. Stefan Erhardt (Edinburgh University) involved the evaluation of several heterocyclic scaffolds with different substitution patterns. The best results were verified for benzimidazole structure (Figure II.7) which conducted to a restricted group of compounds that were selected to be synthesized.

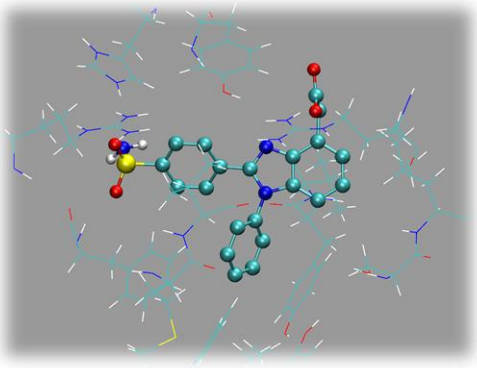


Figure II.7. Proposed docking structure.

The substitution pattern that gave the most promising results was, as expected, the one that contains benzimidazole as the heterocylic core, possessing two aryl substituents at the adjacent *N*-1 and C-2 positions. The aromatic ring in position C-2 should contain a sulfonyl group (sulfonamide or methylsulfone group) in the *para* position, an important feature for activity and selectivity, as already described in Chapter I.

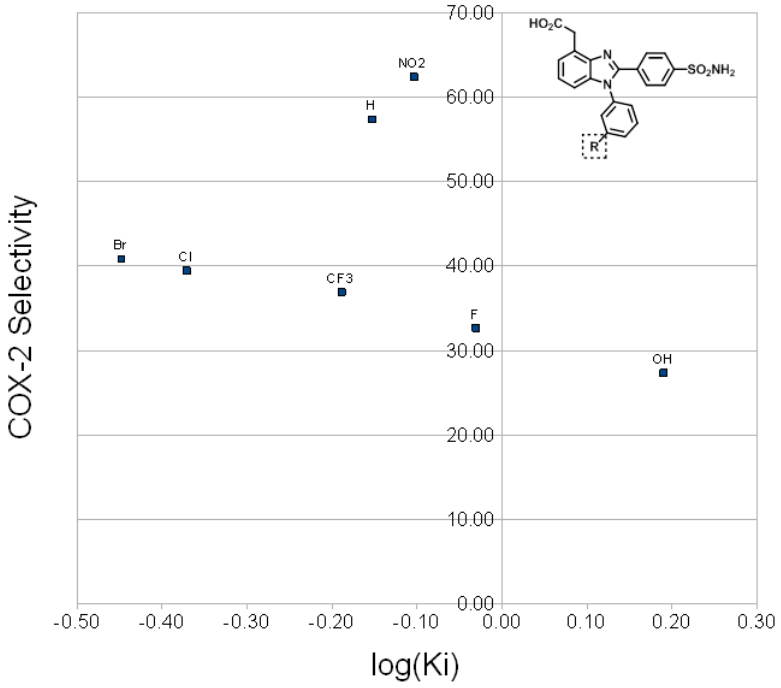


Figure II.8. Docking results based on *meta* substitution (for acid derivatives).

Moreover, it was also proposed that the aromatic ring at *N*-1 must be substituted in *meta* position with a halogen, trifluoromethyl, hydroxyl or nitro group (Figure II.8).

It was also established by the docking studies, that the central benzimidazole core should contain a carboxylic acid, an ester or an amide group in position 4. In fact, it was reported that an additional acetic acid chain, analogous to indomethacin, can lead to an increased inhibitory activity (Figure II.9).[105]

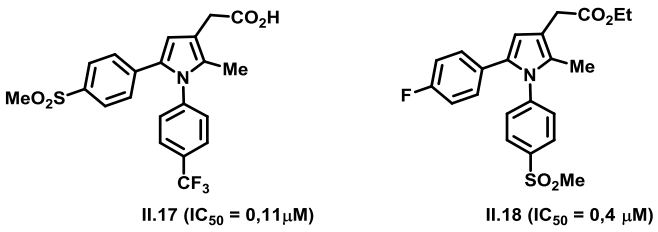


Figure II.9. Pyrrole based structures containing an acetic acid chain and corresponding IC_{50} (COX-2).[105a,b]

Therefore, the proposed general structure for the NSAIDs library is described in Figure II.10.

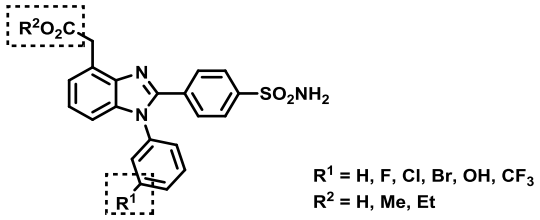


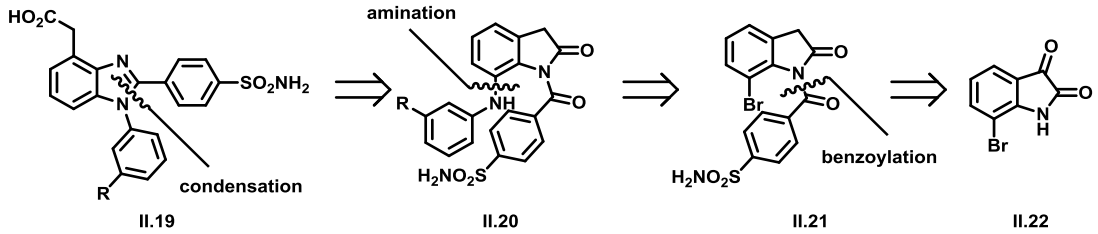
Figure II.10. Proposed benzimidazole structures.

Having in mind the substitution pattern of our goal structures, all the reported synthetic approaches were carefully analysed, as well as the available starting materials, in order to devise an efficient and short-step synthetic plan. Despite the not so evident application in our approach, it was chosen as starting material, the commercially available 7-bromo isatin (**II.22**), which after few synthetic manipulations possesses the key structural features to construct the benzimidazole ring with the desired functionalization. In fact, the manipulation of already existent heretocycles in order to attain new heterocyclic entities with increased structural complexity is a commonly used synthetic approach.

II.3.2 Synthesis of 1,2-Disubstituted benzimidazole library

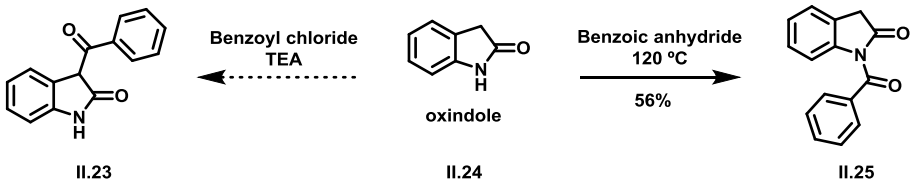
II.3.2.1 Preliminary experiments

In the preliminary retrosynthetic plan to generate the 1,2-disubstituted benzimidazoles library it was envisaged that compounds of general structure **II.19** could be obtained by condensation with concomitant ring opening of **II.20**, which could be prepared by arylamination of **II.21** *via* a metal-catalyzed C-N cross coupling. The retrosynthetic plan included the preparation of **II.21** by *N*-benzoylation of oxindole which in turn, was obtained from the reduction of 7-bromo isatin (**II.22**) (Scheme II.24).



Scheme II.24. Preliminary retrosynthetic plan.

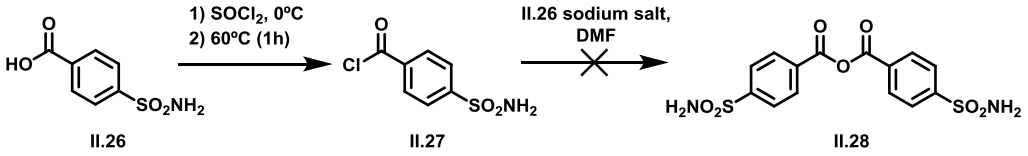
Despite its low nucleophilicity, the *N*-benzoylation of oxindole (**II.24**) was performed using benzoic anhydride in order to obtain compound **II.25** (Scheme II.25). Moreover, the benzoylation on position C-3 (to give **II.23**) was not observed. In fact, the formation of **II.23** could be favoured using benzoyl chloride as acylation agent, in the presence of triethylamine, which can elicit the corresponding *O*-benzoylated enol intermediate that rearranges to form **II.23**.



Scheme II.25. *N*-Benzoylation of oxindole **II.24**.

As a result, it was attempted to synthesize the desired anhydride substituted in the *para* position with the sulfonamide group (**II.28**).

Thus, the corresponding acyl chloride (**II.27**) was synthesized from the commercially available 4-sulfamoyl benzoic acid (**II.26**) (Scheme II.26). After a brief optimization step, it was observed that thionyl chloride needed to be previously placed in an ice bath and vigorously stirred before the slow addition of **II.26**, otherwise side products were obtained. After heating for 1 h at 60ºC, $SOCl_2$ was co-evaporated with dried toluene, to afford **II.27** as a white solid further characterized by IR.



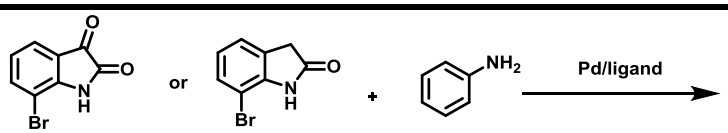
Scheme II.26. Synthesis of acyl chloride **II.27** and attempt to prepare the anhydride **II.28**.

The preparation of the corresponding sulfamoyl benzoic anhydride was then tried reacting the benzoyl chloride **II.27** with the corresponding sodium salt of the acid **II.26**, in DMF. However, product **II.28** was not attained, fact that can be explained by the low solubility of both **II.26** and **II.27**, allied to benzoyl chloride instability. Consequently, this preliminary approach was abandoned.

Since the Pd-catalyzed arylamination of heterocycles was already described with good results,[106] this methodology was also tested for 7-bromo isatin as well as 7-bromo oxindole (Table II.3). A preliminar test was made between **II.22** and aniline in the absence of any Pd salt, and no reaction ocurred.

The BrettPhos system,[107] initially described by Buchwald *et al.* as an excellent ligand to couple anilines with aryl chlorides at low catalyst loadings (e.g. 0.01 mol%), was chosen due to its good results in the cross-coupling of aromatic amines with heterocyclic structures.[106]

Table II.3. Conditions and observations of arylamination of 7-Br isatin (**II.22**) and 7-Br oxindole (**II.29**).[a]



Entry	Compound	System	Solvent	Temperature	TLC
1	**II.22**	BrettPhosPd/BrettPhos precatalyst; LiHMDS (5 equiv)	THF	65ºC	Complex mixture
2	**II.22**	BrettPhosPd/BrettPhos precatalyst; LiHMDS (3.4 equiv)	THF	65ºC	Complex mixture
3	**II.22**	BrettPhosPd/BrettPhos precatalyst; LiHMDS (2.2 equiv)	THF	65ºC	No reaction
4	**II.29**	BrettPhosPd/BrettPhos precatalyst; LiHMDS (5equiv)	THF	65ºC	No reaction
5[b]	**II.29**	Pd_2dba_3/BINAP; Cs_2CO_3[b]	dioxane	90ºC	Complex mixture

[a] BrettPhos Pd (1 mol %), BrettPhos precatalyst (1 mol %), aniline (1.2 equiv); [b] Pd_2dba_3 (5mol%), BINAP (7.5 mol%); Cs_2CO_3 (3 equiv).

However, employing the same procedure for **II.22** resulted in a complex mixture that was insoluble during the work up procedure (entries 1 and 2). This could be attributed to the intermolecular arylamination to form a polymeric structure. Decreasing the amount of base to 2.2 equivalents prevented the reaction to occur.

BrettPhos system was also employed to **II.29** without obtaining the expected results (entry 4). Using Pd_2dba_3/BINAP in the presence of Cs_2CO_3 resulted in a complex TLC (entry 5). The reaction was not further explored for **II.29** due to the possible intermolecular C-arylation in position 3, since this type of oxindole arylations was already reported by Buchwald group.[108]

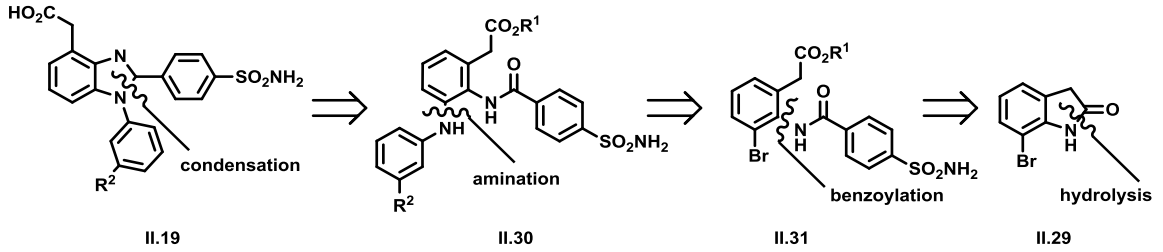
Since the preliminary approach failed, an improved retrosynthetic plan was proposed.

II.3.2.2 First retrosynthetic approach

As in the preliminary experiments, 7-bromo isatin (**II.22**) was chosen as starting material. The retrosynthetic analysis is depicted in Scheme II.27.

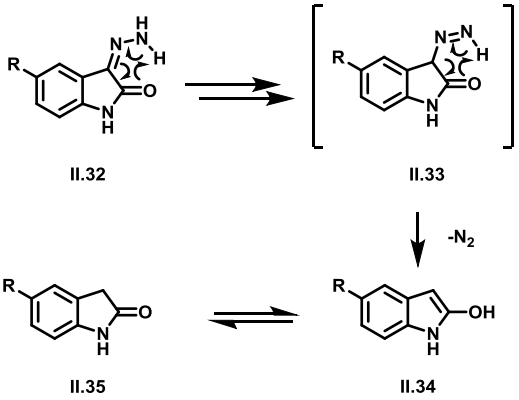
It was anticipated that compounds of general structure **II.19** could be obtained from **II.30** by an acid catalyzed cyclization and carboxylic acid deprotection. Derivative **II.30** could be conceivably produced by Pd-catalyzed arylamination of **II.31** with several *meta*-substituted anilines.

The synthetic intermediate **II.31** could be prepared from 7-bromo oxindole (**II.29**) by basic hydrolysis, carboxylic acid protection and benzoylation with sulfamoyl benzoyl chloride (**II.27**). 7-Bromo oxindole (**II.29**) can be readily attained by reduction of **II.22**.



Scheme II.27. First retrosynthetic plan to prepare benzimidazole of general structure **II.19**.

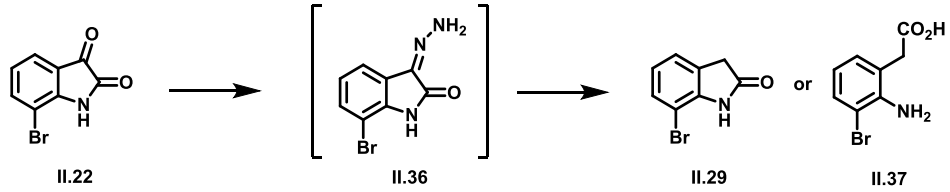
The first synthetic step involved the reduction of the 7-bromo isatin (**II.22**) to compound **II.29**, which can be performed using different procedures.[109] Hence, it was adopted the method described by Saladino *et al.* [110] In this method, that describes a mild Wolff-Kishner reduction of isatins, it was expected to obtain the desired product in a very fast manner and in good yields without isolation of the corresponding hydrazone. It was proposed that the reduction is more efficient than the usual Wolff-Kishner procedure since α-ketoamide can form an intramolecular hydrogen bonding with the hydrazine proton, creating a six member ring intermediate (**II.32**). Proton tranfer can proceed through the enol form to product **II.33**, that after a second similar process with subsequent loss of a nitrogen molecule yields the final product **II.35** (Scheme I.28).



Scheme II.28. Proposed mechanism for reduction of isatins by Crestini and Saladino.[110]

However, the ^{1}H NMR of the crude mixture showed a complex mixture of the hydrazone (**II.36**), the product **II.29** and other uncharacterized products (Table II.4, entries 1 and 2).

Table II.4. Conditions used for Wolff-Kishner reduction and corresponding observations.

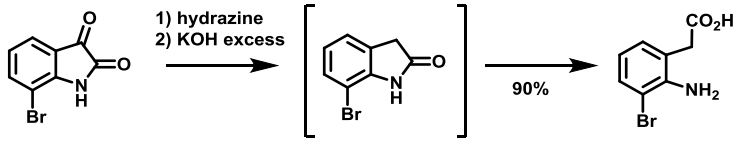


Entry	Conditions	Solvent	Temperature/Time	Observation/ Yield (%)
1	Hydrazine hydrate 65%	---	Reflux (30 min).	Mixture
2	Hydrazine hydrate 99-100%	---	Reflux (30 min).	Mixture
3	1) Hydrazine hydrate 99-100% 2) KOH (5 equiv)	DEG	1) 80 ºC (1h) (hydrazone isolation) 2) 80 ºC (1h)	**II.29** + **II.36**
4	1) Hydrazine hydrate 99-100% 2) KOH (5 equiv)	DEG	1) 80 ºC (1h) 2) 80 ºC (1h)	Product not characterized
5	1) Hydrazine hydrate 99-100% 2) KOH (5 equiv)	DEG	1) 80 ºC (1h) 2) 80 ºC (overnight)	Product not characterized
6	1) Hydrazine hydrate 99-100% 2) NaOEt (3 equiv)/ethanol	EtOH	1) 80 ºC (1h) (hydrazone isolation) 2) 120 ºC (2h)	**II.22** + Product not characterized
7	1) Hydrazine hydrate 99-100% 2) KOH (5 equiv)	DEG	1) 80 ºC (1h) 2) 120 ºC (2h)	**II.29** (82%)
8	1) Hydrazine hydrate 99-100% 2) KOH (17 equiv)	DEG	1) 80 ºC (1h) 2) 120 ºC (2h)	**II.37** (90%)

In this way it was adopted the classical Wolff-Kishner conditions; in a first step the formation of **II.36** using hydrazine hydrate in diethyleneglycol (DEG), followed by a basic hydrolysis with KOH (entries 3-5). Thus, compound **II.36** was formed by heating the isatin solution in DEG with hydrazine hydrate at 80ºC. The formation of **II.36** could be visually monitored: the translucid red solution corresponding to **II.22** progressed towards a strong yellow precipitate (hydrazone). It can be also pointed out that the water content of hydrazine highly influenced the reaction outcome. The hydrazone was then isolated and further hydrolysed under basic conditions, at 80ºC, to yield a mixture of **II.36** and **II.29** (entry 3). When hydrazone was not isolated an uncharacterized product was obtained (entry 4 and 5).

Facing these problems it was then explored a similar procedure where the hydrolysis step was performed at 120ºC for 2 h using sodium ethoxide (NaOEt) in ethanol (entry 6).[111] However, it was obtained a mixture of starting material and an uncharacterized product. Gladly, applying these same temperature and time conditions to the previous method (KOH/DEG) the product **II.29** was isolated in good yield (entry 7).

The next step consisted on the amide bond hydrolysis of **II.29** that could be made after the purification of this compound. Given that the Wolff-Kishner reaction evolves under harsh basic conditions, it was verified that using an excess of KOH (17 equiv), the ring opening product (**II.37**) could be easily obtained (Table II.4, entry 8, and Scheme II.29).



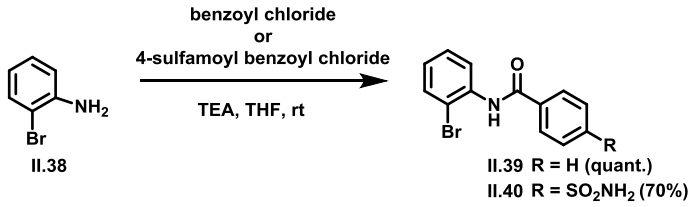
Scheme II.29. Cascade reaction to obtain compound **II.37**.

In fact this compound was easily purified in the work-up procedure; performing several extractions with ethyl acetate before neutralization of the reaction mixture, allowed the removal of all undesired organic compounds. A careful neutralization using a 1M HCl solution (performed in an ice bath), allowed the product to precipitate. Filtration and washing with cold water afforded **II.37** in almost quantitative yield.

II.3.2.3 Arylamination experiments

Encouraged by the early studies, developed by Ma and Buchwald that reported the *o*-acetanilide group effect on arylamination, we devised that similar molecules could aid the construction of the benzimidazole library.[50a,b] Thus, in order to perform preliminary studies on the arylamination reaction, two *N*-benzoylated model structures **II.39** and **II.40** were synthesized from 2-bromo

aniline (**II.38**). Thus, **II.38** was reacted with benzoyl and the 4-sulfamoylbenzoyl chlorides, to obtain the compounds **II.39** and **II.40**, respectively (Scheme II.30).



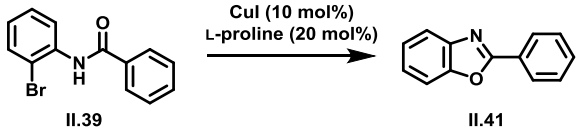
Scheme II.30. Benzoylation of **II.38** with the corresponding acid chlorides to yield **II.39** and **II.40**.

Both copper and the palladium salts were already described as efficient catalysts in C-N cross-coupling reactions. Moreover, both were described for the amination of 2-haloacetanilides (*see* Schemes II.14 and II.15). However, copper salts as well as their ligands are readily available and much cheaper than the palladium counterparts that require particular ligands in order to attain enhanced yields.

Thus, the first approach to the arylamination reaction involved a copper catalysis previously described by Ma *et al.*[50b] In this work, it was demonstrated that NHCOR groups provide an *ortho*-substituent effect in the amination of 2-haloacetanilides, allowing the reaction to proceed under very mild conditions. This amination reaction was catalyzed by CuI/L-proline under K_2CO_3 conditions in DMSO (*see* Scheme II.14).

The reaction was then tested using the model structure **II.39** and aniline under the conditions reported by Ma *et al*. (Table II.5).

Table II.5. Conditions and observations to the copper catalyzed arylamination of compound **II.39**.[a]



Entry	Base	Solvent	Temperature	Yield (%)
1	K_2CO_3	DMSO	rt → 40ºC → 60ºC [b]	23
2	K_2CO_3	DMSO	70ºC	22
3	K_3PO_4	DMSO	65ºC	59
4	K_2CO_3	toluene	reflux	61
5	K_3PO_4	toluene	reflux	94

[a] CuI (10 mol %), L-proline (20 mol %), K_2CO_3 (2 equiv), aniline (1.5 equiv), overnight; [b] 3 days.

However, using the reported conditions (at 40ºC) the reaction did not proceed towards the desired product (monitored by TLC). When the temperature was further raised to 60ºC, a small amount of benzoxazole (**II.41**) was isolated (entry 1).

The same result was obtained when the reaction was carried at 70ºC (entry 2). Using K_3PO_4 as base, a slightly increase of benzoxazole quantity was observed but without any product formation (entry 3). Changing the solvent to toluene, increased the benzoxazole amount either using K_2CO_3 or K_3PO_4 (entries 4 and 5). Moreover, K_3PO_4 seems to potentiate **II.41** formation.

It was assumed that copper-catalyzed amination reaction only works for alkyl amines, requiring moderate temperatures for these derivatives. The less nucleophilic aniline hinders the reaction, and an increase in temperature gives the corresponding benzoxazole despite the several reaction conditions used. Moreover, Ma *et al.* employed the more reactive aryl iodides instead of aryl bromides, which requires higher temperatures and longer times, promoting the benzoxazole formation. The observed results establish that the conditions used by Ma *et al.* cannot be employed for these substrates, which tend to form the corresponding benzoxazoles by cyclization of the corresponding amides under copper conditions.[112]

Since catalysis using the system CuI/L-proline did not give the expected results, it was adopted a Pd-catalyzed arylamination method reported by Buchwald using the BrettPhos precatalyst with the corresponding phosphine ligand.[5] These new palladium based systems, mostly developed on the last decade, allowed to extend the C-N cross coupling to a wide scope of reactants (Figure II.11).[113]

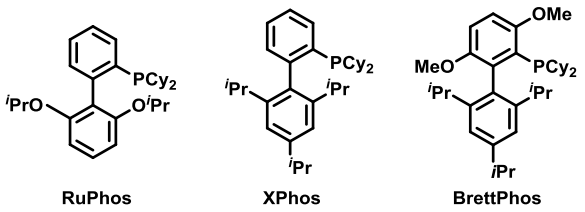
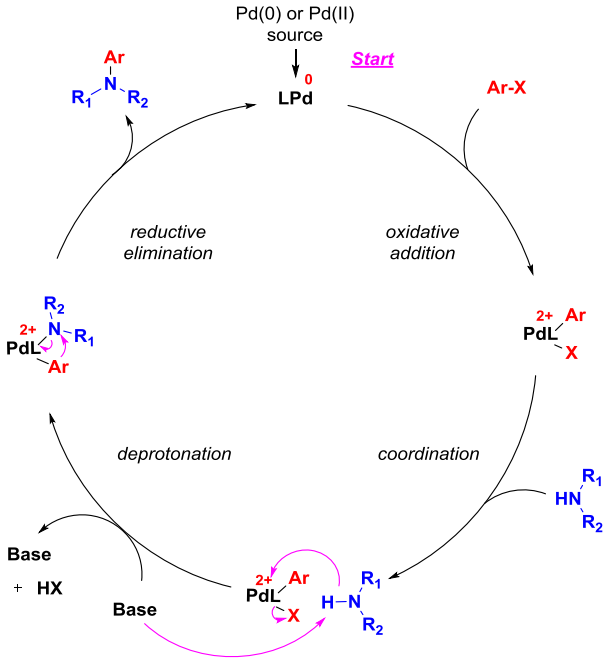


Figure II.11. Some of the most widely used Buchwald ligands.

The Buchwald-Hartwig amination (independently reported by Buchwald and Hartwig),[114] is a cross-coupling reaction of an aryl halide with an amine using a palladium salt as catalyst in the presence of a strong base.

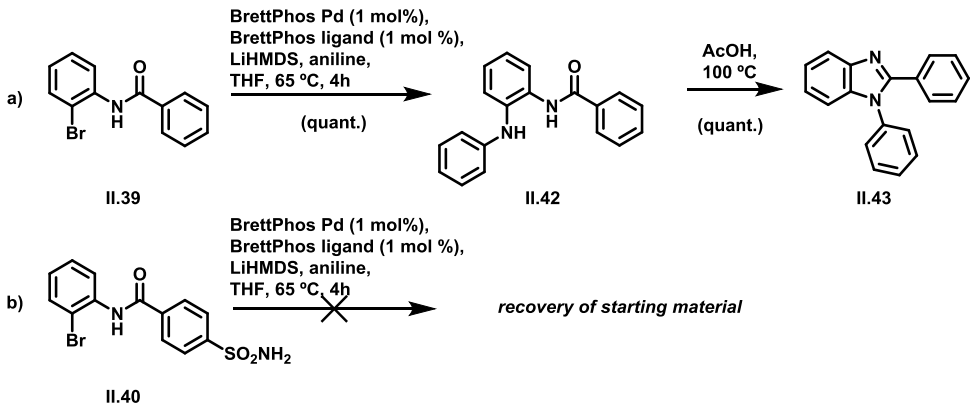
The active catalyst is commonly achieved using a Pd(0) source, such as Pd_2dba_3 or by reducing a Pd(II) salt *in situ* [e.g. $Pd(OAc)_2$]. The mechanism starts with the oxidative addition of the aryl halide to Pd(0) which is followed by coordination of the amine to the palladium (Scheme II.31). A strong base then abstracts a proton from the amine, which in turn attacks the palladium expelling the halide as a leaving group. Finally, reductive elimination yields the aryl amine product and regenerates the catalyst.



Scheme II.31. Catalytic cyclic for Pd-catalyzed amination reaction.

Indeed, the first experiment using BrettPhos catalytic system was very encouraging since the C-N cross coupling reaction involving the model compound **II.39** yielded the derivative **II.42** in quantitative yield (after chromatographic isolation). After cyclization under acidic conditions (acetic acid under reflux), it was obtained the corresponding 1,2-disubstituted benzimidazole **II.43** (Scheme II.32.a).

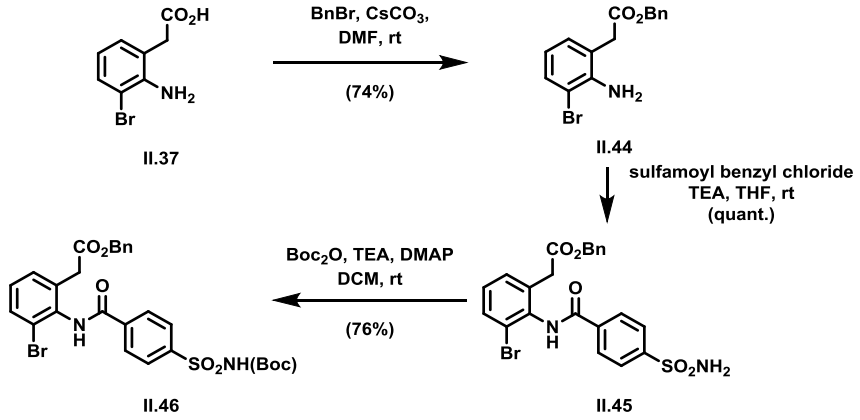
The same procedure was also employed for sulfonamide derivative **II.40** though without success, maybe due to the poor solubility of compound **II.40** in THF (Scheme II.32.b).



Scheme II.32. Arylamination experiments using BrettPhos system to achieve the benzimidazole core.

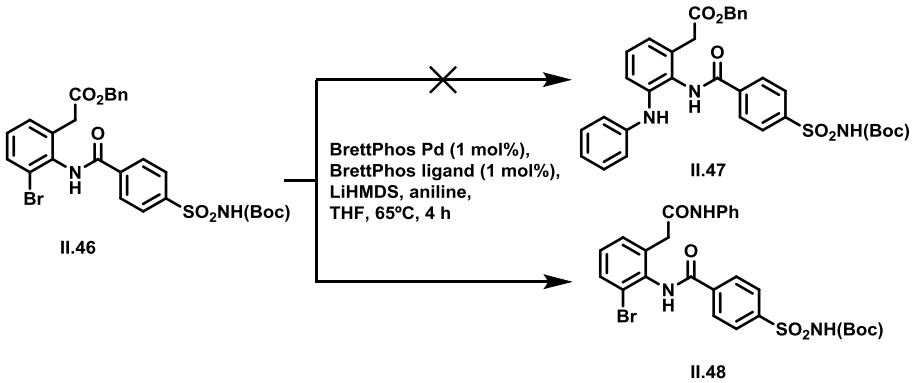
Having the preliminary information about the BrettPhos catalyzed cross-coupling reaction, the key intermediate **II.37** was further modified in order to proceed with the synthesis.

The first step consisted on carboxylic acid moiety modification to the corresponding benzyl ester, which was expected to survive under the amination conditions while increasing the compound solubility. Thus, compound **II.37** was reacted with benzyl bromide in DMF in the presence of Cs_2CO_3 at room temperature to give **II.44** in 74% yield. Compound **II.44** was further *N*-benzoylated to obtain **II.45** quantitatively. Moreover, the sulfonamide group was protected using the *tert*-butyloxycarbonyl (Boc) group expecting to reduce the solubility issue. Thus product **II.46** was obtained with a global yield of 56% (Scheme II.33).



Scheme II.33. Synthesis of derivative **II.46**.

The arylamination of derivative **II.46** with aniline was firstly attempted using the BrettPhos system conditions. After four hours of reaction at 65ºC, it was verified the total consumption of starting material, although compound **II.48** was formed with 84% yield instead of the desired **II.47** (Scheme II.34). This fact was confirmed by mass analysis and by ^{1}H RMN that clearly showed the absence of the signal corresponding to the benzylic CH_2 (5.1 ppm). Moreover, the aromatic signals corresponding to aniline were identified, along with the observation of the signal corresponding to the formed amide NH at 10.1 ppm (acetone-d_6).

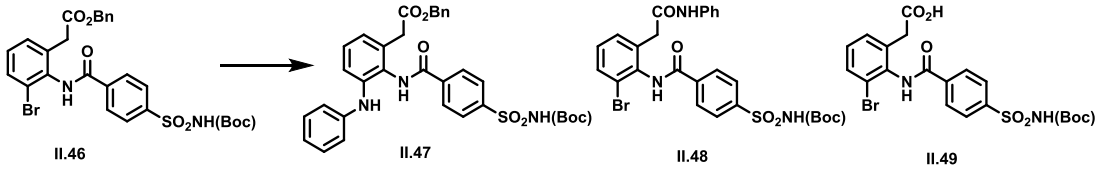


Scheme II.34. BrettPhos catalyzed arylamination of derivative **II.46.**

It was further made an exhaustive study in order to establish the optimal conditions for the arylamination reaction without the loss of the benzyl group (*see* Table II.6).

Decreasing the reaction temperature from 65 ºC to 40 ºC did not change the reaction outcome, since after 4 h it was observed the formation of compound **II.48**, although with low conversion (entry 2). Changing the solvent to toluene and using the same catalytic system, it was verified that after 2 h at 65 ºC there was no product formation. Increasing the temperature to 100 ºC gave **II.48** in 71 % yield (entry 3).

Table II.6. Reaction conditions and observations of Pd-catalyzed arylamination of **II.46** with aniline.[a]



Entry	Catalyst (mol %)	Base	Solvent	Temperature	Yield (%)
1	BrettPhos Pd (1 mol%) + BrettPhos ligand (1 mol%)	LiHMDS (3.4 equiv)	THF	65 ºC	84 (**II.48**)
2	BrettPhos Pd (1 mol%) + BrettPhos ligand (1 mol%)	LiHMDS (3.4 equiv)	THF	40 ºC	58 (**II.48**)
3	BrettPhos Pd (1 mol%) + BrettPhos ligand (1 mol%)	LiHMDS (3.4 equiv)	Toluene	1) 65 ºC [c] 2) 100 ºC [c]	71 (**II.48**)
4	BrettPhos Pd (1 mol%) + BrettPhos ligand (1 mol%)	Cs_2CO_3 (2.5 equiv)	Toluene	1) 65 ºC [c] 2) 100 ºC [c]	0 [b]
5	BrettPhos Pd (1 mol%) + BrettPhos ligand (1 mol%)	NaO^tBu (3.2 equiv)	Toluene	1) 80 ºC [c] 2) 100 ºC [c]	0 [b]
6	BrettPhos Pd (10 mol%) + BrettPhos ligand (10 mol%)	NaO^tBu (3.2 equiv)	Toluene	1) 80 ºC [c] 2) 100 ºC [d]	62 (**II.49**)
7	$Pd_2(dba)_3$ (2 mol%) + BrettPhos ligand (8 mol%)	K_3PO_4 (2.5 equiv)	^{t}BuOH	110ºC	66 (**II.49**)
8	$Pd_2(dba)_3$ (8 mol%) + BINAP (12 mol%)	Cs_2CO_3 (1.4 equiv)	dioxane	100ºC	Complex mixture

[a] aniline (1.5 equiv); [b] starting material recovery; [c] 2 h; [d] 18 h.

Changing the base to Cs_2CO_3, a base not as strong as LiHDMS, the starting material was recovered without formation of any product (entry 4). The same was verified when NaO^tBu was employed despite the high temperature used overnight (entry 5). Increasing the mol% of BrettPhos Pd and ligand from 1 mol% to 10 mol% while using NaO^tBu, it was verified that after 20 h the debenzylated product **II.49** was obtained without arylamination reaction (entry 6). These results indicate that the BrettPhos system is not promoting the arylamination reaction, while other side reactions are taking place such as ester hydrolysis or amidation. Other catalytic systems were used, such as Pd_2dba_3 (2 mol%) and BrettPhos (8 mol%) in the presence of K_3PO_4 in ^{t}BuOH, which conducted to a mixture of product **II.49** and starting material (entry 7), or Pd_2dba_3 (8 mol%)/BINAP (12 mol%), although a complex mixture was obtained in this case (entry8).

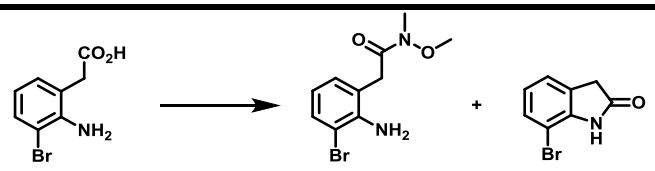
II.3.2.4 Protection of carboxylic acid

In order to solve the debenzylation problem verified in the arylamination reaction, some attempts were made to protect the carboxylic acid with different protecting groups. Despite its known lability, groups like the *tert*-butyl dimethylsilane (TBDMS) or the trimethylsilane ether (TMSE) were tested. However, the conditions used for the insertion of these groups promoted the ring closing to obtain mainly the oxindole **II.29**. It was also attempted the protection of **II.37** with trytil group, however with poor yields.

Since the first attempts to protect **II.37** failed, it was tried to mask the carboxylic acid moiety forming the corresponding Weinreb amide (**II.50**). Weinreb amides are important intermediates in organic chemistry,[115] and usually cleaved under basic conditions.[116] Although in less extension, it was also expected the intramolecular attack of amine to the activated acid specie. Thus, some efforts were made to avoid this lateral reaction and to enhance the product formation (Table II.7).

Thus, **II.37** was activated with DCC in DCM (at -10ºC) followed by the addition of the free *N*,*O*-dimethylhydroxylamine. However, it was observed by ^{1}H NMR that besides the desired product **II.50** it was also formed the corresponding oxindole that unhappily possessed the same retardation factor (*Rf*) of **II.50** (entry 1). This fact made the reaction monitoring and the product purification difficult tasks.

Table II.7. Conditions and observations of Weinreb amide formation (**II.50**).



II.37 **II.50** **II.29**

Entry	Conditions	Solvent	Temperature	Ratio [a]
1	1) **II.37** + DCC (10 min.) 2) hydroxylamine (1.1 equiv) + TEA (10 min.)	DCM	1) – 10 ºC 2) rt	1:1
2	1) **II.37** + DCC (10 min.) 2) hydroxylamine (2 equiv) + TEA (30 min.)	DCM	1) – 25 ºC 2) rt	1.5:1
3	1) **II.37** + DCC (5 min.) 2) hydroxylamine (5 equiv) + TEA (20 min.)	DCM	1) 0ºC 2) rt	1.6:1
4	1) **II.37** + DCC (15 min.) 2) hydroxylamine (1.1 equiv) + TEA (10 min.)	THF	1) -10ºC 2) rt	0.4:1
5	1) **II.37** + hydroxylamine (1.1 equiv) + TEA 2) DCC	DCM	1) 0 ºC 2) rt	1.4:1

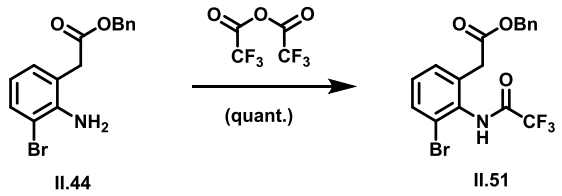
[a] verified by ^{1}H RMN.

When the amount of hydroxylamine was increased, a small increase of **II.50** was achieved (entries 2 and 3). In THF, the yield was very poor since the *N*,*O*-dimethylhydroxylamine hydrochloride was very insoluble (entry 4). Since the ring closing is promoted by the initial acid

activation, the addition order was inverted. Thus, a solution of DCC in DCM was added to a mixture of starting material, hydroxylamine and triethylamine in DCM, however without improvement (entry 5).

II.3.2.5 Trifluoroacetyl group in *ortho*- position

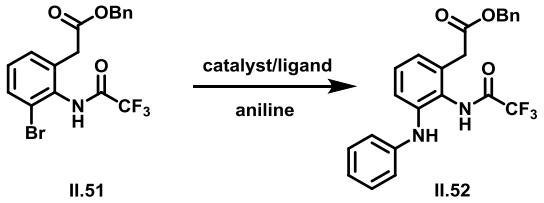
Due to the failure of the previous attempts, it was envisioned that trifluoroacetyl group could be a valuable *ortho*-auxiliary group in arylamination.[50b,117] Thus, compound **II.51** was prepared reacting **II.44** with the corresponding trifluoroacetic anhydride in quantitative yield (Scheme II.35).



Scheme II.35. Trifluoroacetylation of derivative **II.44.**

Several arylamination experiments were made using compound **II.51** as described in Table II.8. Despite using the reported conditions for these type of compounds ($Pd_2(dba)_3$/RuPhos/K_3PO_4), the reaction did not proceed (entry 1).[50a]

Table II.8. Arylamination attempts using derivative **II.51**.[a]



Entry	Conditions	Base	Solvent	Temperature	Observations
1	$Pd_2(dba)_3$ (2 mol%), RuPhos (8 mol%)	K_3PO_4 (2.5 equiv)	^{t}BuOH	110 ºC (18h)	Complex mixture
2	$Pd_2(dba)_3$ (2 mol%), RuPhos (8 mol%)	LiHMDS (2.5 equiv)	THF	80 ºC (18h)	Complex mixture
3	$Pd_2(dba)_3$ (8 mol%), BINAP (12 mol%)	Cs_2CO_3 (1.4 equiv)	Toluene	60ºC (3.5h)	-[b]
4	$Pd_2(dba)_3$ (8 mol%), BINAP (12 mol%)	Cs_2CO_3 (1.4 equiv), TEA (0.5 equiv)	Toluene	90 ºC (18h)	-[b]
5	$Pd_2(dba)_3$ (10 mol%), BINAP (15 mol%)	NaOtBu (2.1 equiv)	dioxane	1) 65 ºC (2h) 2) 100 ºC (18h)	-[b]

[a] aniline (1.2 – 1.4 equiv); [b] starting material recovery.

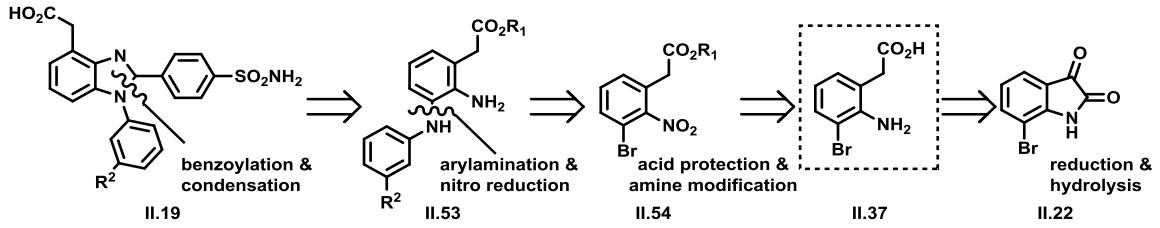
Employing a stronger base such as LiHMDS, a complex mixture was verified by TLC (entry 2). It was then envisaged the use of the $Pd_2(dba)_3$/BINAP system, but once more complex mixtures were obtained (entries 3-5).

The obtained results can be attributed to the substitution pattern of this molecule. It can be suggested that the presence of the benzyl ester moiety is impeding the arylamination reaction.

II.3.2.6 Second retrosynthetic approach

As discussed in the previous sections, there were several handicaps that hampered the first strategies to assemble the desired benzimidazole structures. A new plan was formulated as depicted in Scheme II.36. In this new retrosynthetic plan, it was envisaged that amine group should be masked in order to affect neither the acid protection nor the arylamination step. Moreover, the introduction of an adequate group at this position can highly favor the arylamination reaction.

Thus, benzimidazoles **II.19** could be obtained by benzoylation of derivative **II.53** followed by an acid catalyzed condensation. Synthon **II.53** could be attained by arylamination of **II.54** with different *meta* substituted anilines. Additionally, an extra synthetic (reductive) step is needed to recover the amine moiety. Derivative **II.54** can be prepared by amine oxidation followed by esterification of intermediate **II.37**.

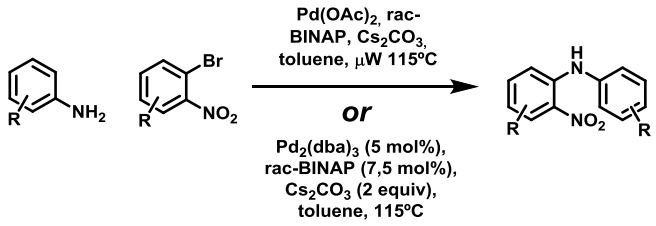


Scheme II.36. Second retrosynthetic plan to assemble benzimidazoles of general structure **II.19**.

It is noteworthy that compound **II.37** [obtained from the reduction 7-bromo isatin (**II.22**) with concomitant amide bond hydrolysis] is the key molecule to construct the desired benzimidazole core. This structure contains the key elements to assemble the benzimidazole core with the acetic acid moiety in position 4.

II.3.2.7 Benzimidazole library synthesis through the nitro intermediate

Due to the observed difficulties to accomplish the arylamination reaction using the above mentioned methods, it was devised the amine modification to a nitro group. In fact, it seems that the introduction of a nitro group can favor the arylamination reaction, as previously reported for *o*-halo nitro arenes (Scheme II.37).[118]

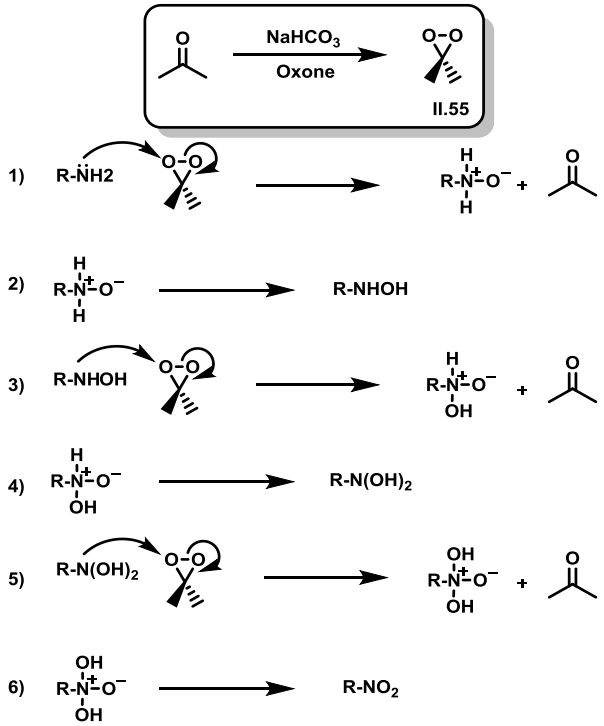


Scheme II.37. Previous reported strategies of arylamination using anilines.[118]

Thus, it was envisaged that the amine moiety of derivative **II.37** could be readily oxidized to nitro group in order to obtain derivative **II.56**.

Direct oxidation of aromatic amines to nitro compounds can be achieved using several reagents.[119] However, some of these procedures usually lead to the formation of side products formed mainly from the partial oxidation of the amines.

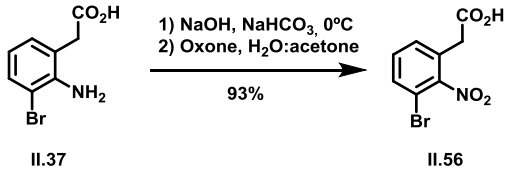
In this work, it was adopted a method previously described in the literature for anilines,[120] that uses Oxone® as the oxidizing agent – a triple salt of $2KHSO_5 \cdot KHSO_4 \cdot K_2SO_4$ – where the active component is the potassium peroxymonosulfate. This salt, when placed in a buffered aqueous acetone medium, forms the active and unstable intermediate dimethyldioxirane **II.55** that will act as the oxidant specie of amine group (Scheme II.38).



Scheme II.38. Formation of the intermediate dimethyldioxirane (**II.55**) and proposed oxidation mechanism.

Compound **II.37** was firstly stirred in a basic solution of NaOH and $NaHCO_3$ placed in an ice bath protected from light. Then, Oxone® was added to the solution followed by the slow addition

of a water/acetone mixture. After quenching with an acidic solution and extraction with ethyl acetate, the product was afforded in 93% yield (Scheme II.39). Since the chromatographic isolation of compound **II.56** was problematic (due to the strong affinity to silica-gel), it was chosen to use it directly in the next reaction step, without any purification.



II.37 II.56

Scheme II.39. Preparation of nitro derivative **II.56.**

Due to the preliminary problems faced with the choice of the carboxylic acid protecting group, it was prepared the methyl ester derivative (**II.57**) in order to be further used in the arylamination reaction. To prepare the compound **II.57**, several standard methods were examined: 1) formation of the corresponding acyl chloride by using $SOCl_2$ in MeOH; 2) acid activation with DCC followed by methanol addition; 3) methylation using MeI or diazomethane, or 4) esterification in MeOH/HCl. In Table II.9 are resumed the results for the different methods.

Table II.9. Conditions for esterification of compound **II.56**.

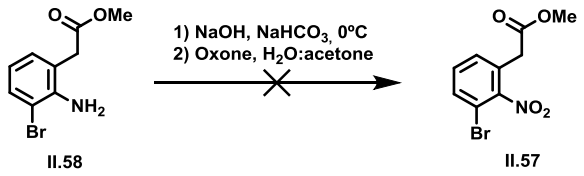
II.56 II.57

Entry	Conditions	Solvent	Temperature/Reaction time	Yield (%)
1	$SOCl_2$	MeOH	rt/overnight	19
2	1) DCC; 2) MeOH	DCM	rt/3h	48
3	MeI, K_2CO_3	DMF	rt/overnight	Mixture
4	1) NaH; 2) MeI	DMF	rt/overnight	Mixture
5	Diazomethane	DCM	rt/1h	58
6	MeOH/HCl	MeOH	reflux/3h	67

It was verified that the yields were globally not high, which can be explained by the use of the crude mixture of **II.56**. Other explanation could be attributed to the presence of the nitro group since it could form intramolecular hydrogen bonds with the hydroxyl group of carboxlic acid, decreasing its reactivity on the reaction.

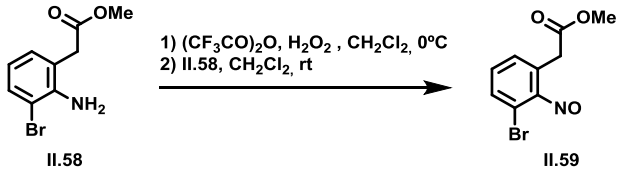
Esterification methods like $SOCl_2$/MeOH or the DCC activation followed by the addition of MeOH, presented poor yields (entries 1 and 2). It was also verified on TLC – by comparison with the isolated product, that methylation with MeI did not form **II.57**, yielding a mixture of products (entries 3 and 4). Reaction with the freshly prepared diazomethane yielded the product in 58% yield (entry 5) while the esterification with MeOH in aqueous HCl afforded the product in 67% yield (entry 6, Table II.9). Thus, this last method was further used to prepare **II.57**.

The synthesis of compound **II.57** was also attempted through oxidation of compound **II.58** with Oxone® (Schemes II.40). However, in these conditions it was obtained the 7-bromo oxindole and a compound that could be the nitroso derivative **II.59** (as suggested by IR).



Scheme II.40. Oxidation of derivative **II.58**.[121]

Consequently, an alternative oxidation methodology was employed (Schemes II.41); a solution of trifluoroacetic peracid in DCM was prepared by the addition of 50% hydrogen peroxide to a solution of trifluoroacetic anhydride in DCM, followed by the addition of compound **II.58**. However, it was verified the formation of the same compound **II.59**.[121]



Scheme II.41. Oxidation of derivative **II.58**.

II.3.2.8 Preliminar synthesis – synthesis of *meta*-substituted anilines with NHBoc, OAc and OMe groups and arylamination optimization

In order to obtain the final benzimidazole structures where the phenyl ring in position *N*-1 possesses a hydroxyl or nitro group in *meta* position (Figure II.12), it would be necessary to perform the arylamination of **II.57** with the corresponding *meta*-substituted anilines. However, due to the incompatibility of these groups (-OH and -NO_2) with the subsequent synthetic steps, it was necessary to prepare the corresponding anilines where these groups were masked.

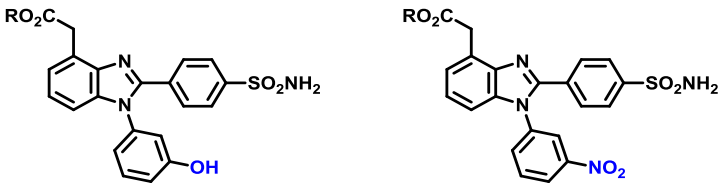
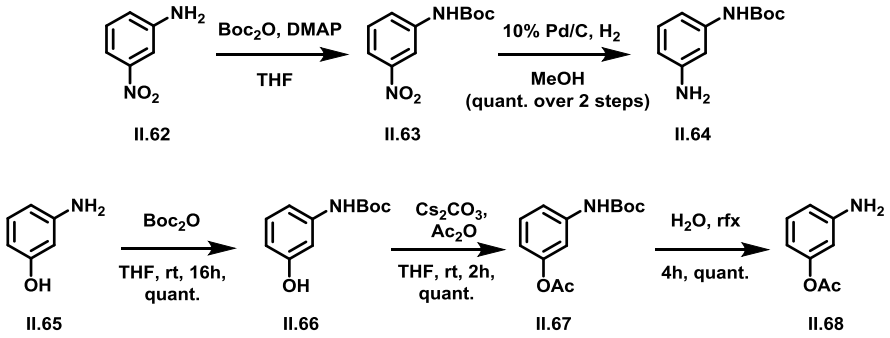


Figure II.12. Benzimidazole structures where *N*-phenyl ring is *meta*-substituted with the hydroxyl or nitro groups.

Therefore, 3-nitro aniline (**II.62**) was protected with Boc group followed by the nitro reduction with 10% Pd/C under H_2 atmosphere in methanol, to form the aniline **II.64** in quantitative yield over two steps (Scheme II.42).[122]

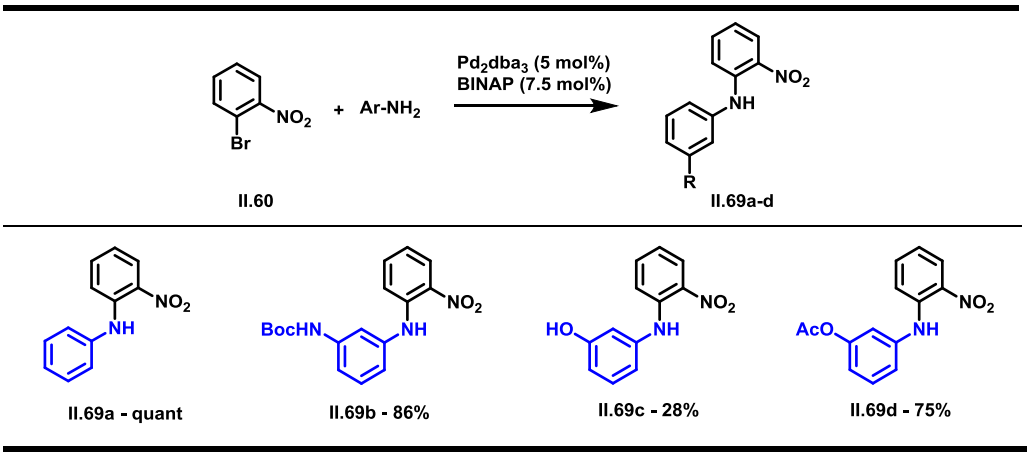


Scheme II.42. Synthesis of *meta*-substituted anilines with NHBoc (**II.64**) and OAc (**II.68**) groups.

Using 3-amino phenol (**II.65**) directly for the arylamination of **II.60** (used as a model structure) (*see* Table II.10), afforded the arylation product in 28% yield. It was then chosen to protect the hydroxyl group of **II.65** with the acetyl group, which could easily removed in a later stage. Thus, hydroxyl group was protected in a sequential protection/deprotection strategy: the amine group was Boc protected followed by acetylation of hydroxyl group to afford **II.67**. The last step consisted on the Boc removal in water to form **II.68** (Scheme II.42). It was verified that this aniline was very unstable even when stored for short periods of time, hence being prepared just before used.

The preliminary arylamination trials were performed using **II.60**, aniline and the prepared anilines, *tert*-butyl 3-aminophenyl carbamate (**II.64**), the amino-phenol (**II.65**) and the *O*-acetylated compound (**II.68**), to attain the compounds **II.69a-d**, according to the Table II.10.

Table II.10 Cross-coupling of **II.60** with the desired anilines.[a]



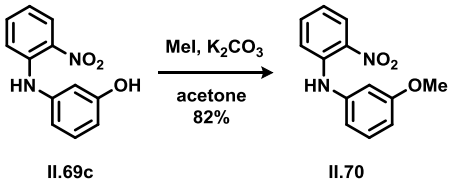
[a] reaction conditions: Pd_2dba_3 (5 mol%), BINAP (7.5 mol%), Cs_2CO_3 (2 equiv), aniline (2 equiv), toluene (0.25 M) in a sealed tube under an argon atmosphere, 90 ºC, 3 – 6 h.

As expected, the arylamination of **II.60** with aniline yielded **II.69a** quantitatively. The reaction with **II.64** afforded compound **II.69b** in good yield, without cleavage of the Boc group (that could occur due to the high temperature employed).

Since, arylamination of **II.60** with **II.65** rendered compound **II.69c** in a poor yield, along high amounts of unreacted starting material, it was stipulated that hydroxyl protection was needed. It is worth mentioning that *O*-arylation product was not expected, since chemoselectivity of Pd-catalyzed cross-coupling procedure involving aminophenols was already described, yielding the *N*-arylated derivative.[123]

In the arylamination with **II.68**, it was verified the total consumption of starting material. However, it was observed on TLC the partial hydrolysis of acetyl group, to form a mixture of acetylated (**II.69d**) and the free hydroxyl product (**II.69c**). This mixture could be fully acetylated with Ac_2O in the presence of Cs_2CO_3 in toluene to attain **II.69d** in quantitative yield.

Since **II.69d** was unstable, it was further proposed to transform the hydroxyl in a methoxyl group.

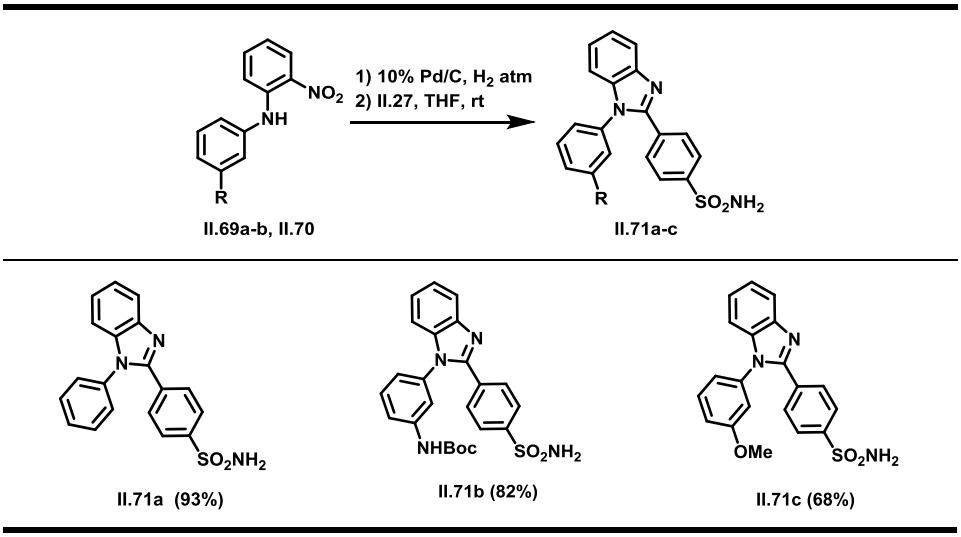


Scheme II.43. Preparation of methoxylated product **II.70**.

Thus, compound **II.69c** was placed in acetone and reacted with MeI in the presence of K_2CO_3 at room temperature, to yield the methoxylated compound (**II.70**) in 82% yield (Scheme II.43).

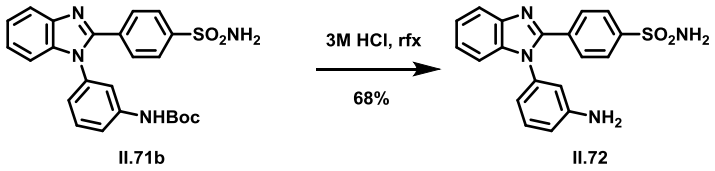
In order to test the viability of these groups, compounds **II.69a, II.69b** and **II.70** were reduced under standard conditions (10% Pd/C, H_2 atmosphere) and benzoylated with sulfamoyl benzoyl chloride (**II.27**) with concomitant cyclization to afford benzimidazoles **II.71a-c** (Table II.11).

Table II.11. Reduction and benzoylation/cyclization of **II.69a-b** and **II.70** to afford benzimidazoles **II.71a-c**.



All the benzimidazole structures were achieved in good to excellent yields (although trace amounts of the corresponding ring opening products were observed). Moreover it was verified that benzimidazole **II.71b** can be attained without cleavage of Boc group. Compound **II.71c** was also achieved in good yield.

It was further investigated the cleavage of Boc group. Despite the good results observed for compound **II.67** (*see* Scheme II.42), the Boc cleavage under water reflux conditions did not occur for benzimidazole structure **II.71b**. This compound was also subjected to acidic conditions (acetic acid in acetonitrile) under reflux for 5 h, however without good results. When stronger acidic conditions were used (refluxing **II.71b** in a 3M HCl solution) Boc was successfully removed, yielding the corresponding product (**II.72**) along with (uncharacterized) side products (Scheme II.44).

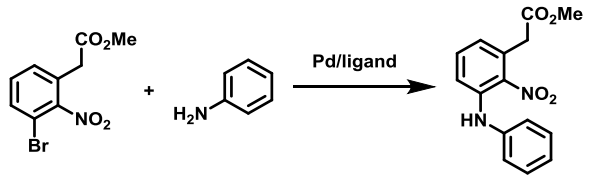


Scheme II.44. Deprotection of Boc group for compound **II.71b**.

II.3.2.9 Arylamination reaction

The optimization of arylamination reaction was performed using the intermediate **II.57** and aniline. Several palladium based catalytic systems were tested, and the results are presented in Table II.12.

Table II.12. Optimization of arylamination of **II.57** with aniline.



II.57 **II.73a**

Entry	Conditions	Base	Solvent	Temperature/time	Yield (%)
1[a]	$Pd_2(dba)_3$ (5 mol%) + BINAP (7.5 mol%)	Cs_2CO_3 (2 equiv)	Toluene	110 ºC (4.5 h)	73
2[a]	$Pd_2(dba)_3$ (5 mol%) + BINAP (7.5 mol%)	Cs_2CO_3 (2 equiv)	Toluene	1) 70ºC (2 h) 2) 90ºC (3 h)	83
3[b]	$Pd(OAc)_2$ (2 mol%) + BINAP (3 mol%)	Cs_2CO_3 (3 equiv)	Toluene	65 ºC (overnight)	66
4[c]	BrettPhos (1 mol%) + BrettPhos precatalyst (1 mol%)	LiHMDS (3.4 equiv)	THF	65ºC (4 h)	47
5[d]	BrettPhos (1 mol%) + BrettPhos precatalyst (1 mol%)	Cs_2CO_3 (2 equiv)	Toluene	1) 65ºC (5 h) 2) 90ºC (overnight)	45

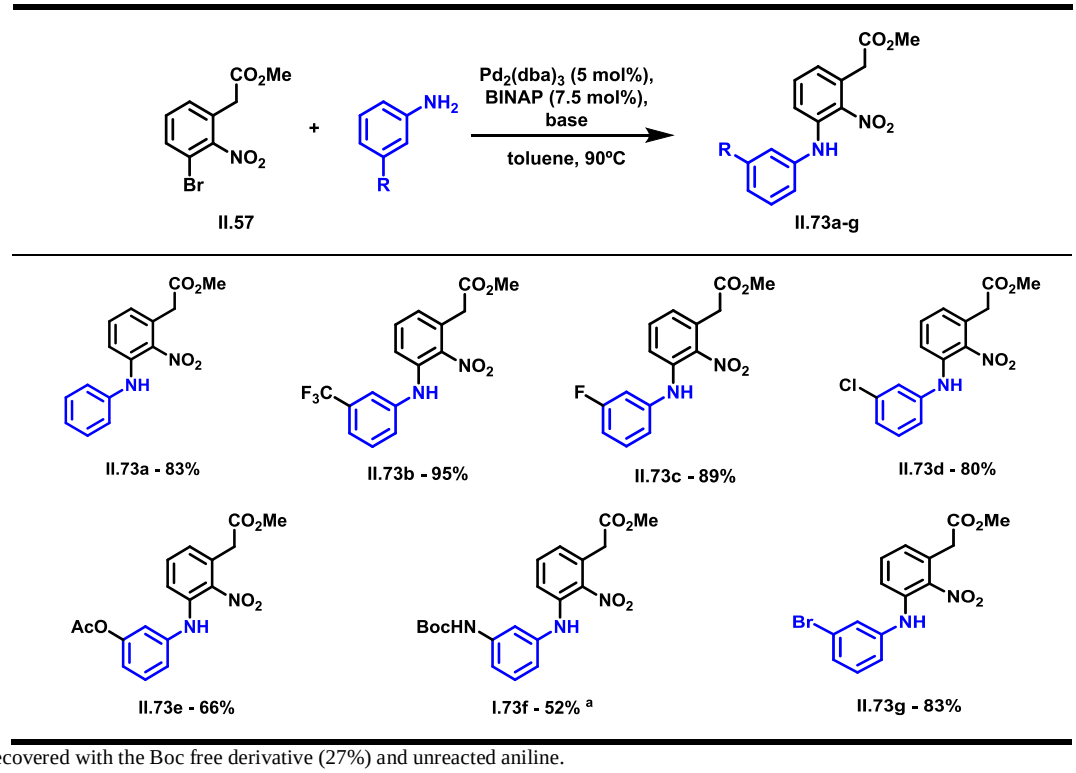
[a] aniline (3 equiv), toluene (0.25 M); [b] aniline (2 equiv), toluene (0.25 M); [c] aniline (1.2 equiv); [d] aniline (2 equiv), solvent (0.25 M);

As previously mentioned, derivatives containing a nitro group in an adjacent position to the halide were successfully submitted to arylamination using $Pd_2(dba)_3$ (5 mol%) and BINAP (7.5 mol%) in toluene.[118a,b] These conditions were tested for derivative **II.57**, yielding the product **II.73a** in excellent yields (entries 1 and 2, Table II.12). It was also verified that a slight decrease in temperature improved the yield, maybe due to less decomposition.

The system that comprises a Pd(II) salt, such as $Pd(OAc)_2$/BINAP in the presence of Cs_2CO_3, yielded the product in 66% (entry 3).[118c] The BrettPhos system[107] was also examined, proving its inadequacy for these type of derivatives, either with LiHMDS or with a milder base such as Cs_2CO_3 (entries 4 and 5, Table II.12).

Therefore, the $Pd_2(dba)_3$/BINAP system was chosen for the arylamination of **II.57** with the required anilines, to obtain the corresponding products **II.73a-g** in excellent yields (Table II.13). However, derivative **II.73f** was obtained in moderate yield (52%) due to the Boc deprotection that occurred during the reaction. The corresponding Boc free derivative was recovered in 27% along with a minor portion of unreacted aniline.

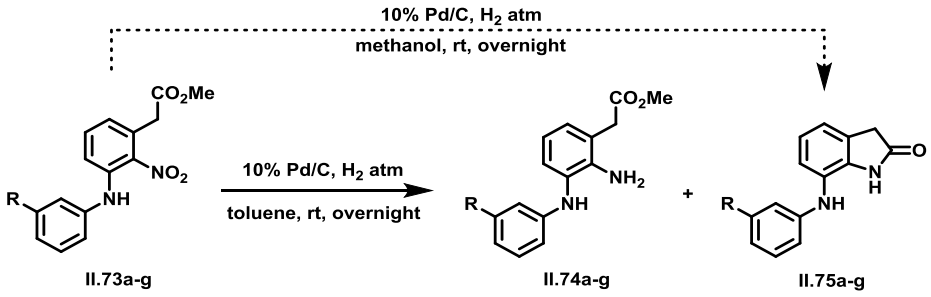
Table II.13. Cross-coupling of **II.57** with the several anilines to obtain the derivatives **II.73a-g**.



[a] recovered with the Boc free derivative (27%) and unreacted aniline.

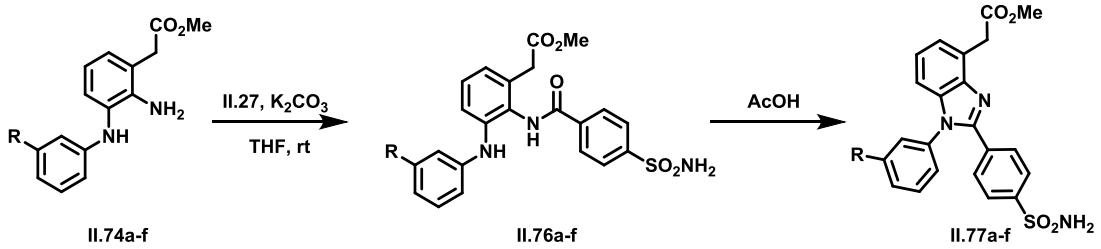
Having the arylamination products (**II.73a-g**) in hands, the next step involved the reduction of nitro group of to obtain the derivatives **II.74a-g**. However, several concerns were raised due to the deliverance of a free amine that could be prone to an unsought cyclization.

The reduction of nitro derivatives was firstly performed using the usual hydrogenation conditions with 10% Pd/C in methanol under H_2 atmosphere (1 h). To our distress, these conditions promoted the ring closing reaction to afford the 7-substituted oxindole structures **II.75**. Gladly, when the reaction was performed in toluene, the desired products **II.74** were obtained as the major compounds, although with a small amount of the corresponding oxindole structures (Scheme II.45). These compounds were simply filtered and used in the following step without further purification.



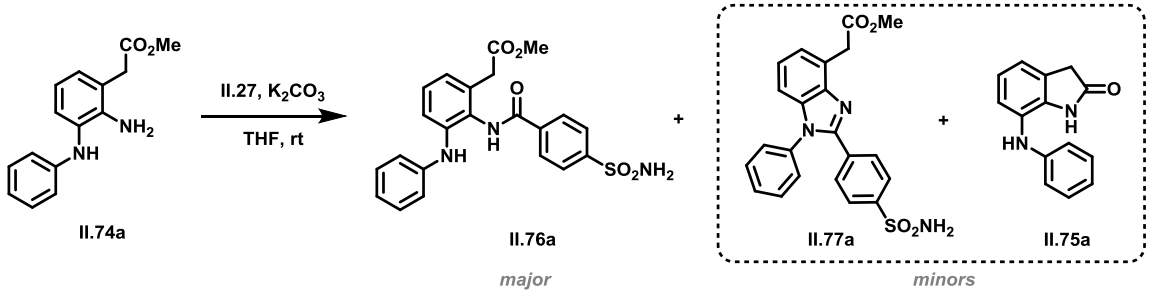
Scheme II.45. Nitro group reduction of compounds **II.73a-g**.

The next step involved the *N*-benzoylation of derivatives **II.74a-f** with the previously synthesized sulfamoyl benzoyl chloride (**II.27**). As a result, it was envisaged that the final compounds could be easily attained after this step, through an acid promoted cyclization using acetic acid, as described by Ma *et al*. (Scheme II.46).[50b]



Scheme II.46. Preparation of the final products using Ma *et al*. procedure.

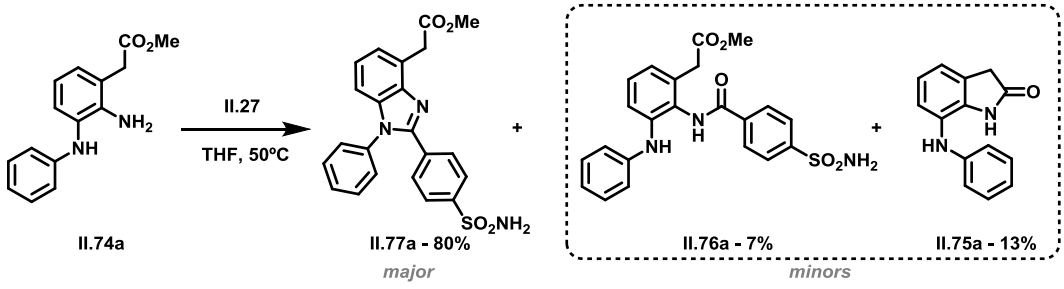
The reaction was initially performed by adding derivative **II.74a** to a cold solution of **II.27** (freshly prepared) in THF in the presence of K_2CO_3. The reaction was then stirred at room temperature overnight. However, under these conditions it was observed (by TLC) the formation of **II.76a** as the major product along with the corresponding benzimidazole **II.77a** and oxindole **II.75a** (amounts not quantified) (Scheme II.47).



Scheme II.47. *N*-Benzoylation of derivative **II.74a** under basic conditions.

These two products – **II.76a** and **II.77a** – despite their easy isolation from other side-products, such as the corresponding oxindole, were very difficult to isolate from each other, since they are strongly retained in silica-gel and overlap.

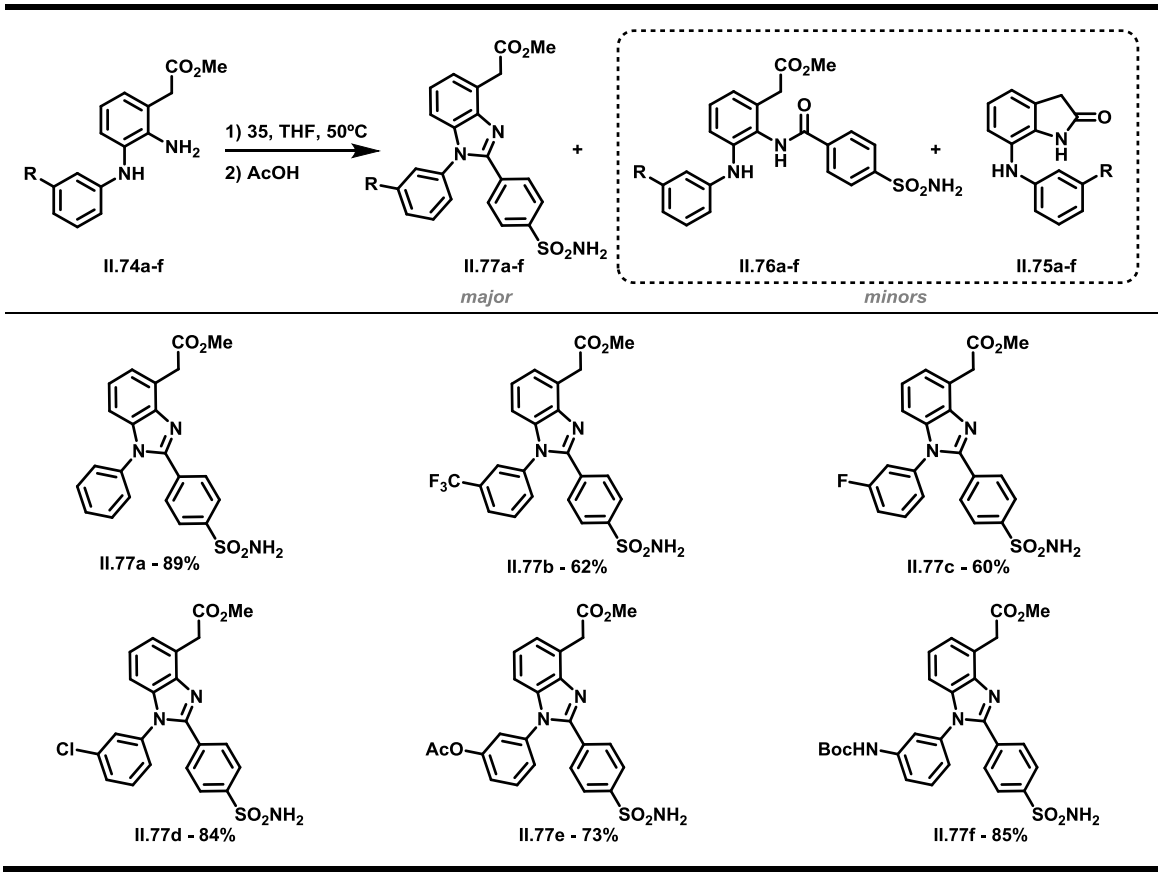
The observation of the benzimidazole structure **II.77a** and the need of an acidic medium to achieve the cyclization, gave us the input to the next experiment.



Scheme II.48. Consecutive *N*-benzoylation and cyclization steps to prepare **II.77a.**

In the second attempt, it was added **II.74a** to a solution of **II.27** in THF without the addition of K_2CO_3, and the mixture was stirred at 50ºC. This reaction evolved in a fast way, with total consumption of starting material after 3 h (monitored by TLC). As expected, this trial led to the *N*-benzoylation of **II.74a** with a concomitant cyclization to directly attain the benzimidazole **II.77a** as the major product (Scheme II.48).

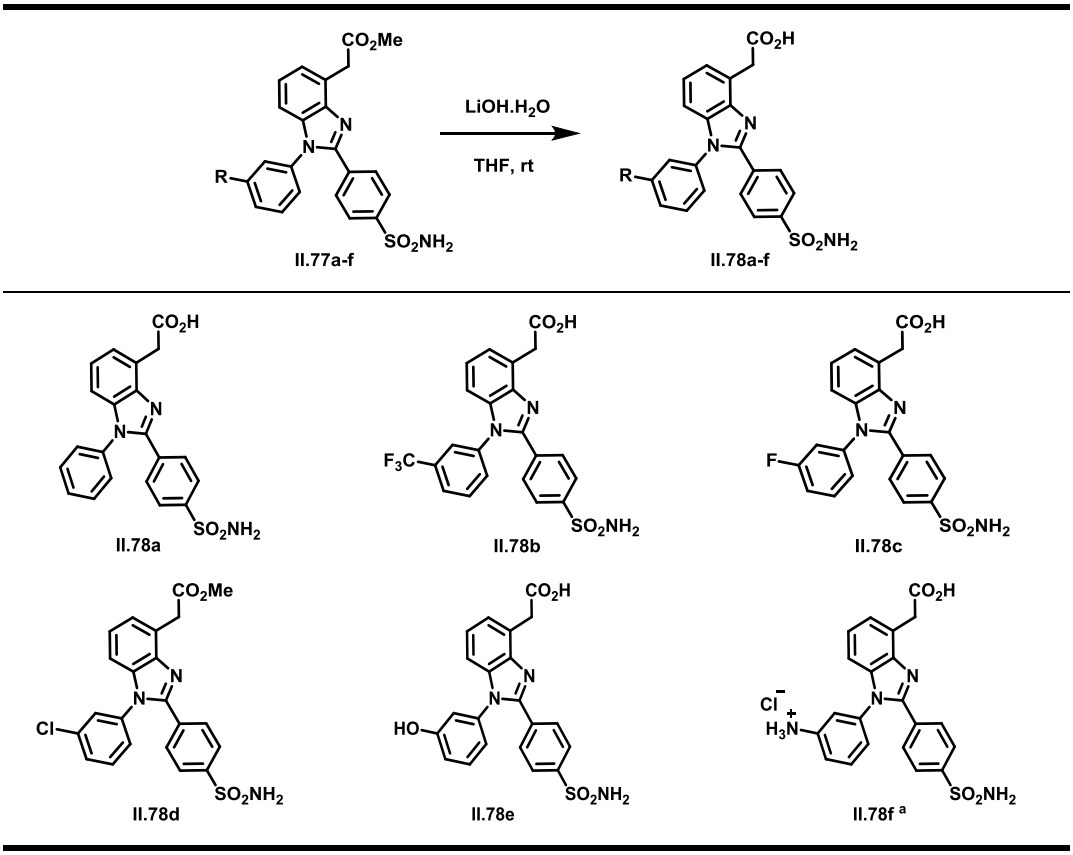
Table II.14. Sequential *N*-benzoylation and condensation/cyclization steps to prepare derivatives **II.77a-f**.



Under these conditions, the ring open product **II.76a** as well as the oxindole **II.75a** were also formed and isolated in 7% and 13 % yield, respectively. In order to maximize the amount of benzimidazole **II.77a**, the crude mixture was subjected to a further step of cyclization in AcOH in order to fully convert derivative **II.76a** into benzimidazole **II.77a**. The process was repeated for derivatives **II.74a-f** (Table II.14).

The acid derivatives were obtained by hydrolysis of the methyl ester group with $LiOH.H_2O$, a fast (1 h) and smooth process that yielded the target benzimidazoles (**II.78a-f**) quantitatively (Table II.15).

Table II.15. Ester hydrolysis to prepare derivatives **II.78a-f**.



[a] hydrolysis performed with 3M HCl aqueous solution at 80ºC, 4h.

It is noteworthy that the acetyl protection of derivative **II.77e** was also cleaved under the hydrolysis step, affording the corresponding hydroxylated derivative **II.78e**. In order to cleave the Boc group, derivative **II.77f** was placed in a 3M HCl aqueous solution at 80ºC for 4 h, yielding compound **II.78f** quantitatively.

In order to perform the biological evaluation, is required a high degree of purity of the synthesized compounds. Thus, all the ester derivatives **II.77a-e** as well as the acid compounds **II.78a-e** were analyzed by HPLC. Despite their high levels of purity – above 96% – it was verified that the ester compounds had small amounts of the corresponding acid derivatives (<3%).

Having in hands the purified benzimidazole compounds, the next step consisted on the biological evaluation in order to verify their potential as efficient COX inhibitors, and the STD-NMR experiments to study their binding mechanism within COX.

II.3.3 Evaluation of the synthesized library

The inhibitory activity of the synthesized compounds was tested against COX-1 and COX-2 isoenzymes in order to verify their selectivity. The chosen method to evaluate this activity was the human WBA.

As above mentioned the WBA involves three tasks. However, the sample preparation procedure is slightly different for the different COX isoforms, affecting the time spent in the two assays (excluding the ELISA test). While for COX-1 assay, the incubation takes place within less than 3 h (incubation times excluding in-between operational tasks), for COX-2 assay is required a longer period of incubation to attain the desired plasma, which takes approximately 6 h.

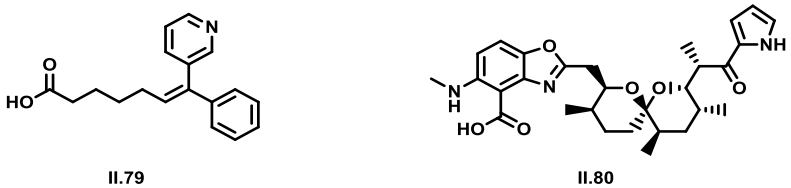
The inhibitory activity studies were performed for the ester and acid compounds (**II.77a-e** and **78a-e**, respectively) at different concentrations, starting at 100 μM, and gradually decreasing until the compounds showed inexpressive inhibitory activity. Desirably, each study must correspond to at least three (reproducible) experiments for each concentration. Due to solubility issues and experimental strategy, the compounds were not tested within the same range of concentrations.

II.3.3.1 Evaluation of inhibitory activity towards COX-1

In the COX-1 assay, the blood stimulation was achieved by COX stimulation using a calcium ionophore (**II.80**) following to the exposure to the analyzed compound at a specific concentration, in the presence a thromboxane synthetase inhibitor (TXBSI) (**II.79**) (*see* Experimental for details).

As the name suggests, the TXBSI is used to inhibit the thromboxane synthase, an enzyme that converts the AA derivative – PGH_2 – to TXA_2, which in turn degrades into the stable TXB_2. Since PGE_2 is produced in substantially lower amounts compared to TXB_2 (only 1–2 %), the introduction of TXBSI will amplify the PGE_2 content. The TXBSI used in this work is the (*E*)-7-phenyl-7-(3-pyridyl)-6-heptenoic acid (**II.79**) also known by CV-4151, a selective TXA_2 synthase inhibitor with an IC_{50} of 0.026 μ M (Figure II.13).[99,124]

Calcium ionophore A23187 (**II.80**), also known as Calcimycin, is an antibiotic and a mobile ion-carrier that forms stable complexes with divalent cations (such as, Mn^{2+}, Ca^{2+} and Mg^{2+}), allowing these ions to cross cell membranes, which are usually impermeable to them.[125] It is also used to stimulate prostanoids formation through a COX-1 and COX-2 pathway, as previously reported.[126]



II.79 II.80

Figure II.13. TXBSI (**II.79**) and calcium ionophore (**II.80**).

For each compound, solutions of desired concentrations were freshly prepared in a buffer from the compound stock solution (4 mM) which was prepared in DMSO. Although its unfavorable interferences with blood, DMSO can be used in low concentrations (< 1%) being used as vehicle in most WBAs.[99]

The solubility of the studied compounds was analyzed for the tested concentrations, and the insolubility of some compounds was observed at higher concentrations. After a period of incubation in the presence of all assay components, the blood samples were centrifuged and the plasma was taken for the ELISA test.

The employed PGE_2 EIA kit is a colorimetric competitive enzyme immunoassay kit that measures the levels of downstream PGE_2 of an assay exposed to the different concentrations of COX inhibitors. A low PGE_2 content conducts to a higher absorbance read for the sample (at a given concentration), which means a high inhibitory activity (and *vise-versa*). The percentage of inhibition is obtained from standard equations described on literature.[97]

The majority of the tested compounds were found to inhibit COX-1 as observed in Table II.16. Inhibition of COX-1 by the synthesized compounds is expressed as the percent inhibition of COX-1 activity and the known COX inhibitor indomethacin was used as positive control.

It was neatly observed that the ester derivatives (**II.77a-e**) showed higher inhibition values when compared to acid related structures (**II.78a-e**), inhibiting COX-1 in a concentration-dependent manner. In fact, only the acid derivatives **II.78a**, **II.78b** and **II.78d** showed inhibitory activities, e.g. 52 ± 9 %, 25 ± 2 % and 23 ± 8 %, respectively, for 50 μM. Moreover, these compounds showed high values of SEM making difficult to achieve a concentration-dependent effect.

Indomethacin inhibitory percentages were 88 ± 8 % (1 μM) and 55 ± 12 % at 0.625 μM, thus having higher potency towards COX-1 than the studied compounds.

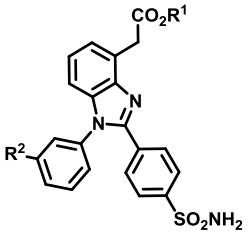
The inhibition percentages that displayed high values of SEM were considered inconclusive.

As observed in Table II.16 and Figure II.14, all the ester derivatives (**II.77a-e**) presented high inhibitory values (e.g. 74 ± 5 % to 99.85 ± 0.05 % at 50 μM) while the acids where poor inhibitors or fail to inhibit COX-1. The inhibition differences between acids and esters derivatives can be attributed to the high affinity of acids to a different region in the protein which does not influence its activity.

In fact, it was already described that some acid containing inhibitors possess a time-dependent effect that can be attributed to an allosteric effect or due to chemical modification of the active site.[127] However, transforming the carboxylic acid moiety in a methyl ester group modifies its character, conferring to these compounds the reversible ability to inhibit the protein.[127]

More recently, it was also described that pyrrole based structures containing an acetic ester chain at C-3 possess an increased activity toward COX-2 than the corresponding acids.[105c]

Table II.16. Percent inhibition of COX-1 activity of synthesized compounds determined by human WBA.

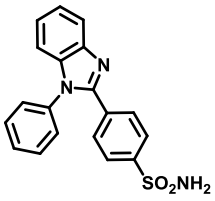


esters R^1 = Me

R^2 = H	II.77a
R^2 = CF_3	II.77b
R^2 = F	II.77c
R^2 = Cl	II.77d
R^2 = OAc	II.77e

acids R^1 = H

R^2 = H	II.78a
R^2 = CF_3	II.78b
R^2 = F	II.78c
R^2 = Cl	II.78d
R^2 = OH	II.78e



II.71a

Compound	Conc (%)							
	100 μM	50 μM	25 μM	12.5 μM	5 μM	2.5 μM	1μM	0.625μM
II.77a	[a]	99.25 ±0.35	[a]	93 ±2	80 ±3	60 ±4	[a]	15.20 ±0.42
II.77b	92 ±2	77 ±6	[b]	61 ±3	2.50 ±2.50	[a]	[a]	[a]
II.77c	[a]	97 ±1	92 ±2	81 ±6	68 ±6	42 ±11	[b]	32 ±5
II.77d	[a]	99.85 ±0.05	[a]	94 ±2	86 ±5	64±8	[b]	41 ±14
II.77e	94.77 ±0.47	74 ±5	59 ±9	24 ±2	0.50 ±0.50	[a]	[a]	[a]
II.78a	68 ±16	52 ±9	[a]	[a]	[b]	[a]	[a]	[a]
II.78b	[b]	25 ±2	[a]	[a]	[b]	[a]	[a]	[a]
II.78c	[b]	[b]	[a]	[a]	[b]	[a]	[a]	[a]
II.78d	50 ±15	23 ±8	[a]	[a]	[b]	[a]	[a]	[a]
II.78e	[b]	[b]	[a]	[a]	[b]	[a]	[a]	[a]
II.71a	[b]	32 ±14	[a]	[a]	[b]	[a]	[a]	[a]
Indomethacin	[a]	[a]	[a]	[a]	[a]	[a]	88 ±8	55 ±12

[a] not performed; [b] inconclusive

Within the ester compounds, it can be observed a clear distinction for compounds **II.77a**, **II.77c** and **II.77d** when compared to **II.77b** (R = CF_3) and **II.77e** (R = OH). While the first three compounds presented 99.25 ± 0.35 %, 97 ± 1 % and 99.85 ± 0.05 %, at 50 μM, compounds **II.77b** and **II.77e** presented 77 ± 6 % and 74 ± 5 %, respectively. The same trend was verified for lower concentrations and below 5 μM, **II.77b** and **II.77e** displayed inhibition values less than 10%, which were considered not significant. These facts, allows one to consider **II.77a, II.77c** and **II.77d** the most active compounds towards COX-1.

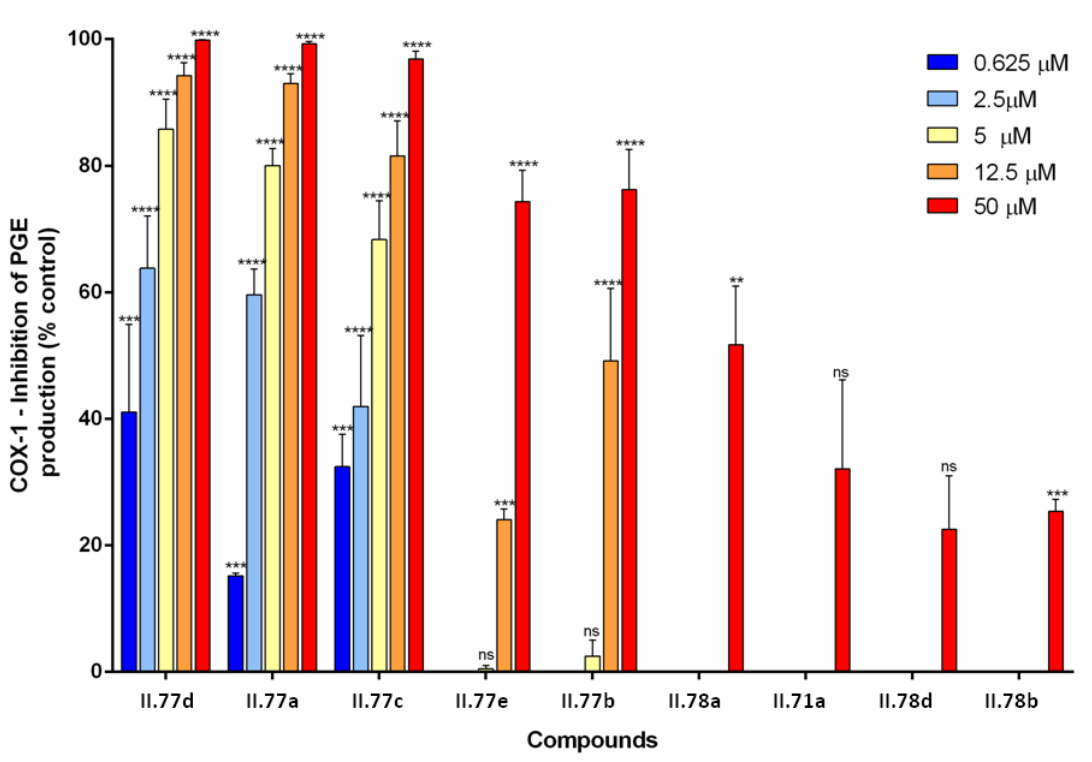


Figure II.14. COX-1 inhibitory activity.

It is interesting to observe that small structural differences confer discrepant inhibitory activities against COX-1. Compound **II.77a** – which does not have any substituent in aromatic ring – has similar values to compounds **II.77c** and **II.77d** that possess a halogen atom in *meta* position, F and Cl, respectively. For compounds **II.77b** and **II.77e**, the presence of a *m*-CF_3 or a *m*-OAc seems to have an unfavorable effect.

It appears that the halogen atom has an important role in the inhibitory activity, being verified a correlation in the activities and the halogen present in the molecule, e.g. F < Cl. Unfortunately *m*-bromine compound was not possible to achieve, but it was expected that this structure could possess the higher inhibition percentages toward COX, as confirmed by docking studies.

Compound **II.71a** was also evaluated in order to understand the impact of the acetic acid anchor on the inhibitory activity. This compound presented poor inhibition values towards COX-1, similar to the obtained for the acid derivatives. This result suggests that none of these compounds bind COX catalytic site, and that the acetic acid side chain do not contribute to improve compounds activity. However, one cannot say that the free acetic acid chain moiety is dispensable, since the acid moiety can interact differently with protein leading to the similar (poor) results observed for **II.71a**.

Unfortunately, despite the several inhibition percentages taken for all the compounds, there weren't enough data to calculate the corresponding IC_{50} values for each compound. Nevertheless, a dose-response curve was constructed for all the ester derivatives, showing the unfeasibility of IC_{50} assignment (Figure II.15).

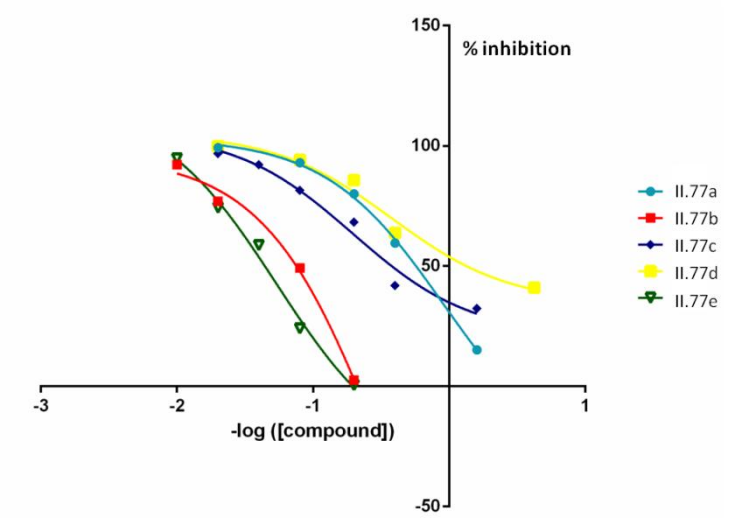


Figure II.15. Dose-response curve for COX-1 (fitting performed in GraphPad Prism 6).

As can be seen, the curves did not show the desired top and bottom plateaus, corresponding to the maximum and minimum inhibition concentrations achieved, respectively. Evidently, more points would be needed in order to obtain a correct fitting and to determine the IC_{50} values, however due to time and costs limitations it couldn´t be taken.

II.3.3.2 Evaluation of inhibitory activity towards COX-2

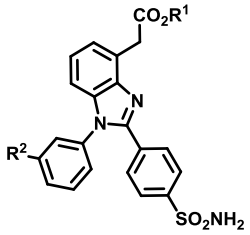
For the evaluation of inhibitory activity towards COX-2, the blood stimulation included a selective inhibition of COX-1 activity using acetylsalicylic acid, the use of TXBSI as described above and the COX-2 induction *via* lipopolysachacaride (LPS) stimuli.[128]

The majority of the tested compounds were found to inhibit COX-2 as observed in Table II.17.

As observed for COX-1 assay, while the acid derivatives (**II.78a-e**) showed poor inhibitory activities, the ester derivatives (**II.77a-e**) inhibit COX-2 in a same level as Celecoxib, the selective COX-2 inhibitor used as control.

Within the acids group, **II.78a** and **II.78d** exhibited the higher inhibitory activities, 30 ± 3 % and 18 ± 2 %, respectively, at 50 μM, following the previous trend. The other acid compounds displayed inhibition values less than 10%, which conducted to high values of SEM thus not being considered.

Table II.17. Percent inhibition of COX-2 activity of synthesized compounds, determined by human WBA.

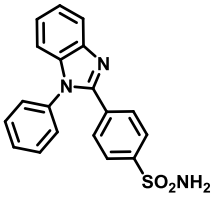


esters R^1 = Me

R^2 = H	II.77a
R^2 = CF_3	II.77b
R^2 = F	II.77c
R^2 = Cl	II.77d
R^2 = OAc	II.77e

acids R^1 = H

R^2 = H	II.78a
R^2 = CF_3	II.78b
R^2 = F	II.78c
R^2 = Cl	II.78d
R^2 = OH	II.78e



II.71a

Compound	Conc (%)							
	100 μM	50 μM	25 μM	12.5μM	5 μM	1.25 μM	0.5 μM	0.125 μM
II.77a	[b]	88 ±3	--		81 ±5	59 ±4	30 ±2	[a]
II.77b	[b]	78.94 ±0.13	[a]	[a]	65 ±7	31 ±3	7 ±2	[a]
II.77c	[b]	88 ±2	[a]	[a]	81 ±2	82 ±3	42 ±8	[a]
II.77d	[b]	84 ±3	[a]	[a]	81 ±2	83 ±3	60 ±8	21±2
II.77e	88 ±3	83 ±5	80 ±6	40 ±4	5 ±2	[a]	[a]	[a]
II.78a	[b]	30 ±3	[a]	4 ±4	[a]	[a]	[a]	[a]
II.78b	[b]	[b]	[a]	[b]	[a]	[a]	[a]	[a]
II.78c	[b]	7 ±7	[a]	0.33 ±0.33	[a]	[a]	[a]	[a]
II.78d	[b]	18 ±2	[a]	0.85 ±0.85	[a]	[a]	[a]	[a]
II.78e	[b]	7 ±7	[a]	1 ±1	[a]	[a]	[a]	[a]
II.71a	[a]	46 ±1		3 ±3	4 ±4	[a]	[a]	[a]
Celecoxib	[a]	[a]	[a]	[a]	72 ±10	[a]	[a]	[a]

[a] not performed; [b] inconclusive

Compound **II.71a** was also evaluated for COX-2, but besides its lower inhibition, no selectivity was found for this compound, that presented 46 ± 1 % at 50 μM (32 ± 14 % at the same concentration for COX-1). It is interesting to compare **II.71a** with the already reported benzimidazoles structures, **I.1** and **I.2**, both highly potent and selective towards COX-2 (Figure II.16). None of these compounds possess the acid or ester anchor in position 4, while the substituents in aromatic ring in position 1, appear as an important feature for the inhibitory activity.

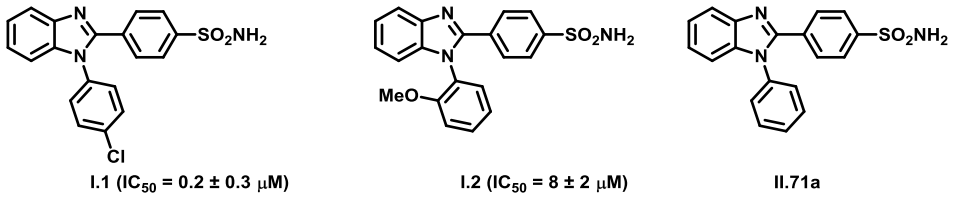


Figure II.16. Benzimidazoles showing inhibitory activity towards COX-2. [35a]

The ester compounds **II.77a-e** presented high inhibitory values towards COX-2 (Figure II.17). As already verified, compounds **II.77a**, **II.77c** and **II.77d** were the most potent, showing a similar pattern and inhibiting COX-2 by 88 ± 3 %, 88 ± 2 % and 84 ± 3 (50 μM), respectively. At the same concentration, compound **II.77b** and **II.77e** exhibit 78.94 ± 0.13% and 83 ± 5 %, respectively. However, below 5 μM, **II.77e** displayed inhibition values less than 10%, while for **II.77b** the same was verified below 0.5 μM. Thus, the less potent compound was **II.77e** possessing an acetyl group at the *meta* position.

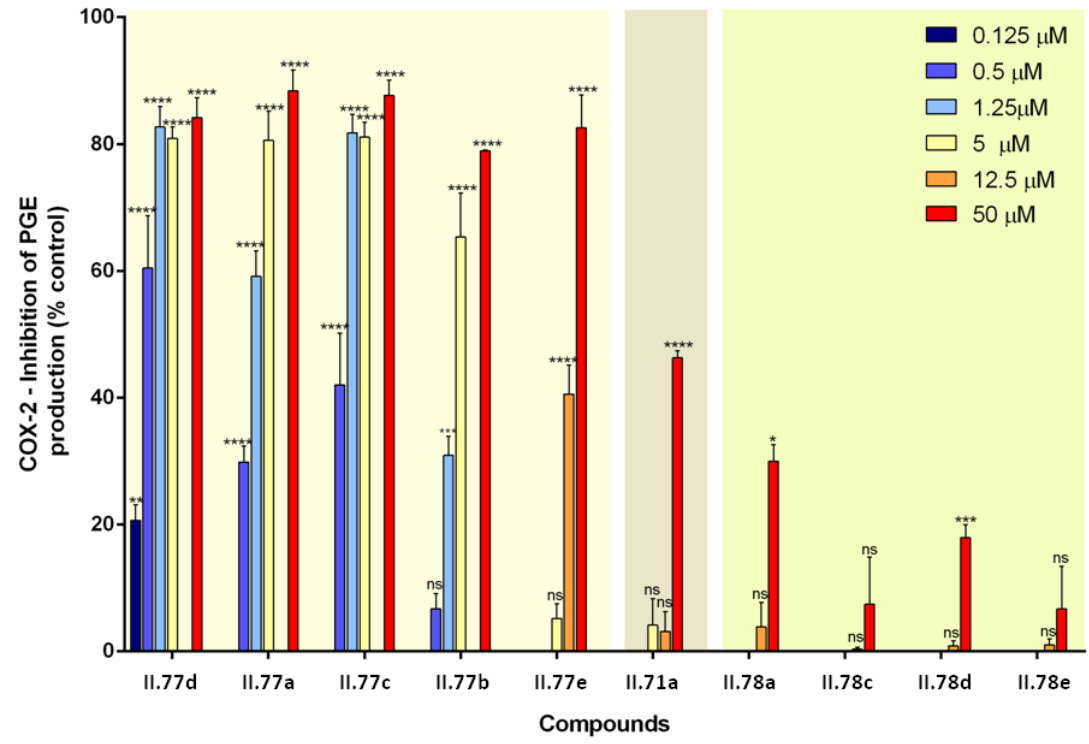


Figure II.17. COX-2 inhibitory activity.

Once more, compound **II.77d –** which have a chlorine atom at *meta* position, showed the higher potency, inhibiting 21 ± 2 % in 0.125 μM. Remarkably, this compound seems to be even more active than Celecoxib which inhibitory percentage was 72 ± 10 % at 5 μM. As described before, it was expected that *m*-bromine compound presented the higher inhibitions toward COX-2, as predicted by the preliminary docking studies (Figure II.18).

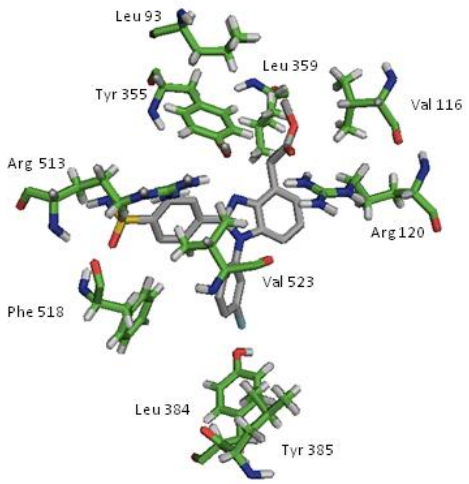


Figure II.18. Docking structure of compound **II.77d** (using AutoDock 4.2.5.1).

This difference between acid and esters derivatives was previously observed for pyrrole based structures. As reported by Biava *et al.*, pyrroles containing an acetic ester chain at C-3 possess an increased activity toward COX-2 than the corresponding acids.[105] Moreover, it is proved that the type of ester, e.g. insertion of isopropyl or butyl moiety, have high influence in the inhibitory activity (Figure II.19). [105c]

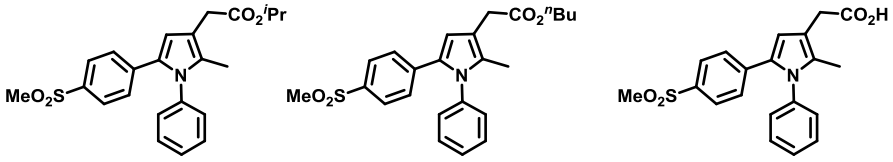


Figure II.19. Pyrrole structures and corresponding IC_{50} obtained by WBA for COX-2 (Biava *et al.*).[105c]

Biava also proposed that the ester anchor creates very important hydrophobic interactions with specific residues such as Leu93, Val116, Tyr355, and Leu359 contributing to the observed enhanced COX-2 inhibition (Figure II.20). [105c]

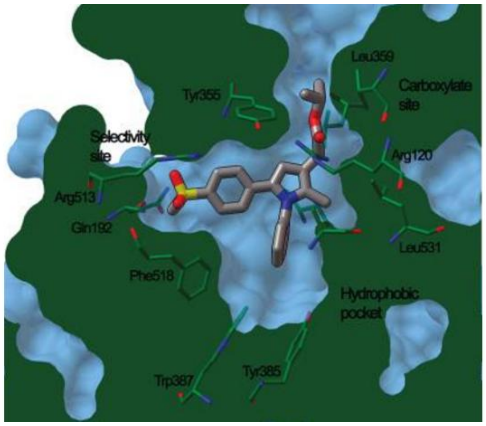


Figure II.20. Representation of the binding mode of **II.81** within the COX-2 binding site (adapted from Biava *et al.*). [105c]

These results are according to the inhibitory activities observed for the ester compounds. Moreover our docking studies also predict that ester anchor can perform important interactions with the same Leu93, Val116, Tyr355, and Leu359 residues.

As discussed for COX-1 evaluation, despite the several inhibition percentages taken for all the compounds, there weren't enough data to calculate the corresponding IC_{50} values for each compound. Once more, a dose-response curve was constructed for all the ester derivatives, to demonstrate the unfeasibility of this assignment (Figure II.21).

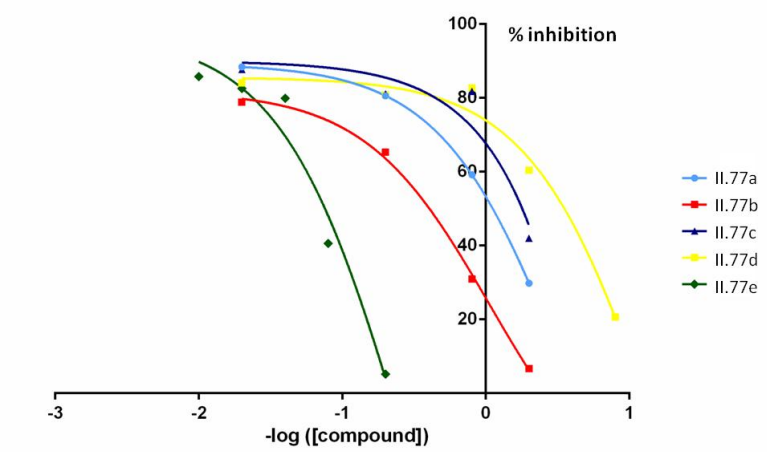


Figure II.21. Dose-response curve for COX-2 (fitting performed in GraphPad Prism 6).

It can be said that both esters and acid derivatives presented a similar inhibition pattern for COX-1 and COX-2 isoforms: while the acid derivatives did not show or presented low inhibitory activities, the ester compounds were able to inhibit both COX isoforms in a high and concentration-dependent way. Within the esters group, the chlorine derivative **II.77d** showed the best results, having a comparable inhibitory activity to Celecoxib.

The results obtained for acid derivatives (**II.78a-e**) are against the preliminary docking studies that predicted an elevated potency and selectivity toward COX-2 for this benzimidazole library. Moreover, despite their low selectivity it was simply expected that the acid derivatives should be good inhibitors toward COXs. An explanation can reside in different binding locations in protein that docking studies cannot predict. These higher affinity sites for the acid derivatives can prevent their function as inhibitors.

For ester derivatives, despite their high inhibitory activity, it was demonstrated a moderate COX-2 selectivity. It is known that the residues drive the affinity of inhibitors affecting their orientation inside the catalytic active site. Thus, it is suggested that the orientation adopted inside the active site places the sulfonamide pointed towards the side pocket, as predicted. However, the

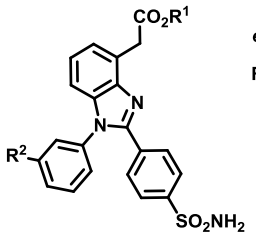
aromatic ring in *N*-1 is pointed upwards to the top of the active site while the ester anchor forms interactions with the residues at the entrance of the active site, i.e. Leu93, Arg 120, Val116, Tyr355, and Leu359. This observation is consistent for the general non-selective NSAIDs. Diclofenac and lumiracoxib have their carboxylates pointed at the top of the active site, which may be a required position to be a COX-2 selective inhibitor.

In order to clarify the binding mode of these compounds within protein, several STD-NMR studies were undertaken.

II.3.4 STD-NMR studies

To investigate the relationship between the inhibitory activities found for benzimidazoles (against COX-2) and their binding mode to protein, the epitope mapping of different compounds was examined by STD-NMR. Moreover, STD-NMR competitive experiments were also conducted in the presence of known drugs in order to understand if the designed ester and acid compounds bind on the same location or have different binding sites.

Unfortunately, it was verified that the ester derivatives had a poor solubility in the buffer solution used in the NMR studies. Consequently, the STD-NMR studies were performed for compounds **II.78a-e** and for the ester **II.77a** (soluble on the studied conditions) (Figure II.22).



esters R^1 = Me

R^2 = H II.77a

acids R^1 = H

R^2 = H II.78a
R^2 = CF_3 II.78b
R^2 = F II.78c
R^2 = Cl II.78d
R^2 = OH II.78e

Figure II.22. Compounds examined by STD-NMR.

The STD-NMR experiments were acquired at 37 °C using commercial ovine (oCOX-2) and human (hCOX-2) COX-2 buffered solutions. It can be referred that the majority of studies were performed with oCOX-2 due to the expensive costs associated to hCOX-2. This enzyme was only used for one of the most promising ester derivatives, **II.77a**, in order to validate the preliminary STD-NMR studies and the biological experiments.

In the previous STD-NMR studies, it was observed that the spectra were dominated by strong signals in the aliphatic zone from the Tris buffer and the additives (Tween 20 and DDC), showing that the surfactant also interacts with the protein.[104] These results were not unexpected, since the surfactant is added to stabilize and solubilize the protein and some degree of association between the enzyme and the surfactant is expected to occur. The same was observed in the present work, where the additives signals overlap with some ligand resonance peaks making the complete epitope mapping not possible. Earlier attempts to resuspend the protein in different buffers resulted in precipitation. Thus the studies were conducted with the proteins as supplied.

II.3.4.1 Epitope mapping of ligands

Initially, the compounds were examined individually in order to establish their binding interaction with COX-2. The region of the ligand having the closest/strongest contact to the protein would show the most intense NMR signals allowing the epitope mapping of the ligand.

The experiments were performed using a protein:ligand ratio of 1:100. All the acid based compounds were soluble in the buffer solution used, while the ester derivatives showed a low solubility within the used range of concentrations. Thus, only compound **II.77a** was tested. The corresponding STD-NMR spectra (expanded aromatic region) are depicted in Figure II.23.

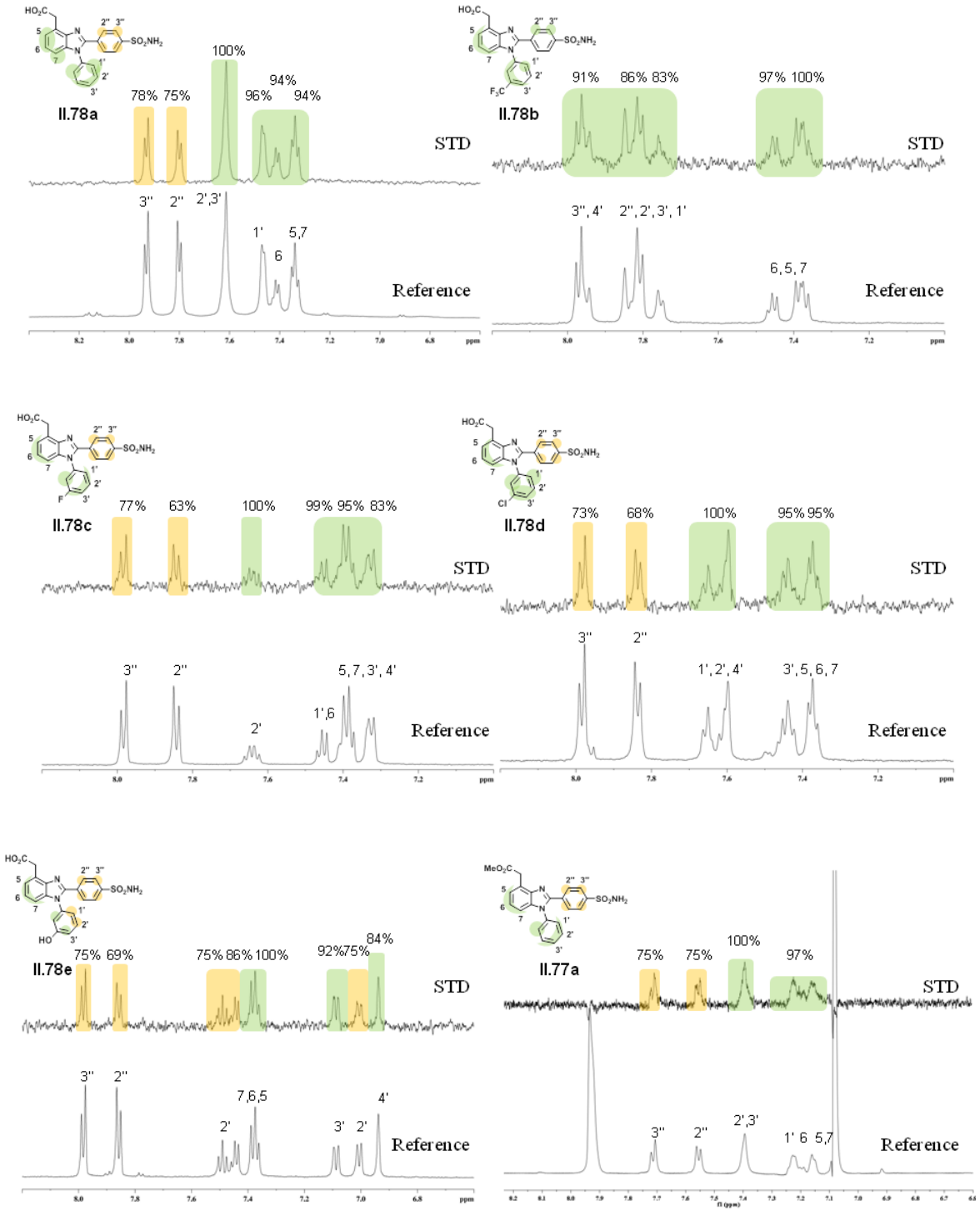


Figure II.23. Expansion of the aromatic region of the reference and ^{1}H STD-NMR spectra of **II.78a-e** (300μM) in the presence of oCOX-2 (3 μM) and **II.77a** (115μM) in the presence of hCOX-2 (1.15 μM), at 600 MHz and 37 °C.

For all the analyzed acid compounds (**II.78a-e**), the aromatic protons showed strong STD responses, indicating reversible binding to the enzyme. It reveals that in all cases the drug is in close contact with the protein. In the aromatic region it is possible to integrate the resonances of the resolved proton signals and determine the relative STD intensities (Figure II.23). Due to the strong signals in the aliphatic zone from the buffer and the additives that overlaps with the ligand resonance peaks, it is not possible to verify the interaction between the acetic acid anchor and protein, e.g. CH_2 STD percentages.

The related STD percentages are similar for all studied compounds. This is not unexpected, since the studied compounds belong to the same family having structural similarities. The STD percentages observed for the aromatic protons of the heterocyclic core are above 86 % for all the studied compounds. It is also seen that the aromatic ring containing the sulfonamide group has lower STD interaction when compared with the other aromatic protons. This can indicate that the molecules possess an orientation in protein binding site where this ring is pointed outwards the protein or is inserted in a hollow place.

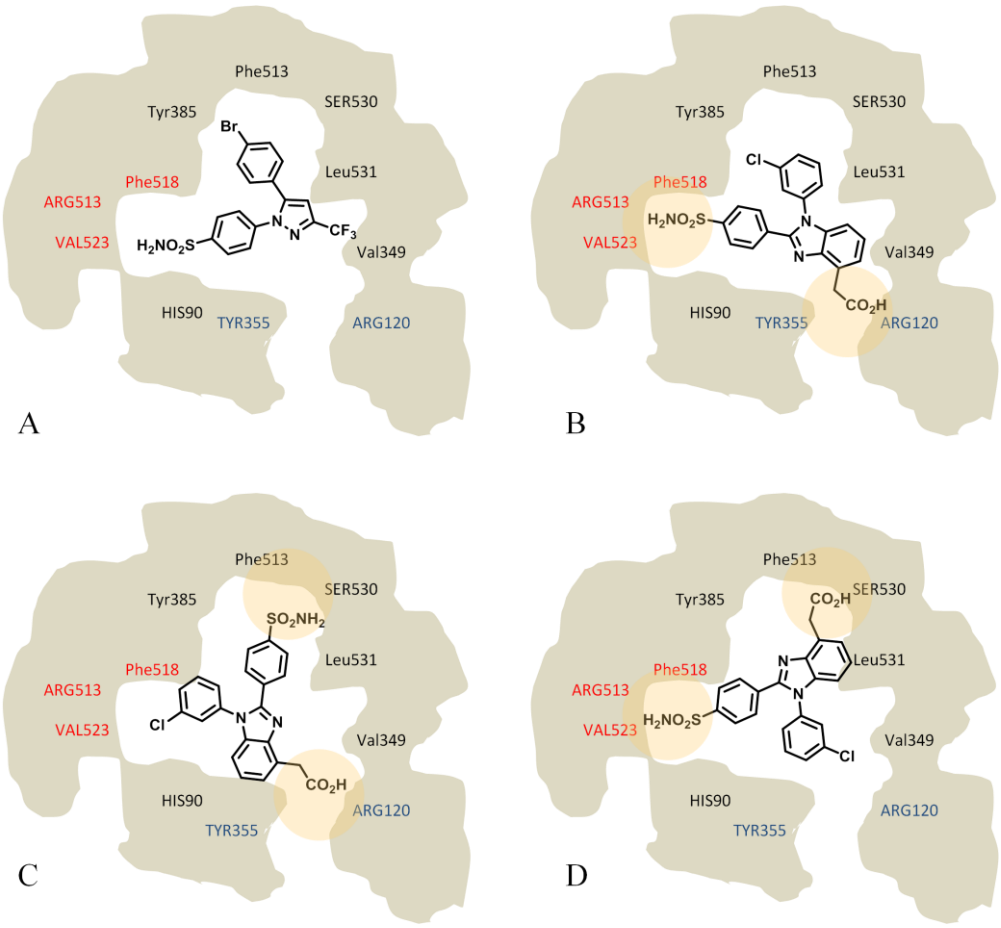


Figure II.24. A. Orientation of SC-558 inside COX-2 active site; B – D. Possible orientations for the studied compounds inside COX-2 active site.

According to the previous STD-NMR studies performed for diclofenac and ibuprofen, it was suggested that the ligand moieties with higher STD response were directed to Arg120 and Tyr355 residues, while the moieties receiving less saturation transfer were pointed towards Ser530 and Tyr385.[104] Based on these observations, the sulfonamide group would be directed to the top of COX-2 active site, i.e. towards Ser530 and Tyr385 (Figure II.24, c). However, it was anticipated that the compounds should have an orientation similar to the already reported coxibs (Figure II.24, a), result that was also predicted by the docking studies (Figure II.24, b). Moreover, it was also expected that the carboxylate group could particularly interact with Arg120 and Tyr355 residues, as verified for naproxen or indomethacin (*see* Figure I.10). Other possible orientation places the carboxylic acid moiety at the top of the active site, forming hydrogen bonds with Ser530 and Tyr385, similarly to lumiracoxib and diclofenac (*see* Figure I.10). Unfortunately, it was not possible to verify the STD response for the acetic acid chain, which could confirm the orientation of the studied molecules within the COX-2 binding site.

It can be inferred that the results obtained for the previously studied NSAIDs cannot be extrapolated for the presently studied compounds, which share the y shape structure of coxibs. Thus, one can say that these were the first STD-NMR studies performed for coxib-like molecules, and that further studies are still required (using a well-known coxib) in order to confirm the obtained results.

It can be said that the strong STD signals observed for all the compounds reflects a reversible binding to enzyme sites and a fast exchange on the NMR time scale. However one cannot conclude about their binding site on protein without additional STD-NMR studies.

II.3.4.2 Competitive STD-NMR Studies

In order to have a deeper insight of the mechanisms of action for the designed compounds, competitive STD-NMR experiments were carried using ibuprofen, diclofenac and naproxen as spy molecules.

The choice of these drugs is related to the way these drugs bind the protein. The time-independent inhibitor ibuprofen binds to both COX active sites, E_{cat} and E_{allo}, while time-dependent inhibitors such as naproxen and diclofenac bind preferentially to a single monomer.

Diclofenac binds E_{cat} and compete with AA for the catalytic site which conducts to the complete COX inhibition, while naproxen is an allosteric regulator that binds to E_{allo}, causing an incomplete inhibition (Figure II.25). [10] According to the reported IC_{50} values, diclofenac (IC_{50} of 38 nM) is a more potent inhibitor of COX-2 than ibuprofen (IC_{50} = 7.2 μM) and naproxen (IC_{50} = 28 μM). [96]

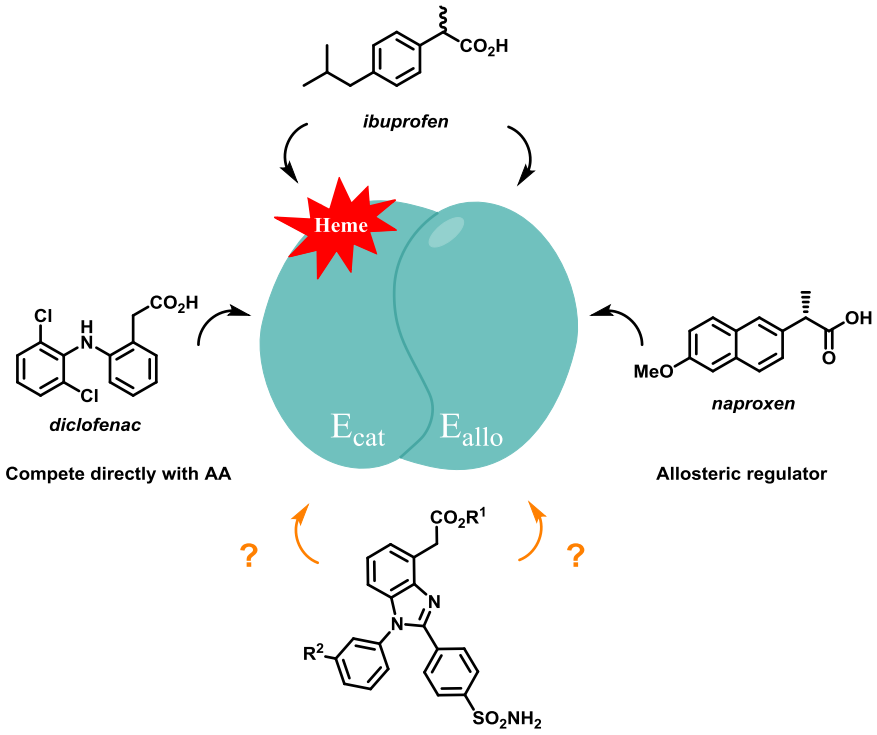


Figure II.25. Competitive experiments to understand the binding mechanism of the synthesized compounds.

The first experiment consisted on the competition between the known drugs: ibuprofen, naproxen and diclofenac (Figure II.26). The first two superimposed spectra corresponds to the reference and STD-NMR of ibuprofen (7.20-7.35 ppm) (Figure II.26, a).

To the sample containing ibuprofen (1:100, protein:ligand ratio) an excess of naproxen was added (2 equiv. relatively to ibuprofen). Both drugs displayed STD interactions; however naproxen signals overlapped with those belonging to ibuprofen making the analysis a difficult task (Figure II.26, b). Nevertheless, it can be seen a decrease of naproxen STD response of approximately 1/2. This corroborates the fact that while naproxen binds to one of the monomers, ibuprofen can bind to both. This result can also indicate that both ibuprofen and naproxen possess similar binding patterns, allowing ibuprofen to compete for E_{allo}. Ibuprofen binds both monomers possessing a reversible, poor binding behavior, which can conduct to a high STD response.

The next step consisted on the addition of two equivalents (relatively to ibuprofen) of diclofenac to the previous mixture (Figure II.26, c). It is worth to notice that a competitive experiment was already conducted between diclofenac and ibuprofen, where the STD response of ibuprofen was about 1/2 of diclofenac.[104]

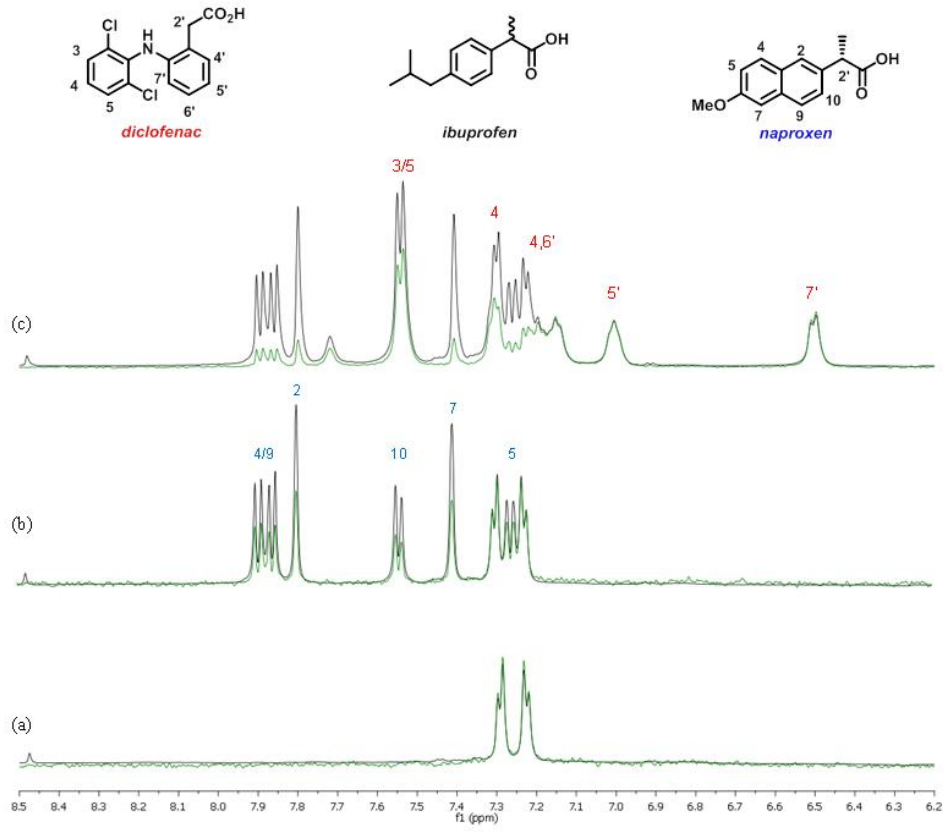


Figure II.26 Expansion of the aromatic region of the reference (black) and ^{1}H STD-NMR spectra (green) of a) ibuprofen (326 μM) and competition experiments with b) naproxen (652 μM) and c) naproxen and diclofenac (652 μM) in the presence of oCOX-2 (3.26 μM), at 600 MHz and 37 °C.

In our experiment it was expected that the presence of both naproxen and diclofenac would suppress almost completely the STD response from ibuprofen because of its lower IC_{50} compared to those drugs. This would prove its binding character to both E_{cat} and E_{allo}. Unfortunately, due to spectral overlap in the ibuprofen region (7.20-7.35 ppm), it is difficult to separate the STD responses from ibuprofen, naproxen and diclofenac in order to compare with the individual experiments. It seems that ibuprofen signals are attenuated, however one cannot confirm the disappearance of ibuprofen signals. Thus only the latter drugs were compared.

In this case, a clear alteration in STD spectrum is observed. Both diclofenac and naproxen possess STD interactions; however naproxen signals are significantly attenuated. This can indicate three different binding mechanisms; a) diclofenac (which is a strong time-dependent inhibitor) binds preferentially to E_{cat} and ibuprofen will in turn compete with naproxen by E_{allo}, leading to the observed results; b) diclofenac binds to E_{cat} and its presence can modulate E_{allo}, impeding naproxen to bind its site; or c) diclofenac also competes for E_{allo}. These two last proposals would be in opposition to the previously reported results.[10] Moreover, it seems that the presence of naproxen (which binds to E_{allo}) does not have influence in the binding mode of diclofenac.

II.3.4.3 Compound II.78a *vs* ibuprofen, diclofenac and naproxen

The existence of STD signals for compounds **II.78a-e** – although the poor results for biological evaluation – suggests that these derivatives might bind to a third location which do not influence the COX activity. In order to elucidate this hypothesis, competitive STD experiments were carried.

The initial competition experiments with the acid derivative **II.78a** were performed using ibuprofen as spy molecule. The experiment depicted in Figure II.28 consisted on titration of ibuprofen in the presence of **II.78a** (protein:ligand, 1:25).

In case of ligand competition for the same binding site, it was expected that ibuprofen would suppress almost completely the STD response from **II.78a** due to this compound poor inhibitory activity (*see* Chapter II.2, page 65) when compared to ibuprofen. What can be observed in Figure II.27 is a similar STD response for both ibuprofen and **II.78a** when the compounds are in 1:1 ratio (Figure II.27, c). When ibuprofen is in 4-fold excess to **II.78a**, the acid derivative still maintains similar STD intensities, showing that the increase of ibuprofen does not affect the binding of **II.78a** to the protein (Figure II.27, d). This could indicate that both compounds bind to E_{allo} and E_{cat}; while ibuprofen was binding to one monomer, **II.78a** could bind to the other and *vise-versa*. However, due to the poor inhibitory activity presented by **II.78a** it is not possible to exclude a third binding site that does not take part in the mechanism of inhibition.

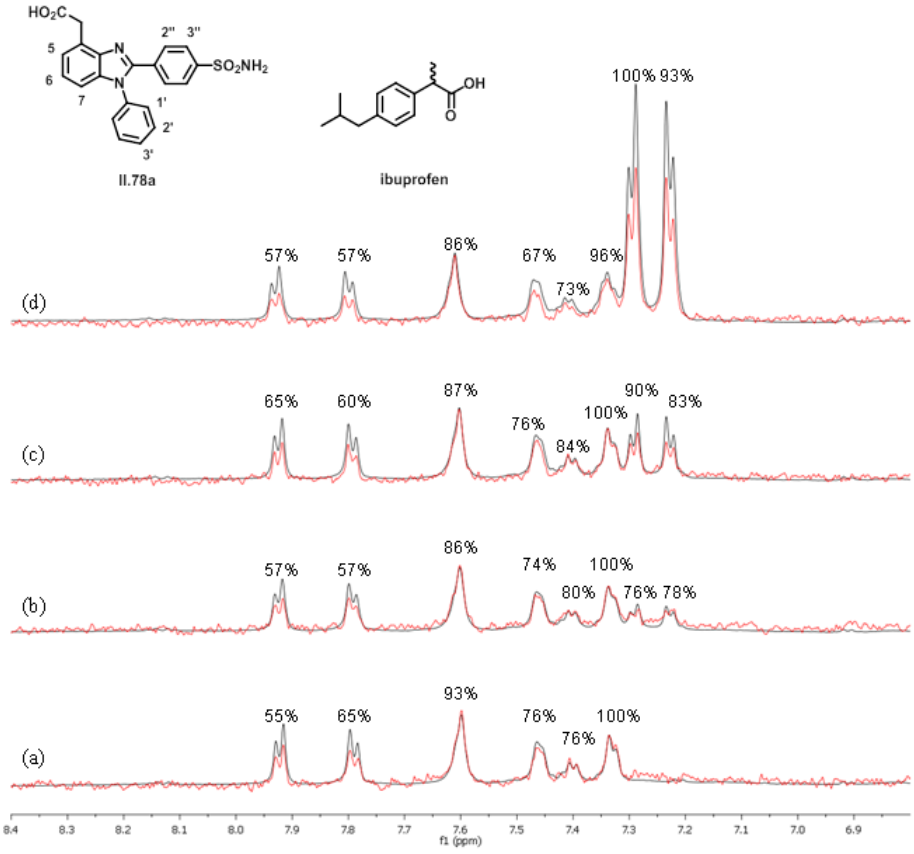


Figure II.27 Expansion of the aromatic region of the reference (black) and ^{1}H STD-NMR spectra (red) of: a) **II.78a** (81.5 μM), and with different ibuprofen concentrations: b) 32.6 μM; c) 81.5 μM; d) 326 μM, in the presence of oCOX-2 (3.26 μM), at 600 MHz and 37 °C.

The same trend was verified for all the acid compounds **II78b-e** (*see* Appendix, page 255).

The competition studies between **II78a** and naproxen are represented in Figure II.28. Unfortunately, because of spectral overlap in the aromatic region, it is difficult to separate the STD responses from **II.78a** and naproxen in order to individually compare both compounds. Thus, only the signals corresponding to $H_{2'}$ and $H_{3'}$ from **II.78a**, and H_5 from naproxen were evaluated and compared. Taking the **II.78a** signal – corresponding to $H_{2'/3'}$ – as reference it is observed that despite the successive increasing of naproxen concentration, the STD response of **II.78a** is maintained. This can indicate that naproxen is not competing for the same binding site of **II.78a**. However, for 1:1 ratio, naproxen STD response seems to be ½ the **II.78a** response (Figure II.28, d), indicating that while naproxen is only binding one monomer (E_{allo}), **II.78a** could bind both E_{allo} and E_{cat}. Other possibility is that the binding of **II.78a** to E_{cat} or to a third site, can influence the naproxen binding to E_{allo}.

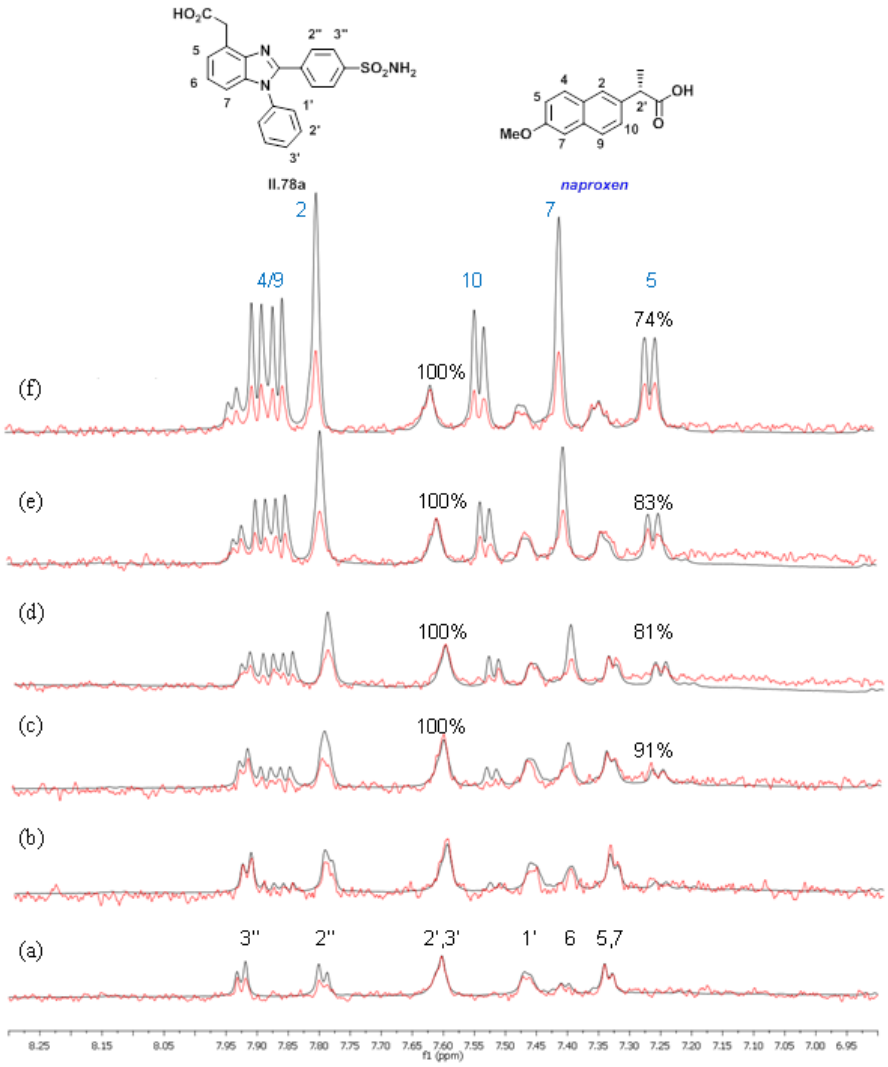


Figure II.28 Expansion of the aromatic region of the reference (black) and ^{1}H STD-NMR spectra (red) of: a) **II.78a** (163 μM) and with different naproxen concentrations: b) 32.6 μM; c) 81.5 μM; d) 163 μM; e) 326 μM); f) 652 μM, in the presence of oCOX-2 (3.26 μM), at 600 MHz and 37 °C.

The competitive STD-NMR experiment between **II.78a** (protein:ligand, 1:400) and diclofenac, shows that the increase of diclofenac amount decreases the **II.78a** STD response (Figure II.29). In fact, comparing the signal corresponding to $H_{2',3'}$ (**II.78a**) with the signal of $H_{3/5}$ (diclofenac), allows one to approximately quantify the STD response of **II.78a** as 1/2 of that corresponding to diclofenac.

This result is similar to the previously reported competitive studies between diclofenac and ibuprofen, where was concluded that the existence of STD response for ibuprofen in the presence of diclofenac could indicate that the inhibitors were not competing for the same binding site.

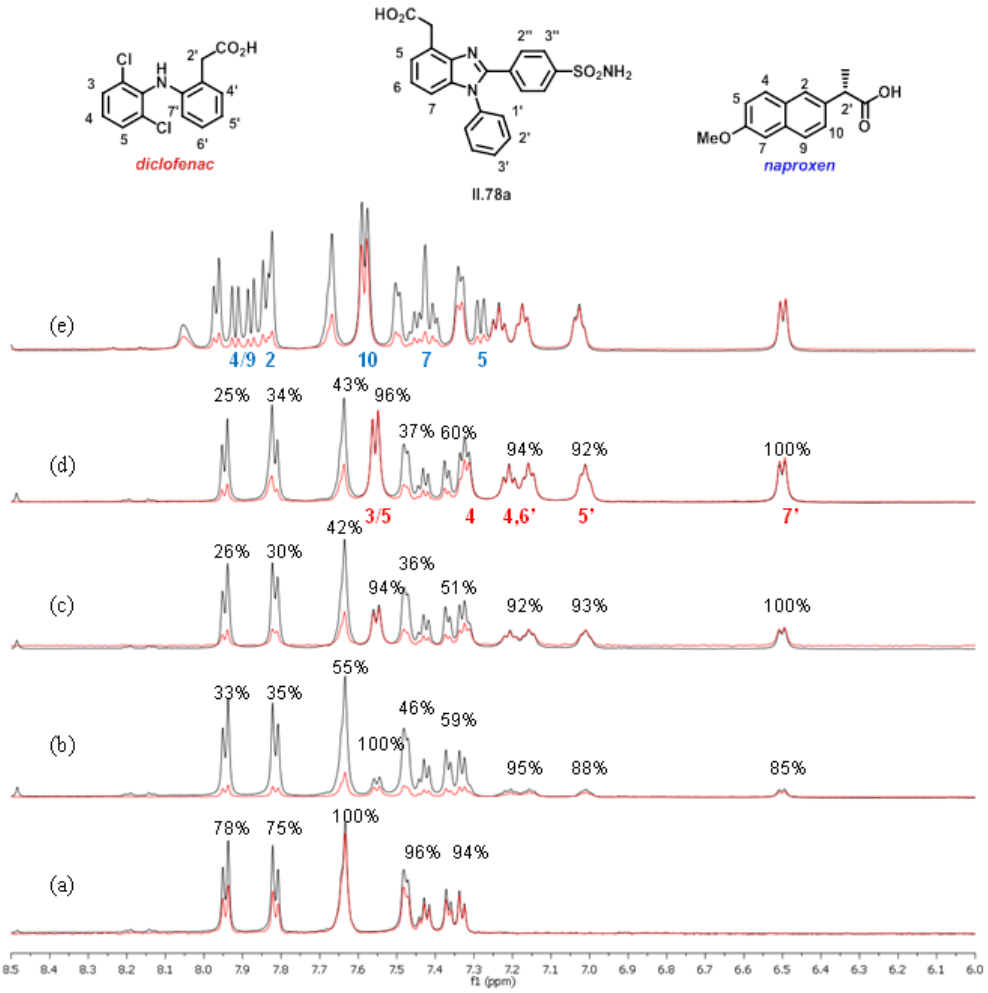


Figure II.29 Expansion of the aromatic region of the reference (black) ^{1}H STD-NMR spectra (red) of: a) **II.78a** (1.304 mM) and with different diclofenac concentrations: b) 326 μM, c) 652 μM and d) 1.304 mM, and e) with naproxen (1304 μM) in the presence of oCOX-2 (3.26 μM), at 600 MHz and 37 °C .

Thus, the results suggest that **II.78a** is not competing neither for the naproxen binding site, E_{allo}, nor for E_{cat}, but that diclofenac attachment to E_{cat} affects the way that **II.78a** binds COX-2. The STD-NMR studies indicate that the acids **II.78a-e** might have higher affinity to a third binding site, that is influenced by the way diclofenac binds to E_{cat}.

II.3.4.4 Compound II.77a *vs* naproxen and diclofenac

Similarly to the studies undertaken for the acid derivatives, competitive STD experiments were carried for esters. Due to the higher inhibitory activity detected by biological assays, our interest was to verify whether these compounds bind E_{cat} and/or E_{allo}.

The competitive STD-NMR studies were conducted using **II.77a**, soluble on the experimental conditions. Nevertheless, **II.77a** yielded poor resolution STD-NMR spectra, which can be due to its high affinity towards COX-2, i.e. high Ki.

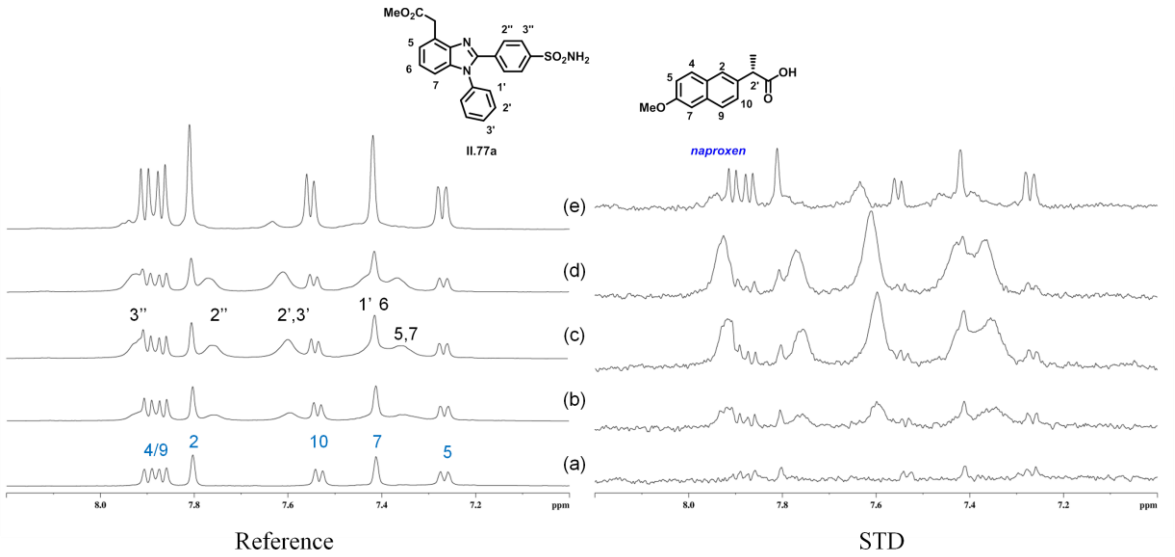


Figure II.30 Expansion of the aromatic region of the reference (left) and ^{1}H STD-NMR (right) spectra of: a) naproxen (163 μM) and with different **II.77a** concentrations: b) 81.5 μM; c) 163 μM; d) 245 μM and e) addition of naproxen (326 μM), in the presence of oCOX-2 (3.26 μM), at 600 MHz and 37 °C.

In Figure II.30 are represented the spectra of naproxen (protein:ligand, 1:50) and the competitive experiments with **II.77a** (at different concentrations – Figure II.29, b-d). Initially, for a naproxen:**II.77a** ratio of 1:0.5 (Figure II.30, b), it was observed that the STD response of **II.77a** was higher than the response of naproxen (Figure II.30, c). When both drugs were in 1:1 ratio, **II.77a** also demonstrated higher STD signals compared to naproxen. Unfortunately the signals overlap and do not allow the determination of STD percentages.

This result suggests two different mechanisms. The first mechanism would indicate the existence of a competition of **II.77a** for the same binding site of naproxen (which preferentially binds to E_{allo}) (Figure II.31-A). In addition, the result can suggest that compound **II.77a** has a stronger affinity than naproxen, corroborating the results obtained by the biological evaluation. However, naproxen signals never disappeared suggesting that the inhibitor is being pushed to E_{cat} or that it can still bind to E_{allo} thus presenting a moderate STD response.

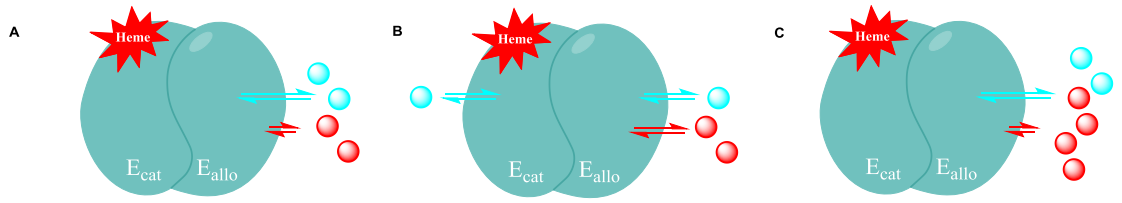


Figure II.31. Competitive experiments between **II.77a** (blue) and naproxen (red) and proposed binding mechanisms.

The second mechanism could indicate that **II.77a** can bind both E_{cat} and E_{allo} having a time-independent, ibuprofen-like behavior, while naproxen still binds to E_{allo} (Figure II.31-B).

When the order of addition is reversed, i.e. naproxen concentration is twice the **II.77a** (Figure II.30, e), is clear the reduction of **II.77a** STD response, suggesting that naproxen is expelling this compound from its binding site (Figure II.31-C).

These competitive STD-NMR experiments were also made with hCOX-2 (protein:ligand, 1:100). In this case, STD-NMR spectra of **II.77a** had enhanced resolution compared to oCOX-2 counterpart, despite the small protein concentration used (Figure II.32).

When one equivalent of diclofenac was added to **II.77a** both compounds showed STD response with similar intensities, demonstrating that the compounds do not compete for the same binding place (Figure II.32, b). Moreover, if **II.77a** is binding E_{allo}, that is not influencing the binding mode of diclofenac to E_{cat}.

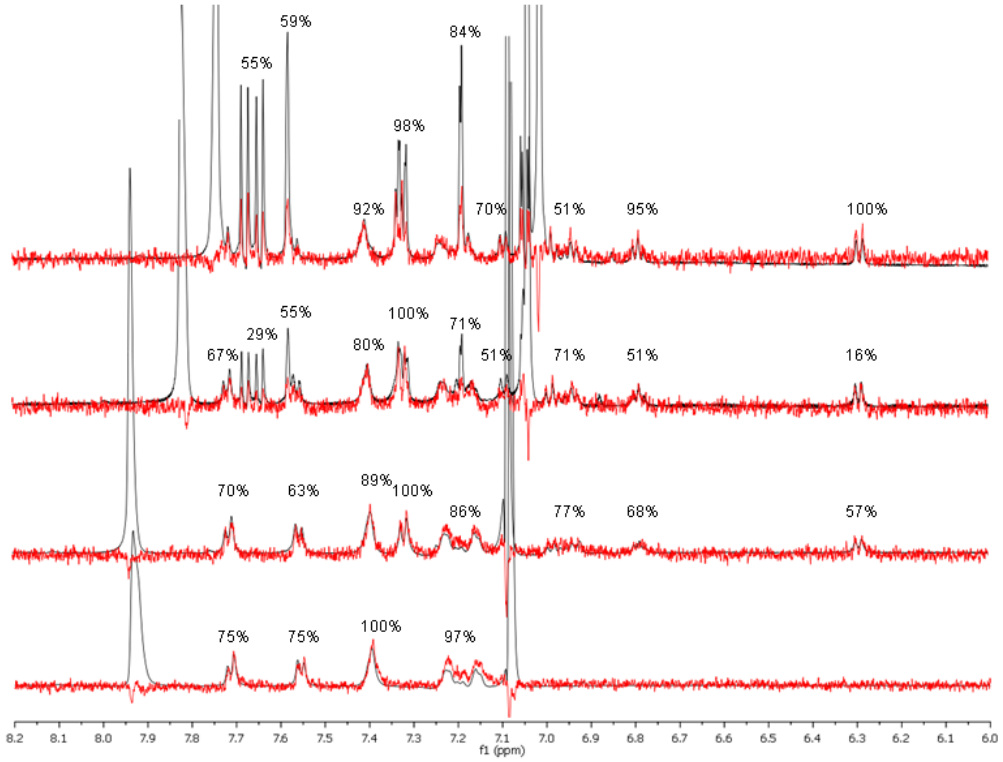


Figure II.32 Expansion of the aromatic region of the reference (left) and ^{1}H STD-NMR (right) spectra of: a) **II.77a** (115 μM) with b) diclofenac (115 μM) and c) naproxen (115 μM), d) naproxen (345 μM) in the presence of hCOX-2 (1,15 μM), at 600 MHz and 37 °C.

Then, one equivalent of naproxen was added to the previous mixture. It can be observed on STD spectrum a slight decrease of naproxen STD response compared to **II.77a**, while diclofenac intensities were maintained (Figure II.32, c). This result suggests the competition of **II.77a** for the binding site of naproxen.

It was then expected from the reverse order addition experiment (in opposition to experiment depicted on Figure II.30), i.e. when three equivalents of naproxen were added – that the STD response of **II.77a** would disappear. However that was not observed (Figure II.32, d). Since diclofenac has a tight, slow binding mode (and IC_{50} higher than naproxen and **II.77a**), the result suggests that **II.77a** still binds to E_{allo} having a binding behavior compared to naproxen. However one cannot rule out the existence of a third binding location for **II.77a** only by these experiments.

II.4 Conclusions

Taking advantage of a rational drug design approach which included the insights obtained from the previously synthesized indole based library,[129] the herein reported second generation of heterocyclic structures was successfully synthesized and evaluated, confirming the improvement achieved on the development of novel highly potent COX inhibitors. It was also established that the iterative and cooperative strategy between the several groups, was a fast and effective methodology to construct biologically relevant targets.

Despite the initial drawbacks, a synthetic route was successfully conceived, allowing a direct access to the benzimidazole structures with the desired substitution pattern. Taking 7-bromo isatin as starting material, twelve new benzimidazole based compounds were synthesized in good yields.

From these compounds, ten were biologically evaluated against COX-1 and COX-2. It was verified that the acid derivatives were very poor COX inhibitors, while the ester compounds inhibited both COX isoforms in a high and concentration-dependent way. Compound **II.77d** (Figure II.33) was the most promising inhibitor, showing a COX-2 inhibitory activity of 81 ± 2%, at 5 μM, comparable to Celecoxib which showed 72 ± 10% of inhibition, at the same concentration. As anticipated by the docking studies, the compounds having a halogen at *meta* position displayed a superior inhibitory activity. It was expected that bromine derivative would be the most potent of the esters group. Unfortunately, it was not possible to attain this compound.

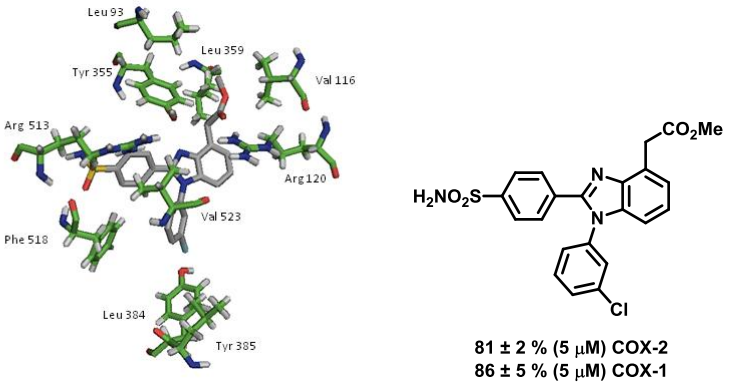


Figure II.33. Docking and chemical structure of compound **II.77d**.

It was concluded that the biological results do not corroborate the docking predictions that envisaged high inhibitory activities and COX-2 selectivity for all the studied compounds. Only the ester derivatives showed a poor COX-2 selectivity at lower concentrations: 41 ± 14 % (at 0.625 μM) for COX-1 and 60 ± 8 % (at 0.5 μM) for COX-2.

The performed STD-NMR studies support that the acid derivatives (**II.78a-e**) most likely possess a third binding site on COX-2 which do not contribute for its activity. Although the poor inhibitory activity obtained on the biological assays, it was verified that all the acid compounds possessed good STD response that proves its interaction with the protein. The competitive STD-

NMR studies carried between **II.78a** and ibuprofen, naproxen and/or diclofenac also support that the compound do not compete for the same binding sites of the NSAIDs. It is concluded that there is different binding locations in protein that docking studies cannot predict. These higher affinity sites for the acid derivatives can prevent their function as inhibitors.

The ester **II.77a**, which possesses excellent inhibitory percentages towards COX-2, also presented good STD response. In this case, the performed competitive experiments suggest that **II.77a** compete with naproxen for E_{allo}.

It can be said that small substituent changes – even within the same scaffold – can produce significant differences on the inhibition or selectivity results mainly due to different molecular interactions within the protein. Although the moderate COX-2 selectivity achieved, the present compounds possess enhanced inhibitory activities compared to the first generation of indolic structures.[129]

Despite the lack of data to determine the IC_{50} for our compounds, it can be inferred by the results obtained for COX-1 and COX-2, that the compounds showing the best inhibitory activities (**II.77a**, **II.77c** and **II.77d**) can be placed in the blue zone of the graphical representation (Figure II.34).

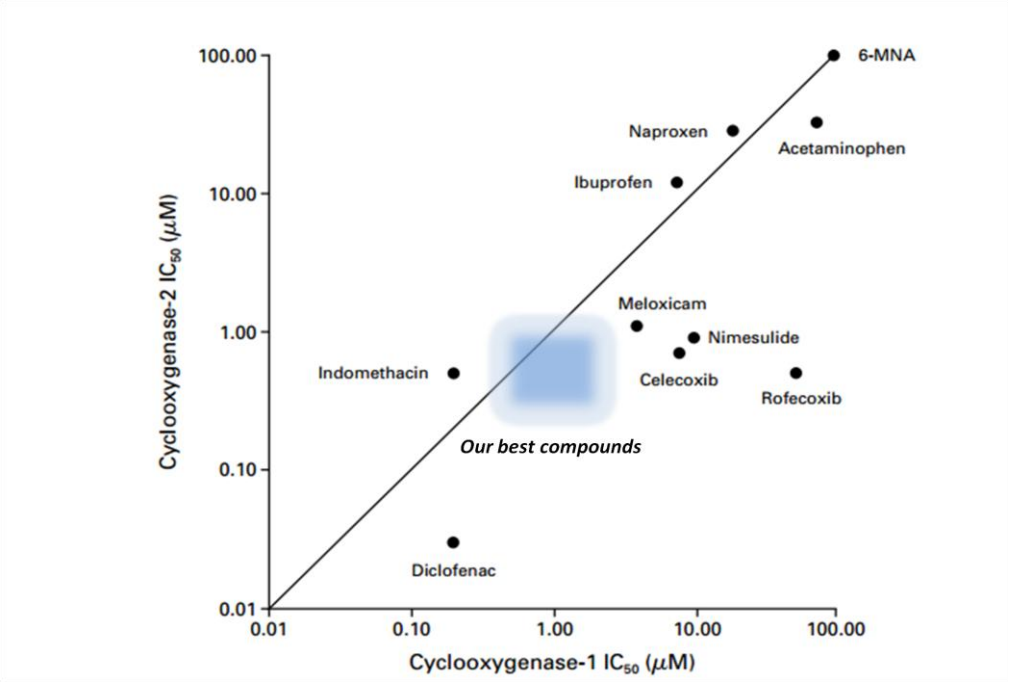


Figure II.34. IC_{50} of several drugs determined by WBA. Drugs below the diagonal line are more potent inhibitors of COX-2 than drugs on or above the line.[2] Our best candidates are within the blue zone.

In conclusion, the ester derivatives show higher potencies and almost similar selectivity to a vast range of commercially available NSAIDs, conferring some commercial viability. It can be said that due to the ester moiety, these drugs could not be used in an oral regimen, but could be administrated for example, in an intravenous way.

II.5 Experimental

II.5.1 Benzimidazole Library Synthesis

II.5.1.1 General information

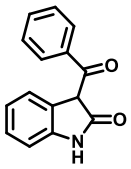
All commercially obtained reagents were used without further purification unless specified. All the mentioned solvents used in the reactions were dried by usual methods.[130] Molecular sieves 4Å were activated by heating at 300 ºC for 3 h.

Column chromatography was carried out with silica gel 60 Å (220-440 mesh) using the described eluent for each case. Preparative and analytical TLC was performed with silica gel 60 plates of 1 mm, 0.5 mm and 0.25 mm, respectively.

IR spectra were recorded using a Perkin-Elmer Spectrum 1000 FT-IR. The IR bands are classified as strong (s), medium (m), or weak (w). NMR spectra were recorded with a Brucker ARX 400, Brucker Avance 400 and Brucker Avance 600 spectrometers using $CDCl_3$, DMSO-d_6, acetone-d_6 and D_2O as solvents using their corresponding $CHCl_3$, DMSO, acetone and water signals as reference, respectively. Melting points were determined using melting point apparatus Reichert Thermovar equipped with a Kofler plate and uncorrected. Mass spectra were obtained on a Micromass AutoSpecQ and a Micromass GTC (MALDI-TOF-MS, Matrix: α-Cyano-4-hydroxy-cinnamic acid).

HPLC was performed using a Merck HITACHI La Chrom chromatograph equipped with a DAD detector L-7450A and a LiChrospher 100 RP-18 (10 µm) LiChroCART® 250-4 column; injection volume: 20 µL; A: water (pH 2.5), B: methanol; gradient elution (time, %A, %B): 0 min., 50:50; 5 min., 30:70; 20min. 10:90; 30min. 0:100; 35min, 0:100; 38 min. 50:50. Measured at 270 nm.

II.5.1.2 3-Benzoyl oxindole (II.23)[131]



To an oxindole solution (100 mg, 0.751 mmol) in toluene (3 mL, 0.25 M) was added benzoyl chloride (87 μL, 0.751 mmol). The mixture was placed in an oil bath and heated to 70 ºC. Then, a triethylamine (0.105 mL, 0.751 mmol) solution in toluene (1 mL) was added dropwise. The mixture was allowed to stir overnight at 70 ºC. The reaction was quenched with saturated aqueous $NaHCO_3$ (15 mL), extracted with EtOAc and washed with *brine*. The combined organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. Purification by flash column chromatography using a gradient system from hexane to hexane:EtOAc (9:1), afforded **II.23** as a white solid (110 mg, 62 %). *Rf* = 0.7, hexane:EtOAc (4:1).

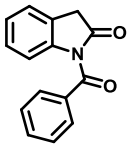
MP: 162-165 ºC;

IR (KBr) ν_{max} (cm^{-1}): 3364 (s, *N-H*), 1732 (s, *C=O*), 1450 (w), 1263 (s), 1066 (m), 702 (m);

^{1}H NMR (400 MHz, acetone-d_6) δ: 10.58 (s, N*H*, 1H), 8.21 (d, *J* = 8.0 Hz, Ar*H*, 2H), 7.76 (t, *J* = 7.4 Hz, Ar*H*, 1H), 7.63 (dd, *J* = 7.6 Hz, Ar*H*, 2H), 7.54 (d, *J* = 7.7 Hz, Ar*H*, 1H), 7.38 (d, *J* = 7.9 Hz, Ar*H*, 1H), 7.09 (m, Ar*H*, 2H), 6.36 (s, H_3, 1H);

^{13}C NMR (100 MHz, acetone-d_6) δ: 163.9 (*C*O), 144.0 (*C*Ar), 135.0 (*C*Ar), 132.6 (*C*Ar), 130.9 (*C*Ar), 129.8 (*C*Ar), 129.6 (*C*Ar), 128.1 (*C*Ar), 122.0 (*C*Ar), 120.9 (*C*Ar), 120.6 (*C*Ar), 111.7 (*C*Ar), 88.8 (*C*H).

II.5.1.3 *N*-Benzoyl oxindole (II.25)[132]



To a round bottom flask were added benzoic anhydride (255 mg, 1.13 mmol) and oxindole (100 mg, 0.75 mmol). The mixture was placed in an oil bath and was allowed to stir overnight at 120 ºC. After cooling, the residue was purified by flash column chromatography to give **II.25** as a white solid (100 g, 56 %). *Rf* = 0.6, hexane:ether (3:2).

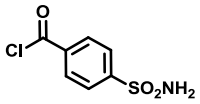
MP: 135-138ºC;

IR (KBr) ν_{max} (cm^{-1}): 1755 (s, *C=O*), 1682 (s), 1350 (m), 1297 (s) 756 (w);

^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.81 – 7.75 (m, Ar*H*, 3H), 7.60 (t, *J* = 7.4 Hz, Ar*H*, 1H), 7.47 (t, *J* = 7.6 Hz, Ar*H*, 2H), 7.37 – 7.32 (m, Ar*H*, 2H), 7.20 (t, *J* = 7.5 Hz, Ar*H*, 1H), 3.76 (s, CH_2, 2H).

^{13}C NMR (400 MHz, $CDCl_3$) δ: 174.4 (*C*O), 169.6 (*C*O), 133.1 (*C*Ar), 130.3 (*C*Ar), 129.5 (*C*Ar), 128.4 (*C*Ar), 124.9 (*C*Ar), 124.5 (*C*Ar), 115.1 (*C*Ar), 36.7 (CH_2).

II.5.1.4 4-Sulfamoylbenzoyl chloride (II.27)[133]



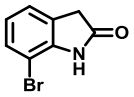
A sealed tube containing $SOCl_2$ (2.2 mL, 29.8 mmol) was placed in an ice bath. The $SOCl_2$ was stirred while the oven dried potassium salt of 4-sulfamoylbenzoyl acid (500 mg, 2.49 mmol) was added portion wise. The mixture was refluxed for 1 h. It was then was allowed to cool to room temperature and transferred to a round bottom flask, where $SOCl_2$ was evaporated until dryness. The obtained crude was recrystallized from benzene, to give **II.27** as a white solid (400 mg, 73%).

MP: 138-140 ºC;

IR (KBr) ν_{max} (cm^{-1}): 3332 (s, *N-H*), 3251 (s, *N-H*), 1723 (s, *C=O*), 1349 (s, *S-O*), 1161 (s, *S-O*), 879 (m), 741 (m);

^{1}H NMR (400 MHz, $CDCl_3$ + 2% DMSO-d_6) δ: 8.10 (d, *J* = 7.5 Hz, 2H), 7.90 (d, *J* = 7.5 Hz, 2H), 7,28 (bs, NH_2, 2H).

II.5.1.5 7-Bromo oxindole (II.29)[134]



To a 7-bromo isatin solution (50 mg, 0.221 mmol) in DEG (0.5 mL) was added hydrazine hydrate 99-100% (0.103 mL, 3.32 mmol). The mixture was heated at 80 ºC for 1 h until it turned strong yellow. The mixture was allowed to cool down and a KOH aqueous solution (1.11 mmol, 5 equiv) was added. The mixture was heated at 80 ºC for an additional hour. The aqueous layer was neutralized with 1M HCl solution and extracted several times with EtOAc. The combined organic layer was washed with saturated aqueous $NaHCO_3$ and *brine*, dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by preparative thin layer chromatography to give **II.29** as a white solid (38 g, 81%). *Rf* = 0.5, hexane:EtOAc (3:2).

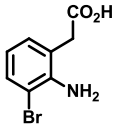
MP: 201ºC (decomp.);

IR (KBr) ν_{max} (cm^{-1}): 3209 (w), 1700 (s, *C=O*), 1617 (m), 1322 (w), 1123 (w), 705 (w);

^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.48 (s, N*H*, 1H), 7.34 (d, *J* = 8.2 Hz, ArH_6, 1H), 7.15 (d, *J* = 7.2 Hz, ArH_4, 1H), 6.90 (t, *J* = 7.8 Hz, ArH_5, 1H), 3.65 (s, CH_2, 2H);

^{13}C NMR (100 MHz, $CDCl_3$) δ: 176.0 (*C*O), 142.0 (C_q), 130.8 (*C*Ar), 126.5 (C_q), 123.7 (*C*Ar), 123.6 (*C*Ar), 102.8 (C_q), 37.4 (CH_2).

II.5.1.6 2-(2-Amino-3-bromophenyl)acetic acid (II.37)



To a 7-bromo-isatin solution (250 mg, 1.11 mmol) in DEG (2.5 mL) was added hydrazine hydrate 99-100% (0.81 mL, 17 mmol). The mixture was heated at 80 ºC for 1 h until it turned strong yellow. The mixture was allowed to cool down and a KOH solution (1 g, 18.8 mmol, 17 equiv) was added. The mixture was heated at 120 ºC for additional 2 h. The reaction was diluted with water and washed with EtOAc to remove any unreacted oxindole. The aqueous layer was carefully neutralized with 1M HCl solution (in an ice bath), and the product precipitated as a light brown solid. The residue was filtrated and washed with cold water to give **II.37** as a light brown solid (230 mg, 90%). *Rf* = 0.2, hexane:EtOAc (3:2).

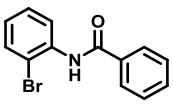
MP: 206 ºC (decomp.);

IR (KBr) ν_{max} (cm^{-1}): 3366 (s, *N-H*), 3301 (s, *N-H*), 1695 (s), 1458 (s), 1251 (s), 1229 (s), 743 (w);

^{1}H NMR (400 MHz, acetone-d_6) δ: 7.34 (d, *J* = 8.0 Hz, Ar*H*, 1H), 7.10 (d, *J* = 7.4 Hz, Ar*H*, 1H), 6.58 (t, *J* = 7.7 Hz, Ar*H*, 1H), 4.90 (bs, NH_2, 1H), 3.63 (s, CH_2, 2H);

^{13}C NMR (100 MHz, acetone-d_6) δ: 172.6 (*C*O), 132.2 (*C*Ar), 131.3 (*C*Ar), 131.0 (*C*Ar), 124.8 (*C*Ar), 119.2 (*C*Ar), 38.8 (CH_2).

II.5.1.7 *N*-(2-Bromophenyl)benzamide (II.39)[135]



To a 2-bromo aniline solution (100 mg, 0.580 mmol) in THF (1.7 mL, 0.35 M) was added triethylamine (0.126 mL, 0.640 mmol). The mixture was placed on an ice bath and benzoyl chloride (74 μL, 0.640 mmol) was added dropwise. The mixture was filtrated through a celite pad and the residue was purified by silica preparative to give **II.39** as a white solid (150 mg, 93%). *Rf* = 0.6, hexane:ether (2:1).

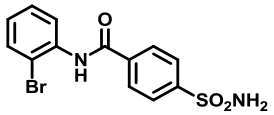
MP: 99-101ºC;

IR (KBr) ν_{max} (cm^{-1}): 3276 (m, *N-H*), 1651 (s), 1533 (s), 1436 (m), 1310 (m), 1027 (w), 750 (m);

^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.56 (dd, *J* = 8.2 Hz, Ar*H*, 1H), 8.47 (bs, N*H* 1H), 7.94 (d, *J* = 7.4 Hz, Ar*H*, 2H), 7.61 – 7.51 (m, Ar*H*, 4H), 7.38 (t, *J* = 7.8 Hz, Ar*H*, 1H), 7.03 (t, *J* = 7.7 Hz, Ar*H*, 1H);

^{13}C NMR (400 MHz, $CDCl_3$) δ: 165.4 (*C*O), 136.0 (C_q), 134.8 (C_q), 132.4 (*C*Ar), 132.3 (*C*Ar), 129.1 (2x*C*Ar), 128.7 (*C*Ar), 127.2 (2x*C*Ar), 125.4 (*C*Ar), 120.0 (*C*Ar), 113.9 (C_q).

II.5.1.8 *N*-(2-Bromophenyl)-4-sulfamoylbenzamide (II.40)



To a 2-bromo aniline solution (50 mg, 0.29 mmol) in THF (0.8 mL) was added triethylamine (61 μL, 0.44 mmol). The mixture was placed in an ice bath and a 4-sulfamoylbenzoyl chloride (96 mg, 0.44 mmol) solution in THF (0.3 mL) was added dropwise. The mixture was stirred overnight at room temperature. The reaction was quenched with saturated aqueous $NaHCO_3$, extracted with EtOAc and washed with *brine*. The combined organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was recrystallized to give **II.40** as a white solid (36 mg, 70% based on starting material recovered). *Rf* = 0.2, hexane:EtOAc (3:2).

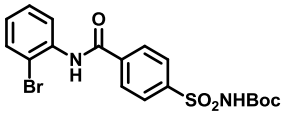
MP: 248-253 ºC;

IR (KBr) ν_{max} (cm^{-1}): 3362 (s, *N-H*), 3332 (s, *N-H*), 3066 (m), 2925 (m), 1658 (s), 1524 (s), 1335 (s, *S-O*), 1164 (s, *S-O*), 758 (m), 614 (m);

^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.28 (bs, N*H*), 8.15 (d, *J* = 8.2 Hz, Ar*H*, 2H), 7.98 (d, *J* = 8.3 Hz, Ar*H*, 2H), 7.75 (d, *J* = 8.0 Hz, Ar*H*, 1H), 7.58 – 7.44 (m, Ar*H*, NH_2, 4H), 7.27 (t, *J* = 7.7 Hz, Ar*H*, 1H);

^{13}C NMR (100 MHz, DMSO-d_6) δ: 164.4 (*C*O), 146.7 (C_q), 136.8 (C_q), 136.1 (C_q), 132.7 (*C*Ar), 128.9 (*C*Ar), 128.2 (2x*C*Ar), 128.2 (*C*Ar), 128.1 (*C*Ar), 125.7 (2x*C*Ar), 120.6 (C_q).

II.5.1.9 *tert*-Butyl 4-(2-bromophenylcarbamoyl)phenylsulfonylcarbamate



In a two neck flask equipped with a magnetic stir bar, was placed *N*-(2-bromophenyl)-4-sulfamoylbenzamide (10 mg, 0.028 mmol), triethylamine (4.6 μL, 0.033 mmol), DMAP (1.7 mg, 0.014 mmol) and DCM (0.5 mL). The vial was suba-sealed, evacuated and backfilled with argon. $(Boc)_2O$ (18 μL, 0.084 mmol) was added *via* syringe. The reaction mixture was stirred at room temperature for 4 h. The reaction was diluted with water, extracted with DCM and washed with *brine*. The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by silica flash chromatography (hexane:EtOAc 4:1) to give the product as a white solid (12 mg, quant.). *Rf* = 0.5, hexane:EtOAc (4:1).

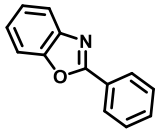
MP: 118-120 ºC;

IR (KBr) ν_{max} (cm^{-1}): 3234 (m), 2982 (m), 1746 (s, *C*=*O* carbamate), 1667 (s, *C*=*O* amide), 1520 (s), 1436 (s, *S*-*O*), 1148 (s, *S*-*O*);

^{1}H NMR (400 MHz, CH_3OD) δ: 8.16 (d, *J* = 8.6 Hz, Ar*H*, 2H), 8.12 (d, *J* = 8.7 Hz, Ar*H*, 2H), 7.71 (dd, *J* = 8.0, 1.3 Hz, Ar*H*, 2H), 7.43 (td, *J* = 7.9, 1.3 Hz, Ar*H*, 1H), 7.22 (td, *J* = 8.0, 1.5 Hz, Ar*H*, 1H), 1.38 (s, C(CH_3)$_3$, 9H);

^{13}C NMR (100 MHz, CH_3OD) δ: 167.3 (CO), 151.6 (*C*OC(CH_3)$_3$), 144.1 (C_q), 140.1 (C_q), 137.0 (C_q), 134.1 (*C*Ar), 129.3 (2x*C*Ar), 129.3 (2x*C*Ar), 129.3 (2x*C*Ar), 129.0 (*C*Ar), 121.0 (C_q), 84.3 (*C*(CH_3)$_3$), 28.1 (C(*C*H_3)$_3$).

II.5.1.10 2-Phenyl-benzoxazole (II.41)[135]



A screw-cap test-tube, equipped with a magnetic stir bar, was charged with *N*-(2-bromofenil)benzamide (0.1 mmol), CuI (10 mol %), L-proline (20 mol%) and the desired base (0.2 mmol). The vial was sealed with a teflon screw-cap, evacuated and backfilled with argon. The solvent (0.1 mL) was added *via* syringe, followed by aniline (0.12 mmol). The reaction mixture was heated (*see* Table II.5 for temperature and reaction time). The solution was allowed to cool to room temperature, quenched by the addition of NH_4Cl solution, diluted with EtOAc and poured into sat. $NaHCO_3$. After extracting 3 times with EtOAc, the combined organic layers were washed with brine, dried over $MgSO_4$, and then concentrated. The crude product was purified by silica flash chromatography to give **II.41** as a white solid. *Rf* = 0.8, hexane:ether (2:1).

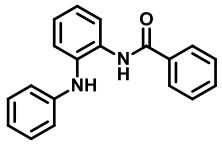
MP: 100-103ºC;

IR (KBr) ν_{max} (cm^{-1}): 1552 (m), 1447 (m), 1242 (m), 1053 (m), 745 (s);

^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.28 – 8.26 (m, Ar*H*, 2H), 7,79 – 7.77 (m, Ar*H*, 1H), 7,60 – 7.52 (m, Ar*H*, 4H), 7,36 – 7.34 (m, Ar*H*, 2H);

^{13}C NMR (100 MHz, $CDCl_3$) δ: 163.1 (C_q), 150.9 (C_q), 142.2 (C_q), 131.6 (*C*Ar), 129.0 (*C*Ar), 127.7 (*C*Ar), 127.3 (C_q), 125.2 (*C*Ar), 124.7 (*C*Ar), 120.1 (*C*Ar), 110.7 (*C*Ar).

II.5.1.11 *N*-(2-(Phenylamino)phenyl)benzamide (II.42)



A screw-cap test-tube, equipped with a magnetic stir bar, was charged with BrettPhos ligand (2.8 mg, 1 mol %), BrettPhos precatalyst (4.0 mg, 1 mol %) and *N*-(2-bromophenyl)benzamide (70 mg, 0.253 mmol). The vial was sealed with a teflon screw-cap, evacuated and backfilled with argon. LiHMDS (1M in THF) (0.9 mL, 0.86 mmol, 3.4 equiv) was added *via* syringe, followed by aniline (29 μL, 0.304 mmol, 1.2 equiv). The reaction mixture was heated at 65 °C for 4 h. The solution was allowed to cool to room temperature, quenched by the addition of 1M HCl solution (1 mL), diluted with EtOAc and poured into sat. $NaHCO_3$. After extracting with 3 portions of EtOAc, the combined organic layers were washed with brine, dried over $MgSO_4$, and then concentrated. The crude product was purified by pTLC, eluting with ether:hexane (1:4), to provide **II.42** as a light brown solid (70 mg, quant.). *Rf* = 0.2, hexane:Et_2O (4:1).

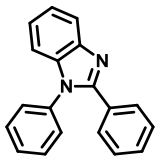
MP: 134-136ºC;

IR (KBr) ν_{max} (cm^{-1}): 3371 (m, *N-H*), 3326 (m, *N-H*), 1644 (s), 1600 (s), 1520 (s), 1453 (s), 1312 (s), 741 (m), 689 (m);

^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.49 (bs, N*H*, 1H), 8.21 (d, *J* = 7.5 Hz, Ar*H*, 1H), 7.68 (d, *J* = 7.3 Hz, Ar*H*, 2H), 7.51 – 7.17 (m, Ar*H*, 8H), 6.94 – 6.84 (m, Ar*H*, 3H), 5.77 (bs, *NH*, 1H);

^{13}C NMR (100 MHz, $CDCl_3$) δ: 165.9 (*C*O), 144.9 (*C*Ar), 134.7 (*C*Ar), 133.5 (*C*Ar), 132.7 (*C*Ar), 131.9 (*C*Ar), 129.6 (2x*C*Ar), 128.8 (2x*C*Ar), 127.1 (2x*C*Ar), 125.5 (*C*Ar), 125.3 (*C*Ar), 124.4 (*C*Ar), 122.3 (*C*Ar), 120.5 (*C*Ar), 116.3 (*C*Ar).

II.5.1.12 1,2-Diphenyl-1H-benzimidazole (II.43)[74]



To a *N*-(2-(phenylamino)phenyl)benzamide (**II.42**) solution (10 mg, 0.035 mmol) in dry DMSO (0.1 mL, 0.35 M) was added AcOH (0.4 mL, 6.94 mmol). The mixture was stirred overnight at 85ºC. The mixture was co-evaporated with toluene, diluted with EtOAc and washed with water and *brine*. The combined organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The brown residue was recrystallized to give **II.43** as a white solid (9 mg, quant.). *Rf* = 0.3, hexane:Et_2O (4:1).

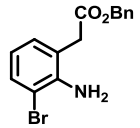
MP:110-113ºC;

IR (KBr) ν_{max} (cm^{-1}): 3433 (w), 3049 (w), 1594 (w), 1492 (s), 1476 (s), 1455 (m), 1382 (s), 764 (S), 750 (s), 704 (s);

^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.92 (d, *J* = 7.5 Hz, Ar*H*, 1H), 7.61 – 7.51 (m, Ar*H*, 5H), 7.35 – 7.29 (m, Ar*H*, 8H);

^{13}C NMR (100 MHz, $CDCl_3$) δ: 152.3 (*C*Ar), 143.0 (*C*Ar), 137.1 (*C*Ar), 136.8 (*C*Ar), 129.9 (*C*Ar), 129.8 (*C*Ar), 129.4 (*C*Ar), 129.4 (*C*Ar), 128.5 (*C*Ar), 128.2 (*C*Ar), 127.4 (*C*Ar), 123.3 (*C*Ar), 122.9 (*C*Ar), 119.8 (*C*Ar), 110.4 (*C*Ar).

II.5.1.13 Benzyl 2-(2-amino-3-bromophenyl)acetate (II.44)



To a 2-(2-amino-3-bromophenyl)acetic acid solution (**II.37**) (15 mg, 0.065 mmol) in DMF (0.4 mL, 0.2 M) was added benzyl bromide (8 μL, 0.065 mmol) and Cs_2CO_3 (21 mg, 0.065 mmol). The mixture was stirred overnight at room temperature. The reaction was quenched with water (10 mL), extracted with EtOAc (20 mL) and washed with *brine*. The combined organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by silica flash chromatography to give **II.44** as a yellow solid (15 mg, 72%). *Rf* = 0.8, hexane:Et_2O (4:1).

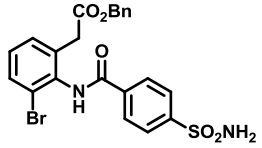
MP: 70-75ºC;

IR (KBr) ν_{max} (cm^{-1}): 3443 (s, *NH*$_2$), 3363 (s, *NH*$_2$), 3026 (w), 2926 (w), 1721 (s, *C*=*O* ester), 1629 (s), 1457 (m), 1330 (m), 1161 (s), 969 (w), 737 (w), 696 (w);

^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.39 – 7.29 (m, Ar*H*, 6H), 7.04 (dd, *J* = 10.4, 3.8 Hz, Ar*H*, 1H), 6.61 (t, *J* = 7.8 Hz, Ar*H*, 1H), 5.13 (s, C*H*$_2$Ph, 1H), 3.65 (s, C*H*$_2$, 1H);

^{13}C NMR (100 MHz, $CDCl_3$) δ: 171.3 (*C*O_2Bn), 135.5 (*C*$_q$), 132.1 (*C*Ar), 130.5 (*C*Ar), 128.8 (2x*C*Ar), 128.5 (*C*Ar), 128.3 (2x*C*Ar), 127.1 (*C*$_q$), 119.5 (*C*$_q$), 67.2 (C*H*$_2$Ph,), 39.3 (C*H*$_2$,).

II.5.1.14 Benzyl 2-(3-bromo-2-(4-sulfamoylbenzamido)phenyl)acetate (II.45)



To an ice cold suspension of sulfamoylbenzyl chloride (485 mg, 2.2 mmol, 1.5 equiv) and K_2CO_3 (203mg, 1.47 mmol) in THF (3 mL) was added dropwise a benzyl 2-(2-amino-3-bromophenyl)acetate solution (**II.44**) (470 mg, 1.47 mmol) in THF (2 mL). The mixture was stirred overnight at room temperature. The reaction was quenched with water (15 mL), extracted with EtOAc and washed with *brine*. The combined organic layer was dried over Na_2SO_4 and

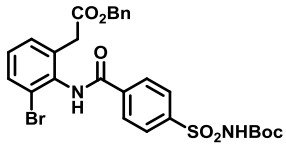
concentrated *in vacuo*. The residue was purified by silica flash chromatography (EtOAc:hexane 2:3) to give **II.45** as a white solid (690 mg, 93%). *Rf* = 0.2, hexane:EtOAc (1:1).

IR (KBr) ν_{max} (cm^{-1}): 3393 (s, *N-H*), 3278 (s, *N-H*), 3200 (m), 1730 (s, *C=O* ester), 1640 (s), 1535 (m), 1328 (s, *S-O*), 1159 (s, *S-O*), 745 (w);

^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.34 (bs, N*H*, 1H), 8.09 (d, *J* = 8.4 Hz, Ar*H*, 2H), 7.94 (d, *J* = 8.4 Hz, Ar*H*, 2H), 7.68 (d, *J* = 7.6 Hz, Ar*H*, 1H), 7.53 (s, Ar*H*, 2H), 7.43 (d, *J* = 7.6 Hz, Ar*H*, 1H), 7.30 – 7.26 (m, Ar*H*, 4H), 7,19 – 7.18 (m, Ar*H*, 2H), 5.01 (s, CH_2Ph, 2H), 3.74 (s, CH_2, 2H);

^{13}C NMR (100 MHz, DMSO-d_6) δ: 170.6 (*C*O_2Bn), 164.8 (*C*ONHR), 147.2 (C_q), 137.1 (C_q), 136.2, 135.9 (C_q), 132.1 (*C*Ar), 131.1 (*C*Ar), 129.4 (C_q), 128.8 (4x*C*Ar), 128.5 (*C*Ar), 128.4 (2x*C*Ar), 126.2 (2x*C*Ar), 123.9 (C_q), 66.4 (*C*H_2Ph), 38.2 (*C*H_2).

II.5.1.15 Benzyl 2-(3-bromo-2-(4-(N-(tert-butoxycarbonyl)sulfamoyl)benzamido) phenyl)acetate (II.46)



To a two neck flask equipped with a magnetic stir bar, was added benzyl 2-(3-bromo-2-(4-sulfamoylbenzamido)phenyl)acetate (**II.45**) (100 mg, 0.199 mmol), triethylamine (56 μL, 0.398 mmol, 2 equiv), DMAP (12 mg, 0.10 mmol, 0.5 equiv) and DCM (2 mL, 0.1 M). The vial was suba-sealed, evacuated and backfilled with argon. $(Boc)_2O$ (85 μL, 0.398 mmol, 2 equiv) was added *via* syringe. The reaction mixture was stirred at room temperature for 1 h. The reaction was quenched with water, extracted with DCM and washed *brine*. The combined organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by silica flash chromatography (EtOAc:hexane 1:1 gradient to EtOAc:acetone 1%) to give **II.46** as a white solid (91 mg, 76%). *Rf* = 0.6, 2x hexane:EtOAc (1:1).

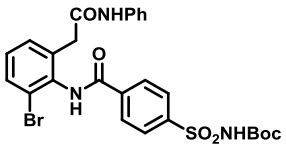
MP: 154-157ºC;

IR (KBr) ν_{max} (cm^{-1}): 3211 (s), 1755 (s, *C=O* carbamate), 1733 (s, *C=O* ester), 1653 (s), 1522 (m), 1432 (m), 1342 (m), 1163 (s), 1140 (s), 733 (m), 607 (m);

^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.28 (bs, N*H*, 1H), 8.07 – 7.94 (m, Ar*H*, 4H), 7.64 (s, Ar*H*, 1H), 7.34 – 7.20 (m, Ar*H*, 8H), 5.11 (s, CH_2Ph, 2H), 3.77 (s, CH_2, 2H), 1.41 (s, C(CH_3)$_3$, 9H);

^{13}C NMR (100 MHz, CDCl3) δ: 171.2 (*C*O_2Bn), 164.0 (*C*ONHR), 148.9 (*C*OC(CH_3)$_3$), 141.9 (C_q), 138.3 (C_q), 135.2 (C_q), 134.6 (C_q), 134.0 (*C*Ar), 132.7 (*C*Ar), 130.3 (*C*Ar), 129.1 (*C*Ar), 128.7 (2x*C*Ar), 128.6 (2x*C*Ar), 128.3 (2x*C*Ar), 128.0 (2x*C*Ar), 123.4 (C_q), 84.5 (*C*(CH_3)$_3$), 67.2 (*C*H_2Ph), 39.3 (*C*H_2), 27.9 (C(*C*H_3)$_3$).

II.5.1.16 *t*-Butyl 4-(2-bromo-6-(2-oxo-2-(phenylamino)ethyl)phenylcarbamoyl) phenyl sulfonylcarbamate (II.48)



Following the general arylamination procedure used for **II.42**, were used **II.46** (50 mg, 0.083 mmol), aniline (10 μL, 0.12 mmol) and LiHMDS (0.28 mL, 0.28 mmol). The crude product was purified *via* chromatography on silica, eluting with a gradient from EtOAc:hexane 1:1 to EtOAc:MeOH 5%, to provide **II.48** as a white solid (*see* Table II.7 for yields). *Rf* = 0.2, 2x(hexane:EtOAc, 3:2).

MP: > 200ºC (decomposition);

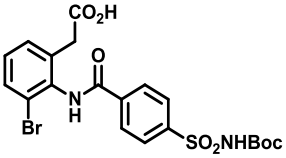
IV (KBr) ν_{max} (cm^{-1}): 3301 (m), 3262 (m), 1746 (s, *C*=*O* carbamate), 1664 (s), 1444 (m), 1148 (m);

^{1}H NMR (400 MHz, acetone-d_6) δ: 10.1 (bs, N*H*, 1H), 9.49 (bs, N*H*, 1H), 8.35 (d, *J* = 7.9 Hz, Ar*H*, 2H), 8.18 (d, *J* = 7.9 Hz, Ar*H*, 2H), 7.73 – 7.54 (m, Ar*H*, 4H), 7.34 – 7.33 (m, Ar*H*, 3H), 7.11 (m, Ar*H*, 1H), 3.91 (s, CH_2, 2H), 1.37 (s, C(CH_3)$_3$, 9H);

^{13}C NMR (100 MHz, acetone-d_6) δ: 169.7 (CO), 165.5 (*C*ONHR), 150.5 (*C*OC(CH$_3$)$_3$), 143.7 (C_q), 140.1 (C_q), 139.5 (C_q), 137.6 (C_q), 136.4 (C_q), 132.7 (*C*Ar), 130.9 (*C*Ar), 129.8 (*C*Ar), 129.5 (2x*C*Ar), 129.3 (2x*C*Ar), 129.0 (2x*C*Ar), 124.4 (*C*Ar), 120.2 (*C*Ar), 83.6 (*C*(CH$_3$)$_3$), 41.9 (*C*H$_2$), 28.0 (C(*C*H$_3$)$_3$);

HRMS: calcd for $C_{26}H_{27}BrN_3O_6S$ $[M + Na]^+$: 588.0798, found 588.0795.

II.5.1.17 2-(3-Bromo-2-(4-(N-(tert-butoxycarbonyl)sulfamoyl)benzamido) phenyl)acetic acid (II.49)



Following general arylamination procedure used for **II.42**, were used **II.46** (25 mg, 0.041 mmol), aniline (6 μL, 0.061 mmol), NaO^{t}Bu (13 mg, 0.131 mmol), BrettPhos precatalyst (3 mg, 10 mol%) and BrettPhos ligand (2 mg, 10 mol%). The crude product was purified *via* chromatography on silica, eluting with a gradient from EtOAc:hexane 2:1 to EtOAc:MeOH 5%, to provide the **II.49** as a white solid (13 mg, 62%). *Rf* = 0, hexane:EtOAc (3:2).

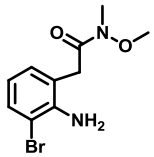
MP: 198-202ºC;

IR (KBr) ν_{max} (cm^{-1}): 3358 (m), 3260 (m), 1731 (s), 1646 (m), 1515 (m), 1319 (m), 1163 (s);

^{1}H NMR (400 MHz, acetone-d_6) δ: 9.65 (bs, N*H*, 1H), 8.28 (d, *J* = 8.2 Hz, Ar*H*, 2H), 8.15 (d, *J* = 8.2 Hz, Ar*H*, 2H), 7.67 (d, *J* = 7.7 Hz, Ar*H*, 1H), 7.46 (d, *J* = 7.2 Hz, Ar*H*, 1H), 7.30 (m, Ar*H*, 1H), 3.77 (s, CH_2, 2H), 1.38 (s, C(CH_3)$_3$, 9H);

^{13}C NMR (100 MHz, acetone-d_6) δ: 172.3 (*C*O_2H), 165.3 (*C*ONHR), 150.4 (*C*OC(CH$_3$)$_3$), 143.6 (C_q), 139.7 (C_q), 137.2 (C_q), 136.4 (C_q), 132.6 (*C*Ar), 131.3 (*C*Ar), 129.8 (*C*Ar), 129.2 (2x*C*Ar), 128.9 (2x*C*Ar), 124.3 (C_q), 83.6 (*C*(CH$_3$)$_3$), 38.8 (*C*H$_2$), 28.0 (C(*C*H$_3$)$_3$).

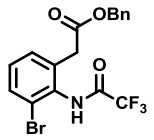
II.5.1.18 2-(2-amino-3-bromophenyl)-N-methoxy-N-methylacetamide (II.50)



To a hydroxylamine hydrochloride (21 mg, 0.215 mmol) solution in DCM (0.5 mL), was added triethylamine (30 μL, 0.215 mmol) and the mixture was allowed to stir for 30 min. In a separate flask placed in an ice bath, was added **II.37** (45mg, 0.195 mmol), DCM (0.5 mL) and DCC (44 mg, 0.215 mmol). To this last mixture was added the hydroxylamine solution and the mixture was stirred for 4h at room temperature. The reaction was quenched with water (15 mL), extracted with DCM and washed with *brine*. The combined organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by silica flash chromatography to give a mixture of product and oxindole, 1:1 (40 mg).

^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.31 (dd, *J* = 8.0, 1.2 Hz, 1H), 7.09 (d, *J* = 7.3 Hz, 1H), 6.54 (t, *J* = 7.7 Hz, 1H), 5.14 (bs, 2H), 3.76 (s, 3H), 3.73 (s, 2H), 3.14 (s, 3H).

II.5.1.19 Benzyl 2-(3-bromo-2-(2,2,2-trifluoroacetamido)phenyl)acetate (II.51)



To a **II.44** (25 mg, 0.078 mmol) and triethylamine (15 μL, 0.109 mmol) solution in DCM (0.5 mL, 0.15 M) in a ice bath, was added dropwise trifluoroacetic anhydride (14 μL, 0.094 mmol). The reaction mixture was stirred at room temperature for 3 h. The mixture was evaporated and the crude was purified, to give **II.51** (32 mg, quant.) as a white solid. *Rf* = 0.2, hexane:EtOAc (8:1).

MP: 23-28ºC;

IR (KBr) ν_{max} (cm^{-1}): 3272 (m, *N-H*), 1728 (s, *C=O*, amide and ester), 1532 (m), 1343 (m), 1236 (s), 1199 (s), 1165 (s), 736 (m);

^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.51 (bs, 1H, N*H*), 7.65 (dd, *J* = 7.9, 1.3 Hz, 1H, Ar*H*), 7.43 – 7.22 (m, 7H, Ar*H*), 5.18 (s, 2H, CH_2), 3.73 (s, 2H, CH_2);

^{13}C NMR (100 MHz, $CDCl_3$) δ: 171.1 (*C*OOBn), 155.2 (q, *J* = 37.8 Hz, *C*OCF_3), 135.0 (C_qAr), 133.5 (C_qAr), 133.0 (*C*Ar), 132.2 (C_qAr), 130.3 (*C*Ar), 129.9 (*C*Ar), 128.7 (2x*C*Ar), 128.6 (*C*Ar), 128.4 (2x*C*Ar), 123.0 (C_qAr), 115.83 (q, *J* = 288.6 Hz, CF_3), 67.5 (*C*H_2), 38.8 (*C*H_2).

II.5.1.20 2-(3-Bromo-2-nitrophenyl)acetic acid (II.56)[134]

To a NaOH (24 mg, 0.62 mmol) solution at 0ºC were added **II.37** (200 mg, 0.87 mmol) and $NaHCO_3$ (730 mg, 8.7 mmol). The mixture was protected from light and allowed to stir for 10 min. A solution of Oxone (1.34 g, 2.18 mmol) in EDTA solution ($4x10^{-4}$ M, 8 mL) was then added followed by a solution of H_2O:acetone 1:1 (8 mL). The mixture was stirred for 4 h in an ice/water bath. The mixture was quenched by a saturated $NaHSO_4$ solution until a suspension was formed. Then, was extracted three times with EtOAc (25 mL), washed with water and brine, dried over Na_2SO_4 and evaporated. The yellow residue was used in the next step without further purification (210 mg, 93%). *Rf* = 0.2, 2x(hexane:EtOAc, 3:2).

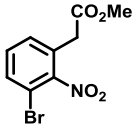
MP: 144-147ºC;

IR (KBr) ν_{max} (cm^{-1}): 3075 (b, w), 2929 (w), 1719 (s, *C=O*), 1534 (m), 1277 (m), 1229 (m), 942 (w);

^{1}H NMR (400 MHz, acetone-d_6) δ: 7,78 (d, *J* = 7.9 Hz, Ar*H*, 1H), 7,61 (d, *J* = 7.5 Hz, Ar*H*, 1H), 7,53 (t, *J* = 7.8 Hz, Ar*H*, 1H), 3,77 (s, CH_2, 2H);

^{13}C NMR (100 MHz, acetone d_6) δ: 172.3 (*C*O), 152.7 ($C_q$$NO_2$), 133.9 (*C*Ar), 133.3 (*C*Ar), 133.1 (*C*Ar), 131.3 (C_qAr), 113.5 (C_qAr), 38.4 (*C*H_2).

II.5.1.21 Methyl 2-(3-bromo-2-nitrophenyl)acetate (II.57)



To a **II.56** (30 mg, 0.078 mmol) solution in DCM (1 mL) in a ice bath, was added dropwise diazomethane (0.25 mL, 0.2 mmol). The reaction mixture was stirred at room temperature for 1 h. The mixture was evaporated and the crude was purified, to give **II.57** (18 mg, 60%) as a light yellow solid. *Rf* = 0.7, hexane:EtOAc (3:2).

Mp: 53-56ºC;

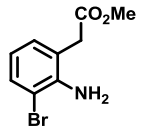
IR (KBr) ν_{max} (cm^{-1}): 3075 (w), 2954 (w), 2888 (w), 1737 (s, *C=O* ester), 1529 (s), 1438 (m), 1368 (m), 1338 (m), 1218 (m), 1162 (m), 1000 (w), 764 (w);

^{1}H NMR (400 MHz, acetone-d_6) δ: 7.81 (d, *J* = 7.0 Hz, Ar*H*, 1H), 7.63–7.54 (m, Ar*H*, 2H), 3.80 (s, CH_2, 2H), 3.67 (s, CO_2CH_3, 3H);

^{13}C NMR (100 MHz, acetone-d_6) δ: 170.6 (*C*O), 152.7 (C_qNO_2), 134.3 (*C*Ar), 133.2 (2x*C*Ar), 130.3 (C_qAr), 113.8 (C_qAr), 53.0 (CH_3), 37.8 (CH_2);

HRMS: calcd for $C_9H_8BrNO_4$ $[M + Na]^+$: 295.9529, found 295.9527.

II.5.1.22 Methyl 2-(2-amino-3-bromophenyl)acetate (II.58)



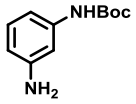
To a **II.56** (100 mg, 0.43 mmol) solution in DMF (2 mL, 0.2 M) placed in a ice bath, was added dropwise MeI (33 μL, 0.52 mmol) and K_2CO_3 (33 μL, 0.52 mmol). The reaction mixture was stirred at room temperature for 2 h. The mixture was evaporated and the crude was purified by flash column chromatography (EtOAc:hexane, 1:5) to give **II.58** (92 mg, 87 %) as a light yellow solid. *Rf* = 0.7, hexane:EtOAc (3:2).

MP: 62-64ºC;

IR (KBr) ν_{max} (cm^{-1}): 3450 (w), 3376 (w), 2851 (w), 1728 (s, *C=O* ester), 1624 (m), 1460 (m), 1250 (m), 1159 (m), 751 (w);

^{1}H NMR (400 MHz, acetone-d_6) δ: 7.35 (d, *J* = 7.7 Hz, Ar*H*, 1H), 7.08 (d, *J* = 7.1 Hz, Ar*H*, 1H), 6.57 (t, *J* = 7.6 Hz, Ar*H*, 1H), 4.87 (bs, NH_2, 2H), 3.65 (s, CH_2, 5H).

II.5.1.23 *tert*-Butyl 3-aminophenylcarbamate (II.64)[122]



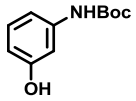
Compound **II.64** was prepared according to the literature and obtained as a white solid (quant). *Rf* = 0.2, EtOAc:hexane (2:3).

MP: 106-109ºC;

IR (KBr) ν_{max} (cm^{-1}): 3487 (m, *N-H*), 3385 (m, *N-H*), 3328 (s, *N-H*), 2981, 1690 (s, *C=O*), 1533 (m), 1247 (m), 1158 (s);

^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.04 (dd, *J* = 8.0 Hz, Ar*H*, 1H), 6.98 (bs, N*H*, 1H), 6.57 (dd, *J* = 8.0, 1.3 Hz, Ar*H*, 1H), 6.43 (s, Ar*H*, 1H), 6.38 (dd, *J* = 7.9, 1.7 Hz, Ar*H*, 1H), 3.66 (bs, NH_2, 2H), 1.51 (s, $C(CH_3)_3$, 9H).

II.5.1.24 *tert*-Butyl 3-hydroxyphenylcarbamate (II.66)[136]



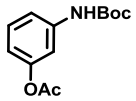
To a 3-aminophenol (400 mg, 3.67 mmol) solution in THF (4 mL) was added Boc_2O (880 mg, 4.03 mmol). The mixture was stirred overnight at room temperature. The mixture was diluted with EtOAc and washed with water and brine. The combined organic fractions were combined and evaporated. The crude product was purified by flash column chromatography using a gradient (EtOAc:hexane, 1:7 to 1:3) to give **II.66** (765 mg, quant.) as a white solid. *Rf* = 0.5, EtOAc:hexane (2:3).

MP: 135º-137ºC;

IR (KBr) ν_{max} (cm^{-1}): 3382 (m), 3314 (m), 1694 (s, *C=O*), 1598 (m), 1525 (m), 1443 (s), 1150 (m);

^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.12 – 7.06 (m, Ar*H*, 2H), 6.75 (dd, *J* = 8.0 Hz, Ar*H*, 1H), 6.67 (s, N*H*, 1H), 6.54 (dd, *J* = 8.1, 1.8 Hz, Ar*H*, 1H), 1.50 (s, $C(CH_3)_3$, 9H).

II.5.1.25 3-(*tert*-Butoxycarbonylamino)phenyl acetate (II.67)



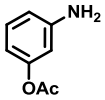
To a *tert*-butyl 3-hydroxyphenylcarbamate (**II.66**) (300 mg, 1.43 mmol) solution in THF (14 mL, 0.1 M) was added Cs_2CO_3 (932 mg, 2.86 mmol). Then, Ac_2O (0.15 mL, 1.58 mmol) was added. The mixture was allowed to stir for 2 h at room temperature. The mixture was quenched with water and washed with water and brine. The combined organic fractions were combined and evaporated. The crude product was purified by flash column chromatography (EtOAc:hexane, 1:5) to give **II.67** (360 mg, quant) as a light brown oil. *Rf* = 0.3, 2x(ether:hexane, 1:4).

IR (KBr) ν_{max} (cm^{-1}): 3349 (m, *N-H*), 2925 (s, *C-H*), 1767 (s, *C=O*), 1729 (s, *C=O*), 1538 (m), 1151 (s);

^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.34 (bs, N*H*, 1H), 7.21 (dd, *J* = 8.1 Hz, Ar*H*, 1H), 7.03 (dd, *J* = 8.1, 1.1 Hz, Ar*H*, 1H), 6.82 (s, Ar*H*, 1H), 6.73 (dd, *J* = 8.0, 1.5 Hz, Ar*H*, 1H), 2.24 (s, $COCH_3$, 3H), 1.49 (s, $C(CH_3)_3$, 9H);

^{13}C NMR (100 MHz, $CDCl_3$) δ: 169.5 (*C*OCH_3), 152.6 (*C*$OC(CH_3)_3$), 151.2 (*C*Ar), 139.7 (*C*Ar), 129.5 (*C*Ar), 115.9 (*C*Ar), 115.7 (C_qAr), 111.9 (C_qAr), 28.3 ($C(CH_3)_3$) , 21.1 ($COCH_3$).

II.5.1.26 3-Aminophenyl acetate (II.68)[137]



A 3-(*tert*-butoxycarbonylamino)phenyl acetate (**II.67**) (170 mg, 0.68 mmol) solution in water (14 mL) was refluxed for 3 h. The mixture was extracted with EtOAc and washed with brine. The combined organic fractions were combined, dried over Na_2SO_4 and evaporated. The crude was purified by flash column chromatography (EtOAc:hexane, 1:4 → 1:2) to give **II.68** (42 mg, 41%) as light orange solid. *Rf* = 0.6, EtOAc:hexane (2:3).

MP: 52-55ºC;

IR (KBr) ν_{max} (cm^{-1}): 3437 (m, *N-H*), 3354 (m, *N-H*), 1734 (s, *C=O*), 1625 (m), 1223 (s), 1143 (m);

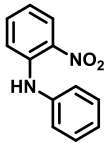
^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.13 (dd, *J* = 8.0 Hz, Ar*H*, 1H), 6.52 (dd, *J* = 8.0, 1.4 Hz, Ar*H*, 1H), 6.46 (dd, *J* = 8.0, 1.4 Hz, Ar*H*, 1H), 6.40 (dd, *J* = 2.1 Hz, Ar*H*, 1H), 3.72 (bs, NH_2, 2H), 2.27 (s, $COCH_3$, 3H);

^{13}C NMR (100 MHz, $CDCl_3$) δ: 169.7 (*C*OCH_3), 151.7 (C_qAr), 147.8 (C_qAr), 130.1 (*C*Ar), 112.7 (*C*Ar), 111.3 (*C*Ar), 108.3 (*C*Ar), 21.2 ($COCH_3$).

General procedure for arylation:

To a screw-cap test-tube equipped with a magnetic stir bar, was added Pd_2dba_3 (5 mol%), BINAP (7.5 mol%) and Cs_2CO_3 (2 equiv). The vial was sealed with a suba-seal, evacuated and backfilled with argon. A solution of 1-bromo 2-nitro benzene (1 equiv) in dry toluene (0.25 M) was then added *via* syringe, and several cycles vacuum/argon were performed. Then was added the aniline (3 equiv) and the suba-seal was replaced by the teflon screw-cap. The reaction mixture was heated at 90 °C for 3 to 6 h. The solution was allowed to cool to room temperature, quenched by the addition of 1 mL HCl (1M) and diluted with EtOAc and water. The mixture was extracted with EtOAc and washed with water and brine. The combined organic layers were dried over Na_2SO_4, filtered and concentrated in vacuo.

II.5.1.27 2-Nitro-*N*-phenylaniline (II.69a)[118a]



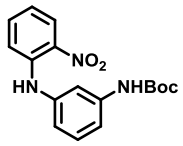
The crude product was purified by flash column chromatography (Et_2O:hexane, 1:6) to give **II.69a** (84 mg, quantitative yield) as red oil. *Rf* = 0.7, Et_2O:hexane (1:4).

IR (KBr) ν_{max} (cm^{-1}): 3355 (m, *N-H*), 3063 (w), 1618 (m), 1595 (m), 1573 (m), 1504 (s, *N=O*), 1351 (m, *N=O*), 1263 (s).

^{1}H NMR (400 MHz, acetone-d_6) δ: 9.44 (bs, N*H*, 1H), 8.17 (dd, *J* = 8.4, 0.9 Hz, Ar*H*, 1H), 7.50 – 7.24 (m, Ar*H*, 7H), 6.86 (t, *J* = 7.4 Hz, Ar*H*, 1H).

^{13}C NMR (100 MHz, acetone-d_6) δ: 143.6 (C_qAr), 140.0 (C_qAr), 136.6 (*C*Ar), 134.4 (C_qAr), 130.4 (*C*Ar), 127.1 (*C*Ar), 126.1 (*C*Ar), 125.0 (*C*Ar), 118.4 (*C*Ar), 117.1 (*C*Ar).

II.5.1.28 *tert*-Butyl (3-((2-nitrophenyl)amino)phenyl)carbamate (II.69b)



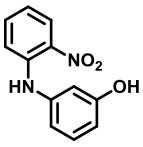
The crude product was purified by flash column chromatography (Et_2O:hexane, 1:7) to give **II.69b** (70 mg, 86%) as yellow solid. *Rf* = 0.6, Et_2O:hexane (1:4).

MP: 171-173ºC;

IR (KBr) ν_{max} (cm^{-1}): 3340 (m, *N-H*), 2974 (w), 1742 (s, *C=O*), 1505 (s), 1256 (s), 1145 (s);

^{1}H NMR (400 MHz, acetone-d_6) δ: 9.44 (s, N*H*, 1H), 8.18 (d, *J* = 8.5 Hz, Ar*H*, 1H), 7.50 (t, *J* = 7.7 Hz, Ar*H*, 2H), 7.38 – 7.20 (m, Ar*H*, 4H), 6.90 (t, *J* = 7.7 Hz, Ar*H*, 1H), 1.47 (s, C(CH_3)$_3$, 9H).

II.5.1.29 3-(2-Nitrophenylamino)phenol (II.69c)[138]



The crude product was purified by flash column chromatography (Et_2O:hexane, 1:4 to 1:1) to give **II.69c** (16 mg, 28%) as red solid. *Rf* = 0.3, Et_2O:hexane (1:4).

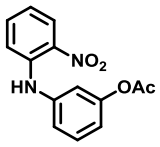
Mp: 137-140ºC (lit. 139-141ºC);

IR (KBr) ν_{max} (cm^{-1}): 3352 (m), 1598 (m), 1573 (m), 1505 (s), 1259 (m), 1155 (m);

^{1}H NMR (400 MHz, acetone-d_6) δ: 9.35 (bs, N*H*, 1H), 8.64 (s, Ar*H*, 1H), 8.15 (dd, *J* = 8.5, 1.2 Hz, Ar*H*, 1H), 7.50 (t, *J* = 7.1 Hz, Ar*H*, 1H), 7.33 – 7.23 (m, Ar*H*, 2H), 6.87 – 6.72 (m, Ar*H*, 4H);

^{13}C NMR (100 MHz, acetone-d_6) δ: 159.4 (C_qAr), 143.6 (C_qAr), 141.2 (C_qAr), 136.6 (*C*Ar), 134.4 (C_qAr), 131.2 (*C*Ar), 127.1 (*C*Ar), 118.5 (*C*Ar), 117.5 (*C*Ar), 115.9 (*C*Ar), 113.3 (*C*Ar), 111.8 (*C*Ar).

II.5.1.30 3-(2-Nitrophenylamino)phenyl acetate (II.69d)



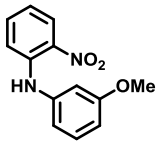
The crude product was purified by flash column chromatography (Et_2O:hexane, 1:7 to 1:1) to give **II.69d** (21 mg, 31%) as red oil. *Rf* = 0.6, 2x(Et_2O:hexane, 1:4).

IR (KBr) ν_{max} (cm^{-1}): 3350 (w, N-H), 2924 (w), 1765 (s, C=O), 1602 (s), 1501 (s), 1205 (s);

^{1}H NMR (400 MHz, acetone-d_6) δ: 9.41 (bs, N*H*, 1H), 8.17 (dd, *J* = 8.5, 1.4 Hz, Ar*H*, 1H), 7.56 – 7.49 (m, Ar*H*, 1H), 7.48 – 7.42 (m, Ar*H*, 1H), 7.34 (dd, *J* = 7.6 Hz, Ar*H*, 1H), 7.27 (dd, *J* = 10.0, 8.8 Hz, Ar*H*, 1H), 7.16 (t, *J* = 2.0 Hz, Ar*H*, 1H), 6.98 (dd, *J* = 8.1, 1.5 Hz, Ar*H*, 1H), 6.95 – 6.89 (m, Ar*H*, 1H), 2.26 (s, COCH_3, 3H);

^{13}C NMR (100 MHz, acetone-d_6) δ: 169.6 (*C*OCH$_3$)), 152.8 (C_qAr), 143.4 (C_qAr), 142.9 (C_qAr), 141.3 (C_qAr), 136.6 (*C*Ar), 131.17, 131.0 (*C*Ar), 129.8 (C_qAr), 129.2 (C_qAr), 127.1 (*C*Ar), 126.5 (*C*Ar), 121.7 (*C*Ar), 119.2 (*C*Ar), 119.1 (*C*Ar), 118.1 (*C*Ar), 117.5 (*C*Ar), 20.9 (CO*C*H$_3$).

II.5.1.31 *N*-(3-methoxyphenyl)-2-nitroaniline (II.70)[118a]



In a two neck flask was added **II.69c** (75 mg, 0.325 mmol), K_2CO_3 (68 mg, 0.487 mmol, 1.5 equiv) and acetone (1.1 mL, 0.3 M). The flask was placed in an ice bath and MeI (28 μL, 0.456 mmol, 1.4 equiv) was added dropwise *via* syringe. The reaction mixture was stirred at room temperature overnight. Then it was evaporated to dryness and dissolved in EtOAc. The organic layer was washed with water and brine, dried over Na_2SO_4, filtered and concentrated in vacuo. The crude product was purified by flash column chromatography (Et_2O:hexane, 1:8) to give **II.70** (65 mg, 82%) as red oil. *Rf* = 0.6, Et_2O:hexane (1:4).

IR (KBr) ν_{max} (cm^{-1}): 3351 (m, *N-H*), 2940 (w), 1617 (m), 1600 (s), 1504 (s), 1259 (s);

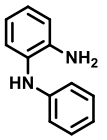
^{1}H NMR (400 MHz, acetone-d_6) δ: 9.39 (s, N*H*, 1H), 8.16 (dd, *J* = 8.1 Hz, Ar*H*, 1H), 7.51 (t, *J* = 7.3 Hz, Ar*H*, 1H), 7.34 (m, Ar*H*, 2H), 6.96 – 6.80 (m, Ar*H*, 4H), 3.82 (s, OCH_3, 3H);

^{13}C NMR (100 MHz, acetone-d_6) δ: 161.8 (C_qAr), 143.3 (C_qAr), 141.3 (C_qAr), 136.6 (*C*Ar), 134.5 (C_qAr), 131.1 (*C*Ar), 127.1 (*C*Ar), 118.7 (*C*Ar), 117.5 (*C*Ar), 116.8 (*C*Ar), 111.8 (*C*Ar), 110.4 (*C*Ar), 55.7 (O*C*H_3).

General procedure for the nitro group reduction:

To compounds **II.69a, II.69b, II.69d** and **II.70** (1 equiv) in EtOAc:ethanol (1:1, 0.2 M), was added 10 mol% Pd/C (1 mol%) and the mixture was allowed to stir at room temperature over an H_2 atmosphere for 2 to 4 h (until total consumption of starting material, monitored by TLC). The mixture was filtered and concentrated *in vacuo*. The reduced compounds were immediately used without further purification.

II.5.1.32 N-phenylbenzene-1,2-diamine [139]



The residue was purified by flash column chromatography (hexane/ethyl acetate, 8:1) to give the compound as a light orange solid (133 mg, 97%). *Rf* = 0.4 EtOAc:hexane (1:6).

Mp: 77-79 ºC;

IR (KBr) ν_{max} (cm^{-1}): 3448 (m), 3387 (m), 3363 (m), 3014 (w), 1615 (s), 1597 (s), 1504 (s), 1316 (m);

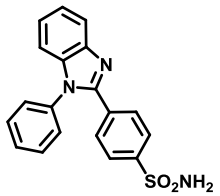
^{1}H NMR (400 MHz, acetone-d_6) δ: 7,17 – 7,08 (m, Ar*H*, 3H), 6,96 – 6,61 (m, Ar*H*, 6H), 6,42 (bs, N*H*, 1H), 4,43 (bs, NH_2, 2H);

^{13}C NMR (100 MHz, acetone-d_6) δ: 147.3 (C_qAr), 144.1 (C_qAr), 129.7 (2x*C*Ar), 128.9 (C_qAr), 125.9 (*C*Ar), 125.5 (*C*Ar), 118.9 (*C*Ar), 118.2 (*C*Ar), 116.3 (*C*Ar), 115.5 (2x*C*Ar).

General procedure for the benzoylation/cyclization:

To a sulfamoylbenzyl chloride (2.5 equiv) suspension in dry THF (0.5 M) placed in an ice bath, was added the corresponding amine solution (1equiv) drop wise. The mixture was stirred for 1 h at room temperature and then heated at 50ºC until total consumption of starting material (benzimidazole products show a distinctive blue spot visible in UV light). The reaction was quenched with water, extracted with EtOAc and washed with *brine*. The combined organic layer was dried over Na_2SO_4 and concentrated *in vacuo*.

II.5.1.33 4-(1-Phenyl-1H-benzo[d]imidazol-2-yl)benzenesulfonamide (II.71a)[133]



The crude was isolated by pTLC (eluted twice with EtOAc:hexane 2:3 and EtOAc:hexane 1:1) to give **II.71a** as a white solid (150 mg, 93%). *Rf* = 0.2, ether:hexane (1:4).

MP: 262-265 ºC;

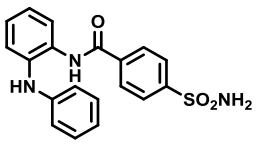
IR (KBr) ν_{max} (cm^{-1}): 3281 (b, m), 3034 (b, m), 2924 (w), 1597 (w), 1500 (m), 1456 (m), 1409 (m), 1336 (s, *S=O*), 1164 (s, *S=O*);

^{1}H NMR (400 MHz, acetone-d_6) δ: 7.87 – 7.55 (m, Ar*H*, 5H), 7.66 – 7.58 (m, Ar*H*, 3H), 7.49 – 7.47 (m, Ar*H*, 2H), 7.35-7.24 (m, Ar*H*, 3H), 6.73 (bs, -SO_2NH_2, 2H);

^{13}C NMR (100 MHz, acetone-d_6) δ: 151.5 (C_qAr), 145.5 (C_qAr), 144.0 (C_qAr), 138.5 (C_qAr), 137.7 (C_qAr), 134.5 (C_qAr), 131.0 (2x*C*Ar), 130.5 (2x*C*Ar), 128.8 (*C*Ar), 128.5 (2x*C*Ar), 126.7 (2xCAr), 124.5 (*C*Ar), 123.8 (*C*Ar), 120.7 (*C*Ar), 111.4 (*C*Ar).

HPLC: RT = 12, 88 min. Purity 98 %.

II.5.1.34 *N*-(2-(Phenylamino)phenyl)-4-sulfamoylbenzamide[140]



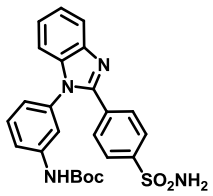
The compound was isolated as a white solid (4 mg, 2 %). *Rf* = 0.3, ether:hexane (1:4).

MP: 197-200 ºC;

IR (KBr) ν_{max} (cm^{-1}): 3436 (b, s), 3363 (s), 2925 (w), 1640 (m), 1599 (m), 1326 (s, *S=O*), 1160 (*S=O*);

^{1}H NMR (400 MHz, acetone-d_6) δ: 9.33 (bs, N*H*, 1H), 8.02 – 7.89 (m, Ar*H*, 5H), 7.37 (dd, *J* = 7.5 Hz, Ar*H*, 1H), 7.21 – 7.07 (m, Ar*H*, 4H), 6.92 – 6.81 (m, Ar*H*, 3H), 6.72 (bs, -SO_2NH_2, 1H).

II.5.1.35 *tert*-Butyl 3-(2-(4-sulfamoylphenyl)-1H-benzo[d]imidazol-1-yl)phenylcarbamate (II.71b)

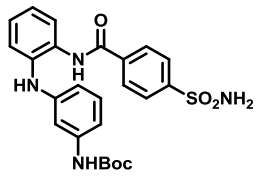


The crude was isolated by pTLC (eluted twice with EtOAc:hexane 1:1) to give **II.71b** as a light brown solid (89 mg, 82 %). *Rf* = 0.3, EtOAc:hexane (3:2).

IR (KBr) ν_{max} (cm^{-1}): 3337 (b, w), 2978 (w), 1725 (m), 1705 (m), 1603 (m), 1335 (m, *S=O*), 1162 (s, *S=O*);

^{1}H NMR (400 MHz, acetone-d_6) δ: 8.78 (bs, N*H*, 1H), 7.87 (d, *J* = 8.7 Hz, Ar*H*, 2H), 7.83 – 7.73 (m, Ar*H*, 5H), 7.49 (t, *J* = 8.3 Hz, Ar*H*, 1H), 7.34 – 7.31 (m, Ar*H*, 3H), 7.04 – 7.00 (m, Ar*H*, 1H), 6.76 (bs, -SO_2NH_2, 2H), 1.46 (s, 9H).

II.5.1.36 *tert*-Butyl 3-(2-(4-sulfamoylbenzamido)phenylamino)phenylcarbamate

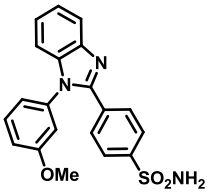


The compound was isolated as a white solid (4 mg, 4 %). *Rf* = 0.4, EtOAc:hexane (3:2).

IR (KBr) ν_{max} (cm^{-1}): 3340 (b, w), 1735 (m), 1336 (m, *S=O*), 1160 (s, *S=O*);

^{1}H NMR (400 MHz, acetone-d_6) δ: 9.34 (s, N*H*, 1H), 8.32 (s, N*H*, 1H), 8.04 (d, *J* = 8.4 Hz, Ar*H*, 2H), 7.97 (d, *J* = 8.7 Hz, Ar*H*, 2H), 7.88 (d, Ar*H*, 1H), 7.39 (dd, *J* = 7.5 Hz, Ar*H*, 1H), 7.24 – 7.17 (m, Ar*H*, 2H), 7.12 – 7.06 (m, Ar*H*, 2H), 6.98 (dd, *J* = 8.8 Hz, Ar*H*, 1H), 6.75 (bs, -SO_2NH_2, 2H), 6.53 (dd, *J* = 6.9 Hz, Ar*H*, 1H), 1.45 (s, 9H).

II.5.1.37 4-(1-(3-Methoxyphenyl)-1H-benzo[d]imidazol-2-yl)benzenesulfonamide (II.71c)



The crude was isolated by pTLC (eluted twice 3 x EtOAc:hexane 1:1) to give **II.71c** as a light pink solid (60 mg, 68 %). *Rf* = 0.3, EtOAc:hexane (3:2).

MP: 261-264ºC;

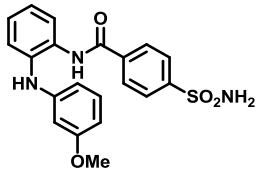
IR (KBr) ν_{max} (cm^{-1}): 3271 (b, m), 3006 (b, m), 1602 (m), 1493 (m), 1336 (s, *S=O*), 1163 (s, *S=O*), 747 (m);

^{1}H NMR (400 MHz, acetone-d_6) δ: 7.87 (d, *J* = 8.7 Hz, Ar*H*, 2H), 7.80 (d, *J* = 8.7 Hz, Ar*H*, 2H), 7.52 (t, *J* = 8.1 Hz, Ar*H*, 1H), 7.33 – 7.29 (m, Ar*H*, 3H), 7.15 (ddd, *J* = 8.4, 2.5, 0.8 Hz, Ar*H*,

1H), 7.09 (t, *J* = 2.2 Hz, Ar*H*, 1H), 7.01 (ddd, *J* = 7.8, 1.9, 0.9 Hz, Ar*H*, 1H), 6.71 (bs, -SO_2NH_2, 2H), 3.83 (s, OCH_3, 3H);

^{13}C NMR (100 MHz, acetone-d_6) δ: 161.8 (C_qAr), 151.5 (C_qAr), 145.8 (C_qAr), 144.0 (C_qAr), 138.7 (C_qAr), 138.5 (C_qAr), 134.4 (C_qAr), 131.7 (*C*Ar), 130.4 (2x*C*Ar), 126.7 (2x*C*Ar), 124.5 (*C*Ar), 123.7 (*C*Ar), 120.6 (*C*Ar), 120.5 (*C*Ar), 115.6 (*C*Ar), 114.0 (*C*Ar), 111.5 (*C*Ar), 56.0 (OCH_3).

II.5.1.38 *N*-(2-(3-Methoxyphenylamino)phenyl)-4-sulfamoylbenzamide



The compound was isolated as a white solid (6 mg, 7 %). *Rf* = 0.4, EtOAc:hexane (3:2).

IR (KBr) ν_{max} (cm^{-1}): 3344 (b, m), 3250 (m), 1646 (m), 1597 (s), 1522 (s), 1456 (m), 1337 (m, *S*=*O*), 1158 (s, *S*=*O*), 756 (w);

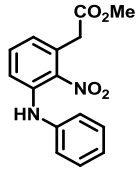
^{1}H NMR (400 MHz, acetone-d_6) δ: 9.35 (bs, N*H*, 1H), 8.03 – 7.87 (m, Ar*H*, 5H), 7.39 (dd, *J* = 7.5 Hz, Ar*H*, 1H), 7.22 – 7.08 (m, Ar*H*, 3H), 6.76 (bs, -SO_2NH_2, 1H), 6.49 – 6.39 (m, Ar*H*, 3H), 3.70 (s, OCH_3, 3H);

^{13}C NMR (100 MHz, acetone-d_6) δ: 165.3 (*C*O), 161.8 (C_qAr), 147.7 (C_qAr), 147.0 (C_qAr), 138.8 (C_qAr), 136.6 (C_qAr), 131.8 (C_qAr), 130.8 (*C*Ar), 128.9 (*C*Ar), 127.0 (*C*Ar), 126.7 (*C*Ar), 125.3 (*C*Ar), 124.0 (*C*Ar), 123.3 (*C*Ar), 109.7 (*C*Ar), 106.1 (*C*Ar), 102.9 (*C*Ar), 55.3 (OCH_3).

General procedure for arylamination:

To a screw-cap test-tube equipped with a magnetic stir bar, was added Pd_2dba_3 (5 mol %), BINAP (7.5 mol %) and Cs_2CO_3 (2 equiv). The tube was sealed with a suba-seal, evacuated and backfilled with argon. A solution of the aryl halide (1 equiv) in dry toluene (0.25 M) was then added *via* syringe, and several cycles vacuum/argon were performed. Then was added the aniline (3 equiv) and the suba-seal was replaced by the teflon screw-cap. The reaction mixture was heated at 90 °C for 4 h. The solution was allowed to cool to room temperature, quenched by the addition of HCl solution (1M) and diluted with EtOAc and water. After extracting with 3 portions of EtOAc, the combined organic layers were washed with water, brine, dried over Na_2SO_4, filtered and concentrated in vacuo.

II.5.1.39 Methyl 2-(2-nitro-3-(phenylamino)phenyl)acetate (II.73a)



The crude product was purified by pTLC (Et_2O:hexane, 1:4) to give **II.73a** (86 mg, 83 %) as red oil; *Rf* = 0.5, EtOAc:hexane (1:4).

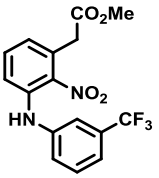
IR (NaCl) ν_{max} (cm^{-1}): 3381 (m, *N-H*), 2952 (w), 1738 (s, *C=O* ester), 1592 (s), 1504 (s), 1280 (s), 1175 (s), 1065 (w), 752 (w);

^{1}H NMR (400 MHz, acetone-d_6) δ: 8.15 (bs, N*H*, 1H), 7.41 – 7.27 (m, Ar*H*, 6H), 7.12 (t, *J* = 7.1 Hz, Ar*H*, 1H), 6.91 (d, *J* = 7.1 Hz, Ar*H*, 1H), 3.93 (s, CH_2, 2H), 3.67 (s, CO_2CH_3, 3H);

^{13}C NMR (100 MHz, acetone-d_6) δ: 171.0 (*C*O), 141.8 (C_qAr), 141.0 (C_qAr), 133.5 (*C*Ar), 131.7 (C_qAr), 130.4 (2x*C*Ar), 124.6 (*C*Ar), 124.2 (*C*Ar), 122.7 (2x*C*Ar), 118.3 (*C*Ar), 52.2 (CO_2CH_3), 39.6 (CH_2);

HRMS: calcd for $C_{15}H_{14}N_2O_4$ $[M + Na]^+$ 309.0846, found 309.0842.

II.5.1.40 Methyl 2-(2-nitro-3-(phenylamino)phenyl)acetate (II.73b)



The crude product was purified by pTLC (Et_2O:hexane, 1:6) to give **II.73b** (80 mg, 95%) as a red solid. *Rf* = 0.5, 2x(Et_2O:hexane, 1:4).

MP: 69-73ºC;

IR (NaCl) ν_{max} (cm^{-1}): 3379 (w, *N-H*), 2957 (w), 1739 (s, *C=O* ester), 1593 (s), 1503 (s), 1330 (s), 1166 (m), 1123 (m), 782 (w);

^{1}H NMR (400 MHz, acetone-d_6) δ: 8,15 (s, N*H*, 1H), 7,55 – 7,33 (m, Ar*H*, 6H), 7,07 (d, *J* = 6.9 Hz, Ar*H*, 1H), 3,92 (s, CH_2, 2H), 3,67 (s, CO_2CH_3, 3H);

^{13}C NMR (100 MHz, acetone-d_6) δ: 170.9 (*C*O), 143.9 (C_qAr), 138.9 (C_qAr), 133.4 (*C*Ar), 131.3 (*C*Ar), 126.1 (*C*Ar), 124.2 (*C*Ar), 120.3 (*C*Ar), 119.7 (*C*Ar), 117.4 (*C*Ar), 52.3 (CO_2CH_3), 39.1 (CH_2);

HRMS: calcd for $C_{16}H_{13}F_3N_2O_4$ $[M + Na]^+$: 377.0720, found 377.0725.

II.5.1.41 Methyl 2-(3-(3-fluorophenylamino)-2-nitrophenyl)acetate (II.73c)

The crude product was purified by silica flash chromatography using a gradient from Et_2O:hexane, 1:6 to 1:5 to give **II.73c** (79 mg, 89%) as red solid. *Rf* = 0.5, 2x(Et_2O:hexane, 1:4).

MP: 88-91ºC;

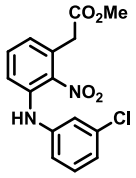
IR (NaCl) ν_{max} (cm^{-1}): 3379 (s, *N-H*), 2949 (w), 1742 (s, *C=O* ester), 1600 (s), 1505 (s), 1357 (m), 1168 (m), 784 (w);

^{1}H NMR (400 MHz, acetone-d_6) δ: 8,03 (1H, bs, N*H*), 7,48 – 7,32 (3H, Ar*H*, m), 7,03 – 6,99 (3H, Ar*H*, m), 6,78 (1H, Ar*H*, m), 3,91 (2H, CH_2, s), 3,66 (3H, CO_2CH_3, s);

^{13}C NMR (100 MHz, acetone-d_6) δ: 170.9 (*C*O), 164.52 (d, *J* = 243.1 Hz, *CF*), 144.8 (C_qAr), 142.0 (C_qAr), 139.2 (C_qAr), 133.4 (C_qAr, *J* = 113.0 Hz), 133.4 (*C*Ar), 131.8 (d, *J* = 9.7 Hz, *C*Ar), 131.3 (C_qAr), 125.8 (*C*Ar), 120.3 (*C*Ar), 116.8 (*C*Ar), 110.0 (d, *J* = 21.4 Hz, *C*Ar), 107.9 (d, *J* = 24.7 Hz, *C*Ar), 52.3 (CO_2CH_3), 39.1 (CH_2);

HRMS: calcd for $C_{15}H_{13}FN_2O_4$ $[M + Na]^+$: 327.0752, found 327.0748.

II.5.1.42 Methyl 2-(3-(3-chlorophenylamino)-2-nitrophenyl)acetate (II.73d)



The crude product was purified by pTLC (CH_2Cl_2:hexane:MeOH = 1:5:0.1) to give **II.73d** (75 mg, 80%) as red oil. *Rf* = 0.5, 2x(EtOAc:hexane, 1:4).

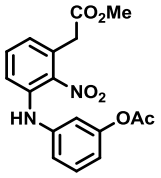
IR (NaCl) ν_{max} (cm^{-1}): 3383 (w, *N-H*), 2949 (w), 1739 (s, *C=O* ester), 1587 (s), 1494 (s), 1352 (s), 1284 (s), 1176 (m), 774 (w);

^{1}H NMR (400 MHz, acetone-d_6) δ: 8,03 (s, N*H*, 1H), 7,48 – 7,02 (m, Ar*H*, 7H), 3,91 (s, CH_2, 2H), 3,66 (s, CO_2CH_3, 3H);

^{13}C NMR (100 MHz, acetone-d_6) δ: 171.0 (*C*O), 144.3 (C_qAr), 141.8 (C_qAr), 139.2 (C_qAr), 135.4 (C_qAr), 133.4 (*C*Ar), 131.7 (*C*Ar), 131.4 (C_qAr), 125.8 (*C*Ar), 123.4 (*C*Ar), 121.0 (*C*Ar), 120.2 (*C*Ar), 119.5 (*C*Ar), 52.3 (CO_2CH_3), 39.2 (CH_2);

HRMS: calcd for $C_{15}H_{13}ClN_2O_4$ $[M + Na]^+$: 343.0456, found 343.0453.

II.5.1.43 Methyl 2-(3-(3-acetoxyphenylamino)-2-nitrophenyl)acetate (II.73e)



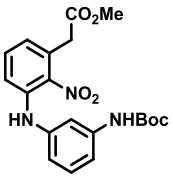
The crude product was purified by pTLC (Et_2O:hexane, 1:3 to 1:2) to give **II.73e** (105 mg, 66% yield) as red oil. *Rf* = 0.5, EtOAc:hexane (2:3).

IR (NaCl) ν_{max} (cm^{-1}): 2954 (w), 1745 (b, s, *C=O* ester), 1538 (s), 1368 (m), 1239 (m), 1173 (m), 849 (w);

^{1}H NMR (400 MHz, acetone-d_6) δ: 8.08 (bs, 1H), 7.45 – 7.33 (m, Ar*H*, 3H), 7.12 (dd, *J* = 7.9, 1.7 Hz, Ar*H*, 1H), 7.02 – 6.99 (m, Ar*H*, 2H), 6.82 (dd, *J* = 8.1, 1.5 Hz, Ar*H*, 1H), 3.91 (s, CH_2, 2H), 3.66 (s, CO_2CH_3, 3H), 2.24 (s, OCH_3, 3H);

^{13}C NMR (100 MHz, acetone-d_6) δ: 171.0 (*C*O), 169.7 (*C*OCH_3), 153.0 (C_qAr), 143.4 (C_qAr), 140.0 (C_qAr), 133.5 (*C*Ar), 131.6 (C_qAr), 131.0 (*C*Ar), 125.2 (*C*Ar), 119.4 (*C*Ar), 119.0 (*C*Ar), 117.4 (*C*Ar), 115.3 (*C*Ar), 52.3 (CO_2CH_3), 39.4 (CH_2), 21.1 ($COCH_3$).

II.5.1.44 Methyl 2-(3-(3-(tert-butoxycarbonylamino)phenylamino)-2-nitrophenyl) acetate (II.73f)



The crude product was purified by silica flash chromatography using a gradient of Et_2O:hexane from 1:4 to 1:1, to give **II.73f** (100 mg, 52% yield) as red solid. *Rf* = 0.5, EtOAc:hexane (2:3).

MP: 43-45ºC;

IR (KBr) ν_{max} (cm^{-1}): 3391 (b, m, *N-H*), 2978 (w), 1728 (b, s, *C=O* ester, carbamate), 1596 (s), 1159 (s), 772 (w);

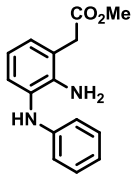
^{1}H NMR (400 MHz, acetone-d_6) δ: 8.45 (bs, N*H*, 1H), 8.12 (bs, N*H*, 1H), 7.55 (s, Ar*H*, 1H), 7.43 – 7.33 (m, Ar*H*, 2H), 7.26 – 7.25 (m, Ar*H*, 2H), 6.93 – 6.88 (m, Ar*H*, 2H), 3.91 (s, CH_2, 2H), 3.66 (s, CO_2CH_3, 3H), 1.47 (s, $C(CH_3)_3$, 9H);

^{13}C NMR (100 MHz, acetone-d_6) δ: 171.1 (*C*O), 153.7 (*C*$OC(CH_3)_3$), 142.3 (C_qAr), 141.9 (C_qAr), 140.9 (C_qAr), 133.5 (*C*Ar), 131.7 (C_qAr), 130.6 (*C*Ar), 124.3 (*C*Ar), 118.9 (*C*Ar), 116.4 (*C*Ar), 114.5 (*C*Ar), 112.2 (*C*Ar), 80.2 (*C*$(CH_3)_3$), 52.2 (CO_2CH_3), 39.6 (CH_2), 28.6 ($C(CH_3)_3$).

General procedure for the nitro group reduction:

To compounds **II.73a-f** (1 equiv) in toluene (0.2 M), was added 10% Pd/C (1 mol%) and the mixture was allowed to stir at room temperature over an H_2 atmosphere overnight (until total consumption of starting material, monitored by TLC). The mixture was filtered and concentrated *in vacuo*. The reduced compounds were immediately used without further purification.

II.5.1.45 Methyl 2-(2-amino-3-bromophenyl)acetate (II.73a)



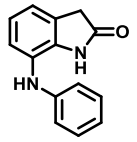
The residue was purified by silica flash chromatography (eluted with hexane to EtOAc:hexane 1:2) to give **II.73a** as yellow oil (20 mg, 74%). *Rf* = 0.3, EtOAc:hexane (1:4).

IR (KBr) ν_{max} (cm^{-1}): 3436 (b, w, *N-H*), 3363 (m, *N-H*), 3038 (w), 1727 (s, *C=O* ester), 1591 (s), 1496 (s), 1310 (m), 1243 (m), 1154 (m), 750 (m);

^{1}H NMR (400 MHz, acetone-d_6) δ: 7.13 – 6.92 (m, Ar*H*, 4H), 6.74 – 6.49 (m, Ar*H*, N*H*, 5H), 4.47 (bs, NH_2, 2H), 3.65 – 3.63 (m, CH_2, CO_2CH_3, 5H);

^{13}C NMR (100 MHz, acetone-d_6) δ: 172.6 (CO), 147.5 (C_qAr), 143.1 (C_qAr), 130.0 (C_qAr), 129.8 (2x*C*Ar), 128.2 (*C*Ar), 125.1 (*C*Ar), 121.1 (C_qAr), 119.1 (*C*Ar), 118.3 (*C*Ar), 115.7 (2x*C*Ar), 52.2 (CO_2CH_3), 38.4 (CH_2).

II.5.1.46 7-(phenylamino)indolin-2-one (II.75a)[141]



The compound was isolated (as side product) as a white solid (4 mg, 13 %). *Rf* = 0.3, 2x (EtOAc:hexane, 2:3).

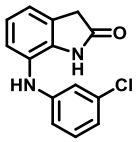
MP: 158-161ºC;

IR (KBr) ν_{max} (cm^{-1}): 3395 (w, *N-H*), 3054 (w), 1698 (s, *C=O*), 1593 (w), 1498 (w), 1330 (w), 751 (m);

^{1}H NMR (400 MHz, acetone-d_6) δ: 9,09 (bs, N*H*, 1H), 7,21-7,12 (m, Ar*H*, 3H), 6,96-6,77 (m, Ar*H*, 5H), 3,50 (s, CH_2, 2H);

^{13}C NMR (100 MHz, acetone-d_6) δ: 176.3 (*C*O), 145.6 (C_qAr), 137.2 (C_qAr), 130.0 (2x*C*Ar), 127.8 (C_qAr), 126.4 (C_qAr), 122.8 (*C*Ar), 121.2 (*C*Ar), 120.1 (*C*Ar), 119.8 (*C*Ar), 116.4 (2x*C*Ar), 36.8 (CH_2).

II.5.1.47 7-(3-Chlorophenylamino)indolin-2-one (II.75d)



The compound was isolated (as side product) as a white solid. *Rf* = 0.5, 2x(EtOAc:hexane, 2:3).

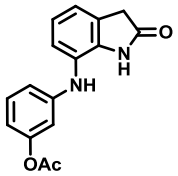
MP: 147-151 ºC;

IR (KBr) ν_{max} (cm^{-1}): 3344 (m), 1690 (s, *C=O*), 1593 (s), 1331 (m), 758 (m);

^{1}H NMR (400 MHz, acetone-d_6) δ: 9.27 (bs, N*H*, 1H), 7.20 – 6.71 (m, Ar*H*, 7H), 3.50 – 3.49 (s, 2H);

^{13}C NMR (100 MHz, acetone-d_6) δ: 176.4 (CO), 147.8 (*C*Ar), 135.1 (*C*Ar), 131.3 (*C*Ar), 129.9 (*C*Ar), 123.3 (*C*Ar), 122.8 (*C*Ar), 121.2 (*C*Ar), 120.0 (*C*Ar), 119.0 (*C*Ar), 116.3 (*C*Ar), 114.9 (*C*Ar), 113.8 (*C*Ar), 36.8 (*C*H$_2$).

II.5.1.48 3-(2-oxoindolin-7-ylamino)phenyl acetate (II.75e)



The compound was isolated (as side product) as a white solid. *Rf* = 0.4, EtOAc:hexane, (3:2).

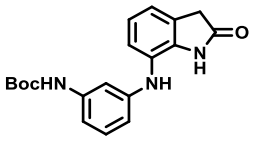
MP: 62-65ºC;

IR (KBr) ν_{max} (cm^{-1}): 3350 (b, m, *N-H*), 2924 (w), 1764 (m), 1698 (s, *C=O*), 1598 (m), 1491 (m), 1204 (s), 759 (w);

^{1}H NMR (400 MHz, acetone-d_6) δ: 9.26 (bs, N*H*, 1H), 7.19 – 7.12 (m, Ar*H*, 2H), 7.02 – 6.93 (m, Ar*H*, 2H), 6.70 (dd, *J* = 8.2, 1.2 Hz, Ar*H*, 1H), 6.57 (m, Ar*H*, 1H), 6.50 (dd, *J* = 7.8, 1.7 Hz, Ar*H*, 1H), 3.50 (s, C*H*$_2$, 2H), 2.20 (s, COC*H*$_3$, 3H);

^{13}C NMR (100 MHz, acetone-d_6) δ: 176.5 (*C*O), 169.7 (*C*OCH$_3$), 153.1 (C_qAr), 147.4 (C_qAr), 138.2 (C_qAr), 130.6 (*C*Ar), 128.0 (C_qAr), 125.7 (C_qAr), 122.9 (*C*Ar), 122.5 (*C*Ar), 120.7 (*C*Ar), 113.2 (*C*Ar), 112.9 (*C*Ar), 109.2 (*C*Ar), 36.9 (*C*H$_2$), 21.1 (COC*H*$_3$).

II.5.1.49 *tert*-Butyl 3-(2-oxoindolin-7-ylamino)phenylcarbamate (II.75f)



The compound was isolated (as side product) as a light brown solid. *Rf* = 0.5, EtOAc:hexane, (3:2).

IR (KBr) ν_{max} (cm^{-1}): 3344 (b, m, *N-H*), 2976 (w), 1698 (s, *C=O*), 1598 (m), 1160 (s), 767 (w);

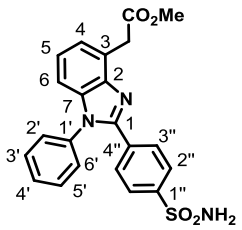
^{1}H NMR (400 MHz, acetone-d_6) δ: 9.11 (bs, N*H*, 1H), 8.28 (bs, N*H*, 1H), 7.21 – 7.14 (m, Ar*H*, 2H), 7.07 (t, *J* = 8.0 Hz, Ar*H*, 1H), 6.95 – 6.91 (m, Ar*H*, 3H), 6.81 (bs, N*H*, 1H), 6.51 (d, *J* = 7.9 Hz, Ar*H*, 1H), 3.49 (s, CH_2, 2H), 1.46 (s, C(CH_2)$_3$, 9H);

^{13}C NMR (100 MHz, acetone-d_6) δ: 176.4 (*C*O), 153.7 (*C*OC(CH$_3$)$_3$), 146.2 (C_qAr), 141.6 (C_qAr), 130.2 (*C*Ar), 127.8 (C_qAr), 126.5 (C_qAr), 122.8 (*C*Ar), 121.4 (C_qAr), 119.8 (*C*Ar), 110.3 (*C*Ar), 106.5 (*C*Ar), 79.8 (*C*(*C*H$_3$)$_3$), 36.9 (*C*H$_2$), 28.6 (C(*C*H$_3$)$_3$).

General procedure for the benzoylation/cyclization:

To a sulfamoylbenzyl chloride (2.5 equiv) suspension in dry THF (0.5 M) placed in an ice bath, was added the corresponding **II.73a-e** (1 equiv) solution in THF (0.5M), dropwise. The mixture was stirred for 1h at 0 ºC and then at 50 ºC overnight. The reaction was quenched with water (15 mL), extracted with EtOAc and washed with *brine*. The combined organic layer was dried over Na_2SO_4 and concentrated *in vacuo* to obtain the below mentioned products **II.77a-e**.

II.5.1.50 Methyl 2-(1-phenyl-2-(4-sulfamoylphenyl)-1H-benzo[d]imidazol-4-yl)acetate (II.77a)



The residue was purified by pTLC [EtOAc:hexane (2:3)] to give **II.77a** as a light pink solid (51 mg, 89%). *Rf* = 0.2, 2x(EtOAc:hexane 2:3).

MP: 199-200ºC;

IR (KBr) ν_{max} (cm^{-1}): 3383 (w, *N-H*), 3270 (w, *N-H*), 1724 (s, *C=O* ester), 1500 (w), 1329 (s, *S-O*), 1170 (s, *S-O*), 767 (w);

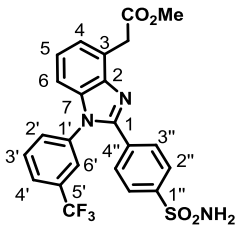
^{1}H NMR (400 MHz, acetone-d_6) δ: 7.87 (d, *J* = 8.0 Hz, $H_{2''}$, 2H), 7.76 (d, *J* = 8.0 Hz, $H_{3''}$, 2H), 7.64 – 7.61 (m, $H_{3'}$, $H_{4'}$, 3H), 7.52 – 7.49 (m, $H_{2'}$, 2H), 7.29 – 7.27 (m, H_4, H_5, 2H), 7.16 (dd, H_6, 1H), 6.69 (bs, NH_2, 2H), 4.18 (s, CH_2, 2H), 3.70 (s, CO_2CH_3, 3H);

^{13}C NMR (100 MHz, acetone-d_6) δ: 172.3 (COCH$_3$), 151.3 ($C_{1''}$), 145.3 (C_1), 143.2 (C_2), 138.4 ($C_{1'}$), 137.8 (C_7), 134.4 ($C_{4''}$), 131.1 ($C_{3'}$), 130.7 ($C_{3''}$), 130.0 ($C_{4'}$), 128.6 ($C_{2'}$), 127.3 (C_3), 126.9 ($C_{2''}$), 124.6 (C_5), 124.5 (C_4), 110.3 (C_6), 52.1 (CO_2CH_3), 36.2 (CH_2);

HRMS *m/z* calcd for $C_{22}H_{20}N_3O_4S$ $[M+H]^+$: 422.1169, found 422.1162;

HPLC Purity: RT = 12,8 min., Purity 98%.

II.5.1.51 Methyl-2-(2-(4-sulfamoylphenyl)-1-(3-(trifluoromethyl)phenyl)-1H-benzo[d]imidazol-4-yl) acetate (II.77b)



The residue was purified by pTLC (2x EtOAc:hexane 1:2) and recrystallized from ethanol to give **II.77b** as a white solid (65 mg, 62 %); *Rf* = 0.2, EtOAc:hexane (1:1).

MP: 259-261ºC;

IR (KBr) ν_{max} (cm^{-1}): 3310 (m, *N-H*), 1716 (s, *C=O* ester), 1345 (s), 1317 (m), 1172 (s), 1130 (m), 845 (w);

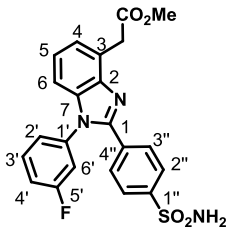
^{1}H NMR (400 MHz, acetone-d_6) δ: 7.98 (s, $H_{6'}$,1H), 7.95 (dd, *J* = 7.9 Hz, $H_{4'}$,1H), 7.89 – 7.87 (m, $H_{3'}$, $H_{2''}$, 3H), 7.82 (dd, *J* = 8.0 Hz, $H_{2'}$, 1H), 7.77 (d, *J* = 8.5 Hz, $H_{3''}$, 2H), 7.31 – 7.30 (m, H_4, H_6, 2H), 7.20 – 7.18 (m, H_5, 1H), 6.72 (bs, NH_2, 2H), 4.17 (s, CH_2, 2H), 3.70 (s, CO_2CH_3, 3H);

^{13}C NMR (100 MHz, acetone-d_6) δ: 172.2 (CO_2CH$_3$), 151.3 ($C_{1''}$), 145.8 (C_1), 143.2 (C_2), 138.5 ($C_{1'}$), 138.0 (C_7), 134.2 ($C_{4'}$), 132.7 ($C_{2'}$), 132.6 (q, *J* = 32.9 Hz, $C_{5'}$), 132.2 ($C_{3'}$), 130.8 ($C_{3''}$), 127.4 (C_3), 126.9 ($C_{2''}$), 126.6 (d, *J* = 3.8 Hz, $C_{4'}$), 125.5 (d, *J* = 3.9 Hz, $C_{6'}$), 124.8 (C_5) 124.7 (C_4), 124.6 (q, *J* = 270.7 Hz, CF$_3$), 110.1 (C_6), 52.1 (CO_2CH_3), 36.1 (CH_2);

HRMS: *m/z* calcd for $C_{23}H_{19}F_3N_3O_4S$ $[M+Na^+]^+$: 490.1043, found 490.1051;

HPLC Purity: 97% (RT = 15,3 min.).

II.5.1.52 Methyl 2-(1-(3-fluorophenyl)-2-(4-sulfamoylphenyl)-1H-benzo[d]imidazol-4-yl)acetate (II.77c)



The residue was purified by pTLC [EtOAc:hexane (2:3)] to give **II.77c** as a white solid (36 mg, 59%); *Rf* = 0.4, hexane:EtOAc (2:3).

MP: 179-181ºC;

IR (KBr) ν_{max} (cm^{-1}): 3368 (b, m, *N-H*), 1732 (s, *C=O* ester), 1596 (m), 1492 (m), 1338 (s, *S=O*), 1166 (s, *S=O*);

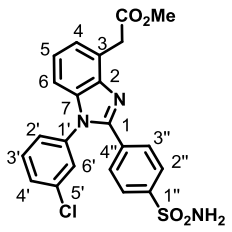
^{1}H NMR (400 MHz, acetone-d_6) δ: 7.89 (d, *J* = 8.6 Hz, $H_{2''}$, 2H), 7.78 (d, *J* = 8.6 Hz, $H_{3''}$, 2H), 7.67 (dd, *J* = 14.5, 8.1 Hz, $H_{3'}$, 1H), 7.43 – 7.29 (m, $H_{2'}$, $H_{4'}$, $H_{6'}$, 3H), 7.30 – 7.29 (m, H_4, H_5, 2H), 7.22 – 7.20 (m, H_6, 1H), 6.72 (bs, NH_2, 2H), 4.17 (s, CH_2, 2H), 3.70 (s, CO_2CH_3, 3H);

^{13}C NMR (100 MHz, acetone-d_6) δ: 172.2 (CO_2CH_3), 164.0 (d, *J* = 247.3 Hz, $C_{5'}$), 151.2 ($C_{1''}$), 145.8 (C_1), 143.1 (C_2), 139.2 (d, *J* = 10.1 Hz, $C_{1'}$), 138.1 (C_7), 134.3 ($C_{4''}$), 132.7 (d, *J* = 9.3 Hz $C_{3'}$), 130.7 ($C_{3''}$), 127.4 (C_3), 126.9 ($C_{2''}$), 124.8 (d, *J* = 3.3 Hz, $C_{2'}$), 124.8 (C_5), 124.7 (C_4), 116.91 (d, *J* = 21.1 Hz, $C_{6'}$), 116.01 (d, *J* = 23.7 Hz, $C_{4'}$), 110.2 (C_6), 52.1 (CO_2CH_3), 36.1 (CH_2);

HPLC Purity: 96% (RT =12,9 min.);

HRMS: *m/z* Calculated for $C_{22}H_{18}N_3O_4FS$ $[M+Na]^+$: 439.1002, found: 439.1004.

II.5.1.53 Methyl 2-(1-(3-chlorophenyl)-2-(4-sulfamoylphenyl)-1H-benzo[d]imidazol-4-yl)acetate (II.77d)



The residue was purified by pTLC [2x EtOAc:hexane (2:3)] to give **II.77d** as a white solid (85 mg, 84%). *Rf* = 0.2, hexane:EtOAc (3:2).

MP: 197-199ºC;

IR (KBr) ν_{max} (cm^{-1}): 3368 (w, N-H), 2949 (w), 1734 (s, *C=O* ester), 1590 (w), 1339 (s, *S-O*), 1165 (s, *S-O*), 757 (w);

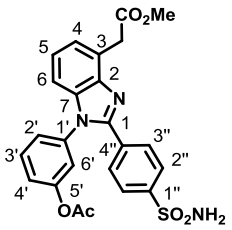
^{1}H NMR (400 MHz, acetone-d_6) δ: 7.90 (d, *J* = 8.7 Hz, $H_{2''}$, 2H), 7.80 (d, *J* = 8.7 Hz, $H_{3''}$, 2H), 7.66 – 7.64 (m, $H_{2'}$, $H_{3'}$, $H_{6'}$, 3H), 7.49 – 7.47 (m, $H_{4'}$, 1H), 7.30 – 7.28 (m, H_4, H_5, 2H), 7.20 – 7.18 (m, H_6, 1H), 6.73 (bs, NH_2, 2H), 4.17 (s, CH_2, 2H), 3.69 (s, CO_2CH_3, 3H);

^{13}C NMR (100 MHz, acetone-d_6) δ: 172.2 (CO_2CH_3), 151.2 ($C_{1''}$), 145.8 (C_1), 143.2 (C_2), 139.1 ($C_{1'}$), 138.1 (C_7), 135.8 ($C_{5'}$), 134.3 ($C_{4''}$), 132.4 ($C_{3'}$), 130.7 ($C_{3''}$), 130.1 ($C_{6'}$), 128.6 ($C_{2'}$), 127.4 ($C_{4'}$, C_3), 126.9 ($C_{2''}$), 124.8 (C_5), 124.7 (C_4), 110.2 (C_6), 52.0 (CO_2CH_3), 36.1 (CH_2);

HPLC Purity: 96% (RT =14,5 min.);

HRMS: *m/z* Calculated for $C_{22}H_{18}N_3O_4ClS$ $[M+Na]^+$: 455.0707, found: 455.0717.

II.5.1.54 Methyl 2-(1-(3-acetoxyphenyl)-2-(4-sulfamoylphenyl)-1H-benzo[d]imidazol-4-yl)acetate (II.77e)



The residue was purified by pTLC [(2x EtOAc:hexane (2:3)] to give **II.77e** as a white solid (95 mg, 73%). *Rf* = 0.25, hexane:EtOAc (3:2).

MP: 167-169ºC;

IR (KBr) ν_{max} (cm^{-1}): 3317 (m, *N-H*), 2956 (w), 1768 (s, *C=O* acetyl), 1718 (s, C=O ester), 1596 (m), 1342 (s, *S-O*), 1165 (s, *S-O*), 758 (w);

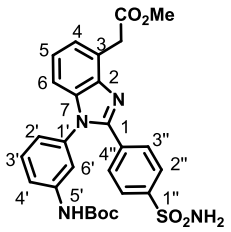
^{1}H NMR (400 MHz, acetone-d_6) δ: 7.88 (d, *J* = 8.4 Hz, $H_{2''}$, 2H), 7.80 (d, *J* = 8.5 Hz, $H_{3''}$, 1H), 7.64 (t, *J* = 8.1 Hz, $H_{3'}$, 1H), 7.41 – 7.33 (m, $H_{2'}$, $H_{4'}$, $H_{6'}$, 3H), 7.30 – 7.27 (m, H_4, H_5, 2H), 7.20 – 7.18 (m, H_6, 1H), 6.72 (bs, NH_2, 1H), 4.17 (s, CH_2, 2H), 3.69 (s, CO_2CH_3, 3H), 2.28 (s, $COCH_3$, 3H);

^{13}C NMR (151 MHz, acetone-d_6) δ: 172.2 (CO_2CH_3), 169.5 ($COCH_3$), 152.8 ($C_{5'}$), 151.1 ($C_{1''}$), 145.6 (C_1), 143.1 (C_2), 138.4 ($C_{1'}$), 138.2 (C_7), 134.3 ($C_{4''}$), 131.7 ($C_{3'}$), 130.6 ($C_{3''}$), 127.3 (C_3), 126.8 ($C_{2''}$), 125.8 (C_5), 124.7 (C_4), 124.6 ($C_{4'}$), 123.4 ($C_{2'}$), 122.4 ($C_{6'}$), 110.2 (C_6), 52.0 (CO_2CH_3), 36.1 (CH_2), 20.9 ($COCH_3$);

HRMS: *m/z* Calculated for $C_{24}H_{21}N_3O_6S$ $[M+Na]^+$: 479.1151, found: 479.1154.

HPLC Purity: 94%, (RT = 11,1 min.).

II.5.1.55 Methyl 2-(1-(3-(tert-butoxycarbonylamino)phenyl)-2-(4-sulfamoylphenyl)-1H-benzo[d]imida- zol-4-yl)acetate (II.77f)



The residue was purified by pTLC [2x hexane:EtOAc (3:2)] to give **II.77f** as a light pink solid (44 mg, 85%); *Rf* = 0.3, hexane:EtOAc (3:2).

MP: 116-118º C;

IR (KBr) ν_{max} (cm^{-1}): 3400 (b), 2926 (w), 1725 (s, *C=O* ester), 1602 (m), 1340 (m), 1162 (s, *S-O*);

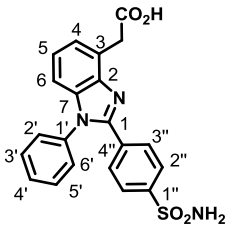
^{1}H NMR (400 MHz, acetone-d_6) δ: 8.76 (s, N*H*, 1H), 7.88 (d, *J* = 8.6 Hz, $H_{2''}$, 2H), 7.83 (d, *J* = 8.6 Hz, $H_{3''}$, 2H), 7.78 – 7.73 (m, $H_{2'}$, $H_{6'}$, 2H), 7.50 (t, *J* = 8.1 Hz, $H_{3'}$, 1H), 7.29 – 7.28 (m, H_4, H_5, 2H), 7.23 – 7.21 (m, H_6, 1H), 7.05 (dd, *J* = 7.8, 1.1 Hz, $H_{4'}$, 1H), 6.69 (bs, NH_2, 2H), 4.17 (s, CH_2, 2H), 3.69 (s, CO_2CH_3, 3H), 1.47 (s, $C(CH_3)_3$, 9H);

^{13}C NMR (151 MHz, acetone-d_6) δ: 172.2 (CO_2CH_3), 153.6, 151.1 ($C_{1''}$), 145.6 (C_1), 143.2 (C_2), 142.4 ($C_{5'}$), 138.3 ($C_{1'}$), 138.1(C_7), 134.6 ($C_{4''}$), 131.3 ($C_{3'}$), 130.6 ($C_{3''}$), 127.3 (C_3), 126.9 ($C_{2''}$), 124.6 (C_5), 124.5 (C_4), 122.0 ($C_{4'}$), 119.2 ($C_{6'}$), 117.7 ($C_{2'}$), 110.4 (C_6), 80.6 ($C(CH_3)_3$), 52.0 (CO_2CH_3), 36.1 (CH_2), 28.5 ($C(CH_3)_3$).

General procedure for hydrolysis:

To the **II.77a-e** (1 equiv) solution in a dioxane:THF:water mixture (1:1:1, 0.5 M), was added $LiOH.H_2O$ (5 equiv). The mixture was stirred at room temperature until total conversion verified by TLC (about 1h). The mixture was diluted with EtOAc and washed with water and *brine*. The combined organic layer was dried over Na_2SO_4 and concentrated *in vacuo* to give the below described products **II.78a-e** that were not further purified.

II.5.1.56 2-(1-phenyl-2-(4-sulfamoylphenyl)-1H-benzo[d]imidazol-4-yl)acetic acid (II.78a)



The compound was obtained as a light pink solid (quant.), *Rf* = 0.1, EtOAc: hexane (3:2).

MP: 275-278ºC;

IR (KBr) ν_{max} (cm^{-1}): 3418 (b, s), 3331 (s), 1668 (s), 1338 (m), 1160 (s);

^{1}H NMR (400 MHz, acetone-d_6) δ: 7.87 (d, *J* = 8.3 Hz, $H_{2''}$, 2H), 7.75 (d, *J* = 8.3 Hz, $H_{3''}$, 2H), 7.63 – 7.48 (m, $H_{1'}$, $H_{2'}$, $H_{3'}$, 5H), 7.28 – 7.12 (m, H_4, H_5, H_6, 3H), 7.03 (bs, NH_2, 2H), 4.11 (s, CH_2, 2H);

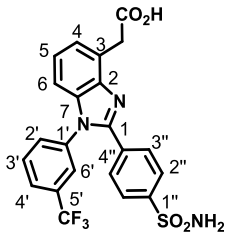
^{1}H NMR (600 MHz, D_2O) δ: 7.99 (d, *J* = 8.3 Hz, $H_{2''}$, 2H), 7.86 (d, *J* = 8.4 Hz, $H_{3''}$, 2H), 7.70 – 7.79 (m, $H_{3'}$, $H_{4'}$, $H_{5'}$, 3H), 7.53 – 7.35 (m, $H_{1'}$, $H_{6'}$, H_4, H_5, H_6, 5H), 4.06 (s, CH_2, 2H).

^{13}C NMR (100 MHz, acetone-d_6) δ: 173.1 (*C*O_2H), 151.1 ($C_{1''}$), 145.9 (C_1), 143.1 (C_2), 138.2 ($C_{1'}$), 137.7 (C_7), 134.2 ($C_{4''}$), 130.9 ($C_{3'}$), 130.4 ($C_{3''}$), 129.8 ($C_{4'}$), 128.4 ($C_{2'}$), 127.8 (C_3), 126.7 ($C_{2''}$), 124.4 (C_4, C_5), 109.9 (C_6), 36.5 (*C*H_2);

HPLC Purity: 97% (RT = 9,60 min.).

HRMS: *m/z* Calculated for $C_{21}H_{17}N_3O_4S$ $[M+Na]^+$: 407.0940, found: 407.0922.

II.5.1.57 2-(1-phenyl-2-(4-sulfamoylphenyl)-1H-benzo[d]imidazol-4-yl)acetic acid (II.78b)



The compound was obtained as a white solid (quant.). *Rf* = 0.1, EtOAc: hexane (3:2).

MP: 265-268ºC;

IV (KBr) ν_{max} (cm^{-1}): 3416 (b, m), 3343 (m), 3078 (m), 1671 (m), 1458 (m), 1338 (s), 1162 (s), 1128 (s), 756 (w);

^{1}H NMR (400 MHz, acetone-d_6) δ: 7.99 (s, $H_{6'}$,1H), 7.95 – 7.85 (m, $H_{2''}$, $H_{3'}$, $H_{4'}$, 4H), 7.82 – 7.75 (m, $H_{3''}$, $H_{2'}$, 3H), 7.33 – 7.28 (m, H_4, H_5, 2H), 7.17 (dd, *J* = 7.3, 1.8 Hz, H_6, 1H), 7.11 (bs, NH_2, 2H), 4.11 (s, CH_2, 2H);

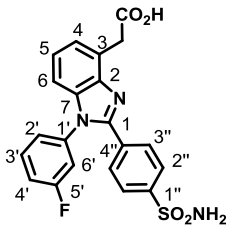
^{1}H NMR (600 MHz, D_2O) δ: 8.08 – 7.95 (m, $H_{2''}$, $H_{6'}$, 3H), 7.92 – 7.81 (m, $H_{3''}$, $H_{3'}$, $H_{4'}$, $H_{2'}$, 5H), 7.50 – 7.35 (m, H_4, H_5, H_6, 3H), 4.06 (s, CH_2, 2H);

^{13}C NMR (100 MHz, acetone-d_6) δ: 173.1 (*C*O_2H), 151.2 ($C_{1''}$), 146.1 (C_1), 143.1 (C_2), 138.4 ($C_{1'}$), 137.9 (C_7), 133.9 ($C_{4'}$), 132.7 ($C_{2'}$), 132.5 (q, *J* = 32.8 Hz, $C_{5'}$), 132.2 ($C_{3'}$), 130.6 ($C_{3''}$), 128.0 (C_3), 126.8 ($C_{2''}$), 126.5 (d, *J* = 3.8 Hz, $C_{4'}$), 125.4 (d, *J* = 3.8 Hz, $C_{6'}$), 124.8 (C_5), 124.7 (C_4), 109.8 (C_6), 36.4 (*C*H_2);

HPLC Purity: 98 % (RT = 13,25 min.).

HRMS: *m/z* Calculated for $C_{22}H_{17}N_3F_3O_4S$ $[M+Na]^+$: 476.0888, found: 476.0886.

II.5.1.58 2-(1-(3-fluorophenyl)-2-(4-sulfamoylphenyl)-1H-benzo[d]imidazol-4-yl)acetic acid (II.78c)



The compound was obtained as a white solid (quant.). *Rf* = 0.1, EtOAc: hexane (3:2).

MP: 256-258ºC;

IV (KBr) ν_{max} (cm^{-1}): 3420 (b, m), 3337 (m), 3084 (m), 1676 (m), 1594 (m), 1341 (m), 1162 (s), 754 (w);

^{1}H RMN (400 MHz, acetone-d_6) δ: 7.90 (d, *J* = 8.5 Hz, $H_{2''}$, 2H), 7.79 (d, *J* = 8.5 Hz, $H_{3''}$, 2H), 7.68 (dd, *J* = 14.5, 8.1 Hz, $H_{3'}$, 1H), 7.45 – 7.29 (m, $H_{2'}$, $H_{4'}$, $H_{6'}$, H_4, H_5, 5H), 7.22 (dd, *J* = 7.3, 1.8 Hz, H_6, 1H), 6.70 (bs, NH_2, 2H), 4.16 (s, CH_2, 2H);

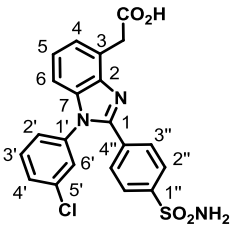
^{1}H NMR (600 MHz, D_2O) δ: 8.01 (d, *J* = 8.4 Hz, $H_{2''}$, 2H), 7.87 (d, *J* = 8.4 Hz, $H_{3''}$, 2H), 7.71 (dd, *J* = 15.0, 8.4 Hz, $H_{3'}$, 1H), 7.52 – 7.37 (m, $H_{2'}$, $H_{4'}$, $H_{6'}$, H_4, H_5, H_6, 6H), 4.06 (s, CH_2, 2H);

^{13}C RMN (100 MHz, acetone-d_6) δ: 172.4 (*C*O_2H), 164.0 (d, *J* = 247.3 Hz, $C_{5'}$), 151.1 ($C_{1''}$), 145.8 (C_1), 143.0 (C_2), 139.2 (d, *J* = 10.2 Hz $C_{1'}$), 138.0 (C_7), 134.2 ($C_{4''}$), 132.6 (d, *J* = 9.4 Hz, $C_{3'}$), 130.7 ($C_{3''}$), 127.5 (C_3), 126.9 ($C_{2''}$), 124.8 (d, *J* = 3.2 Hz, $C_{2'}$), 116.90 (d, *J* = 21.1 Hz, $C_{6'}$), 116.0 (d, *J* = 23.7 Hz, $C_{4'}$), 110.2 (C_6), 36.5 (*C*H_2).

HPLC Purity: 96% (RT = 9,95 min.).

HRMS: *m/z* Calculated for $C_{21}H_{16}N_3O_4FS$ $[M+Na]^+$: 425.0846, found: 425.0843.

II.5.1.59 2-(1-(3-chlorophenyl)-2-(4-sulfamoylphenyl)-1H-benzo[d]imidazol-4-yl)acetic acid (II.78d)



The compound was obtained as a white solid (quant). *Rf* = 0.1, EtOAc: hexane (3:2).

MP: 269-270 ºC;

IV (KBr) ν_{max} (cm^{-1}): 3338 (m), 3068 (m), 1671 (m), 1591 (m), 1338 (m), 1160 (s);

^{1}H RMN (400 MHz, acetone-d_6) δ: 7.90 (d, *J* = 8.4 Hz, $H_{2''}$, 2H), 7.80 (d, *J* = 8.4 Hz, $H_{3''}$, 2H), 7.68 – 7.63 (m, $H_{2'}$, $H_{3'}$, $H_{6'}$, 3H), 7.51 – 7.48 (m, $H_{4'}$, 1H), 7.34 – 7.28 (m, H_4, H_5, 2H), 7.20 (dd, *J* = 7.3, 1.5 Hz, H_6, 1H), 6.71 (bs, NH_2, 2H), 4.16 (s, CH_2, 2H);

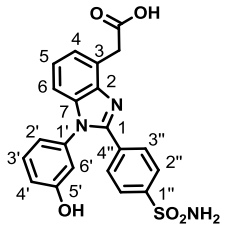
^{1}H NMR (600 MHz, D_2O) δ: 8.01 (d, *J* = 5.9 Hz, $H_{2''}$, 2H), 7.87 (d, *J* = 8.3 Hz, $H_{3''}$, 2H), 7.73 – 7.67 (m, $H_{2'}$, $H_{3'}$, 2H), 7.62 (s, $H_{6'}$, 1H), 7.51 – 7.49 (m, $H_{4'}$, H_5, 2H), 7.44 (d, *J* = 7.2 Hz, H_4, 1H), 7.37 (d, *J* = 8.2 Hz, H_6, 1H), 4.06 (s, CH_2, 2H);

^{13}C RMN (100 MHz, acetone-d_6) δ: 172.5 (*C*O_2H), 151.1 ($C_{1''}$), 145.8 (C_1), 143.0 (C_2), 139.0 ($C_{1'}$), 138.0 (C_7), 135.8 ($C_{5'}$), 134.2 ($C_{4''}$), 132.4 ($C_{3'}$), 130.7 ($C_{3''}$), 130.1 ($C_{6'}$), 128.6 ($C_{2'}$), 127.6 ($C_{4'}$), 127.4 (C_3), 126.9 ($C_{2''}$), 124.8 (C_5), 124.8 (C_4), 110.1 (C_6), 36.5 (*C*H_2);

HPLC Purity: 92% (RT = 12,11 min.).

HRMS: *m/z* Calculated for $C_{21}H_{16}N_3O_4ClS$ $[M+H]^+$: 441.0550, found: 441.0564.

II.5.1.60 2-(1-(3-hydroxyphenyl)-2-(4-sulfamoylphenyl)-1H-benzo[d]imidazol-4-yl)acetic acid (II.78e)



The compound was obtained as a white solid (8 mg, quant.); *Rf* = 0, EtOAc: hexane (3:2).

MP: 245-247 ºC;

IR (KBr) ν_{max} (cm^{-1}): 3368 (b), 1596 (m), 1338 (s), 1161 (s), 757 (w);

^{1}H NMR (400 MHz, acetone-d_6) δ: 7.89 (d, *J* = 8.5 Hz, $H_{2''}$, 2H), 7.82 (d, *J* = 8.5 Hz, $H_{3''}$, 2H), 7.44 (t, *J* = 8.3 Hz, $H_{3'}$, 1H), 7.30 – 7.29 (m, H_4, H_5, 2H), 7.20 (dd, *J* = 6.8, 2.3 Hz, H_6, 1H), 7.06 (dd, *J* = 7.9 Hz, $H_{2'}$, 1H), 6.96 – 6.94 (m, $H_{4'}$, $H_{6'}$, 2H), 6.71 (bs, NH_2, 1H), 4.16 (s, CH_2, 2H);

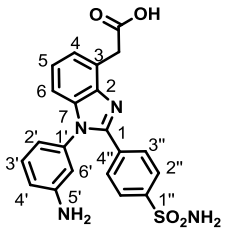
^{1}H NMR (600 MHz, D_2O) δ: 7.98 (d, *J* = 8.2 Hz, $H_{2''}$, 2H), 7.88 (d, *J* = 8.3 Hz, $H_{3''}$, 2H), 7.45 – 7.36 (m, $H_{3'}$, H_4, H_5, H_6, 4H), 6.93 (d, *J* = 9.4 Hz, $H_{2'}$, 1H), 6.78 (s, $H_{6'}$, 1H), 6.73 (d, *J* = 8.2 Hz, $H_{4'}$, 1H), 4.04 (s, CH_2, 2H);

^{13}C NMR (100 MHz, acetone-d_6) δ: 172.4 (*C*O_2H), 159.7 ($C_{5'}$), 151.0 ($C_{1''}$), 145.6 (C_1), 142.9 (C_2), 138.6 ($C_{1'}$), 138.2 (C_7), 134.4 ($C_{4''}$), 131.8 ($C_{3'}$), 130.5 ($C_{3''}$), 127.3 (C_3), 126.8 ($C_{2''}$), 124.6 (C_5), 124.5 (C_4), 119.3 ($C_{4'}$), 117.1 ($C_{2'}$), 115.3 ($C_{6'}$), 110.4 (C_6), 36.7 (*C*H_2);

HPLC Purity: 95% (RT = 7,5 min.).

HRMS: *m/z* Calculated for $C_{21}H_{17}N_3O_5S$ $[M+H^+]^+$: 423.0889, found: 423.0887.

II.5.1.61 2-(1-(3-aminophenyl)-2-(4-sulfamoylphenyl)-1H-benzo[d]imidazol-4-yl)acetic acid (II.78f)



The compound was obtained as a white solid (quant.). *Rf* = 0, EtOAc: hexane (3:2).

MP: 168-171ºC;

IR (KBr) ν_{max} (cm^{-1}): 3141 (b, m), 1557 (m), 1384 (m), 1063 (m), 758 (w);

^{1}H NMR (400 MHz, D_2O) δ: 8.01 (d, *J* = 8.4 Hz, $H_{2''}$, 2H), 7.81 (d, *J* = 8.5 Hz, $H_{3''}$, 2H), 7.75 (t, *J* = 8.1 Hz, $H_{3'}$, 1H), 7.64 – 7.60 (m, H_5, H_4, $H_{2'}$, $H_{4'}$, 4H), 7.53 – 7.51 (m, H_6, $H_{6'}$, 2H), 4.25 (s, CH_2, 2H);

^{13}C NMR (100 MHz, D_2O) δ: 174.8 (CO_2H), 148.6 ($C_{1''}$), 145.1 (C_1), 134.0 ($C_{5'}$), 133.6 ($C_{1'}$), 132.3 ($C_{3'}$), 131.4 ($C_{3''}$), 131.0 (C_2), 128.8 (C_4), 127.5 (C_5), 127.2 ($C_{2'}$), 126.7 ($C_{2''}$), 126.2 ($C_{5'}$), 125.0 ($C_{4'}$), 121.9 (C_7), 121.5 ($C_{6'}$), 112.1 (C_6), 36.3 (CH_2).

HPLC Purity: < 90% (RT = 8 min.).

II.5.2 Evaluation of the inhibitory activity of NSAIDs

Cremophor-EL®, gentamicinin sulfate, calcium ionophore, acetylsalicylic acid and lipopolysaccharides from *E. Coli* 026:B6 were obtained from Sigma Aldrich. PGE_2 ELISA Kit was purchased in Enzo Life Sciences (all components for the PGE_2 ELISA are stable at 4°C until the expiration date; the conjugate and standard must be stored frozen at −20°C. The components have to be prepared as described by the company's manual and must be brought to room temperature for at least 30 min prior to opening). TXBSI was kindly provided by Prof. Dr. Artur Silva (Universidade de Aveiro).

The protocol used in this thesis for whole blood assay was adopted from Laufer and Luik.[97]

II.5.2.1 Samples and Solutions Preparation

II.5.2.1.1 COX-1 Whole Blood Assay

1. Calcium ionophore: 2.5 mg Calcium ionophore is diluted in 1 mL ethanol and stored in single-use aliquots at −20°C.

2. DPBS Buffer: 4.0 g NaCl, 0.1 g KCl, 0.1 g KH_2PO_4, 0.575 g Na_2HPO_4, and 0.5 g glucose are diluted in water (500 ml) and stored at 4°C for a maximum of 7 days.

3. Cremophor-EL/ethanol: 116.9 g Cremophor-EL is mixed with 50.0 g ethanol 99% (250 mL) and stored limitless under cover of darkness.

4. Thromboxane Synthetase Inhibitor (TXBSI) stock solution: 1 mM TXBSI (previously synthesized) in Cremophor-EL/ethanol and stored in single-use aliquots at −20°C.

5. Thromboxane Synthetase Inhibitor (TXBSI) working solution: 81μL DPBS buffer is added to an eppendorf and 9 μL of the TXBSI stock solution is added.

6. Test compounds are dissolved preferentially in 10 mM in Cremophor-EL/ethanol. As compounds sometimes are difficult to be dissolved, stock solutions can be prepared in DMSO that does not interfere with the methodology. Working solutions must be freshly prepared by diluting the stock solution accordingly. Diluted standards should be used within 60 min. of preparation.

7. Dilution of the test compounds, e.g.:

1000 μM	→ 90 μL DPBS buffer + 10 μL of 10 mM Stock test compound solution
100 μM	→ 90 μL DPBS buffer + 10 μL of 1000 μM Test compound
10 μM	→ 90 μL DPBS buffer + 10 μL of 100 μM Test compound
1 μM	→ 90 μL DPBS-buffer + 10 μL of 10 μM Test compound

Test compounds are further diluted when added to blood to give final working concentrations of 10–0.01 μM.

8. Accordant amount of heparinized blood of different donors.

II.5.2.1.2 COX-2 Whole Blood Assay

1. DPBS Buffer: (*see* COX-1).

2. Cremophor-EL/ethanol: (*see* COX-1).

3. Thromboxane Synthetase Inhibitor (TXBSI) stock solution: (*see* COX-1).

6. DPBS–Gentamicin buffer: 12.5 mg Gentamicin is diluted in 50 mL DPBS buffer.

5. Thromboxane Synthetase Inhibitor (TXBSI) working solution: (*see* COX-1).

6. Acetylsalicylic Acid Solution: 2.0 mg Acetylsalicylic acid is diluted in 10.0 mL DPBS–Gentamicin. Working concentration is defined as 10 mg/mL.

7. LPS-Solution: 1.0 mg LPS is diluted in 5 mL DPBS–Gentamicin. Working concentration is defined as 10 mg/mL.

8. Samples: Initial drug concentration range from 10 mM to 10 pM and is prepared by diluting the stock solution in DPBS–Gentamicin. Starting from 10 mM, 1:1000-fold dilution is required to obtain 10 μM.

9. Accordant amount of heparinized blood of different donors.

II.5.2.1.3 PGE2-ELISA

PGE_2 was measured using a commercial ELISA kit from Enzo Life Sciences. Below is a list of the kit's components with a description.

1. This assay is based on the use of a 96-wells plate coated with goat anti-Mouse IgG antibody. The plates are made from break-apart strips coated with goat antibody specific to mouse IgG.

2. Alkaline Phosphatase PGE2 Conjugate: A blue solution of alkaline phosphatase conjugated with PGE_2. Ready to use.

3. PGE_2, EIA antibody: A yellow solution of a monoclonal antibody to PGE2. Ready to use.

4. Assay Buffer Concentrate: Tris buffered saline containing proteins and sodium azide as preservative. The Assay buffer is prepared by diluting 10 mL of the concentrate with 90 mL of bidistillated water.

5. Wash Buffer Concentrate: tris buffer saline containing detergents. The wash buffer is prepared by diluting 5 mL of the supplied concentrate with 95 mL of bidistillated water. This is stored at room temperature until the kit expiration date, or for 3 months, whichever is earlier.

6. Prostaglandin E2 standard: A solution of 50.000 pg/mL PGE2.

7. pNpp Substrate: a solution of *p*-nitrophenyl phosphate in buffer. Ready to use.

8. Stop Solution: a solution of trisodium phosphate in water. Keep tightly capped.

9. PGE_2 Assay Layout Sheet and Plate Sealers.

II.5.2.2 Methods

II.5.2.2.1 COX-1 *Whole Blood Assay*

1. Before starting the assay, the centrifuge was cooled to 4°C and the water bath brought to 37°C;

2. The eppendorf tubes were labeled from 1 to 6 (or 8), and placed in the water bath at 37°C;

3. Six to eight samples can be incubated at the same time. The tip was always washed out three times in the blood suspension. The tip was changed after each step.

4. 500 µL of the heparinized blood of donor was placed into each tube. The remaining blood was stored on the titramax at a low rpm to avoid accumulation of the blood cells.

5. Addition of 5 µL of the TXBSI into each tube. Keep a 15 s rhythm.

6. Addition of 5 µL of the sample dilutions into the corresponding tube. Keep a 15 s rhythm.

7. The blood was carefully mixed with the 1 mL pippet. Start counting exact 15 min. of incubation from the first tube.

8. After 15 min incubation time, 2.5 µL of the calcium ionophor dilution was added to all tubes for starting the reaction. Keep the 15 s rhythm.

9. The blood was carefully mixed with the 1 mL pippet. Start counting exact 15 min. of incubation from the first tube.

10. The tubes were taken out of the water bath and stored on iced water for 5 min. to stop the reaction.

11. The tubes were centrifuged at 1000 × *g* and 4°C for 20 min.

12. The plasma was taken out and put into a fresh tube and stored at −20°C.

13. If the PGE_2-ELISA of the compounds cannot be tested within 1 day, the samples were stored at −10°C.

14. Repeat steps 4 to 6 for the remaining tubes.

15. In the tubes for basal level and for the maximal stimulation data was added 2.5 μL of the DPBS buffer.

16. The blood was carefully mixed with the 1 mL pippet. Start counting exact 15 min. of incubation from the first tube.

17. After 15 min. incubation time, 2.5 μL of the calcium ionophor dilution was added to all tubes for starting the reaction (except the tube for the basal level). Keep the 15 s rhythm.

18. Mix the blood with the 1mL pippet. Start counting exact 15 min. of incubation from the first tube.

19. Handle remaining blood in the exact same manner.

Table II.18. Brief overview of the individual pipetting steps for COX-1 determination.

Basal level	500 μL Blood 5 μL TXBSI 5 μL DPBS-buffer 2.5 μL DPBS-buffer
Maximal stimulation (Control)	500 μL Blood 5 μL TXBSI 5 μL DPBS-buffer 2.5 μL Calciumionophor-dilution
Samples	500 μL Blood 5 μL TXBSI 5 μL Accordant inhibitor-dilution 2.5 μL Calciumionophor-dilution

II.5.2.2.2 COX-1 PGE_2-ELISA

1. The number of wells to be used was previously determined. Usually were used three to four individual strips corresponding to 24 or 32 wells (Figure II.35). The remaining wells (strips) were stored at 4°C. (Note: all the twelve strips can be individually separated from the microplate)

2. The Assay Buffer solution was prepared mixing 1 mL of Assay Buffer concentrate in 9 mL of deionized water. The solution was kept in a dark flask.

2. Preparation of PGE_2 standards:

450 μL of Assay buffer solution was added to the first tube (S1) and 250 μL in the remaining seven (S2-S3). Then, 50 μL of PGE_2 stock solution was placed in S1 and the tube was stirred in the vortex. 250 μL of S1 solution were taken to S2 and the tube was stirred. The same procedure was made until S8.

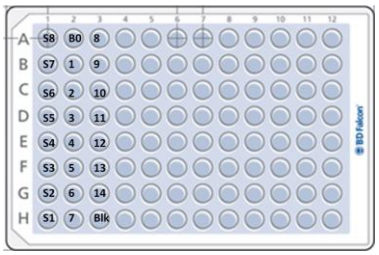
3. Preparation of the samples:

190 μL Assay Buffer was mixed with 10 μL of the corresponding samples. Remaining sample volume was stored at −20°C.

4. 100 μL of PGE_2 standards S1 through S8 were added into the appropriate wells (Figure II.35).

5. 100 μL of Assay Buffer solution were added into the B_0 (0 pg/mL Standard) well (Figure II.35).

6. 100 μL of the samples were added into the corresponding wells (Figure II.35).

a)  b) 

5000 pg/mL (S8)	Bo	8
2500 pg/mL (S7)	1	9
1250 pg/mL (S6)	2	10
625 pg/mL (S5)	3	11
312.5 pg/mL (S4)	4	12
156.25 pg/mL (S3)	5	Basa1 level
78.125 pg/mL (S2)	6	Control
39.1 pg/mL (S1)	7	Blank (B_0)

Figure II.35. a) 96-Well microplate (with 8 rows x 12 strips) used for ELISA test and b) schematic representation of the adopted strategy.

7. Using the multipipet, 50 μL of conjugate was added into each well, except the Blank well.

8. In the same way, 50 μL of the antibody solution were added into each well, except the Blank well. Every well should be green in colour except the Blank well that is empty at this point.

9. The plate was incubated at room temperature on a plate shaker for 120 min at ~500 rpm. The plate was covered with the plate sealer and aluminum foil.

10. The contents of the wells were discarded and the wells were washed by adding 200 μL of wash solution to every well. After each wash, the wells were emptied and the plate tapped on a lint free paper towel. The washing procedure was repeated five more times for a total of six washes.

11. 200 μL of the pNpp substrate solution were added to every well. The plate was incubated in the dark at room temperature for 45 min, without shaking.

12. 50 μL of Stop solution was added to every well, to stop the reaction. The plate should was read immediately.

13. Blank the plate reader against the Blank wells and read the optical density at 405 nm, preferably with correction between 570 and 590 nm. If the plate reader is not able to be blanked against the Blank wells, manually subtract the mean optical density of the Blank wells from all readings.

II.5.2.2.3 COX-1 *Calculation of PGE_2 Concentration*

1. The average of the PGE_2 concentration of stimulation data must be calculated first (**B**).

2. The average of PGE_2 concentration of basal data (**Blk**) has to be subtracted from the average of each sample (including the values for maximal stimulation – $\mathbf{B_0}$).

3. To generate the percentage inhibition, use the following formula

Inhibition [%] = 100 – [Csample($\mathbf{B_{corr}}$)/Cstim($\mathbf{B_{0corr}}$)] x 100,

where C = PGE_2 concentration.

4. Using GraphPad Prism software, plot percent inhibition *versus* concentration of PGE_2 for the standards. Approximate a straight line through the points. The concentration of PGE_2 in the unknowns can be determined by interpolation.

II.5.2.2.4 COX-2 *Whole Blood Assay*

1. 800 μL/well of heparinized blood was placed into a six well plate, and mixed with 10 μL TXBSI per well.
2. 50 μL of acetylsalicylic acid was added at time zero to exclude any contribution of COX-1 to metabolite formation.
3. 100 μL of the sample dilutions were added into the corresponding wells.
4. 100 μL of the DPBS were added into the wells corresponding to maximal stimulation and basal data.
5. The blood was carefully mixed with the 1 mL pippet and incubated in a humidified incubator (37°C, 5% CO_2) for 15 min.
6. 50 μL of LPS was added to sample and maximum stimulation wells to induce COX-2 expression.
7. 50 μL of DPBS–Gentamicin buffer was added into the well for basal data (instead of LPS).

Table II.19. Brief overview of the individual pipetting steps for COX-2 determination.

Basal level	800 µL Blood 10 µL TXBSI 50 µL Acetylsalicylic acid 100 µL DPBS-Gentamicin-buffer
Control (Maximal stimulation)	800 µL Blood 10 µL TXBSI 50 µL Acetylsalicylic acid 100 µL DPBS-Gentamicin-buffer 50 µL LPS-Dilution
Samples	800 µL Blood 10 µL TXBSI 50 µL Acetylsalicylic acid 100 µL Accordant inhibitor dilution 50 µL LPS-Dilution

8. Blood samples were carefully mixed with the 1mL pippet. Samples were incubated in a humidified incubator (37°C, 5% CO_2) for 5 h.

9. Handle remaining blood in the exact same manner.

10. The reaction was stopped by adding iced DPBS buffer (1 mL) into the aliquots and further cooling on ice for 10 min.

11. The blood mixture was placed in Falkon tubes that were centrifuged (1000 × *g*, 4°C, 15 min). Plasma was removed and stored at −20°C.

12. PGE_2 concentration in thawed plasma supernatants is determined by correlate enzyme immunoassay as an indicator of COX-2 activity according to the manufacturers' instructions.

II.5.2.2.5 COX-2 *PGE2-ELISA*

Same procedure used for COX-1.

II.5.2.2.6 COX-2 *Calculation of PGE2 Concentration*

Same procedure used for COX-1.

II.5.3 STD-NMR studies

II.5.3.1 Materials

COX-2 isolated from sheep placenta was purchased from Cayman Chemical (Item number 60120). The protein was supplied in 80 mM Tris-HCl, pH 8.0, 0.1% Tween 20, and 300 μM diethyldithiocarbamate and was used as such.

COX-2 human was purchased from Cayman Chemical (Item number 60120). The protein was supplied in 80 mM Tris-HCl, pH 8.0, 0.1% Tween 20, and 300 μM diethyldithiocarbamate and 10% glycerol and was used as such.

Ibuprofen and diclofenac were purchased from Sigma and Merck, respectively and were used as such. Naproxen was purchased from Sigma.

All STD-NMR experiments were acquired at 37 °C in a Bruker Avance III spectrometer operating at 600 MHz, with a 5 mm triple resonance cryogenic probe head. The STD-NMR spectra were acquired with 1024 transients in a matrix with 32K data points in *t*2 in a spectral window of 12019.23 Hz centered at 2814.60 Hz. Excitation sculpting with gradients was employed to suppress the water proton signals. A spin lock filter ($T1\rho$) with a 2 kHz field and a length of 20 ms was applied to suppress protein background. Selective saturation of protein resonances (on resonance spectrum) was performed by irradiating at −300 Hz using a series of 40 Eburp2.1000 shaped 90° pulses (50 ms, 1 ms delay between pulses) for a total saturation time of 2.0 s. For the reference spectrum (off resonance) the samples were irradiated at 20 000 Hz. Proper control experiments were performed with the reference samples in order to optimize the frequency for protein saturation (−0.5 ppm) and off resonance irradiation, to ensure that the ligand signals were not affected.

II.5.3.2 Sample Preparation

For the STD-NMR experiments, the synthesized compounds, ibuprofen, diclofenac, and naproxen stock solutions (4 mM) were prepared in DMSO-d_6.

From these, an exact amount to attain the desired concentration was added to the COX solution directly in the NMR tube. Then 80 mM Tris-HCl buffer at pH 8.0 was used to adjust the volume to 200 µL. For the binding experiments, the ratio of inhibitors/protein was kept to 100:1.

1. Tris-HCl buffer 80 mM preparation: 80 µL of Tris-HCl 1M buffer solution in 920 µL D_2O.

2. 4 mM ligand stock solutions were prepared dissolving the corresponding amount ligand in 500 µL of DMSO-d_6.

3. Example for one sample for the STD-NMR ligand-based screening experiment: 15 µL of compound stock solution (final concentration: 0.3 mM) and 6 µL COX solution (final

concentration: 3 μM) were added to 180 μL of buffer solution, mixed and transferred to a 3 mm NMR tube. The excess concentration of ligand to protein was 100.

II.5.3.3 STD-NMR Processing

The spectra processing was made according to the procedure described by Viegas *et al.*[101]

II.6 Bibliography

(1) L. Chen, G. Yang, T. Grosser. "Prostanoids and inflammatory pain." *Prostag. Other Lipid Mediat.* **2013**, *104-105*, 58-66.

(2) G. A. FitzGerald, C. Patrono. "The Coxibs, Selective Inhibitors of Cyclooxygenase-2." *N. Engl. J. Med.* **2001**, *345*, 433-442.

(3) T. Miyamoto, N. Ogino, S. Yamamoto, O. Hayaishi. "Purification of prostaglandin endoperoxide synthetase from bovine vesicular gland microsomes." *J. Biol. Chem.* **1976**, *251*, 2629-2636.

(4) a) J. L. Masferrer, K. Seibert, B. Zweifel, P. Needleman. "Endogenous glucocorticoids regulate an inducible cyclooxygenase enzyme." *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 3917-3921; b) M. K. O'Banion, H. B. Sadowski, V. Winn, D. A. Young. "A serum- and glucocorticoid-regulated 4-kilobase mRNA encodes a cyclooxygenase-related protein." *J. Biol. Chem.* **1991**, *266*, 23261-23267.

(5) a) J. R. Vane, Y. S. Bakhle, R. M. Botting. "Cyclooxygenases 1 and 2." *Annu. Rev. Pharmacol. Toxicol.* **1998**, *38*, 97-120; b) M. G. Perrone, A. Scilimati, L. Simone, P. Vitale. "Selective COX-1 inhibition: A therapeutic target to be reconsidered." *Curr. Med. Chem.* **2010**, *17*, 3769-3805.

(6) R. M. Garavito, M. G. Malkowski, D. L. DeWitt. "The structures of prostaglandin endoperoxide H synthases-1 and -2." *Prostag. Other Lipid Mediat.* **2002**, *68–69*, 129-152.

(7) a) D. Picot, P. J. Loll, R. M. Garavito. "The X-ray crystal structure of the membrane protein prostaglandin H2 synthase-1." *Nature* **1994**, *367*, 243-249; b) R. G. Kurumbail, A. M. Stevens, J. K. Gierse, J. J. McDonald, R. A. Stegeman, J. Y. Pak, D. Gildehaus, J. M. Miyashiro, T. D. Penning, K. Seibert, P. C. Isakson, W. C. Stallings. "Structural basis for selective inhibition of cyclooxygenase-2 by anti-inflammatory agents." *Nature* **1996**, *384*, 644-648.

(8) a) A. L. Blobaum, L. J. Marnett. "Structural and functional basis of cyclooxygenase inhibition." *J. Med. Chem.* **2007**, *50*, 1425-1441; b) L. J. Marnett, S. W. Rowlinson, D. C. Goodwin, A. S. Kalgutkar, C. A. Lanzo. "Arachidonic acid oxygenation by COX-1 and COX-2. Mechanisms of catalysis and inhibition." *J. Biol. Chem.* **1999**, *274*, 22903-22906.

(9) W. L. Smith, D. L. DeWitt, R. M. Garavito. "Cyclooxygenases: structural, cellular, and molecular biology." *Annu. Rev. Biochem.* **2000**, *69*, 145-182.

(10) a) C. Yuan, R. S. Sidhu, D. V. Kuklev, Y. Kado, M. Wada, I. Song, W. L. Smith. "Cyclooxygenase Allosterism, Fatty Acid-mediated Cross-talk between Monomers of Cyclooxygenase Homodimers." *J. Biol. Chem.* **2009**, *284*, 10046-10055; b) N. P. Sharma, L. Dong, C. Yuan, K. R. Noon, W. L. Smith. "Asymmetric acetylation of the cyclooxygenase-2 homodimer by aspirin and its effects on the oxygenation of arachidonic, eicosapentaenoic, and docosahexaenoic acids." *Mol. Pharmacol.* **2010**, *77*, 979-986; c) G. Rimon, R. S. Sidhu, D. A.

Lauver, J. Y. Lee, N. P. Sharma, C. Yuan, R. A. Frieler, R. C. Trievel, B. R. Lucchesi, W. L. Smith. "Coxibs interfere with the action of aspirin by binding tightly to one monomer of cyclooxygenase-1." *Proc. Natl. Acad. Sci. U.S.A.* **2010**, *107*, 28-33; d) L. Dong, A. J. Vecchio, N. P. Sharma, B. J. Jurban, M. G. Malkowski, W. L. Smith. "Human cyclooxygenase-2 is a sequence homodimer that functions as a conformational heterodimer." *J. Biol. Chem.* **2011**, *286*, 19035-19046; e) H. Zou, C. Yuan, L. Dong, R. S. Sidhu, Y. H. Hong, D. V. Kuklev, W. L. Smith. "Human cyclooxygenase-1 activity and its responses to COX inhibitors are allosterically regulated by nonsubstrate fatty acids." *J. Lipid Res.* **2012**, *53*, 1336-1347; f) L. Dong, N. P. Sharma, B. J. Jurban, W. L. Smith. "Pre-existent asymmetry in the human cyclooxygenase-2 sequence homodimer." *J. Biol. Chem.* **2013**, *288*, 28641-28655.

(11) a) J. R. Vane. "Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs." *Nature New Biol.* **1971**, *231*, 232-235; b) J. B. Smith, A. L. Willis. "Aspirin selectively inhibits prostaglandin production in human platelets." *Nature New Biol.* **1971**, *231*, 235-237; c) S. H. Ferreira, S. Moncada, J. R. Vane. "Indomethacin and aspirin abolish prostaglandin release from the spleen." *Nature New Biol.* **1971**, *231*, 237-239.

(12) G. G. Graham, M. J. Davies, R. O. Day, A. Mohamudally, K. F. Scott. "The modern pharmacology of paracetamol: therapeutic actions, mechanism of action, metabolism, toxicity and recent pharmacological findings." *Inflammopharmacology* **2013**, *21*, 201-232.

(13) K. D. Rainsford. "Ibuprofen: from invention to an OTC therapeutic mainstay." *Int. J. Clin. Pract.* **2013**, *67*, 9-20.

(14) F. D. Hart, P. L. Boardman. "Indomethacin: A New Non-Steroid Anti-Inflammatory Agent." *Br. Med. J.* **1963**, *2*, 965-970.

(15) A. R. Sallmann. "The history of diclofenac." *Am. J. Med.* **1986**, *80*, 29-33.

(16) K. C. Duggan, M. J. Walters, J. Musee, J. M. Harp, J. R. Kiefer, J. A. Oates, L. J. Marnett. "Molecular basis for cyclooxygenase inhibition by the non-steroidal anti-inflammatory drug naproxen." *J. Biol. Chem.* **2010**, *285*, 34950-34959.

(17) K. D. Rainsford. "Current status of the therapeutic uses and actions of the preferential cyclo-oxygenase-2 NSAID, nimesulide." *Inflammopharmacology* **2006**, *14*, 120-137.

(18) H. E. Vonkeman, M. A. van de Laar. "Nonsteroidal anti-inflammatory drugs: adverse effects and their prevention." *Semin. Arthritis Rheum.* **2010**, *39*, 294-312.

(19) K. R. Gans, W. Galbraith, R. J. Roman, S. B. Haber, J. S. Kerr, W. K. Schmidt, C. Smith, W. E. Hewes, N. R. Ackerman. "Anti-inflammatory and safety profile of DuP 697, a novel orally effective prostaglandin synthesis inhibitor." *J. Pharmacol. Exp. Ther.* **1990**, *254*, 180-187.

(20) N. Futaki, S. Takahashi, M. Yokoyama, I. Arai, S. Higuchi, S. Otomo. "NS-398, a new anti-inflammatory agent, selectively inhibits prostaglandin G/H synthase/cyclooxygenase (COX-2) activity in vitro." *Prostaglandins* **1994**, *47*, 55-59.

(21) T. D. Penning, J. J. Talley, S. R. Bertenshaw, J. S. Carter, P. W. Collins, S. Docter, M. J. Graneto, L. F. Lee, J. W. Malecha, J. M. Miyashiro, R. S. Rogers, D. J. Rogier, S. S. Yu, AndersonGd, E. G. Burton, J. N. Cogburn, S. A. Gregory, C. M. Koboldt, W. E. Perkins, K. Seibert, A. W. Veenhuizen, Y. Y. Zhang, P. C. Isakson. "Synthesis and biological evaluation of the 1,5-diarylpyrazole class of cyclooxygenase-2 inhibitors: identification of 4-[5-(4-methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl]benze nesulfonamide (SC-58635, celecoxib)." *J. Med. Chem.* **1997**, *40*, 1347-1365.

(22) J. L. Hillson, D. E. Furst. "Rofecoxib." *Expert. Opin. Pharmacother.* **2000**, *1*, 1053-1066.

(23) J. J. Talley, D. L. Brown, J. S. Carter, M. J. Graneto, C. M. Koboldt, J. L. Masferrer, W. E. Perkins, R. S. Rogers, A. F. Shaffer, Y. Y. Zhang, B. S. Zweifel, K. Seibert. "4-[5-Methyl-3-phenylisoxazol-4-yl]- benzenesulfonamide, valdecoxib: a potent and selective inhibitor of COX-2." *J. Med. Chem.* **2000**, *43*, 775-777.

(24) a) D. Riendeau, M. D. Percival, C. Brideau, S. Charleson, D. Dubé, D. Ethier, J.-P. Falgueyret, R. W. Friesen, R. Gordon, G. Greig, J. Guay, J. Mancini, M. Ouellet, E. Wong, L. Xu, S. Boyce, D. Visco, Y. Girard, P. Prasit, R. Zamboni, I. W. Rodger, M. Gresser, A. W. Ford-Hutchinson, R. N. Young, C.-C. Chan. "Etoricoxib (MK-0663): Preclinical Profile and Comparison with Other Agents That Selectively Inhibit Cyclooxygenase-2." *J. Pharmacol. Exp. Ther.* **2001**, *296*, 558-566; b) D. J. Cochrane, B. Jarvis, G. M. Keating. "Etoricoxib." *Drugs* **2002**, *62*, 2637-2651; discussion 2652-2633.

(25) a) A. Buvanendran, R. Barkin. "Lumiracoxib." *Drugs Today* **2007**, *43*, 137-147; b) C. M. Rordorf, L. Choi, P. Marshall, J. B. Mangold. "Clinical Pharmacology of Lumiracoxib: A Selective Cyclo-Oxygenase-2 Inhibitor." *Clinical Pharmacokinetics* **2005**, *44*, 1247-1266.

(26) C. A. Harman, M. V. Turman, K. R. Kozak, L. J. Marnett, W. L. Smith, R. M. Garavito. "Structural basis of enantioselective inhibition of cyclooxygenase-1 by S-alpha-substituted indomethacin ethanolamides." *J. Biol. Chem.* **2007**, *282*, 28096-28105.

(27) S. W. Rowlinson, J. R. Kiefer, J. J. Prusakiewicz, J. L. Pawlitz, K. R. Kozak, A. S. Kalgutkar, W. C. Stallings, R. G. Kurumbail, L. J. Marnett. "A novel mechanism of cyclooxygenase-2 inhibition involving interactions with Ser-530 and Tyr-385." *J. Biol. Chem.* **2003**, *278*, 45763-45769.

(28) G. Ermondi, G. Caron, R. Lawrence, D. Longo. "Docking studies on NSAID/COX-2 isozyme complexes using contact statistics analysis." *J. Comput. Aid. Mol. Des.* **2004**, *18*, 683-696.

(29) C. Roubille, J. Martel-Pelletier, J. M. Davy, B. Haraoui, J. P. Pelletier. "Cardiovascular adverse effects of anti-inflammatory drugs." *Antiinflamm. Antiallergy Agents Med. Chem.* **2013**, *12*, 55-67.

(30) C. D. Funk, G. A. FitzGerald. "COX-2 inhibitors and cardiovascular risk." *J. Cardiovasc. Pharmacol.* **2007**, *50*, 470-479.

(31) Y. Yu, E. Ricciotti, R. Scalia, S. Y. Tang, G. Grant, Z. Yu, G. Landesberg, I. Crichton, W. Wu, E. Pure, C. D. Funk, G. A. FitzGerald. "Vascular COX-2 modulates blood pressure and thrombosis in mice." *Sci. Transl. Med.* **2012**, *4*, 132ra154.

(32) L. C. Chen, D. M. Ashcroft. "Risk of myocardial infarction associated with selective COX-2 inhibitors: meta-analysis of randomised controlled trials." *Pharmacoepidemiol. Drug Saf.* **2007**, *16*, 762-772.

(33) R. A. Moore, S. Derry, H. J. McQuay. "Cyclo-oxygenase-2 selective inhibitors and nonsteroidal anti-inflammatory drugs: balancing gastrointestinal and cardiovascular risk." *Bmc Musculoskel. Dis.* **2007**, *8*, 73.

(34) Y. Bansal, O. Silakari. "The therapeutic journey of benzimidazoles: a review." *Bioorg. Med. Chem.* **2012**, *20*, 6208-6236.

(35) a) L. Franke, E. Byvatov, O. Werz, D. Steinhilber, P. Schneider, G. Schneider. "Extraction and visualization of potential pharmacophore points using support vector machines: application to ligand-based virtual screening for COX-2 inhibitors." *J. Med. Chem.* **2005**, *48*, 6997-7004; b) M. Gaba, S. Singh, C. Mohan. "Benzimidazole: An emerging scaffold for analgesic and anti-inflammatory agents." *Eur. J. Med. Chem.* **2014**, *76*, 494-505.

(36) J. A. Asensio, E. M. Sanchez, P. Gomez-Romero. "Proton-conducting membranes based on benzimidazole polymers for high-temperature PEM fuel cells. A chemical quest." *Chem. Soc. Rev.* **2010**, *39*, 3210-3239.

(37) J. B. Wright. "The Chemistry of the Benzimidazoles." *Chem. Rev.* **1951**, *48*, 397-541.

(38) P. Naik, P. Murumkar, R. Giridhar, M. R. Yadav. "Angiotensin II receptor type 1 (AT1) selective nonpeptidic antagonists-a perspective." *Bioorg. Med. Chem.* **2010**, *18*, 8418-8456.

(39) T. Ishida, T. Suzuki, S. Hirashima, K. Mizutani, A. Yoshida, I. Ando, S. Ikeda, T. Adachi, H. Hashimoto. "Benzimidazole inhibitors of hepatitis C virus NS5B polymerase: identification of 2-[(4-diarylmethoxy)phenyl]-benzimidazole." *Bioorg. Med. Chem. Lett.* **2006**, *16*, 1859-1863.

(40) Q. Dang, S. R. Kasibhatla, W. Xiao, Y. Liu, J. Dare, F. Taplin, K. R. Reddy, G. R. Scarlato, T. Gibson, P. D. van Poelje, S. C. Potter, M. D. Erion. "Fructose-1,6-bisphosphatase Inhibitors. 2. Design, synthesis, and structure-activity relationship of a series of phosphonic acid containing benzimidazoles that function as 5'-adenosinemonophosphate (AMP) mimics." *J. Med. Chem.* **2010**, *53*, 441-451.

(41) L. Bielory, K. W. Lien, S. Bigelsen. "Efficacy and tolerability of newer antihistamines in the treatment of allergic conjunctivitis." *Drugs* **2005**, *65*, 215-228.

(42) M. Gaba, D. Singh, S. Singh, V. Sharma, P. Gaba. "Synthesis and pharmacological evaluation of novel 5-substituted-1-(phenylsulfonyl)-2-methylbenzimidazole derivatives as anti-inflammatory and analgesic agents." *Eur. J. Med. Chem.* **2010**, *45*, 2245-2249.

(43) J. F. Miller, E. M. Turner, K. S. Gudmundsson, S. Jenkinson, A. Spaltenstein, M. Thomson, P. Wheelan. "Novel N-substituted benzimidazole CXCR4 antagonists as potential anti-HIV agents." *Bioorg. Med. Chem. Lett.* **2010**, *20*, 2125-2128.

(44) M. Boiani, M. Gonzalez. "Imidazole and benzimidazole derivatives as chemotherapeutic agents." *Mini Rev. Med. Chem.* **2005**, *5*, 409-424.

(45) C. J. Chen, J. J. Yu, C. W. Bi, Y. N. Zhang, J. Q. Xu, J. X. Wang, M. G. Zhou. "Mutations in a beta-tubulin confer resistance of Gibberella zeae to benzimidazole fungicides." *Phytopathology* **2009**, *99*, 1403-1411.

(46) J. Perez-Villanueva, A. Hernandez-Campos, L. Yepez-Mulia, C. Mendez-Cuesta, O. Mendez-Lucio, F. Hernandez-Luis, R. Castillo. "Synthesis and antiprotozoal activity of novel 2-{[2-(1H-imidazol-1-yl)ethyl]sulfanyl}-1H-benzimidazole derivatives." *Bioorg. Med. Chem. Lett.* **2013**, *23*, 4221-4224.

(47) M. J. Plater, P. Barnes, L. K. McDonald, S. Wallace, N. Archer, T. Gelbrich, P. N. Horton, M. B. Hursthouse. "Hidden signatures: new reagents for developing latent fingerprints." *Org. Biomol. Chem.* **2009**, *7*, 1633-1641.

(48) S. Harkal, F. Rataboul, A. Zapf, C. Fuhrmann, T. Riermeier, A. Monsees, M. Beller. "Dialkylphosphinoimidazoles as new ligands for palladium-catalyzed coupling reactions of aryl chlorides." *Adv. Synth. Catal.* **2004**, *346*, 1742-1748.

(49) I. Georgiou, G. Ilyashenko, A. Whiting. "Synthesis of aminoboronic acids and their applications in bifunctional catalysis." *Acc. Chem. Res.* **2009**, *42*, 756-768.

(50) a) N. Zheng, K. W. Anderson, X. H. Huang, H. N. Nguyen, S. L. Buchwald. "A palladium-catalyzed regiospecific synthesis of N-aryl Benzimidazoles." *Angew. Chem. Int. Ed.* **2007**, *46*, 7509-7512; b) B. L. Zou, Q. L. Yuan, D. W. Ma. "Synthesis of 1,2-disubstituted benzimidazoles by a Cu-catalyzed cascade aryl amination/condensation process." *Angew. Chem. Int. Ed.* **2007**, *46*, 2598-2601; c) G. Brasche, S. L. Buchwald. "C-H functionalization/C-N bond formation: Copper-catalyzed synthesis of benzimidazoles from amidines." *Angew. Chem. Int. Ed.* **2008**, *47*, 1932-1934.

(51) C. Gil, S. Brase. "Solid-phase synthesis of biologically active benzoannelated nitrogen heterocycles: an update." *J. Comb. Chem.* **2009**, *11*, 175-197.

(52) a) K. Hirano, A. T. Biju, F. Glorius. "Copper-Catalyzed Synthesis of 2-Unsubstituted, N-Substituted Benzimidazoles." *J. Org. Chem.* **2009**, *74*, 9570-9572; b) M. Shen, T. G. Driver. "Iron(II) bromide-catalyzed synthesis of benzimidazoles from aryl azides." *Org. Lett.* **2008**, *10*, 3367-3370.

(53) P. Wang, G. J. Zheng, Y. P. Wang, X. J. Wang, Y. Li, W. S. Xiang. "A novel and practical synthesis of substituted 2-ethoxy benzimidazole: candesartan cilexetil." *Tetrahedron* **2010**, *66*, 5402-5406.

(54) a) H. G. F. Richter, G. M. Benson, K. H. Bleicher, D. Blum, E. Chaput, N. Clemann, S. Feng, C. Gardes, U. Grether, P. Hartman, B. Kuhn, R. E. Martin, J. M. Plancher, M. G. Rudolph, F. Schuler, S. Taylor. "Optimization of a novel class of benzimidazole-based farnesoid X receptor (FXR) agonists to improve physicochemical and ADME properties." *Bioorg. Med. Chem. Lett.* **2011**, *21*, 1134-1140; b) Z. H. Zhang, J. J. Li, Y. Z. Gao, Y. H. Liu. "Synthesis of 2-substituted benzimidazoles by iodine-mediated condensation of orthoesters with 1,2-phenylenediamines." *J. Heterocyclic Chem.* **2007**, *44*, 1509-1512; c) R. Wang, X. X. Lu, X. Q. Yu, L. Shi, Y. Sun. "Acid-catalyzed solvent-free synthesis of 2-arylbenzimidazoles under microwave irradiation." *J. Mol. Catal. A- Chem.* **2007**, *266*, 198-201.

(55) Y. S. Hsiao, G. S. Yellol, L. H. Chen, C. M. Sun. "Multidisciplinary synthetic approach for rapid combinatorial library synthesis of triaza-fluorenes." *J. Comb. Chem.* **2010**, *12*, 723-732.

(56) R. Ghorbani-Vaghei, H. Veisi. "The application of poly(N, N'-dibromo-N-ethyl-benzene-1,3-disulfonamide) and N, N, N', N'-tetrabromobenzene-1,3-disulfonamide as catalysts for one-pot synthesis of 2-aryl-1-arylmethyl-1H-1,3-benzimidazoles and 1,5-benzodiazepines, and new reagents for synthesis of benzimidazoles." *Mol. Divers.* **2010**, *14*, 249-256.

(57) R. Varala, A. Nasreen, R. Enugala, S. R. Adapa. "L-proline catalyzed selective synthesis of 2-aryl-1-arylmethyl-1H-benzimidazoles." *Tetrahedron Lett.* **2007**, *48*, 69-72.

(58) R. G. Jacob, L. G. Dutra, C. S. Radatz, S. R. Mendes, G. Perin, E. J. Lenardao. "Synthesis of 1,2-disubstitued benzimidazoles using SiO2/ZnCl2." *Tetrahedron Lett.* **2009**, *50*, 1495-1497.

(59) A. J. Blatch, O. V. Chetina, J. A. Howard, L. G. Patrick, C. A. Smethurst, A. Whiting. "Synthesis and structure of bifunctional N-alkylbenzimidazole phenylboronate derivatives." *Org. Biomol. Chem.* **2006**, *4*, 3297-3302.

(60) U. J. Ries, G. Mihm, B. Narr, K. M. Hasselbach, H. Wittneben, M. Entzeroth, J. C. van Meel, W. Wienen, N. H. Hauel. "6-Substituted benzimidazoles as new nonpeptide angiotensin II receptor antagonists: synthesis, biological activity, and structure-activity relationships." *J. Med. Chem.* **1993**, *36*, 4040-4051.

(61) S. Demirayak, I. Kayagil, L. Yurttas. "Microwave supported synthesis of some novel 1,3-diarylpyrazino[1,2-a]benzimidazole derivatives and investigation of their anticancer activities." *Eur. J. Med. Chem.* **2011**, *46*, 411-416.

(62) a) P. K. Sasmal, S. Sasmal, C. Abbineni, B. Venkatesham, P. T. Rao, M. Roshaiah, I. Khanna, V. J. Sebastian, J. Suresh, M. P. Singh, R. Talwar, D. Shashikumar, K. H. Reddy, T. M. Frimurer, O. Rist, L. Elster, T. Hogberg. "Synthesis and SAR studies of benzimidazole derivatives as melanin concentrating hormone receptor 1 (MCHR1) antagonists: Focus to detune hERG inhibition." *Med. Chem. Comm.* **2011**, *2*, 385-389; b) T. Coon, W. J. Moree, B. Li, J. Yu, S. Zamani-Kord, S. Malany, M. A. Santos, L. M. Hernandez, R. E. Petroski, A. Sun, J. Wen, S. Sullivan, J. Haelewyn, M. Hedrick, S. J. Hoare, M. J. Bradbury, P. D. Crowe, G. Beaton. "Brain-penetrating 2-aminobenzimidazole H(1)-antihistamines for the treatment of insomnia." *Bioorg.*

Med. Chem. Lett. **2009**, *19*, 4380-4384; c) X. Wang, P. A. Bhatia, J. F. Daanen, S. P. Latsaw, J. Rohde, T. Kolasa, A. A. Hakeem, M. A. Matulenko, M. Nakane, M. E. Uchic, L. N. Miller, R. Chang, R. B. Moreland, J. D. Brioni, A. O. Stewart. "Synthesis and evaluation of 3-aryl piperidine analogs as potent and efficacious dopamine D4 receptor agonists." *Bioorg. Med. Chem.* **2005**, *13*, 4667-4678; d) Y. Gong, W. He. "Direct synthesis of unprotected 4-aryl phenylalanines via the Suzuki reaction under microwave irradiation." *Org. Lett.* **2002**, *4*, 3803-3805; e) S. M. Allin, W. R. Bowman, R. Karim, S. S. Rahman. "Aromatic homolytic substitution using solid phase synthesis." *Tetrahedron* **2006**, *62*, 4306-4316.

(63) L. Liu, M. Frohn, N. Xi, C. Dominguez, R. Hungate, P. J. Reider. "A soluble base for the copper-catalyzed imidazole N-arylations with aryl halides." *J. Org. Chem.* **2005**, *70*, 10135-10138.

(64) K. W. Anderson, R. E. Tundel, T. Ikawa, R. A. Altman, S. L. Buchwald. "Monodentate phosphines provide highly active catalysts for Pd-catalyzed C-N bond-forming reactions of heteroaromatic halides/amines and (H)N-heterocycles." *Angew. Chem. Int. Ed.* **2006**, *45*, 6523-6527.

(65) X. Lv, W. Bao. "A beta-keto ester as a novel, efficient, and versatile ligand for copper(I)-catalyzed C-N, C-O, and C-S coupling reactions." *J. Org. Chem.* **2007**, *72*, 3863-3867.

(66) D. Yang, H. Fu, L. Hu, Y. Jiang, Y. Zhao. "Copper-catalyzed synthesis of benzimidazoles via cascade reactions of *o*-haloacetanilide derivatives with amidine hydrochlorides." *J. Org. Chem.* **2008**, *73*, 7841-7844.

(67) a) C. T. Brain, J. T. Steer. "An improved procedure for the synthesis of benzimidazoles, using palladium-catalyzed aryl-amination chemistry." *J. Org. Chem.* **2003**, *68*, 6814-6816; b) C. T. Brain, S. A. Brunton. "An intramolecular palladium-catalysed aryl amination reaction to produce benzimidazoles." *Tetrahedron Lett.* **2002**, *43*, 1893-1895.

(68) a) D. S. Surry, S. L. Buchwald. "Biaryl phosphane ligands in palladium-catalyzed amination." *Angew. Chem. Int. Ed.* **2008**, *47*, 6338-6361; b) J. F. Hartwig. "Evolution of a fourth generation catalyst for the amination and thioetherification of aryl halides." *Acc. Chem. Res.* **2008**, *41*, 1534-1544.

(69) a) F. Monnier, M. Taillefer. "Catalytic C-C, C-N, and C-O Ullmann-type coupling reactions." *Angew. Chem. Int. Ed.* **2009**, *48*, 6954-6971; b) Q. Cai, H. Zhang, B. L. Zou, X. Xie, W. Zhu, G. He, J. Wang, X. H. Pan, Y. Chen, Q. Yuan, F. Liu, B. A. Lu, D. W. Ma. "Amino acid-promoted Ullmann-type coupling reactions and their applications in organic synthesis." *Pure Appl. Chem.* **2009**, *81*, 227-234; c) G. Evano, N. Blanchard, M. Toumi. "Copper-mediated coupling reactions and their applications in natural products and designed biomolecules synthesis." *Chem. Rev.* **2008**, *108*, 3054-3131.

(70) X. H. Deng, H. McAllister, N. S. Mani. "CuI-Catalyzed Amination of Arylhalides with Guanidines or Amidines: A Facile Synthesis of 1-H-2-Substituted Benzimidazoles." *J. Org. Chem.* **2009**, *74*, 5742-5745.

(71) X. H. Deng, N. S. Mani. "Reactivity-Controlled Regioselectivity: A Regiospecific Synthesis of 1,2-Disubstituted Benzimidazoles." *Eur. J. Org. Chem.* **2010**, 680-686.

(72) P. Saha, T. Ramana, N. Purkait, M. A. Ali, R. Paul, T. Punniyamurthy. "Ligand-free copper-catalyzed synthesis of substituted benzimidazoles, 2-aminobenzimidazoles, 2-aminobenzothiazoles, and benzoxazoles." *J. Org. Chem.* **2009**, *74*, 8719-8725.

(73) P. Saha, M. A. Ali, P. Ghosh, T. Punniyamurthy. "Cobalt-catalyzed intramolecular C-N and C-O cross-coupling reactions: synthesis of benzimidazoles and benzoxazoles." *Org. Biomol. Chem.* **2010**, *8*, 5692-5699.

(74) J. Peng, M. Ye, C. Zong, F. Hu, L. Feng, X. Wang, Y. Wang, C. Chen. "Copper-catalyzed intramolecular C-N bond formation: a straightforward synthesis of benzimidazole derivatives in water." *J. Org. Chem.* **2011**, *76*, 716-719.

(75) G. Evindar, R. A. Batey. "Copper- and palladium-catalyzed intramolecular aryl guanidinylation: an efficient method for the synthesis of 2-aminobenzimidazoles." *Org. Lett.* **2003**, *5*, 133-136.

(76) B. G. Szczepankiewicz, J. J. Rohde, R. Kurukulasuriya. "Synthesis of purines and other fused imidazoles from acyclic amidines and guanidines." *Org. Lett.* **2005**, *7*, 1833-1835.

(77) X. Lv, W. Bao. "Copper-catalyzed cascade addition/cyclization: an efficient and versatile synthesis of N-substituted 2-heterobenzimidazoles." *J. Org. Chem.* **2009**, *74*, 5618-5621.

(78) G. D. Shen, W. L. Bao. "Synthesis of Benzoxazole and Benzimidazole Derivatives via Ligand-Free Copper(I)-Catalyzed Cross-Coupling Reaction of o-Halophenols or o-Haloanilines with Carbodiimides." *Adv. Synth. Catal.* **2010**, *352*, 981-986.

(79) H. F. He, Z. J. Wang, W. L. Bao. "Copper(II) Acetate/Oxygen-Mediated Nucleophilic Addition and Intramolecular C-H Activation/C-N or C-C Bond Formation: One-Pot Synthesis of Benzimidazoles or Quinazolines." *Adv. Synth. Catal.* **2010**, *352*, 2905-2912.

(80) F. Wang, S. J. Cai, Q. Liao, C. J. Xi. "A Protocol to 2-Aminobenzimidazoles via Copper-Catalyzed Cascade Addition and Cyclization of o-Haloanilines and Carbodiimides." *J. Org. Chem.* **2011**, *76*, 3174-3180.

(81) J. She, Z. Jiang, Y. G. Wang. "One-Pot Synthesis of Functionalized Benzimidazoles and 1H-Pyrimidines via Cascade Reactions of o-Aminoanilines or Naphthalene-1,8-diamine with Alkynes and p-Tolylsulfonyl Azide." *Synlett* **2009**, 2023-2027.

(82) H. W. Jin, X. L. Xu, J. R. Gao, J. H. Zhong, Y. G. Wang. "Copper-Catalyzed One-Pot Synthesis of Substituted Benzimidazoles." *Adv. Synth. Catal.* **2010**, *352*, 347-350.

(83) S. Murru, B. K. Patel, J. Le Bras, J. Muzart. "Copper(I)-Catalyzed Synthesis of Substituted 2-Mercapto Benzimidazoles." *J. Org. Chem.* **2009**, *74*, 2217-2220.

(84) N. Zheng, S. L. Buchwald. "Copper-catalyzed regiospecific synthesis of N-alkylbenzimidazoles." *Org. Lett.* **2007**, *9*, 4749-4751.

(85) N. Ibrahim, M. Legraverend. "Synthesis of 6,7,8-Trisubstituted Purines via a Copper-Catalyzed Amidation Reaction." *J. Org. Chem.* **2009**, *74*, 463-465.

(86) J. Alonso, N. Halland, M. Nazare, O. R'kyek, M. Urmann, A. Lindenschmid. "A Direct, Regioselective Palladium-Catalyzed Synthesis of N-Substituted Benzimidazoles and Imidazopyridines." *Eur. J. Org. Chem.* **2011**, 234-237.

(87) N. T. Jui, S. L. Buchwald. "Cascade Palladium Catalysis: A Predictable and Selectable Regiocontrolled Synthesis of N-Arylbenzimidazoles." *Angew. Chem. Int. Ed.* **2013**, *52*, 11624-11627.

(88) J. T. Zhu, H. B. Xie, Z. X. Chen, S. Li, Y. M. Wu. "Synthesis of N-Substituted 2-Fluoromethylbenzimidazoles via Bis(trifluoroacetoxy)iodobenzene-Mediated Intramolecular Cyclization of N,N '-Disubstituted Fluoroethanimidamides." *Synlett* **2009**, 3299-3302.

(89) J. T. Zhu, H. B. Xie, Z. X. Chen, S. Li, Y. M. Wu. "Synthesis of 2-fluoroalkylbenzimidazoles via copper(I)-catalyzed tandem reactions." *Chem. Commun.* **2009**, 2338-2340.

(90) M. W. Chen, X. G. Zhang, P. Zhong, M. L. Hu. "Copper-Catalyzed Tandem C-N Bond Formation Reaction: Selective Synthesis of 2-(Trifluoromethyl)benzimidazoles." *Synthesis* **2009**, 1431-1436.

(91) W. Shen, T. Kohn, Z. Fu, X. Jiao, S. J. Lai, M. Schmitt. "Synthesis of benzimidazoles from 1,1-dibromoethenes." *Tetrahedron Lett.* **2008**, *49*, 7284-7286.

(92) C. Siddappa, V. Kambappa, A. K. C. Siddegowda, K. S. Rangappa. "One-pot synthesis of benzimidazoles from gem-dibromomethylarenes using o-diaminoarenes." *Tetrahedron Lett.* **2010**, *51*, 6493-6497.

(93) C. S. Cho, J. U. Kim. "Ruthenium-catalyzed synthesis of benzimidazoles from N-alkyl-1,2-diaminobenzenes via alkyl group transfer." *Bull. Korean Chem. Soc.* **2008**, *29*, 1097-1098.

(94) P. Patrignani, D. Volpi, R. Ferrario, L. Romanzini, M. Disomma, C. Patrono. "Effects of Racemic, S-Indobufen and R-Indobufen on Cyclooxygenase and Lipoxygenase Activities in Human Whole-Blood." *Eur. J. Pharmacol.* **1990**, *191*, 83-88.

(95) O. Laneuville, D. K. Breuer, D. L. Dewitt, T. Hla, C. D. Funk, W. L. Smith. "Differential Inhibition of Human Prostaglandin Endoperoxide-H Synthase-1 and Synthase-2 by Nonsteroidal Antiinflammatory Drugs." *J. Pharmacol. Exp. Ther.* **1994**, *271*, 927-934.

(96) T. D. Warner, F. Giuliano, I. Vojnovic, A. Bukasa, J. A. Mitchell, J. R. Vane. "Nonsteroid drug selectivities for cyclo-oxygenase-1 rather than cyclo-oxygenase-2 are associated with human gastrointestinal toxicity: a full in vitro analysis." *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 7563-7568.

(97) S. Laufer, S. Luik. "Different methods for testing potential cyclooxygenase-1 and cyclooxygenase-2 inhibitors." *Methods Mol. Biol.* **2010**, *644*, 91-116.

(98) a) P. Patrignani, M. R. Panara, A. Greco, O. Fusco, C. Natoli, S. Iacobelli, F. Cipollone, A. Ganci, C. Creminon, J. Maclouf, et al. "Biochemical and pharmacological characterization of the

cyclooxygenase activity of human blood prostaglandin endoperoxide synthases." *J. Pharmacol. Exp. Ther.* **1994**, *271*, 1705-1712; b) K. M. Knights, A. A. Mangoni, J. O. Miners. "Defining the COX inhibitor selectivity of NSAIDs: implications for understanding toxicity." *Exp. Rev. Clin. Pharmacol.* **2010**, *3*, 769-776.

(99) S. Laufer, C. Greim, S. Luik, S. S. Ayoub, F. Dehner. "Human whole blood assay for rapid and routine testing of non-steroidal anti-inflammatory drugs (NSAIDs) on cyclo-oxygenase-2 activity." *Inflammopharmacology* **2008**, *16*, 155-161.

(100) M. Mayer, B. Meyer. "Characterization of ligand binding by saturation transfer difference NMR spectroscopy." *Angew. Chem. Int. Ed.* **1999**, *38*, 1784-1788.

(101) A. Viegas, J. Manso, F. L. Nobrega, E. J. Cabrita. "Saturation-Transfer Difference (STD) NMR: A Simple and Fast Method for Ligand Screening and Characterization of Protein Binding." *J. Chem. Educ.* **2011**, *88*, 990-994.

(102) J. L. Wagstaff, S. L. Taylor, M. J. Howard. "Recent developments and applications of saturation transfer difference nuclear magnetic resonance (STD NMR) spectroscopy." *Mol. Biosyst.* **2013**, *9*, 571-577.

(103) a) B. Meyer, T. Peters. "NMR Spectroscopy Techniques for Screening and Identifying Ligand Binding to Protein Receptors." *Angew. Chem. Int. Ed.* **2003**, *42*, 864-890; b) Y. S. Wang, D. Liu, D. F. Wyss. "Competition STD NMR for the detection of high-affinity ligands and NMR-based screening." *Magn Reson Chem.* **2004** , *42*, 485-489.

(104) A. Viegas, J. Manso, M. C. Corvo, M. M. Marques, E. J. Cabrita. "Binding of ibuprofen, ketorolac, and diclofenac to COX-1 and COX-2 studied by saturation transfer difference NMR." *J. Med. Chem.* **2011**, *54*, 8555-8562.

(105) a) M. Biava, G. C. Porretta, A. Cappelli, S. Vomero, F. Manetti, M. Botta, L. Sautebin, A. Rossi, F. Makovec, M. Anzini. "1,5-Diarylpyrrole-3-acetic acids and esters as novel classes of potent and highly selective cyclooxygenase-2 inhibitors." *J. Med. Chem.* **2005**, *48*, 3428-3432; b) M. Biava, G. C. Porretta, G. Poce, S. Supino, S. Forli, M. Rovini, A. Cappelli, F. Manetti, M. Botta, L. Sautebin, A. Rossi, C. Pergola, C. Ghelardini, E. Vivoli, F. Makovec, P. Anzellotti, P. Patrignani, M. Anzini. "Cyclooxygenase-2 inhibitors. 1,5-diarylpyrrol-3-acetic esters with enhanced inhibitory activity toward cyclooxygenase-2 and improved cyclooxygenase-2/cyclooxygenase-1 selectivity." *J. Med. Chem.* **2007**, *50*, 5403-5411; c) M. Biava, G. C. Porretta, G. Poce, C. Battilocchio, F. Manetti, M. Botta, S. Forli, L. Sautebin, A. Rossi, C. Pergola, C. Ghelardini, N. Galeotti, F. Makovec, A. Giordani, P. Anzellotti, P. Patrignani, M. Anzini. "Novel ester and acid derivatives of the 1,5-diarylpyrrole scaffold as anti-inflammatory and analgesic agents. Synthesis and in vitro and in vivo biological evaluation." *J. Med. Chem.* **2010**, *53*, 723-733.

(106) a) J. L. Henderson, S. M. McDermott, S. L. Buchwald. "Palladium-catalyzed amination of unprotected halo-7-azaindoles." *Org. Lett.* **2010**, *12*, 4438-4441; b) J. L. Henderson, S. L.

Buchwald. "Efficient Pd-Catalyzed Amination Reactions for Heterocycle Functionalization." *Org. Lett.* **2010**, *12*, 4442-4445.

(107) B. P. Fors, D. A. Watson, M. R. Biscoe, S. L. Buchwald. "A highly active catalyst for Pd-catalyzed amination reactions: cross-coupling reactions using aryl mesylates and the highly selective monoarylation of primary amines using aryl chlorides." *J. Am. Chem. Soc.* **2008**, *130*, 13552-13554.

(108) R. A. Altman, A. M. Hyde, X. Huang, S. L. Buchwald. "Orthogonal Pd- and Cu-based catalyst systems for C- and N-arylation of oxindoles." *J. Am. Chem. Soc.* **2008**, *130*, 9613-9620.

(109) J. F. M. da Silva, S. J. Garden, A. C. Pinto. "The chemistry of isatins: a review from 1975 to 1999." *J. Braz. Chem. Soc.* **2001**, *12*, 273-U286.

(110) C. Crestini, R. Saladino. "A New Efficient and Mild Synthesis of 2-Oxindoles by One-Pot Wolff-Kishner Like Reduction of Isatin Derivatives." *Synthetic. Commun.* **1994**, *24*, 2835-2841.

(111) D. S. Soriano. "Example of the Wolff-Kishner Reduction Procedure Suitable for an Undergraduate Organic Lab Experiment - Preparation of Oxindole." *J. Chem. Educ.* **1993**, *70*, 332-332.

(112) a) N. Barbero, M. Carril, R. SanMartin, E. Dominguez. "Copper-catalysed intramolecular O-arylation of aryl chlorides and bromides: a straightforward approach to benzo[d]oxazoles in water." *Tetrahedron* **2007**, *63*, 10425-10432; b) R. D. Viirre, G. Evindar, R. A. Batey. "Copper-catalyzed domino annulation approaches to the synthesis of benzoxazoles under microwave-accelerated and conventional thermal conditions." *J. Org. Chem.* **2008**, *73*, 3452-3459.

(113) a) R. J. Lundgren, A. Sappong-Kumankumah, M. Stradiotto. "A highly versatile catalyst system for the cross-coupling of aryl chlorides and amines." *Chem. Eur. J.* **2010**, *16*, 1983-1991; b) R. J. Lundgren, M. Stradiotto. "Addressing challenges in palladium-catalyzed cross-coupling reactions through ligand design." *Chem. Eur. J.* **2012**, *18*, 9758-9769; c) Q. Shen, T. Ogata, J. F. Hartwig. "Highly reactive, general and long-lived catalysts for palladium-catalyzed amination of heteroaryl and aryl chlorides, bromides, and iodides: Scope and structure-activity relationships." *J. Am. Chem. Soc.* **2008**, *130*, 6586-6596.

(114) a) J. Louie, J. F. Hartwig. "Palladium-Catalyzed Synthesis of Arylamines from Aryl Halides - Mechanistic Studies Lead to Coupling in the Absence of Tin Reagents." *Tetrahedron Lett.* **1995**, *36*, 3609-3612; b) A. S. Guram, R. A. Rennels, S. L. Buchwald. "A Simple Catalytic Method for the Conversion of Aryl Bromides to Arylamines." *Angew. Chem. Int. Ed.* **1995**, *34*, 1348-1350.

(115) a) A. R. Katritzky, H. F. Yang, S. M. Zhang, M. Y. Wang. "An efficient conversion of carboxylic acids into Weinreb amides." *Arkivoc* **2002**, 39-44; b) T. Niu, W. Zhang, D. Huang, C. Xu, H. Wang, Y. Hu. "A powerful reagent for synthesis of Weinreb amides directly from carboxylic acids." *Org. Lett.* **2009**, *11*, 4474-4477.

(116) F. A. Jaipuri, M. F. Jofre, K. A. Schwarz, N. L. Pohl. "Microwave-assisted cleavage of Weinreb amide for carboxylate protection in the synthesis of a (R)-3-hydroxyalkanoic acid." *Tetrahedron Lett.* **2004**, *45*, 4149-4152.

(117) D. W. Ma, Q. A. Cai. "Copper/Amino Acid Catalyzed Cross-Couplings of Aryl and Vinyl Halides with Nucleophiles." *Acc. Chem. Res.* **2008**, *41*, 1450-1460.

(118) a) M. Tietze, A. Iglesias, E. Merisor, J. Conrad, I. Klaiber, U. Beifuss. "Efficient methods for the synthesis of 2-hydroxyphenazine based on the Pd-catalyzed N-arylation of aryl bromides." *Org. Lett.* **2005**, *7*, 1549-1552; b) E. Merisor, U. Beifuss. "From the study of naturally occurring N-allylated phenazines towards new Pd-mediated transformations." *Tetrahedron Lett.* **2007**, *48*, 8383-8387; c) Y. X. Gao, L. Chang, H. Shi, B. Liang, K. Wongkhan, D. Chaiyaveij, A. S. Batsanov, T. B. Marder, C. C. Li, Z. Yang, Y. Huang. "A Thiourea-Oxazoline Library with Axial Chirality: Ligand Synthesis and Studies of the Palladium-Catalyzed Enantioselective Bis(methoxycarbonylation) of Terminal Olefins." *Adv. Synth. Catal.* **2010**, *352*, 1955-1966; d) B. D. Palmer, J. B. Smaill, M. Boyd, D. H. Boschelli, A. M. Doherty, J. M. Hamby, S. S. Khatana, J. B. Kramer, A. J. Kraker, R. L. Panek, G. H. Lu, T. K. Dahring, R. T. Winters, H. D. H. Showalter, W. A. Denny. "Structure-activity relationships for 1-phenylbenzimidazoles as selective ATP site inhibitors of the platelet-derived growth factor receptor." *J. Med. Chem.* **1998**, *41*, 5457-5465.

(119) N. Ono. "The nitro group in Organic Synthesis." Wiley-VHC, **2001**.

(120) K. S. Webb, V. Seneviratne. "A Mild Oxidation of Aromatic-Amines." *Tetrahedron Lett.* **1995**, *36*, 2377-2378.

(121) W. D. Emmons. "Peroxytrifluoroacetic Acid .2. The Oxidation of Anilines to Nitrobenzenes." *J. Am. Chem. Soc.* **1954**, *76*, 3470-3472.

(122) L. G. Hamann, R. I. Higuchi, L. Zhi, J. P. Edwards, X. N. Wang, K. B. Marschke, J. W. Kong, L. J. Farmer, T. K. Jones. "Synthesis and biological activity of a novel series of nonsteroidal, peripherally selective androgen receptor antagonists derived from 1,2-dihydropyridono[5,6-g]quinolines." *J. Med. Chem.* **1998**, *41*, 623-639.

(123) D. Maiti, S. L. Buchwald. "Orthogonal Cu- and Pd-based catalyst systems for the O- and N-arylation of aminophenols." *J. Am. Chem. Soc.* **2009**, *131*, 17423-17429.

(124) Z. Terashita, Y. Imura, M. Tanabe, K. Kawazoe, K. Nishikawa, K. Kato, S. Terao. "Cv-4151 a Potent, Selective Thromboxane-A2 Synthetase Inhibitor." *Thromb. Res.* **1986**, *41*, 223-237.

(125) a) X. Wang, K. Sada, S. Yanagi, C. Yang, K. Rezaul, H. Yamamura. "Intracellular calcium dependent activation of p72syk in platelets." *J. Biochem.* **1994**, *116*, 858-861; b) M. Martina, G. Kilic, E. Cherubini. "The effect of intracellular Ca^{2+} on GABA-activated currents in cerebellar granule cells in culture." *J. Membr. Biol.* **1994**, *142*, 209-216; c) B. C. Pressman. "Biological applications of ionophores." *Annu. Ver. Biochem.* **1976**, *45*, 501-530.

(126) W. E. Longo, N. Panesar, J. Mazuski, D. L. Kaminski. "Contribution of cyclooxygenase-1 and cyclooxygenase-2 to prostanoid formation by human enterocytes stimulated by calcium ionophore and inflammatory agents." *Prostaglandins Other Lipid Mediat.* **1998**, *56*, 325-339.

(127) L. H. Rome, W. E. Lands. "Structural requirements for time-dependent inhibition of prostaglandin biosynthesis by anti-inflammatory drugs." *Proc. Natl. Acad. Sci. U.S.A.* **1975**, *72*, 4863-4865.

(128) A. G. Eliopoulos, C. D. Dumitru, C. C. Wang, J. Cho, P. N. Tsichlis. "Induction of COX-2 by LPS in macrophages is regulated by Tpl2-dependent CREB activation signals." *EMBO J.* **2002**, *21*, 4831-4840.

(129) M. S. Estevao, L. C. Carvalho, M. Freitas, A. Gomes, A. Viegas, J. Manso, S. Erhardt, E. Fernandes, E. J. Cabrita, M. M. Marques. "Indole based cyclooxygenase inhibitors: synthesis, biological evaluation, docking and NMR screening." *Eur. J. Med. Chem.* **2012**, *54*, 823-833.

(130) W. L. F. Armarego, D. D. Perrin: *Purification of laboratory chemicals*; Butterworth Heinemann, **1997**.

(131) Y. Nomura, E. Tokunaga, N. Shibata "Inherent Oxygen Preference in Enolate Monofluoromethylation and a Synthetic Entry to Monofluoromethyl Ethers."*Angew. Chem. Int. Ed.* **2011**, *50*, 1885-1889.

(132) L. Marchetti, A. Andreani, *Ann. Chim (Roma)* **1973**, *63*, 681-690

(133) L. L. Winfield, "Synthesis and anti-proliferative effect of benzimidazole derivatives" **2009**, US 20090197889 A1.

(134) D. M. Bender, S. L. Forman, W. D. Jones, D. L. Smith, H. Zarrinmayeh, D. M. Zimmerman, "Heterocyclic sulfonamide derivatives and their use for potentiating glutamate receptor function." **2002**, WO 2002014294 A2.

(135) G. Evindar, R. A. Batey. "Parallel synthesis of a library of benzoxazoles and benzothiazoles using ligand-accelerated copper-catalyzed cyclizations of ortho-halobenzanilides." *J. Org. Chem.* **2006**, *71*, 1802-1808.

(136) S. J. Pastine , S. W. Youn , D. Sames "Pt(IV)-Catalyzed Cyclization of Arene−Alkyne Substrates via Intramolecular Electrophilic Hydroarylation"*Org. Lett.*, **2003**, *5*, 1055–1058.

(137) R. Griera, M. Armengol, A. Reyes, M. Alvarez, A. Palomer, F. Cabré, J. Pascual, M.L. Garcia, D. Mauleón "Synthesis and pharmacological evaluation of new cysLT1 receptor antagonists" *Eur. J. Med. Chem.*, **1997**, *32*, 547-570.

(138) C. W. Bird, M. Latif. "Further-Studies on the Cyclization of 3-Hydroxy-2'-Nitrodiphenyl Ethers and Related-Compounds." *Tetrahedron* **1980**, *36*, 1813-1816.

(139) Q. Sun, R. Wu, S. Cai, Y. Lin, L. Sellers, K. Sakamoto, B. He, B. R. Peterson. "Synthesis and biological evaluation of analogues of AKT (protein kinase B) inhibitor-IV." *J. Med. Chem.* **2011**, *54*, 1126-1139.

(140) Z. Guo, G. Cheng, F. Chu, Faming Zhuanli Shenqing Gongkai Shuomingshu "Diphenyl benzimidazole compound containing sulfonyl group and its preparation process, medicinal composition and use" **2004**, CN 1506355 A.

(141) I. Ueda, Y. Kitaura, N. Konishi, "Phenyl-alkanoic acid derivative and preparation thereof" **1980**, EP 0008226 A2.

III. Pd-catalyzed amination on polyethyleneglycol

My contribution to the work was the methodology optimization and the synthesis of the majority of the compounds.

III.1 Backgroud

III.1.1 Pegylation

PEGylation has been one of the most flourishing strategies employed to the delivery of therapeutic agents in body, such as oligonucleotides, small drugs, proteins and other biomolecules (Figure III.1).[1] It simply involves the covalent attachment of polyethylene glycol (PEG) polymer chains to the target molecules, producing beneficial alterations in the physico-chemical properties of the medicinal agent while maintaining its pharmacological properties.

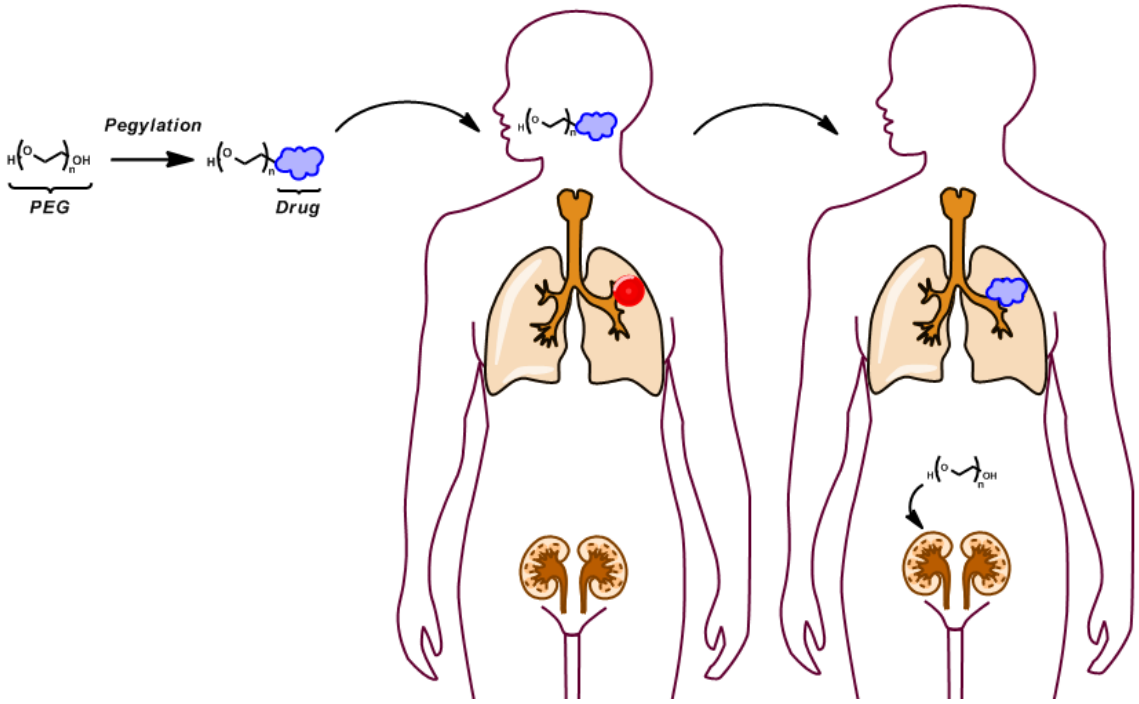


Figure III.1. PEG as vehicle for drug delivery.

Since PEG is a biocompatible, non-toxic and hydrophilic polymer, the modification of a biopharmaceutical compound with PEG provides several advantages. The most relevant one is the increased water solubility afforded to hydrophobic drugs and proteins allowing its easier distribution in the body. Moreover, the increase in the hydrodynamic size of the molecule extends its life cycle by reducing the renal excretion. Pegylation can also influence the binding affinity of the therapeutic moiety to the cell receptors and can alter the absorption and distribution patterns, while protects the agent from the host's immune system contributing for a reduced enzymatic degradation, immunogenicity and antigenic reactions.[2]

All these factors contribute to improve the pharmacokinetics and pharmacodynamic properties of many therapeutic agents, fact that leads to a reduced dosage frequency, increased drug stability maintaining its efficacy and safety.[2-3]

Several aspects, such as molecular weight, shape, reactivity or type of bond established between the therapeutic agent and the PEG moiety are crucial in determining the effect on PEGylated molecules. Consequently a wide number of PEG derivatives and PEG conjugation technologies were developed, in order to design the best PEGylation strategy for each particular application.[4]

Pegylation process usually begins by a proper hydroxyl group modification at one or both PEG terminals in order to attach the desired target molecule. The choice of the functional group for the PEG polymer is based on the type of reactive group on the molecule that will be coupled to the PEG. PEGs that are activated at each terminus with the same reactive moiety are usually known as "homobifunctional", whereas if the functional groups present are different, the PEG derivative is referred as "heterobifunctional" or "heterofunctional".

In contrast with other water soluble biocompatible polymers, the functionality of PEG is usually limited to its two chain termini regardless the molecular weight. However, an increased loading value is usually needed in order to improve the pharmacokinetic and pharmacodynamic effects of therapeutic agents. To this purpose, the preparation of new multifunctional branched PEGs and PEG dendrimers of increased molecular masses have been proposed for small drug delivery.[5] These derivatives have enhanced characteristics, reaching higher drug to polymer ratios, shielding the bioactive molecule better than a linear PEG of the same size, affording a better protection from degradation.[6]

This technology dramatically influences the clinical application of therapeutic biomolecules. Consequently, there is a continuously growing number of PEG conjugated drugs on the market involving new delivery formats and dosing regimens.[4]

III.1.2 PEG as solid support in heterocyclic compounds synthesis

Heterocyclic systems represent an important class of compounds in biologically active pharmaceuticals, natural products, and materials, and therefore the construction and selective functionalization of these molecules is of great interest.[7]

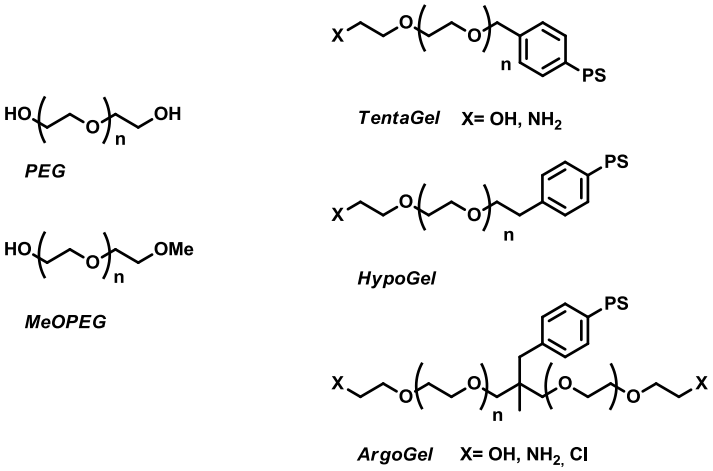
Due to the imperative urgency to prepare new heterocyclic structures with increased structural diversity, solid-phase organic synthesis (SPOS) appeared as a valuable alternative over the synthesis in solution.[8] This methodology simultaneously allows the rapid construction of small libraries of complex derivatives and a high-throughput screening. Although the considerable work that has been performed optimizing not only the SPOS reaction conditions but also the polymeric supports and linkers, it still reveals limitations mainly due to the heterogeneous character of the reactions.

The use of a soluble polymer support in a combinatorial approach tremendously improved heterocyclic compounds preparation, overcoming the difficulty of solid-phase synthesis, while

maintaining its advantages. Moreover, the use of a soluble polymer support allows the reaction monitoring by a simple ^{1}H NMR without cleaving the substrate from the resin while avoiding special NMR techniques used in SPOS, such as high resolution magic angle spinning NMR (HRMAS-NMR).[9]

During the last few years, polyethylene glycol (PEG) was largely used as soluble polymer support in several reactions.[10] Available in a broad range of average molecular weights (the usually used vary from 200 to 4000), PEG is a non-toxic, inexpensive, thermally stable and recoverable polymer that can be used as a reaction medium or solid-liquid phase transfer catalyst.[10] Based on these properties, it appeared as an environmental friendly alternative to the use of volatile, toxic and hazardous organic solvents. In solid phase synthesis, PEG has been described as versatile soluble solid support[11] to assemble oligosaccharides,[12] nucleotides,[2] peptides[13] and heterocycles.[14]

PEG was initially reported as solid support in peptide synthesis.[15] It is soluble in water and in most organic solvents and can be precipitated with solvents such as hexane or diethyl ether. However, due to its water soluble character, inorganic material and organometallic reagents can be difficult to remove. In order to improve its robustness while maintaining its properties, some grafted copolymers were developed. [16]

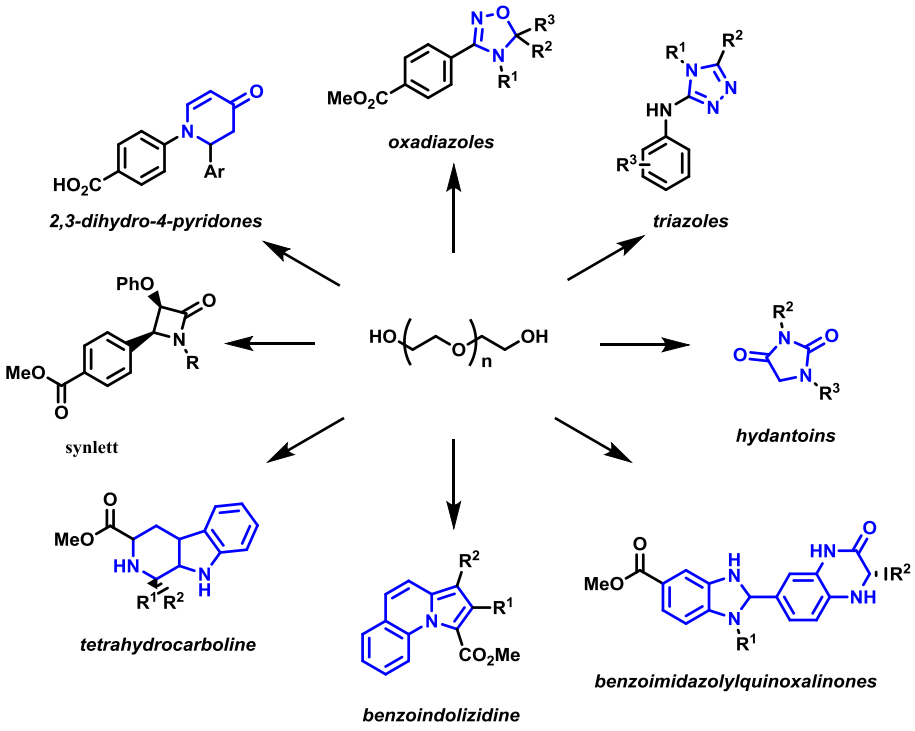


Scheme III.1. PEG based resins.

For example, grafting of PEG to divinylbenzene cross-linked polystyrene improves the handling, isolation, and purification of PEG. Other examples include TentaGel,[17] HypoGel or ArgoGel resins (Scheme III.1).[18] These PEG-based resins exhibit good swelling in polar solvents also allowing good diffusion of the reagents. Their main disadvantages are the loading capacity and the tendency to become sticky and difficult to handle as the synthesis progress.

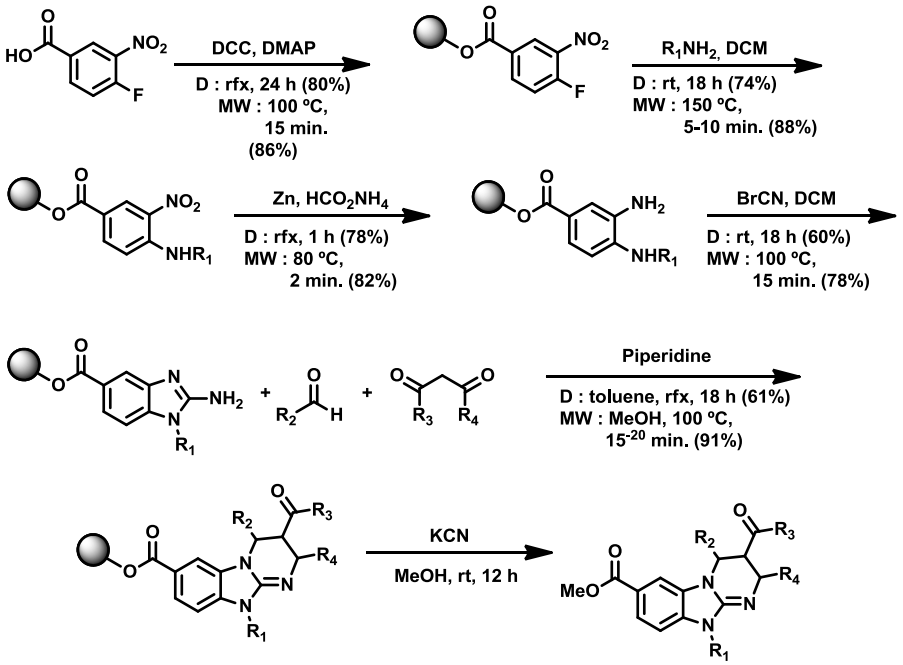
In heterocyclic chemistry, PEG-based resins play an important role.[19] Additionally, the simple and cheaper PEG polymer (with different MWs) has been also used to assemble a large variety of heterocyclic structures, such as 1,2,4-triazoles,[20] 2,3-dihydro-4-pyridones,[21] tetrahydro-β-

carbolines,[22] benzoindolizines,[23] oxadiazoles,[24] hydantoin analogs[25] or benzoimidazoylquinaxolinones (Scheme III.2).[26]



Scheme III.2. Heterocyclic structures synthesized on PEG.

An example of benzimidazoles assembly on PEG was the reported by Sun *et al*.[27] In this work the benzimidazole moiety is assembled by the classical methods using PEG 6000 as the soluble solid support. The process involves the attachment of 4-fluoro-3-nitrobenzoic acid to the solid support and subsequent coupling of a primary amine, by nucleophilic aromatic substitution, to provide the corresponding nitroarene. Subsequent reduction of nitro group to a primary amine followed by cyclization yields the benzimidazole ring. Moreover, microwave-assisted acceleration can be utilized in the synthesis process without prejudice for PEG backbone. In fact, Sun and co-workers adopted a multidisciplinary approach in which a clear comparison between regular and microwave conditions was undertaken (Scheme III.3). It was demonstrated that microwave-assisted synthesis is very effective on speeding up the reaction, and that the reaction monitoring can be easily performed by ^{1}H NMR spectroscopy without cleaving the PEG-bound products.[28]



Scheme III.3. Synthesis of benzimidazole library on PEG by Sun *et al.*[27]

The construction of synthetic complex drugs on PEG would be a huge step forward, taking advantage of PEG as soluble support (in solid phase synthesis) and as polymeric support for pegylated structures. However, until nowadays, and as far is known, there is no reported procedure where a small heterocyclic structure is totally assembled in PEG followed by its application as a pegylated target.

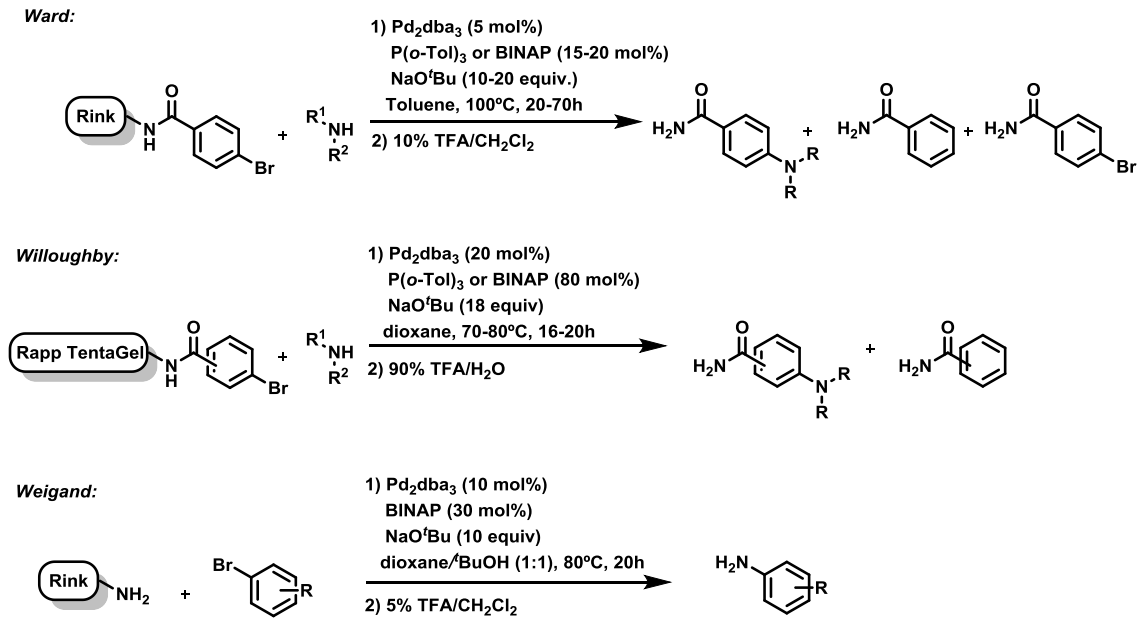
III.1.3 Pd-catalyzed C-N coupling on solid supports

The aryl amine moiety can be found in a wide variety of heterocyclic compounds, and metal catalyzed cross-coupling reactions of anilines with aryl halides constitute the main methods for assembling this type of substructure.[29] In fact, during the last years, there has been a great progress in the development of Pd-catalyzed C-N coupling of aryl halides and amines, leading to the generation of new ligands and catalytic systems.[30] Despite using metal amounts below 1 mol %, these methods are highly dependent on the substrates and reaction conditions. These facts make the cross coupling reactions especially unpredictable, leading in some cases to several side products, laborious purifications and poor yields. Thus, a combinatorial approach would be of tremendous value, in developing and optimizing these reactions.

Since a few years ago, PEG has been successfully employed as solvent or integrated in catalytic systems for some metal catalyzed reactions, such as olefin metathesis,[31] hydroxylation,[32] Suzuki and Sonogashira coupling,[33] Mizoroki–Heck reaction,[34] hydrogenation [35] or oxidations.[36] PEG was

also described as an efficient and reusable soluble support for SPhos, a Buchwald type ligand used for Pd-catalyzed cross-couplings.[37]

The first examples of the Pd-catalyzed amination of aryl halides using solid supports were described in the advent of Buchwald-Hartwig amination developments (Scheme III.4).



Scheme III.4. Previously described arylation on PEG. [38]

In 1996, the procedure was independently described by Ward[38c] and Willoughby[38b] who immobilized the aryl bromides in different types of resins. Few years later, Weigand performed the Pd-mediated amination in an inverted way, immobilizing the amine onto a Rink-resin (Scheme III.4).[38a]

It is noteworthy that a supported copper C-N cross-coupling reaction was also described using arylboronic acids in the presence of $Cu(OAc)_2$ using the Wang resin.[39]

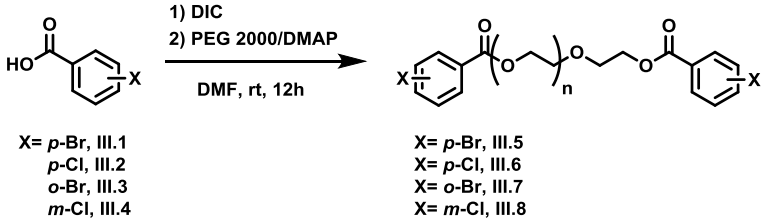
Despite the early described Pd-mediated cross-coupling procedures,[38] it was decided to investigate the possibility of using PEG 2000 as a soluble polymeric support for the preparation of arylated heteroarenes. To the best of our knowledge, so far, PEG has not been employed in this cross-coupling reactions acting as solvent and polymeric support for the halide substrate.

III.2 Results and discussion

By considering the solubility profile and loading capacity of several polymer derivatives, PEG of average molecular weight 2000 was chosen as the polymer support for the synthetic protocol. Moreover, this PEG derivative was also previously reported as ideal solid support for the assembling of heterocyclic structures.[40]

III.2.1 Optimization studies

The preparation of the PEG bound aryl halides (**III.5-III.8**), was performed by a standard esterification, activating the corresponding benzoic acid halide (2 equiv/$PEG_{terminal}$) with DIC in DMF followed by addition of PEG 2000 and DMAP (Scheme III.5). Completeness of the coupling was monitored by the conventional ^1H NMR.



Scheme III.5. Preparation of PEG bound aryl halides (**III.5-III.8**).

The reaction work-up involved a simple filtration over a celite pad in order to remove the formed diisopropylurea. The product was easily obtained by precipitation with cold diethyl ether followed by filtration and washing with an additional amount of cold diethyl ether to remove the remaining DMF. The compounds (**III.5-III.8**) were obtained in quantitative yield as white solids, and characterized by IR and NMR techniques.

In order to optimize the reaction conditions, the *p*-chloro arylated PEG derivative (**III.6**) was reacted with aniline, employing different ligands and solvents as well as using solvent-free conditions, under a range of temperatures and reaction times (Table III.1).

In 2005, Buchwald *et al.* reported the coupling of several anilines to 4,5-dichlorophthalimide derivatives using XPhos/Pd_2dba_3 in the presence of Cs_2CO_3.[41] Interestingly, the amination reaction employing 4-nitroaniline as the nucleophile did not proceeded using Cs_2CO_3 or K_3PO_4 requiring the use of a weaker base such as K_2CO_3. The group also reported similar procedures.

Inspired by this work, the ligand optimization was performed using Pd_2dba_3 as the palladium source, using ^{t}BuOH as solvent. Consequently, several ligands (Figure III.2) were tested while maintaining the catalyst loading (1 mol% Pd and 3 mol% of ligand) (Table III.1 – entries 1 to 5).

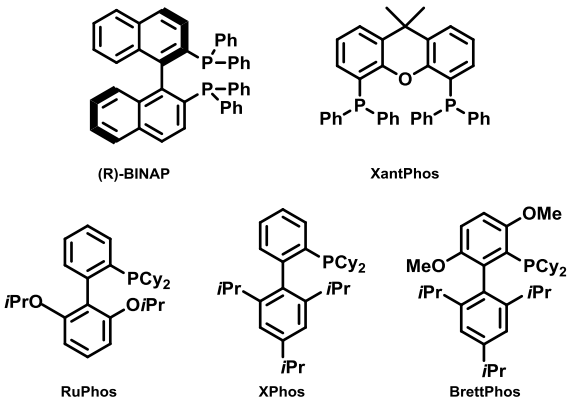
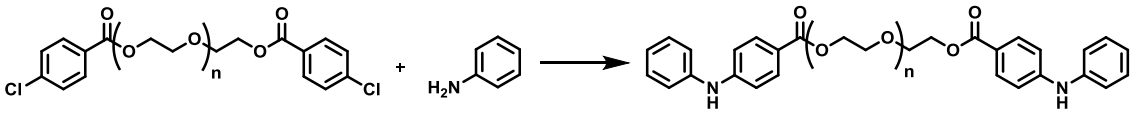


Figure III.2. Ligands used in the optimization of Pd-catalyzed amination on PEG.

Gladly, the use of Pd_2dba_3 and Buchwald ligands,[30c] RuPhos, XPhos and BrettPhos, afforded the product (**III.9**) in good to excellent yields (Table III.1 – entries 3 to 5). It was observed that BINAP and XantPhos were unsuccessful for this type of reaction, as the reaction did not occur.

Since XPhos gave the best results, this ligand was further used to investigate the adequate solvent. In this case, it was verified that ^{t}BuOH, dioxane or toluene, were sufficient to lead the reaction to completion (Table III.1 – entries 5 to 7).

Table III.1 Influence of the reaction conditions for palladium catalyzed direct coupling of PEG bound aryl chloride **III.6** with aniline.[a]



Entry	Ligand	Solvent	Reaction time (h)	Temp. (ºC)	**III.9** (%) [b]
1	BINAP	^{t}BuOH	18	110	0
2	Xantphos	^{t}BuOH	18	110	0
3	BrettPhos	^{t}BuOH	18	110	83
4	RuPhos	^{t}BuOH	18	110	66
5	XPhos	^{t}BuOH	18	110	Quant.
6	XPhos	dioxane	18	110	Quant.
7	XPhos	toluene	18	110	Quant.
8	XPhos	---	18	110	Quant.
9	XPhos	^{t}BuOH	4	110	Quant.
10	XPhos	^{t}BuOH	18	65	0
11	XPhos	---	8	110	94
12	XPhos	---	4	110	90

[a] reaction conditions: Pd_2dba_3 (1 mol%), ligand (3 mol%), K_2CO_3 (1.4 equiv), aniline (1.4 equiv), solvent (0.25 M) in a sealed tube under an argon atmosphere. [b] Verified by ^{1}H NMR.

PEG was already described as an alternative medium in several reactions,[10] thus a solvent-free trial was also performed. Fortunately, the reaction proceeded to completion in the absence of solvent (Table III.1 - entry 8).

The temperature and reaction time were also investigated. It was verified that after 20 h the product was obtained in quantitative yield. The decrease of the reaction time to 8 h or 4 h did not have a strong impact in the yield, since **III.9** was obtained in 94 % and 90 % yield, respectively (Table III.1 - entries 11 and 12).

The reaction could be easily monitored by IR, ^{1}H or ^{13}C NMR, as demonstrated in Figure III.3. In the IR spectrum it was observed a band shift of the corresponding carbonyl group of **III.6** that shifted from 1718 cm^{-1} to 1702 cm^{-1}, matching the carbonyl group of **III.9**.

This product could also be identified by ^{1}H NMR since the CH_2 signals corresponding to the inner chain of PEG resin appeared as a multiplet at 3.63 ppm, and the CH_2 signals corresponding to the ethylene chain direct linked to the benzyl moiety appear respectively at 4.43 and 3.80 ppm. The coupling product appears very clearly in the aromatic region (Figure III.4).

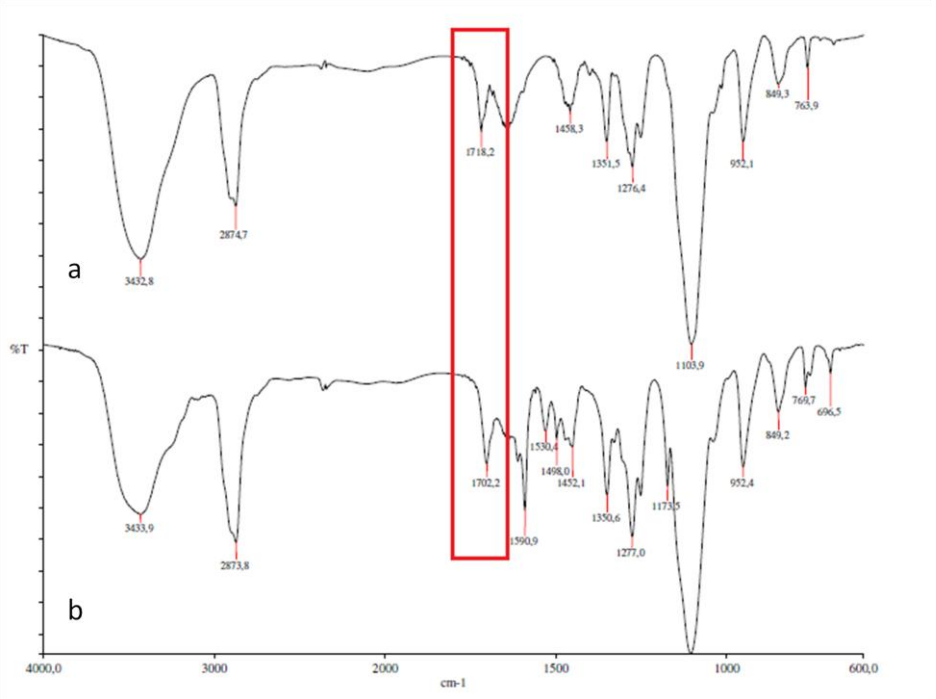


Figure III.3. IR spectra of a) PEG bound aryl chloride (**III.6**) and b) amination product (**III.9**).

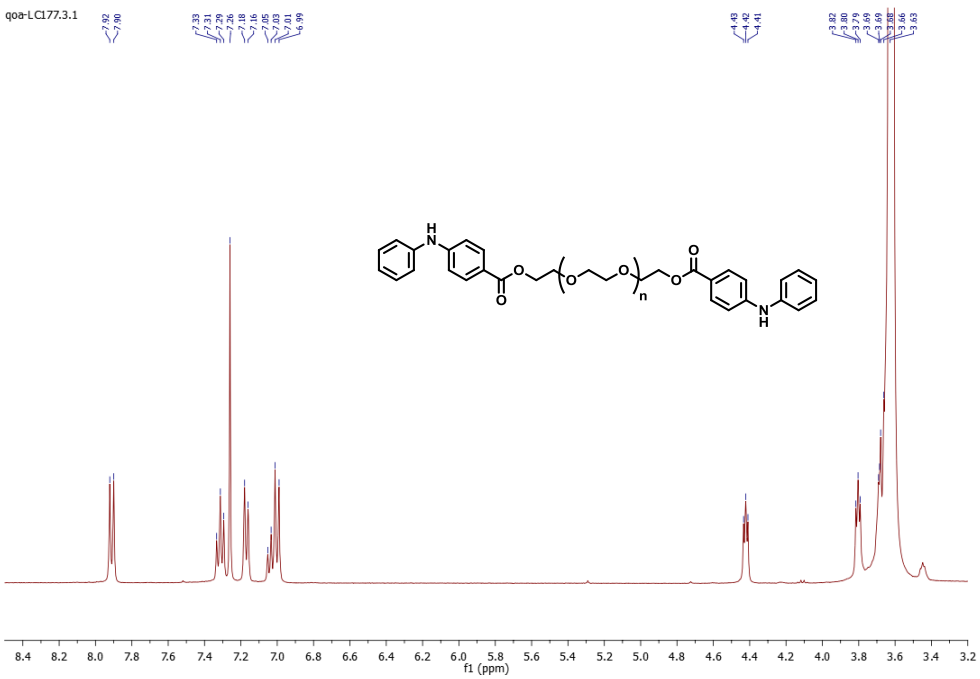


Figure III.4. ^{1}H NMR of **III.9** (Table III.1, entry 5).

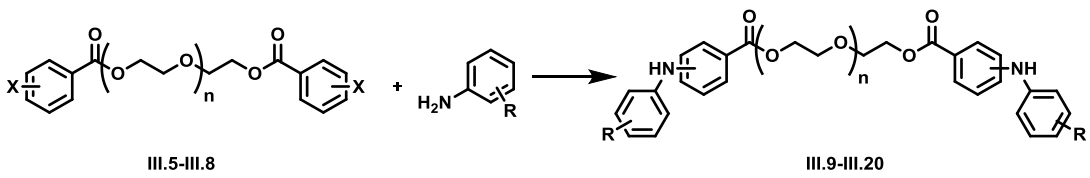
III.2.2 Pd-catalyzed amination of arylated PEG substrates with substituted anilines

Following the optimization condition study, we further investigated the solvent-free Pd-catalyzed amination using different chloro and bromo arylated PEG substrates with several substituted anilines (Table III.2).

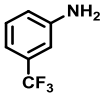
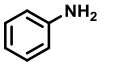
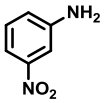
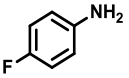
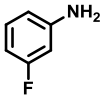
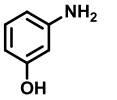
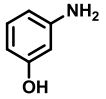
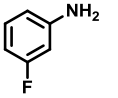
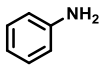
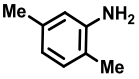
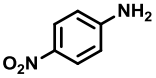
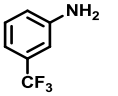
Amination of *p*-chloro arylated PEG substrate with several anilines, led to the corresponding products (**III.10-III.13**) with good to excellent yields under the studied conditions (Table III.2 – entries 1 to 4). As expected, the same behavior was observed for the *p*-bromo arylated PEG compound, either with aniline or with the deactivated *p*-nitro aniline (Table III.2 – entries 5 and 6). However, *o*-bromo substrates revealed to be more difficult to react than the previous substrates, fact that can be explained by steric hindrance (Table III.2 – entries 7 to 10). The amination reaction with aniline performed in 8 h, rendered 50% conversion that was increased up to 66% when the reaction was performed in 20 h (Table III.2 – entry 7). Moreover, the PEG-bounded *m*-haloarenes were also screened and afforded the corresponding products in high yield (Table III.2 – entries 11 and 12).

In the experiments in which quantitative yields were not achieved, the unreacted starting material was recovered along with the reaction product.

Table III.2. Scope of palladium catalyzed direct coupling of PEG bound aryl halides with several anilines.[a]

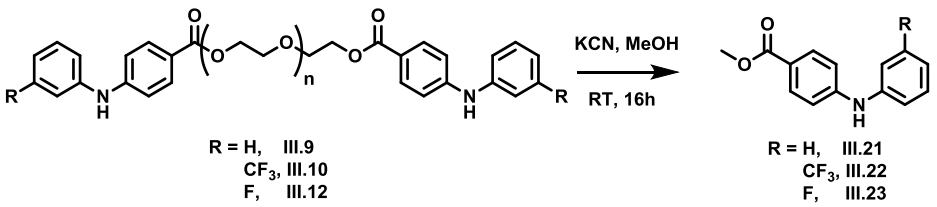


III.5-III.8 III.9-III.20

Entry	Ar-X	$ArNH_2$	Compound (%)[b]	Entry	Ar-X	$ArNH_2$	Compound (%)[b]
1	*p*-Cl		**III.10** (100)	7	*o*-Br		**III.15** (50, 66[c])
2	*p*-Cl		**III.11** (89)	8	*o*-Br		**III.16** (40)
3	*p*-Cl		**III.12** (100)	9	*o*-Br		**III.17** (50)
4	*p*-Cl		**III.13** (100)	10	*o*-Br		**III.18** (80[c])
5	*p*-Br		**III.9** (100)	11	*m*-Cl		**III.19** (100)
6	*p*-Br		**III.14** (90)	12	*m*-Cl		**III.20** (70)

[a] reaction conditions: Pd_2dba_3 (1 mol%), XPhos (3 mol%), K_2CO_3 (1.4 equiv), aniline (1.4 equiv), in a sealed tube under an N_2 atmosphere, 110ºC, 8 h. [b] Verified by ^1H NMR. [c] Reaction performed in 20 h.

It is also important to refer the work-up procedure of this cross-coupling reaction on a soluble solid support. The recovery of the PEG-bound structures consisted on the filtration of the reaction mixture to remove the inorganic salts followed by product precipitation with cold diethyl ether. The product was then thoroughly washed with diethyl ether to remove the amine excess, the catalyst and the ligand. Some structures were difficult to precipitate, forming a viscous slurry that, in these particular cases, made the work-up a delicate and time consuming task.



R = H, III.9
CF_3, III.10
F, III.12

R = H, III.21
CF_3, III.22
F, III.23

Scheme III.6. Cleavage of PEG-bound compounds from the polymer.

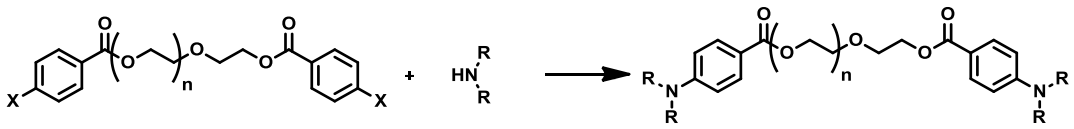
Detachment of the compounds from the solid support could be easily handled by treatment with potassium cyanide in methanol, according to the reported procedure.[17] This trans-esterification method is proved to be simple and efficient, affording the products in high yields and purity. Thus, in order to test the cleavage conditions in our system, some compounds (**III.9**, **III.10** and **III.12**) were subjected to the reported conditions (KCN, MeOH, room temperature, overnight)[17a] and, as expected, the corresponding methyl esters (**III.21-III.23**) were obtained in quantitative yields (Scheme III.6).

III.2.3 Pd-catalyzed amination of arylated PEG substrates with alkyl amines

The Pd-catalyzed amination on PEG using alkyl amines was also investigated, as described in Tables III.3 and III.4.

Initial trials using benzylamine and piperidine showed that the general conditions applied to anilines could not be employed for aliphatic amines (Table III.3).

Table III.3. Optimization of reaction conditions of palladium catalyzed direct coupling of PEG bound aryl halides with benzylamine and piperidine.[a]



Entry	X	Amine	Ligand	Base	Reaction time (h)	Yield (%) [b]
1	*p*-Cl	benzylamine	XPhos	K_2CO_3	20	0
2	*p*-Cl	piperidine	XPhos	Cs_2CO_3	20	0
3	*p*-Cl	benzylamine	BrettPhos [c]	Cs_2CO_3	20	0
4	*p*-Cl	piperidine	BINAP	Cs_2CO_3	20	0
5	*p*-Br	piperidine	XPhos	Cs_2CO_3	20	0
6	*p*-Br	piperidine	BINAP	Cs_2CO_3	20	100
7	*p*-Br	benzylamine	BINAP	Cs_2CO_3	20	100
8	*p*-Br	benzylamine	BINAP	Cs_2CO_3	8	100

[a] reaction conditions: Pd_2dba_3 (1 mol%), ligand (3 mol%), base (1.4 equiv), amine (1.4 equiv) in a sealed tube under an N_2 atmosphere at 110ºC. [b] Verified by ^1H NMR. [c] BrettPhos precatalyst as Pd source.

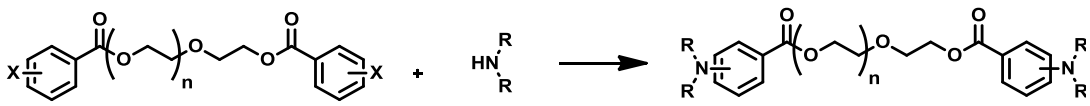
It was observed that the chlorinated derivatives did not undergo the desired amination reaction, although several catalytic systems already described for amination of halo-arenes with aliphatic amines (in solution) were employed (Table III.3, entries 1 – 4). Systems like Pd_2dba_3/Xphos/K_2CO_3

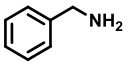
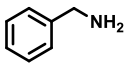
(entry 1), Pd_2dba_3/Xphos/Cs_2CO_3 (entry 2), Brettphos system/Cs_2CO_3 (entry 3) or Pd_2dba_3/BINAP/Cs_2CO_3 (entry 4), were unsuccessful.[42]

However, for bromine derivatives, while XPhos was ineffective as ligand (Table III.3 – entry 5), the system that used BINAP was successful (Table III.3 – entries 6 and 7).[42a] The reaction proceeded in high yield using pegylated bromine arenes, BINAP as ligand and Cs_2CO_3 as base. Moreover, it was verified that for benzylamine the reaction occurred in 8 h in quantitative yield (Table III.3 – entry 8).

After the initial screening, the reaction scope was extended to other amines and PEG bound halides. It was verified that the reaction worked nicely with some primary and secondary amines, such as benzylamine, piperidine, and pyrrolidine. However, the experiments performed proved the failure of this reaction for the studied amines (Table III.4). For pyrrolidine, it was also observed full conversion after 8 h of reaction. Attempts using aromatic amines, such as imidazole or pyrrole were unsuccessful.

Table III.4. Scope of palladium catalyzed direct coupling of PEG bound aryl halides with several amines[a]

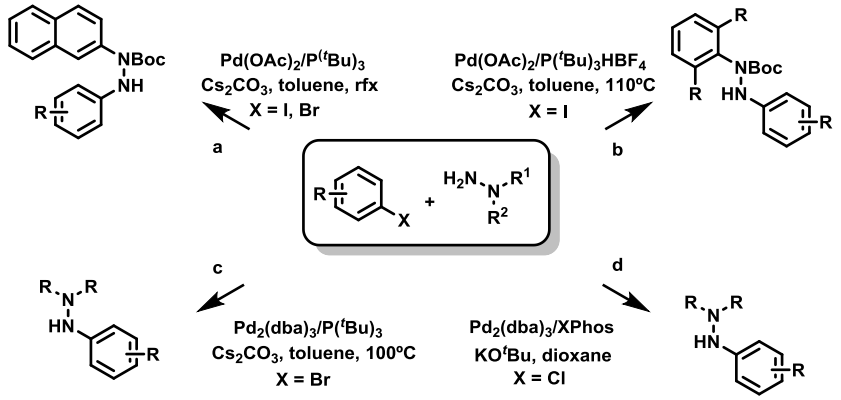


Entry	Ar-X	R_2NH	Compound (%)[b]	Entry	Ar-X	R_2NH	Compound (%)[b]
1	*p*-Br		**III.24** (100)	5	*o*-Br		--
2	*p*-Br		**III.25** (100)	6	*o*-Br		*f*
3	*p*-Br		**III.26** (50, 100[c])	7	*o*-Br		*e*
4	*p*-Br		--	8	*m*-Br		*e*

[a] reaction conditions: Pd_2dba_3 (1 mol%), ligand (3 mol%), Cs_2CO_3 (1.4 equiv), aniline (1.4 equiv), in a sealed tube under an N_2 atmosphere, 110ºC, 8 h. [b] Verified by ^1H NMR. [c] Reaction performed in 20 h. [d] Starting material. [e] NMR spectra inconclusive. [f] Hydrolysis.

III.2.4 Pd-catalyzed amination of arylated PEG substrates with hydrazines

The Pd-catalyzed amination on PEG was also explored with arylhydrazines, since these molecules can produce valuable intermediates in heterocyclic synthesis. Several reports describe the Pd-catalyzed aminations of haloarenes with *N*,*N*-disubstituted hydrazines using different catalytic systems (Scheme III.7).



Scheme III.7. Reported studies on coupling of hydrazines with aryl halides.[43]

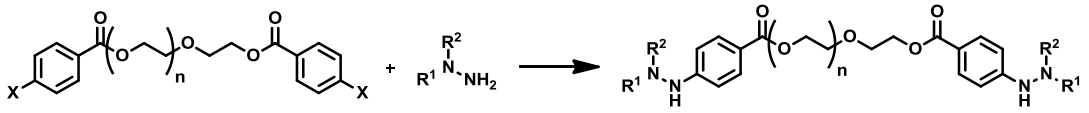
Since the first report by Koie and co-workers of $Pd(OAc)_2/P(^tBu)_3$ system in Pd-catalyzed amination of aryl chlorides,[44] its use have been commonly reported for arylamination reactions involving hydrazines (Scheme III.7 – a, b).[43a,b]

Liu *et al.* described the amination of bromo arenes with *N*,*N*-dialkylhydrazines using $Pd_2(dba)_3/P(^tBu)_3$ as the catalyst system (Scheme III.7 – c).[43c] The base optimization revealed that strong bases such as KO*t*Bu were effective, although functional groups were less tolerated. Both K_2CO_3 and K_3PO_4 demonstrated to be suitable bases. However, Cs_2CO_3 provided the best yield. Other studies include the use of Pd_2dba_3/XPhos in the presence of NaO^tBu and despite K_3PO_4 could be used without big loss of activity, the use of Cs_2CO_3 proved to be unsuccessful (Scheme III.7 – d).[43d]

In our study, the amination of PEG bound bromo arenes with arylhydrazines was attempted using the Pd_2dba_3/XPhos system and K_3PO_4 as base. However, the system proved to be inadequate to the studied arylhydrazines (Table III.5). In some cases, it was possible to recover the starting material (entries 1 and 4), whereas in other experiments the NMR spectra revealed complex reaction mixtures (entries 2 and 3).

Once more, the precipitation of these derivatives with cold diethyl ether was very difficult, yielding gummy (and certainly impure) products. This fact led to a difficult interpretation of the NMR spectra.

Table III.5. Scope of Pd-catalyzed direct coupling of PEG bound aryl halides with some hydrazines.[a]



Entry	Ar-X	$ArNH_2$	Yield (%)[b]	Entry	Ar-X	$ArNH_2$	Yield (%)[b]
1	*p*-Cl	Phenyl hydrazine	--[c]	4	*p*-Br	*N*, *N*-methyl hydrazine	--[c]
2	*p*-Br	Phenyl hydrazine	--[d]	5	*p*-Br	Hydrazine	--[c]
3	*p*-Br	4-nitophenyl hydrazine	--[d]	6	*p*-Br	Benzophenone hydrazone	100

[a] Reaction conditions: Pd_2dba_3 (2 mol%), XPhos (3 mol%), K_3PO_4 (1.4 equiv.), amine (1.2 equiv.), in a sealed tube under an N_2 atmosphere, 80ºC, 20 h. [b] Verified by ^1H NMR. [c] Starting material. [d] NMR spectra inconclusive.

The amination of PEG bound bromo arene using hydrazine and benzophenone hydrazone was also explored (Table III.5 – entries 5 and 6).

In 2010, Lundgren and Stradiotto reported a palladium based catalytic system and reaction conditions that allowed, for the first time, the cross-coupling of aryl chlorides and tosylates with hydrazine.[45] More recently, Stradioto group also reported the monoarylation of hydrazine using a new BippyPhos/$[Pd(cinnamyl)Cl]_2$ system.[46] However, the reported conditions are highly dependent of the catalytic system, fact that could hamper the reaction scope broadening. Very recently, Buchwald describes the coupling of hydrazine with chloro derivatives using several ligands, in which BrettPhos precatalyst proved to be the best catalytic system.[47]

Unfortunately, the cross-coupling with hydrazine was only attempted using the PEG bound bromo derivative, expecting a higher reactivity (entry 5). However, from the reported literature, it seems that the reaction is only efficient for chlorine derivatives. Moreover, the use of BrettPhos system can be the best choice for this reaction on PEG.

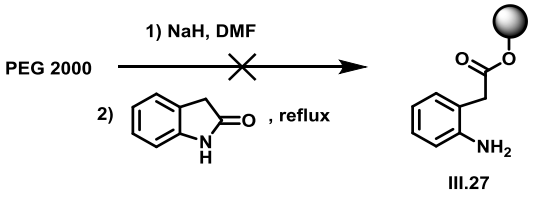
The Pd-catalyzed arylation of benzophenone hydrazone can furnish arylhydrazines that are valuable synthetic intermediates, e.g. Fisher indolization. In the last years, several groups reported cross-coupling procedures involving benzophenone hydrazone.[48]

Thus, it was examined the cross-coupling reaction of PEG *p*-Br arene with benzophenone hydrazone using the Pd_2dba_3/XPhos/K_3PO_4 system. As expected, using phenylhydrazone the reaction was effective yielding the product in quantitative yield.

III.2.5 Preliminary studies to assemble benzimidazoles on PEG using the Pd catalyzed strategy

In order to assemble the benzimidazole structure on PEG taking advantage of the above described procedure and using the key intermediate (**II.56**) synthesized in Chapter II, some preliminary tests were made.

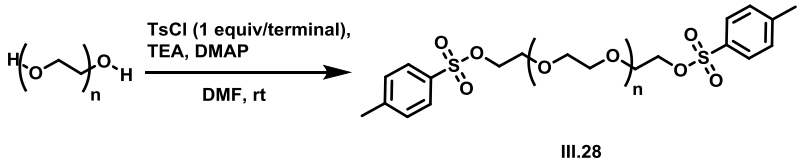
In a first approach it was envisioned that the reaction of the corresponding PEG alkoxide (formed with NaH in DMF) with the oxindole under refluxing conditions, would give the PEG bound intermediate to further functionalization (Scheme III.8). However this approach was unsuccessful.



Scheme III.8. Preliminary attempts to construct benzimidazoles on PEG.

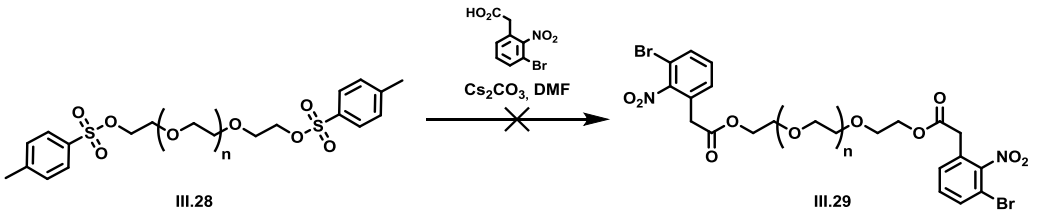
Due to the failure of these preliminary studies, further experiments were performed to attach the key intermediate **II.56** (*see* Chapter II) to PEG.

Thus, the tosylated PEG derivative (**III.28**) was synthesized in order to directly bind the acid **II.56**. Compound **III.28** was easily synthesized in DCM with tosyl chloride in the presence of TEA and DMAP (Scheme III.9).



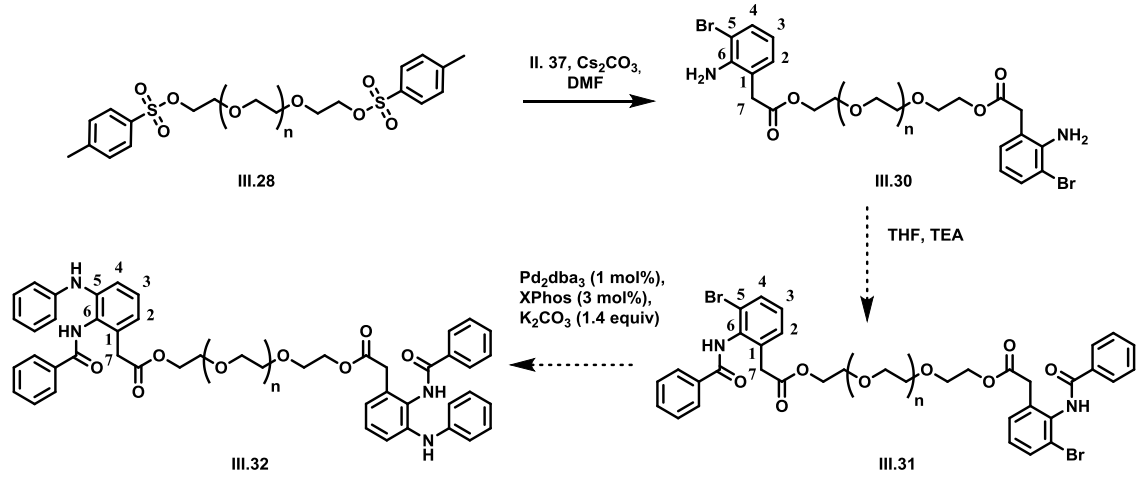
Scheme III.9. Synthesis of tosylated derivative **III.28**.

Hence, it was attempted to achieve compound **III.29** by nucleophilic attack of the carboxylate of compound **II.56** to **III.28**, in the presence of Cs_2CO_3. However, the ^1H NMR of the crude mixture revealed that the major compound was the unreacted PEG polymer, only containing a trace amount of PEG-bound product (Scheme III.10). This observation could be attributed to the presence of hydrogen bridges between the acid and the nitro group, which makes the carboxylate less reactive, thus hindering the reaction.



Scheme III.10. Coupling failure using intermediate **II.56**.

Thus, it was used the amine derivative **II.37** (*see* Chapter II), that was reacted with PEG in the presence of Cs_2CO_3 to afford compound **III.30** (Scheme III.11). However, this product was very difficult to precipitate (even using other solvents such as pentane or hexane) being obtained a brown slurry. This fact could be attributed to the increase of the organic character of the molecule or due to some impurities retained in the polymeric structure that prevents the polymer to precipitate. After several washing steps with cold diethyl ether, a small portion of a light brown solid was attained to further characterization of compound **III.30**.



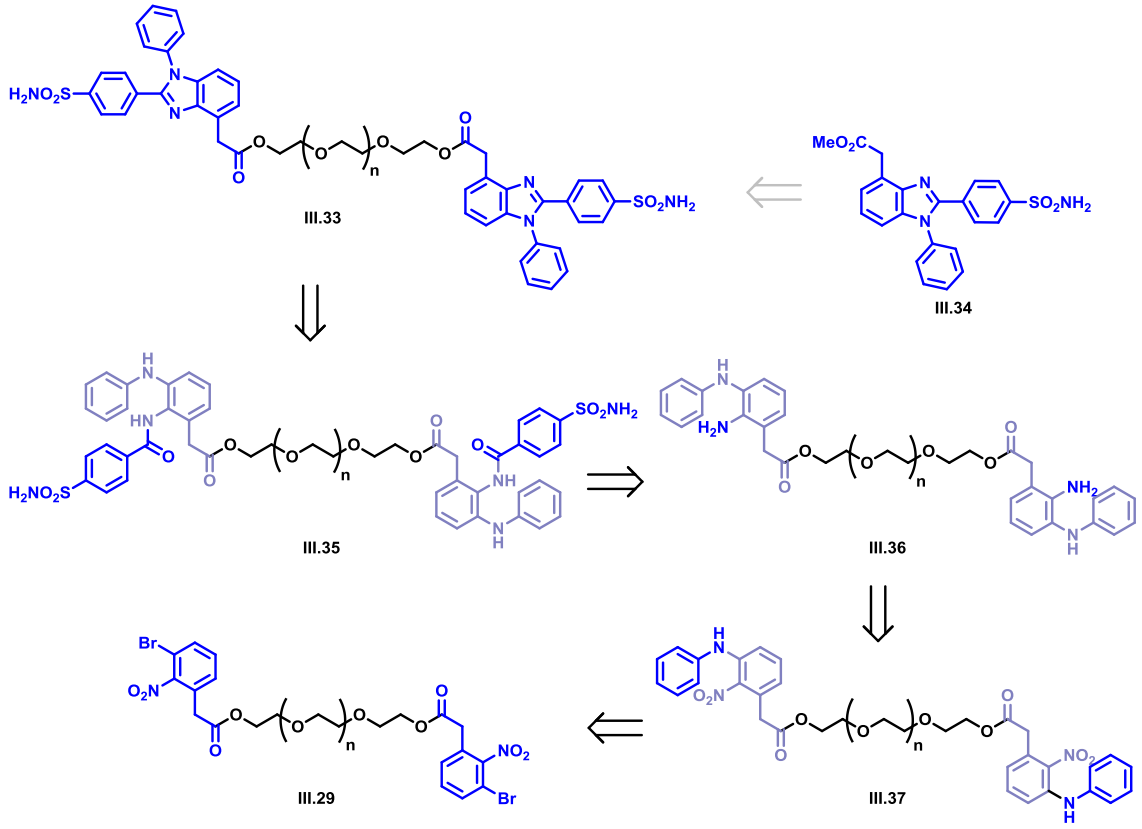
Scheme III.11. Envisioned strategy to prepare compound **III.32**.

Due to the difficult product precipitation, it was envisioned the synthetic progress to *N*-benzoylated compound **III.31** with the compound mixture. Thus, the reaction was repeated and the compound **III.30** slurry was dissolved in THF and reacted with benzoyl chloride in the presence of TEA. Even though the ^{1}H NMR of the crude mixture revealed the *N*-benzoylation product (along other side products) once more the product could not be precipitated as a solid.

Despite the mixture obtained, the next step was attempted. The slurry corresponding to compound **III.31** was further subjected to cross coupling conditions, but in this case the ^{1}H NMR showed a complex mixture of compounds along with free PEG since the intensity of the aromatic signals decreased.

III.3 Conclusions

In order to construct the benzimidazole structures on PEG and obtain a pegylated library, it is necessary to overcome the precipitation issues observed in the preliminary experiments. The use of a PEG polymer with an increased molecular weight, such as PEG 6000, can surpass the lower MWs PEG derivatives while maintaining the same synthetic properties.



Scheme III.12. Proposed retrosynthetic approach to attain the desired PEG bound benzimidazoles.

On the other hand, it would be highly valuable the use of the key intermediate **II.56** containing the nitro group, due to its enhanced properties towards the Pd-catalyzed cross-coupling reaction. Despite the demonstrated issues related to its coupling to PEG, an optimization stage can have a successful outcome. An alternative strategy to attain pegylated benzimidazoles is depicted in Scheme III.12.

Having the compound **III.29** in hands, the Pd-catalyzed amination would be a successful task to attain compound **III.37**. A critical step could be the reduction of nitro group to amine (to obtain **III.36**) and an exhaustive screening of reaction conditions could be necessary in this synthetic stage. The next step involves the benzoylation of **III.36** that can be performed under basic conditions to obtain **III.35**. It is noteworthy, that the absence of base will promote both ring closing and the detachment of the benzimidazole from the polymer. However in this last case the

corresponding ester derivatives would not be achieved. Thus, in order to attain the benzimidazole bound to PEG (**III.33**) a careful analysis of reaction conditions need to be studied. The synthesized benzimidazoles (**III.34**) could be further detached from PEG using the transesterification procedure under MeOH/KCN conditions.

III.4 Experimental

III.4.1 General Information:

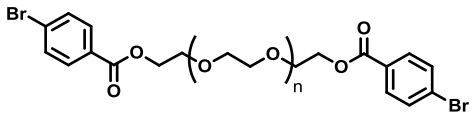
All commercially obtained reagents were used without further purification unless specified. PEG 2000 was purchased from Fluka as white flakes. Pyrrolidine, aniline, 2,5-dimethylaniline were previously distilled. Isopropanol was dried by usual methods.

NMR spectra (400 MHz for ^{1}H and 100 MHz for ^{13}C) were recorded with a Brucker ARX 400 spectrometer using $CDCl_3$ as solvent using the corresponding $CHCl_3$ signal as reference. IR spectra were recorded using a Perkin-Elmer Spectrum 1000 FT-IR. The IR bands are classified as strong (s), medium (m), or weak (w).

III.4.2 General procedure for the preparation of PEG supported aryl halides:

To the corresponding benzoic acid halide (4 equiv) in DMF (0.25 M) was added DIC (4 equiv) and the solution was stirred for 30 min. at room temperature. Then it was added PEG 2000 (1 equiv) and DMAP (0.4 equiv). The mixture was stirred at 50ºC overnight. The mixture was filtered over a celite pad in order to remove the formed urea and the solution was placed in an ice bath. The product was precipitated by the addition of cold diethyl ether. The product was filtered and washed several times with cold diethyl ether in order to remove the DMF. The products were obtained as white solids and were vacuum dried and stored in a desiccator. The products were obtained in quantitative yield, and characterized by IR and NMR spectra.

III.4.2.1 PEG-bis(4-bromobenzoate) (III.5)

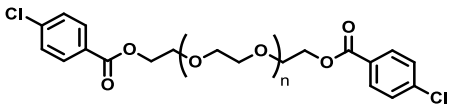


IR (KBr) ν_{max} (cm^{-1}): 2872 (m), 1720 (s, *C*=*O*), 1105 (m);

^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.91 (d, *J* = 8.5 Hz, Ar*H*, 4H), 7.57 (d, *J* = 8.5 Hz, Ar*H*, 4H), 4.47 – 4.44 (m, CH_2 PEG, 4H), 3.83 – 3.63 (m, CH_2 PEG, 192H);

^{13}C NMR (100 MHz, $CDCl_3$) δ: 165.9 (*C*=O), 131.8 (*C*Ar), 131.4 (*C*Ar), 129.1 (*C*-q), 128.2 (*C*-q), 70.8 (*C*H_2 PEG), 70.8 (*C*H_2 PEG), 70.7 (*C*H_2 PEG), 69.2 (*C*H_2 PEG), 64.5 (*C*H_2 PEG).

III.4.2.2 PEG-bis(4-chlorobenzoate) (III.6)

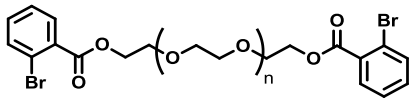


IR (KBr) ν_{max} (cm^{-1}): 2875 (m), 1718 (s, *C*=*O*), 1638 (m), 1104 (m);

^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.98 (d, *J* = 8.5 Hz, Ar*H*, 4H), 7.41 (d, *J* = 8.5 Hz, Ar*H*, 4H), 4.47 – 4.44 (m, C*H*$_2$ PEG, 4H), 3.83 – 3.45 (m, C*H*$_2$ PEG, 216H);

^{13}C NMR (100 MHz, $CDCl_3$) δ: 165.8 (*C*=O), 139.5 (*C*-q), 131.2 (*C*-Ar), 128.8 (*C*-Ar), 128.7 (C-q), 70.8 (*C*H_2 PEG), 70.7 (*C*H_2 PEG), 70.7 (*C*H_2 PEG), 69.3 (*C*H_2 PEG), 64.4 (*C*H_2 PEG).

III.4.2.3 PEG-bis(4-chlorobenzoate) (III.7)

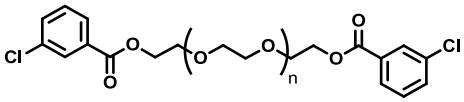


IR (KBr) ν_{max} (cm^{-1}): 2836 (m), 1730 (s, *C*=*O*), 1655 (m), 1114 (m);

^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.79 (dd, *J* = 7.5, 1.9 Hz, Ar*H*, 2H), 7.63 (dd, *J* = 7.7, 1.4 Hz, Ar*H*, 2H), 7.36 – 7.28 (m, Ar*H*, 4H), 4.47 – 4.44 (m, PEG, 4H), 3.81 – 3.43 (m, PEG, 190H);

^{13}C NMR (100 MHz, $CDCl_3$) δ: 166.1 (C=O), 134.3 (*C*Ar), 132.6 (*C*Ar), 132.1 (*C*Ar), 131.5 (*C*Ar), 127.2 (*C*Ar), 121.7 (*C*Ar), 70.7 (CH_2 PEG), 70.6 (CH_2 PEG), 70.6 (CH_2 PEG), 69.0 (CH_2 PEG), 64.6 (CH_2 PEG).

III.4.2.4 PEG-bis(3-chlorobenzoate) (III.8)



IR (KBr) ν_{max} (cm^{-1}): 2882 (m), 1723 (s, *C*=*O*), 1467 (m), 1115 (m);

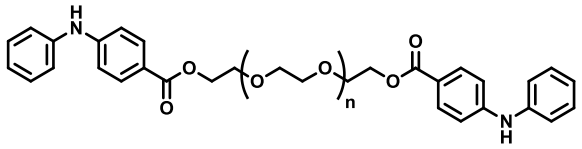
^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.90 (m, Ar*H*, 2H), 7.88 (d, *J* = 7.8 Hz, Ar*H*, 2H), 7.49 – 7.47 (m, Ar*H*, 2H), 7.34 (t, *J* = 7.9 Hz, Ar*H*, 2H), 4.48 – 4.46 (m, C*H*$_2$ PEG, 4H), 3.78 – 3.59 (m, PEG, 208H).

^{13}C NMR (100 MHz, $CDCl_3$) δ: 165.3 (*C*=O), 134.4 (*C*-q), 133.0 (*C*Ar), 131.8 (*C*-q), 129.7 (*C*Ar), 129.7 (*C*Ar), 127.8 (*C*Ar), 70.6 (*C*H_2 PEG), 70.6 (*C*H_2 PEG), 70.5 (*C*H_2 PEG), 69.0 (*C*H_2 PEG), 64.4 (*C*H_2 PEG).

III.4.3 General procedure for Pd-catalyzed amination on PEG

To a screw-cap test-tube equipped with a magnetic stir bar, was added the PEG supported aryl halide (0,1 mmol), Pd_2dba_3 (1 mol %), ligand (3 mol %) and base (1.4 equiv). The vial was sealed with a suba-seal, evacuated and backfilled with argon. Then was added the amine (1.4 equiv) and the suba-seal was replaced by the teflon screw-cap. The reaction mixture was heated at the desired temperature for 8 h (aryl amines) or 20 h (alkyl amines) (see tables). The solution was allowed to cool to room temperature, dissolved with dried isopropanol and filtered over a celite pad. The solution was placed in an ice bath and the product was precipitated by the addition of cold diethyl ether. The product was washed several times with cold diethyl ether. The obtained product was dried over vacuum and stored in a desiccator.

III.4.3.1 PEG-bis(4-(phenylamino)benzoate) (III.9)

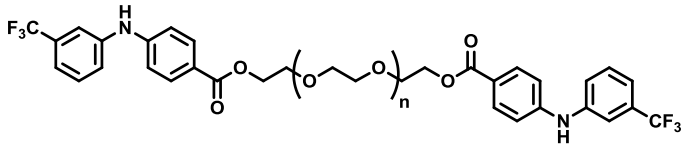


IR (KBr) ν_{max} (cm^{-1}): 2873 (m), 1705 (s, *C=O*), 1591 (m), 1104 (m);

^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.91 (d, *J* = 8.8 Hz, Ar*H*, 4H), 7.31 (t, *J* = 7.9 Hz, Ar*H*, 4H), 7.17 (d, *J* = 7.6 Hz, Ar*H*, 4H), 7.03-7.00 (m, Ar*H*, 6H), 4.43 – 4.41 (m, CH_2 PEG, 4H), 3.82 – 3.63 (m, CH_2 PEG, 218H);

^{13}C NMR (100 MHz, $CDCl_3$) δ: 166.5 (*C*=O), 148.2 (*C*-q), 141.1 (*C*-q), 131.7 (*C*Ar), 129.5 (*C*Ar), 123.1 (*C*Ar), 120.9 (*C*Ar), 120.4 (*C*Ar), 114.8 (*C*Ar), 70.8 (*C*H_2 PEG), 70.7 (CH_2 PEG), 70.6 (*C*H_2 PEG), 69.5 (*C*H_2 PEG), 63.8 (*C*H_2 PEG).

III.4.3.2 PEG-bis(4-(3-(trifluoromethyl)phenylamino)benzoate) (III.10)

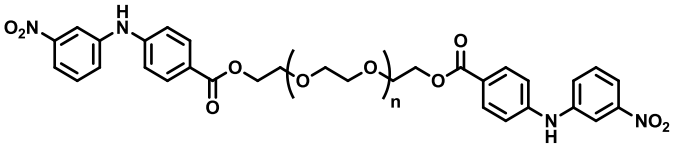


IR (KBr) ν_{max} (cm^{-1}): 2873 (m), 1706 (s, *C=O*), 1596 (m);

^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.96 (d, *J* = 8.6 Hz, Ar*H*, 4H), 7.40 – 7.39 (m, Ar*H*, 6H), 7.23 (d, *J* = 7.5 Hz, Ar*H*, 2H), 7.07 (d, *J* = 8.7 Hz, Ar*H*, 4H), 4.45 – 4.42 (m, CH_2 PEG, 4H), 3.82 – 3.60 (m, CH_2 PEG, 215H);

^{13}C NMR (100 MHz, $CDCl_3$) δ: 166.3 (*C*=O), 147.2 (*C*-q), 142.2 (*C*-q), 131.8 (*C*Ar), 130.1 (*C*Ar), 122.3 (*C*Ar), 122.1 (C-q), 118.8 (*C*Ar), 115.8 (*C*Ar), 115.6 (*C*Ar), 70.9 (*C*H_2 PEG), 70.8 (*C*H_2 PEG), 70.7 (*C*H_2 PEG), 69.5 (*C*H_2 PEG), 63.9 (*C*H_2 PEG).

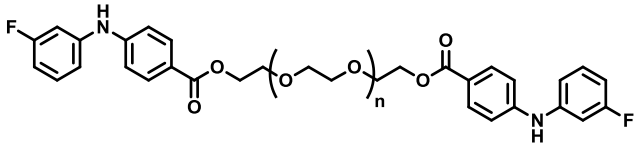
III.4.3.3 PEG-bis(4-(3-nitrophenylamino)benzoate) (III.11)



^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.00 – 7.97 (m, Ar*H*, 6H), 7.79 (d, *J* = 7.8 Hz, Ar*H*, 2H), 7.49 – 7.39 (m, Ar*H*, 4H), 7.13 (d, *J* = 8.7 Hz, Ar*H*, 4H), 4.46 – 4.43 (m, C*H*$_2$ PEG, 4H), 3.83 – 3.59 (m, C*H*$_2$ PEG, 192H).

^{13}C NMR (100 MHz, $CDCl_3$) δ: 166.2 (*C*=O), 149.4 (*C*Ar), 146.5 (*C*Ar), 143.2 (*C*Ar), 131.8 (*C*Ar), 130.3 (*C*Ar), 124.2 (*C*Ar), 122.8 (*C*Ar), 116.3 (*C*Ar), 116.2 (*C*Ar), 112.8 (*C*Ar), 70.9 (*C*H$_2$ PEG), 70.8 (*C*H$_2$ PEG), 70.7 (*C*H$_2$ PEG), 69.4 (*C*H$_2$ PEG), 64.0 (*C*H$_2$ PEG).

III.4.3.4 PEG-bis(4-(3-fluorophenylamino)benzoate) (III.12)

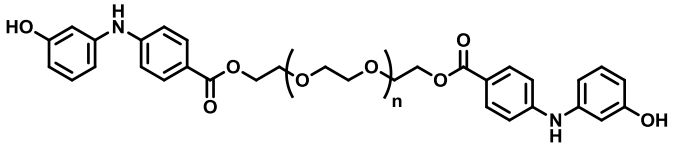


IR (KBr) ν_{max} (cm^{-1}): 2871 (m), 1705 (s, *C*=*O*), 1597 (m), 1106 (m);

^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.94 (d, *J* = 8.7 Hz, Ar*H*, 4H), 7.26 – 7.21 (m, Ar*H*, 2H), 7.06 (d, *J* = 8.7 Hz, Ar*H*, 4H), 6.93 – 6.87 (m, Ar*H*, 4H), 6.68 (td, *J* = 8.5, 2.1 Hz, Ar*H*, 2H), 4.44 – 4.42 (m, C*H*$_2$ PEG, 4H), 3.82 – 3.60 (m, C*H*$_2$ PEG, 210H);

^{13}C NMR (100 MHz, $CDCl_3$) δ: 166.4 (*C*=O), 163.7 (d, *J* = 245.0 Hz, *C*-q), 147.3 (*C*-q), 143.2 (d, *J* = 10.3 Hz, *C*-q), 131.7 (2x *C*Ar), 130.6 (d, *J* = 9.8 Hz, *C*Ar), 121.8 (*C*-q), 115.6 (2x *C*Ar), 114.8 (d, *J* = 2.7 Hz, *C*Ar), 109.0 (d, *J* = 21.3 Hz, *C*Ar), 106.2 (d, *J* = 24.5 Hz, *C*Ar), 70.9 (*C*H$_2$ PEG), 70.8 (*C*H$_2$ PEG), 70.7 (*C*H$_2$ PEG), 69.5 (*C*H$_2$ PEG), 63.9 (*C*H$_2$ PEG).

III.4.3.5 PEG-bis(4-(3-hydroxyphenylamino)benzoate) (III.13)

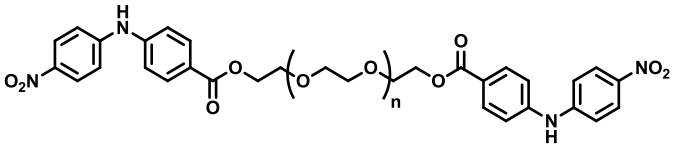


IR (KBr) ν_{max} (cm^{-1}): 2873 (m), 1702 (s, *C*=*O*), 1596 (m), 1105 (m).

^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.89 (d, *J* = 8.7 Hz, Ar*H*, 4H), 7.11 (t, *J* = 8.0 Hz, Ar*H*, 2H), 7.03 (d, *J* = 8.7 Hz, Ar*H*, 4H), 6.73 – 6.68 (m, Ar*H*, 4H), 6.52 (dd, *J* = 8.1, 1.7 Hz, Ar*H*, 2H), 4.43 – 4.41 (m, C*H*$_2$ PEG, 4H), 3.81 – 3.58 (m, C*H*$_2$ PEG, 220H);

^{13}C NMR (100 MHz, $CDCl_3$) δ: 166.6 (*C*=O), 158.0 (*C*-q), 148.5 (*C*-q), 142.4 (*C*-q), 131.6 (2x *C*Ar), 130.2 (*C*Ar), 120.5 (*C*-q), 114.9 (*C*Ar), 111.4 (*C*Ar), 110.2 (*C*Ar), 107.4 (*C*Ar), 71.0 (*C*H_2 PEG), 70.8 (*C*H_2 PEG), 70.6 (*C*H_2 PEG), 69.5 (*C*H_2 PEG), 63.8 (*C*H_2 PEG).

III.4.3.6 PEG-bis(4-chlorobenzoate) (III.14)

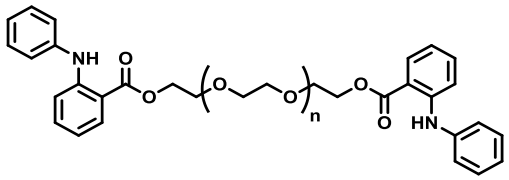


Obs. Product did not precipitate with diethyl ether. It was only observed by ^{1}H NMR along with the *p*-nitro aniline and unreacted starting material.

^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.06 (d, *J* = 9.1 Hz, Ar*H*, 4H), 7.93 (d, *J* = 8.6 Hz, Ar*H*, 4H), 7.31 (d, *J* = 8.7 Hz, Ar*H*, 4H), 7.27 (m, Ar*H*, 4H), 4.42 – 4.40 (m, CH_2 PEG, 4H), 3.80 – 3.57 (m, CH_2 PEG, 218H);

^{13}C NMR (100 MHz, $CDCl_3$) δ: 166.2 (*C*=O), 131.3 (*C*Ar), 131.2 (*C*Ar), 125.8 (*C*Ar), 125.8 (*C*Ar), 118.2 (*C*Ar), 115.7 (*C*Ar), 70.6 (*C*H_2 PEG), 70.3 (*C*H_2 PEG), 69.3 (*C*H_2 PEG), 63.8 (*C*H_2 PEG).

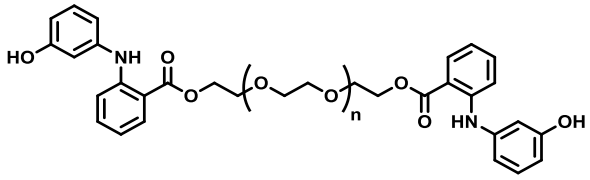
III.4.3.7 PEG-bis(2-(phenylamino)benzoate) (III.15)



Obs. It was observed by ^{1}H NMR along with the unreacted starting material.

^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.40 (s, N*H*, 2H), 7.99 (d, *J* = 8.1 Hz, Ar*H*, 2H), 7.34 –7.26 (m, Ar*H*, 12H), 7.08 (t, *J* = 7.4 Hz, 2H), 6.72 (t, *J* = 7.4 Hz, 2H), 4.46 (m, CH_2 PEG, 4H), 3.84 – 3.45 (m, CH_2 PEG, 300 H).

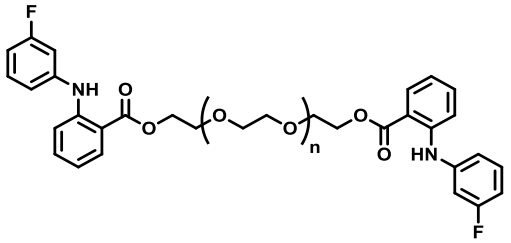
III.4.3.8 PEG-bis(2-((3-hydroxyphenyl)amino)benzoate) (III.17)



Obs. It was observed by ^{1}H NMR along with the unreacted starting material.

^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.19 (s, N*H*, 2H), 7.93 (t, *J* = 6.6 Hz, Ar*H*, 2H), 7.36-7.21 (m, 6H), 7.10 – 7.03 (m, 2H), 6.76 (s, Ar*H*, 2H), 6.65 – 6.53 (m, 4H), 4.53 – 4.41 (m, CH_2 PEG, 4H), 3.88 – 3.48 (m, CH_2 PEG, 254 H).

III.4.3.9 PEG-bis(2-((3-fluorophenyl)amino)benzoate) (III.18)

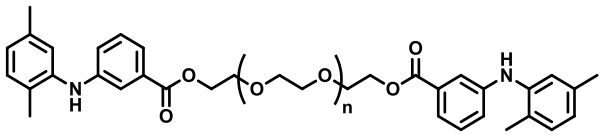


Obs. It was observed by ^{1}H NMR along with the unreacted starting material.

^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.45 (s, N*H*, 2H), 8.00 (d, *J* = 8.0 Hz, Ar*H*, 2H), 7.34 –7.26 (m, Ar*H*, 6H), 6.99–6.76 (m, Ar*H*, 4H), 6.81–6.72 (m, Ar*H*, 4H), 4.46 (t, *J* = 11.0, 6.4 Hz, CH_2 PEG, 4H), 3.84 – 3.45 (m, CH_2 PEG, 246 H);

^{13}C NMR (100 MHz, $CDCl_3$) δ: 168.3 (*C*=O), 163.6 (d, *J* = 245.3 Hz, *C*Ar), 146.9 (*C*Ar), 142.9 (d, *J* = 10.2 Hz, *C*Ar), 134.3 (*C*Ar), 132.0 (*C*Ar), 130.6 (d, *J* = 9.8 Hz, *C*Ar), 118.2 (*C*Ar), 117.2 (d, *J* = 2.7 Hz, *C*Ar), 114.7 (*C*Ar), 113.0 (*C*Ar), 109.8 (d, *J* = 21.3 Hz, *C*Ar), 108.3 (d, *J* = 23.9 Hz, *C*Ar), 70.8 (CH_2 PEG), 70.7 (CH_2 PEG), 70.7 (CH_2 PEG), 69.3 (CH_2 PEG), 64.0 (CH_2 PEG).

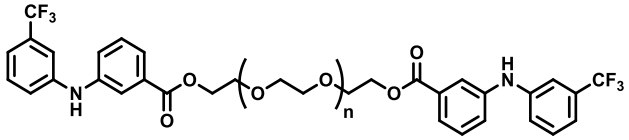
III.4.3.10 PEG-bis(3-(2,5-dimethylphenylamino)benzoate) (III.19)



^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.56 – 7.52 (m, Ar*H*, 4H), 7.26 (m, Ar*H*, 2H), 7.10 – 7.03 (m, Ar*H*, 6H), 6.80 (d, *J* = 7.5 Hz, Ar*H*, 2H), 4.46 – 4.43 (m, CH_2 PEG, 4H), 3.82 – 3.64 (m, CH_2 PEG, 250H), 2.27 (s, CH_3, 6H), 2.19 (s, CH_3, 6H);

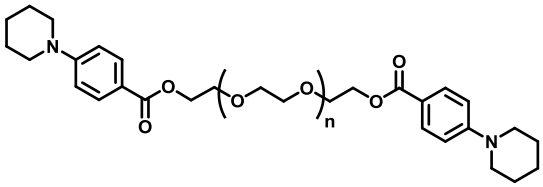
^{13}C NMR (100 MHz, $CDCl_3$) δ: 166.8 (*C*=O), 144.8 (*C*Ar), 140.3 (*C*Ar), 136.7 (*C*Ar), 131.3 (*C*Ar), 131.0 (*C*Ar), 129.3 (*C*Ar), 126.5 (*C*Ar), 123.9 (*C*Ar), 121.2 (*C*Ar), 120.8 (*C*Ar), 120.8 (*C*Ar), 118.0 (*C*Ar), 70.8 (*C*H_2 PEG), 70.7 (*C*H_2 PEG), 69.4 (*C*H_2 PEG), 64.3 (*C*H_2 PEG), 21.3 (*C*H_3), 17.6 (*C*H_3).

III.4.3.11 PEG-bis(3-((3-(trifluoromethyl)phenyl)amino)benzoate) (III.20)



^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.76 (s, Ar*H*, 2H), 7.64 (d, *J* = 7.2 Hz, Ar*H*, 2H), 7.39 – 7.23 (m, Ar*H*, 10H), 7.14 (d, *J* = 7.6 Hz, Ar*H*, 2H), 6.52 (s, Ar*H*, 2H), 4.51 – 4.42 (m, CH_2 PEG, 4H), 3.84 – 3.46 (m, CH_2 PEG, 310 H).

III.4.3.12 PEG-bis(4-(piperidin-1-yl)benzoate) (III.24)

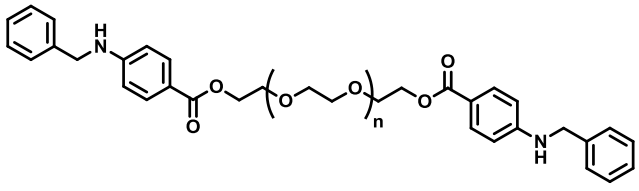


IR (KBr) ν_{max} (cm^{-1}): 2873 (m), 1700 (s, *C*=*O*), 1603 (m), 1104 (m);

^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.88 (d, *J* = 8.6 Hz, Ar*H*, 4H), 6.82 (d, *J* = 8.6 Hz, Ar*H*, 4H), 4.40 – 4.38 (m, CH_2 PEG, 4H), 3.78 – 3.62 (m, CH_2 PEG, 205H), 3.30 (m, CH_2, 8H), 1.63 (m, CH_2, 12H);

^{13}C NMR (100 MHz, $CDCl_3$) δ: 166.7 (*C*=O), 154.6 (*C*Ar), 131.4 (*C*Ar), 118.6 (*C*Ar), 113.6 (*C*Ar), 70.7 (CH_2 PEG), 70.6 (CH_2 PEG), 69.5 (CH_2 PEG), 63.6 (CH_2 PEG), 48.8 (*C*H_2), 25.4 (*C*H_2), 24.4 (*C*H_2).

III.4.3.13 PEG-bis(4-(benzylamino)benzoate) (III.25)

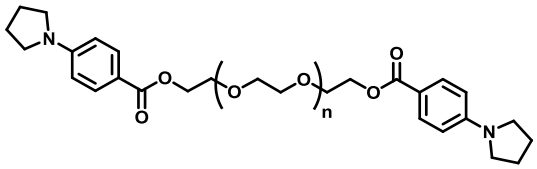


IR (KBr) ν_{max} (cm^{-1}): 2874 (m), 1698 (s, *C*=*O*), 1604 (m), 1104 (m);

^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.85 (d, *J* = 8.7 Hz, Ar*H*, 4H), 7.33 (m, Ar*H*, 10H), 6.57 (d, *J* = 8.7 Hz, Ar*H*, 4H), 4.37 (m, CH_2 benzylic, CH_2 PEG, 8H), 3.79 – 3.62 (m, CH_2 PEG, 282H);

^{13}C NMR (100 MHz, $CDCl_3$) δ: 166.7 (*C*=O), 152.0 (*C*-q), 138.5 (*C*-q), 131.7 (*C*Ar), 128.8 (*C*Ar), 128.6 (*C*Ar), 127.5 (*C*Ar), 127.4 (*C*Ar), 127.2 (*C*-q), 126.9 (*C*Ar), 111.7 (*C*Ar), 70.7 (*C*H_2 PEG), 70.6 (*C*H_2 PEG), 70.4 (*C*H_2 PEG), 69.4 (*C*H_2 PEG), 63.5 (*C*H_2 PEG), 47.6 (*C*H_2).

III.4.3.14 PEG-bis(4-(pyrrolidin-1-yl)benzoate) (III.26)



IR (KBr) ν_{max} (cm^{-1}): 2871 (m), 1696 (s, *C*=*O*), 1605 (m), 1106 (m);

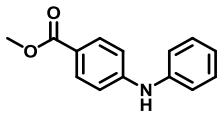
^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.89 (d, *J* = 8.6 Hz, Ar*H*, 4H), 6.49 (d, *J* = 8.6 Hz, Ar*H*, 4H), 4.40 (m, PEG CH_2, 4H), 3.81 – 3.63 (m, PEG CH_2, 240H), 3.34 (m, CH_2, 8H), 2.02 (m, CH_2, 8H);

^{13}C NMR (100 MHz, $CDCl_3$) δ: 167.2 (*C*=O), 151.0 (*C*Ar), 131.6 (*C*Ar), 116.2 (*C*Ar), 110.7 (*C*Ar), 70.8 (*C*H_2 PEG), 70.6 (*C*H_2 PEG), 69.6 (*C*H_2 PEG), 63.5 (*C*H_2 PEG), 47.6 (*C*H_2), 25.6 (*C*H_2).

III.4.4 General procedure for cleavage of compounds from the polymer support:[27]

The PEG bound compound (1 equiv) was dissolved in methanol and a 1 M solution of potassium cyanide (2 equiv) in methanol was added. The mixture was stirred at room temperature for 12 h. After completion of reaction, the inorganic material was removed by filtration and then filtrate was concentrated under reduced pressure. The polymer was precipitated out with excess of cold ether and removed by filtration. The filtrate was evaporated to dryness to obtain white products in good yields (70 - 90%). The spectral data is according to literature.

III.4.4.1 Methyl 4-(phenylamino)benzoate (III.21)[49]

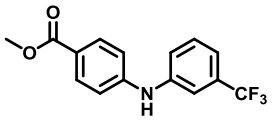


MP: 115-117°C;

IR (NaCl, cm^{-1}): 3334 (m), 1695 (s, *C*=*O*), 1590 (m);

^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.91 (d, *J* = 8.4 Hz, Ar*H*, 2H), 7.34 (m, Ar*H*, 2H), 7.17 (d, *J* = 7.9 Hz, Ar*H*, 2H), 7.06 (t, *J* = 7.3 Hz, Ar*H*, 1H), 6.99 (d, *J* = 8.4 Hz, Ar*H*, 2H), 6.04 (bs, N*H*, 1H), 3.87 (s, CH_3, 3H).

III.4.4.2 Methyl 4-(3-(trifluoromethyl)phenylamino)benzoate (III.22)[50]

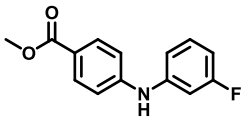


MP: 124-126 ºC;

IR (NaCl, cm^{-1}): 3344 (m), 1694 (s, *C=O*), 1593 (s), 1338 (m);

^{1}H NMR (400 MHz, CDCl$_3$) δ: 7.96 (d, *J* = 8.3 Hz, Ar*H*, 2H), 7.47 – 7.22 (m, Ar*H*, 4H), 7.03 (d, *J* = 8.5 Hz, Ar*H*, 2H), 6.21 (bs, N*H*, 1H), 3.89 (s, CH_3, 3H).

III.4.4.3 Methyl 4-(3-fluorophenylamino)benzoate (III.23)[51]



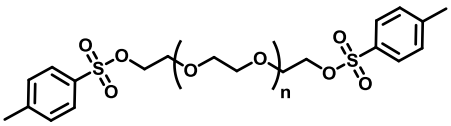
MP: 129-131ºC;

IR (NaCl, cm^{-1}): 3337 (m), 1693 (s, *C=O*), 1597 (m);

^{1}H NMR (400 MHz, CDCl$_3$) δ: 7.94 (d, *J* = 8.5 Hz, Ar*H*, 2H), 7.31 – 7.22 (m, Ar*H*, 1H), 7.04 (d, *J* = 8.5 Hz, Ar*H*, 2H), 6.95 – 6.86 (m, Ar*H*, 2H), 6.72 (t, *J* = 8.3 Hz, Ar*H*, 1H), 3.88 (s, CH_3, 3H).

III.4.5 Other synthesis

III.4.5.1 PEG-bis(4-methylbenzenesulfonate) (III.28)



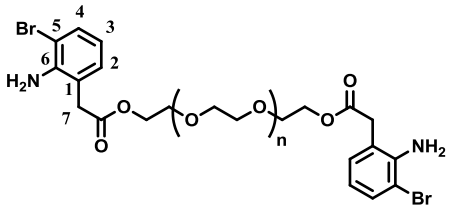
To a PEG 2000 (2 g, 2 mmol) solution in DCM (20 mL) was added triethylamine (0.28 mL, 2 mmol). The flask was placed in an ice bath and TsCl (382 mg, 2 mmol) and DMAP (25 mg, 0.2 mmol) were added. The mixture was allowed to slowly rise to room temperature and stirred at this temperature overnight. The mixture was washed twice with saturated NH_4Cl solution, water, dried over Na_2SO_4 and evaporated to dryness to obtain the compound as a white solid (quant.).

IR (KBr) ν_{max} (cm^{-1}): 3448 (m), 2885 (w), 1638 (s), 1467 (s), 1345 (m), 1115 (m);

^{1}H NMR (400 MHz, CDCl$_3$) δ: 7.76 (d, *J* = 7.9 Hz, Ar*H*, 4H), 7.31 (d, *J* = 7.9 Hz, Ar*H*, 4H), 4.11 (t, *J* = 4.5 Hz, PEG CH_2, 4H), 3.65 – 3.54 (m, PEG CH_2, 210H), 2.41 (s, CH_3, 6H).

^{13}C NMR (100 MHz, CDCl$_3$) δ: 144.8 (*C*Ar), 133.0 (*C*Ar), 129.8 (2x*C*Ar), 128.0 (2x*C*Ar), 70.7 (*C*H_2 PEG), 70.5 (*C*H_2 PEG), 69.2 (*C*H_2 PEG), 68.6 (*C*H_2 PEG), 21.6 (*C*H_3).

III.4.5.2 PEG-bis(2-amino-3-bromophenyl)-3,6,9,12-tetraoxatetradecane-1,14-dione (III.30)



To a PEG tosyl (100 mg, 0.1 mmol) solution in DMF (0.5 mL) was added Cs_2CO_3 (35 mg, 0.1 mmol) and **II.39** (25 mg, 0.11 mmol). The mixture was stirred at room temperature overnight. The mixture was filtered over a celite pad and evaporated to dryness. Then it was dissolved in propanol and precipitated and washed with cold ether, to obtain the compound as a white solid (60 mg, %).

IR (KBr) ν_{max} (cm^{-1}): 3448 (m), 2887 (w), 1736 (s, *C=O*), 1467 (m), 1343 (m), 1148 (m), 1114 (m), 1061 (m), 963 (w), 842 (w);

^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.34 (d, *J* = 7.9 Hz, Ar*H*, 2H), 7.03 (d, *J* = 7.5 Hz, Ar*H*, 2H), 6.57 (t, *J* = 7.7 Hz, Ar*H*, 2H), 4.59 (s, CH_2, 4H), 4.28 – 4.19 (m, PEG CH_2, 4H), 3.84 – 3.42 (m, PEG CH_2, 340H).

^{13}C NMR (100 MHz, $CDCl_3$) δ: 171.3 (C=O), 143.5 (C-6), 132.0 (C-4), 130.5 (C-2), 120.5 (C-1), 119.2 (C-3), 111.0 (C-5), 72.74 (*C*H_2 PEG), 70.7 (*C*H_2 PEG), 70.4 (*C*H_2 PEG), 68.9 (CH_2 PEG), 64.4 (*C*H_2 PEG), 61.8 (*C*H_2 PEG), 39.2 (C-7).

III.5 Bibliography

(1) G. Pasut, F. M. Veronese. "State of the art in PEGylation: the great versatility achieved after forty years of research." *J. Control. Release* **2012**, *161*, 461-472.

(2) E. Markovsky, H. Baabur-Cohen, A. Eldar-Boock, L. Omer, G. Tiram, S. Ferber, P. Ofek, D. Polyak, A. Scomparin, R. Satchi-Fainaro. "Administration, distribution, metabolism and elimination of polymer therapeutics." *J. Control. Release* **2012**, *161*, 446-460.

(3) P. Caliceti, F. M. Veronese. "Pharmacokinetic and biodistribution properties of poly(ethylene glycol)–protein conjugates." *Adv. Drug Del. Rev.* **2003**, *55*, 1261-1277.

(4) J. S. Kang, P. P. Deluca, K. C. Lee. "Emerging PEGylated drugs." *Expert Opin. Emerg. Drugs* **2009**, *14*, 363-380.

(5) M. A. Siyad, G. S. V. Kumar. "Poly(ethylene glycol) grafted polystyrene dendrimer resins: Novel class of supports for solid phase peptide synthesis." *Polymer* **2012**, *53*, 4076-4090.

(6) M. Zacchigna, F. Cateni, S. Drioli, G. M. Bonora. "Multimeric, Multifunctional Derivatives of Poly(ethylene glycol)." *Polymers-Basel* **2011**, *3*, 1076-1090.

(7) a) C. J. Ball, M. C. Willis. "Cascade Palladium- and Copper-Catalysed Aromatic Heterocycle Synthesis: The Emergence of General Precursors." *Eur. J. Org. Chem.* **2013**, *2013*, 425-441; b) S. H. Cho, J. Y. Kim, J. Kwak, S. Chang. "Recent advances in the transition metal-catalyzed twofold oxidative C-H bond activation strategy for C-C and C-N bond formation." *Chem. Soc. Rev.* **2011**, *40*, 5068-5083.

(8) C. Gil, S. Brase. "Solid-phase synthesis of biologically active benzoannelated nitrogen heterocycles: an update." *J. Comb. Chem.* **2009**, *11*, 175-197.

(9) L. R. Carvalho, M. C. Corvo, R. Enugala, M. M. B. Marques, E. J. Cabrita. "Application of HR-MAS NMR in the solid-phase synthesis of a glycopeptide using Sieber amide resin." *Magn. Reson. Chem.* **2010**, *48*, 323-330.

(10) E. Colacino, J. Martinez, F. Lamaty, L. S. Patrikeeva, L. L. Khemchyan, V. P. Ananikov, I. P. Beletskaya. "PEG as an alternative reaction medium in metal-mediated transformations." *Coord. Chem. Rev.* **2012**, *256*, 2893-2920.

(11) a) D. J. Gravert, K. D. Janda. "Organic Synthesis on Soluble Polymer Supports: Liquid-Phase Methodologies." *Chem. Rev.* **1997**, *97*, 489-510; b) P. H. Toy, K. D. Janda. "Soluble Polymer-Supported Organic Synthesis." *Acc. Chem. Res.* **2000**, *33*, 546-554.

(12) J. Etxebarria, S. Serna, A. Beloqui, M. Martin-Lomas, N.-C. Reichardt. "Three-Dimensional Arrays Using GlycoPEG Tags: Glycan Synthesis, Purification and Immobilisation." *Chem. Eur. J.* **2013**, *19*, 4776-4785.

(13) C. Stutz, A. Meszynska, J.-F. Lutz, H. G. Börner. "Convenient Routes to Efficiently N-PEGylated Peptides." *ACS Macro Lett.* **2013**, *2*, 641-644.

(14) W. J. Li, P. Zhan, E. De Clercq, H. X. Lou, X. Y. Liu. "Current drug research on PEGylation with small molecular agents." *Prog. Polym. Sci.* **2013**, *38*, 421-444.

(15) E. Bayer, M. Mutter. "Liquid phase synthesis of peptides." *Nature* **1972**, *237*, 512-513.

(16) F. Garcia-Martin, M. Quintanar-Audelo, Y. Garcia-Ramos, L. J. Cruz, C. Gravel, R. Furic, S. Cote, J. Tulla-Puche, F. Albericio. "ChemMatrix, a poly(ethylene glycol)-based support for the solid-phase synthesis of complex peptides." *J. Comb. Chem.* **2006**, *8*, 213-220.

(17) R. Quarrell, T. D. Claridge, G. W. Weaver, G. Lowe. "Structure and properties of TentaGel resin beads: implications for combinatorial library chemistry." *Mol. Divers.* **1996**, *1*, 223-232.

(18) O. W. Gooding, S. Baudart, T. L. Deegan, K. Heisler, J. W. Labadie, W. S. Newcomb, J. A. Porco, P. van Eikeren. "On the Development of New Poly(styrene−oxyethylene) Graft Copolymer Resin Supports for Solid-Phase Organic Synthesis." *J. Comb. Chem.* **1998**, *1*, 113-122.

(19) N. R. Candeias, L. C. Branco, P. M. Gois, C. A. Afonso, A. F. Trindade. "More sustainable approaches for the synthesis of N-based heterocycles." *Chem. Rev.* **2009**, *109*, 2703-2802.

(20) Y.-X. Zong, J.-K. Wang, G.-R. Yue, L. Feng, Z.-E. Song, H. Song, Y.-Q. Han. "Traceless liquid-phase synthesis of 3-alkylamino-4,5-disubstituted-1,2,4-triazoles on polyethylene glycol (PEG)." *Tetrahedron Lett.* **2005**, *46*, 5139-5141.

(21) H. Guo, K. Ding. "Liquid-phase synthesis of 2,3-dihydro-4-pyridones: one-pot three-component aza Diels–Alder reaction of Danishefsky's diene with aldehydes and PEG-supported amine." *Tetrahedron Lett.* **2003**, *44*, 7103-7106.

(22) W.-B. Yeh, M.-J. Lin, C.-M. Sun. "Liquid-phase parallel synthesis of tetrahydro-β-carbolines." *Tetrahedron Lett.* **2003**, *44*, 4923-4926.

(23) G. Yue, Y. Wan, S. Song, G. Yang, Z. Chen. "Synthesis of a library of benzoindolizines using poly(ethylene glycol) as soluble support." *Bioorg. Med. Chem. Lett.* **2005**, *15*, 453-458.

(24) X.-F. Lin, J. Zhang, Y.-G. Wang. "Parallel synthesis of 4,5-dihydro-1,2,4-oxadiazoles using soluble polymer support." *Tetrahedron Lett.* **2003**, *44*, 4113-4115.

(25) a) M.-J. Lee, C.-M. Sun. "Traceless synthesis of hydantoin by focused microwave irradiation." *Tetrahedron Lett.* **2004**, *45*, 437-440; b) C.-H. Chen, C.-M. Chang, H.-Y. Chen, J.-J. Lai, C.-M. Sun. "Scaffold-Directed Traceless Synthesis of Tetrahydro-β-carbolinehydantoins." *J. Comb. Chem.* **2007**, *9*, 618-626.

(26) K. Chanda, J. Kuo, C.-H. Chen, C.-M. Sun. "Enantioselective Synthesis of Benzimidazolyl Quinoxalinones on Soluble Polymer Support Using Focused Microwave Irradiation." *J. Comb. Chem.* **2009**, *11*, 252-260.

(27) Y. S. Hsiao, G. S. Yellol, L. H. Chen, C. M. Sun. "Multidisciplinary Synthetic Approach for Rapid Combinatorial Library Synthesis of Triaza-Fluorenes." *J. Comb. Chem.* **2010**, *12*, 723-732.

(28) L.-H. Chen, C.-M. Chang, D. B. Salunke, C.-M. Sun. "Divergent Synthesis of Unsymmetrical Annulated Biheterocyclic Compound Libraries: Benzimidazole Linked Indolo-benzodiazepines/quinoxaline." *ACS Comb. Sci.* **2011**, *13*, 391-398.

(29) a) B. L. Zou, Q. L. Yuan, D. W. Ma. "Synthesis of 1,2-disubstituted benzimidazoles by a Cu-catalyzed cascade aryl amination/condensation process." *Angew. Chem. Int. Ed.* **2007**, *46*, 2598-2601; b) N. Zheng, K. W. Anderson, X. H. Huang, H. N. Nguyen, S. L. Buchwald. "A palladium-catalyzed regiospecific synthesis of N-aryl Benzimidazoles." *Angew. Chem. Int. Ed.* **2007**, *46*, 7509-7512.

(30) a) D. S. Surry, S. L. Buchwald. "Dialkylbiaryl Phosphines in Pd-Catalyzed Amination: A User's Guide." *Chem. Sci.* **2011**, *2*, 27-50; b) N. C. Bruno, M. T. Tudge, S. L. Buchwald. "Design and Preparation of New Palladium Precatalysts for C-C and C-N Cross-Coupling Reactions." *Chem. Sci.* **2013**, *4*, 916-920; c) D. S. Surry, S. L. Buchwald. "Biaryl phosphane ligands in palladium-catalyzed amination." *Angew. Chem. Int. Ed.* **2008**, *47*, 6338-6361; d) J. F. Hartwig. "Evolution of a Fourth Generation Catalyst for the Amination and Thioetherification of Aryl Halides." *Acc. Chem. Res.* **2008**, *41*, 1534-1544.

(31) X. Bantreil, M. Sidi-Ykhlef, L. Aringhieri, E. Colacino, J. Martinez, F. Lamaty. "Comprehensive study on olefin metathesis in PEG as an alternative solvent under microwave irradiation." *J. Catal.* **2012**, *294*, 113-118.

(32) J. M. Chen, T. J. Yuan, W. Y. Hao, M. Z. Cai. "Simple and efficient CuI/PEG-400 system for hydroxylation of aryl halides with potassium hydroxide." *Catal. Commun.* **2011**, *12*, 1463-1465.

(33) a) A. Corma, H. Garcia, A. Leyva. "Polyethyleneglycol as scaffold and solvent for reusable C-C coupling homogeneous Pd catalysts." *J. Catal.* **2006**, *240*, 87-99; b) A. Corma, H. Garcia, A. Leyva. "Comparison between polyethylenglycol and imidazolium ionic liquids as solvents for developing a homogeneous and reusable palladium catalytic system for the Suzuki and Sonogashira coupling." *Tetrahedron* **2005**, *61*, 9848-9854.

(34) V. Declerck, E. Colacino, X. Bantreil, J. Martinez, F. Lamaty. "Poly(ethylene glycol) as reaction medium for mild Mizoroki-Heck reaction in a ball-mill." *Chem. Commun.* **2012**, *48*, 11778-11780.

(35) H. F. Zhou, Q. H. Fan, W. J. Tang, L. J. Xu, Y. M. He, G. J. Deng, L. W. Zhao, L. Q. Gu, A. S. C. Chan. "Polyethylene glycol as an environmentally friendly and recyclable reaction medium for enantioselective hydrogenation." *Adv. Synth. Catal.* **2006**, *348*, 2172-2182.

(36) G. Urgoitia, R. SanMartin, M. T. Herrero, E. Dominguez. "Palladium NCN and CNC pincer complexes as exceptionally active catalysts for aerobic oxidation in sustainable media." *Green Chem.* **2011**, *13*, 2161-2166.

(37) A. Leyva, H. Garcia, A. Corma. "A soluble polyethyleneglycol-anchored phosphine as a highly active, reusable ligand for Pd-catalyzed couplings of aryl chlorides: comparison with cross and non-cross-linked polystyrene and silica supports." *Tetrahedron* **2007**, *63*, 7097-7111.

(38) a) K. Weigand, S. Pelka. "Palladium-catalyzed amination of aryl halides on solid support." *Org. Lett.* **2002**, *4*, 4689-4692; b) C. A. Willoughby, K. T. Chapman. "Solid phase synthesis of aryl amines." *Tetrahedron Lett.* **1996**, *37*, 7181-7184; c) Y. D. Ward, V. Farina. "Solid phase synthesis of aryl amines via palladium catalyzed amination of resin-bound aromatic bromides." *Tetrahedron Lett.* **1996**, *37*, 6993-6996.

(39) a) A. P. Combs, S. Tadesse, M. Rafalski, T. S. Haque, P. Y. S. Lam. "N-Arylation of Primary and Secondary Aliphatic Amines on Solid Supports." *J. Comb. Chem.* **2002**, *4*, 179-182; b) A. P. Combs, S. Saubern, M. Rafalski, P. Y. S. Lam. "Solid supported arylheteroaryl C-N cross-coupling reactions." *Tetrahedron Lett.* **1999**, *40*, 1623-1626.

(40) C. Gil, S. Brase. "Solid-Phase Synthesis of Biologically Active Benzoannelated Nitrogen Heterocycles: An Update." *J. Comb. Chem.* **2008**, *11*, 175-197.

(41) E. J. Hennessy, S. L. Buchwald. "Synthesis of 4,5-dianilinophthalimide and related analogues for potential treatment of Alzheimer's disease via palladium-catalyzed amination." *J. Org. Chem.* **2005**, *70*, 7371-7375.

(42) a) J. P. Wolfe, S. L. Buchwald. "Scope and limitations of the Pd/BINAP-catalyzed amination of aryl bromides." *J. Org. Chem.* **2000**, *65*, 1144-1157; b) X. Huang, K. W. Anderson, D. Zim, L. Jiang, A. Klapars, S. L. Buchwald. "Expanding Pd-catalyzed C-N bond-forming processes: the first amidation of aryl sulfonates, aqueous amination, and complementarity with Cu-catalyzed reactions." *J. Am. Chem. Soc.* **2003**, *125*, 6653-6655.

(43) a) Y.-K. Lim, J.-W. Jung, H. Lee, C.-G. Cho. "Acid-Catalyzed Rearrangement of Diaryl Hydrazides for the Efficient Synthesis of Functionalized 2,2‘-Diamino-1,1‘-biaryls." *J. Org. Chem.* **2004**, *69*, 5778-5781; b) H. M. Kang, Y. K. Lim, I. J. Shin, H. Y. Kim, C. G. Cho. "Regioselective [5,5]-sigmatropic rearrangement reactions of aryl hydrazides." *Org. Lett.* **2006**, *8*, 2047-2050; c) X. Liu, M. Barry, H.-R. Tsou. "Palladium-catalyzed arylation of N,N-dialkylhydrazines and the subsequent conversion to anilines." *Tetrahedron Lett.* **2007**, *48*, 8409-8412; d) S. Cacchi, G. Fabrizi, A. Goggiamani, S. Sgalla. "Palladium-Catalyzed N-Arylation of N,N-Dialkylhydrazines with Aryl Chlorides." *Adv. Synth. Catal.* **2007**, *349*, 453-458.

(44) T. Yamamoto, M. Nishiyama, Y. Koie. "Palladium-catalyzed synthesis of triarylamines from aryl halides and diarylamines." *Tetrahedron Lett.* **1998**, *39*, 2367-2370.

(45) R. J. Lundgren, M. Stradiotto. "Palladium-catalyzed cross-coupling of aryl chlorides and tosylates with hydrazine." *Angew. Chem. Int. Ed.* **2010**, *49*, 8686-8690.

(46) S. M. Crawford, C. B. Lavery, M. Stradiotto. "BippyPhos: A Single Ligand With Unprecedented Scope in the Buchwald–Hartwig Amination of (Hetero)aryl Chlorides." *Chem. Eur. J.* **2013**, *19*, 16760-16771.

(47) A. DeAngelis, D. H. Wang, S. L. Buchwald. "Mild and rapid Pd-catalyzed cross-coupling with hydrazine in continuous flow: application to the synthesis of functionalized heterocycles." *Angew. Chem. Int. Ed.* **2013**, *52*, 3434-3437.

(48) a) S. L. Buchwald, C. Mauger, G. Mignani, U. Scholz. "Industrial-Scale Palladium-Catalyzed Coupling of Aryl Halides and Amines - A Personal Account." *Adv. Synth. Catal.* **2006**, *348*, 23-39; b) C. Mauger, G. Mignani. "The Synthesis of Important Pharmaceutical Building Blocks by Palladium-Catalyzed Coupling Reaction: Access to Various Arylhydrazines." *Adv. Synth. Catal.* **2005**, *347*, 773-782; c) J. F. Hartwig. "Synthesis, structure, and reactivity of a palladium hydrazonato complex: A new type of reductive elimination reaction to form C-N bonds and catalytic arylation of benzophenone hydrazone." *Angew. Chem. Int. Ed.* **1998**, *37*, 2090-2093.

(49) L. Ackermann, R. Sandmann, W. Song. "Palladium- and Nickel-Catalyzed Aminations of Aryl Imidazolylsulfonates and Sulfamates." *Org. Lett.* **2011**, *13*, 1784-1786.

(50) A. O. Adeniji, B. M. Twenter, M. C. Byrns, Y. Jin, M. Chen, J. D. Winkler, T. M. Penning. "Development of Potent and Selective Inhibitors of Aldo–Keto Reductase 1C3 (Type 5 17 β-Hydroxysteroid Dehydrogenase) Based on *N*-Phenyl-Aminobenzoates and Their Structure–Activity Relationships." *J. Med. Chem.* **2012**, *55*, 2311-2323.

(51) G. Griffioen, T. Van Dooren, V. Rojas de la Parra, S. Allasia, A. Marchand, A. Kilonda, P. Chaltin, **2012**, WO 2012080221A1.

IV. Amide Electrophilic Activation and Aza-Claisen

My contribution to the work was the synthesis of all the compounds reported on the experimental chapter and the methodology development.

The work described in this chapter was developed in Maulide's former lab at Max Planck Institute für Kohlenforshung from 1st October 2012 until 20th December 2012.

IV.1 Background

IV.1.1 Claisen Rearrangement

Claisen rearrangement is one of the most well known organic reactions, a [3,3] sigmatropic rearrangement early studied in the first years of organic chemistry classes.[1] Firstly reported in 1912 by Ludwig Claisen,[2] this reaction can be defined as a suprafacial, concerted and nonsynchronous pericyclic process.[3] The constant innovations turned it into an efficient and versatile procedure, proceeding with high degrees of chemo-, regio- and enantioselective control which allowed the construction of highly functionalized molecules applied in natural product and medicinal chemistry areas.[4]

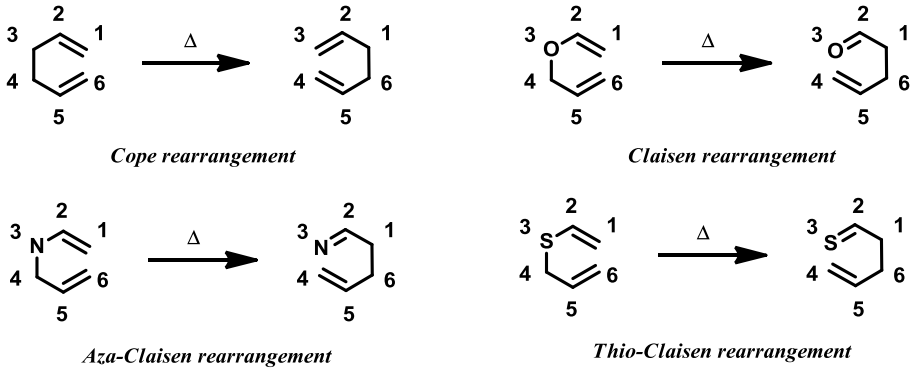


Figure IV.1. Cope and Claisen rearrangement and its variants.

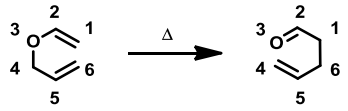
The Claisen rearrangement may be viewed as the oxy-variant of the Cope rearrangement.[5] Systems displaying other heteroatoms in position 3 are known as hetero Claisen or hetero Cope rearrangements (Figure IV.1). When the heteroatom is nitrogen, these types of rearrangements are usually called as aza- or amino-Claisen or 3-aza-Cope rearrangement. Moreover, one of the double bonds can be replaced by an aromatic ring (aromatic-Claisen rearrangement), an acetylenic group or other moieties.

The majority of classical Claisen-type rearrangement reactions described to date are rough processes that require high temperatures (usually above 100 °C), usually leading to competitive side reactions and decomposition of the starting materials and/or products. In fact, during the last years, several efforts were made to transform this reaction into a synthetically versatile procedure.[1] Substances such as transition-metal complexes, Lewis acids, and also the physical parameters have been changed or introduced to catalyze the Claisen rearrangement, accelerating the reaction rate.[6]

IV.1.1.1 Factors influencing the Claisen reaction

An important feature is the effect of the substituents present in the skeleton of the substrate.[1] These effects have been extensively studied, and are currently recognized which groups accelerates or decelerates the Claisen reaction based on their electronic attributes (Table IV.1).

Table IV.1. Influence of substituents on the Claisen reaction.[1]



EWG acceleration		*EDG acceleration*	
Position	**Group**	**Position**	**Group**
2	-CN, $-CO_2$, - CO_2CH_3, $-CF_3$	1	-O, $-NH_2$,-F, CH_3
4	-CN, $-CF_3$	2	-OSi(CH3)3, - CH_3, -F
5	-CN	4	- $COCH_3$, $-CH_3$
		6	- $COCH_3$, $-CH_3$
EWG deceleration		*EDG deceleration*	
1	-CN, - CO_2CF_3	5	- OCH_3, $-CH_3$
6	-CN		

Other factors include the use of polar solvents which increases the rearrangement rate, enabling the reaction to proceed under smooth conditions.[1] Additionally it was also verified that microwave irradiation strongly accelerates the reaction, reducing the thermal treatments imposed by the classical conditions.[7] In fact, the combined effect of microwave irradiation, temperature and solvent allows a considerable decrease in the reaction time.

Positively charged intermediates have also been reported as valuable compounds that accelerates the rearrangement. This type of charge acceleration can be induced by an appropriate catalyst or a Lewis acid.[8]

Besides the Lewis acid catalysis, transition metal catalysts such as Hg, Au, Cu or Pd salts are also employed in Claisen rearrangements.[9] These reactions proceed by a "cyclization-induced rearrangement", which explains the effect of the metal catalysts in the reaction.

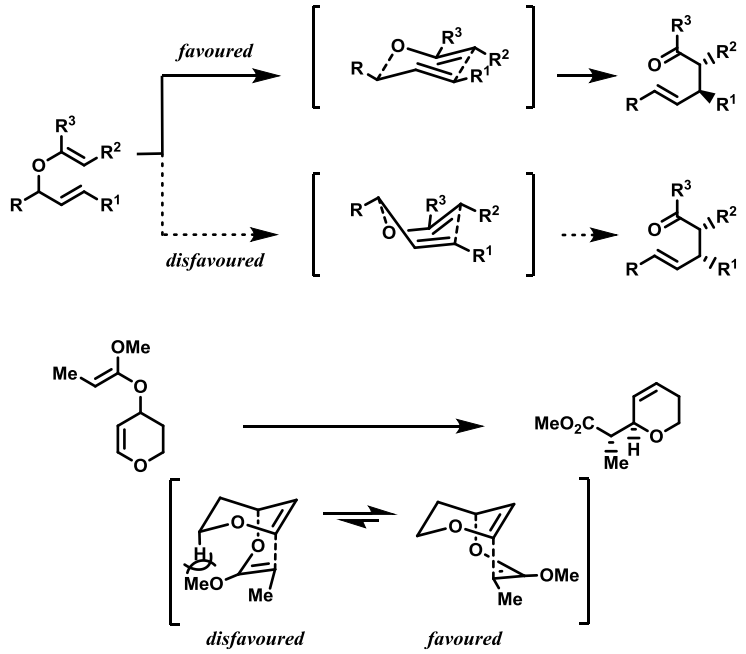
IV.1.1.2 Claisen rearrangement stereocontrol

The stereochemical outcome of a Claisen reaction could be predicted since this [3,3]-sigmatropic rearrangement follows highly ordered cyclic six membered transition states and obey to the orbital symmetry rules.

The cyclic transition state of the Claisen rearrangement proceed *via* a chair- or a boat-like conformation, which leads to diastereomerically divergent products. These conformations can be

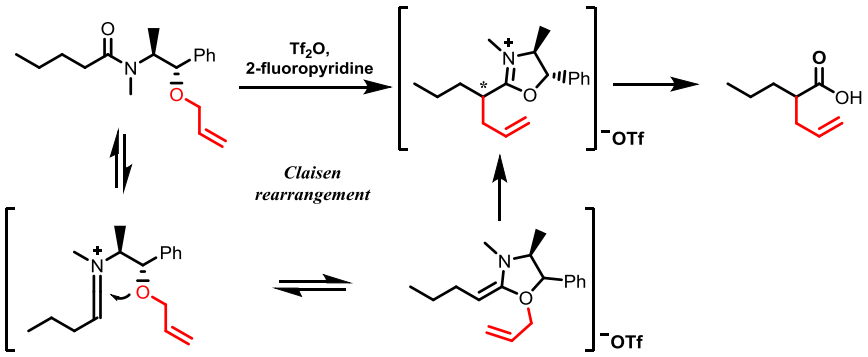
controlled by the structural features of the system, namely steric and electronic properties of the substrate or by the reaction conditions.

In acyclic substrates the chair-like transition state is usually favored, a consequence of energetic minimization of torsional and transannular interactions (Scheme IV.1). On the other hand, this trend is not always verified when the allyl feature is incorporated in a cyclic system, and the Claisen rearrangement can also proceed through a boat-like transition state in order to minimize it energy.[10]



Scheme IV.1. Claisen rearrangement transitions states for acyclic and cyclic substrates.

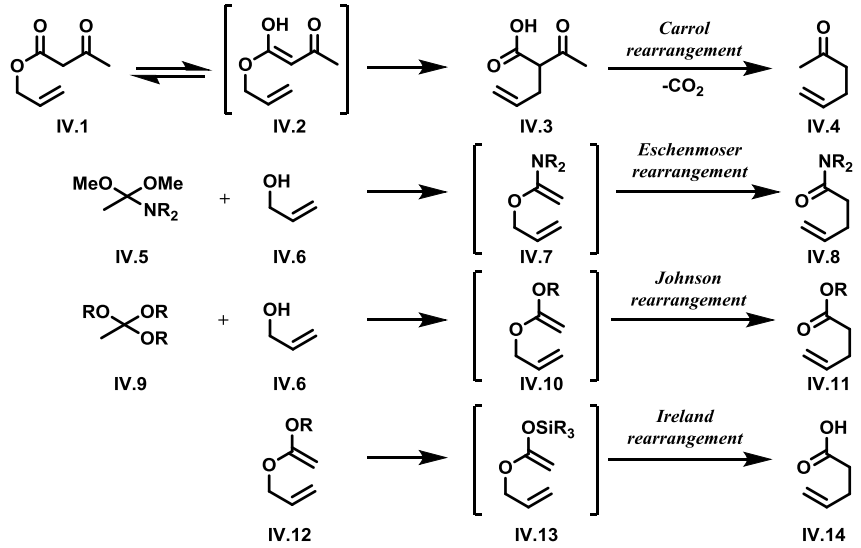
Several approaches using external asymmetric promoters have been exploit in [3,3]-sigmatropic rearrangements,[11] such the use of Lewis-acid based catalysts, or the asymmetric induction using a removable auxiliary to transfer the stereochemical information, like the work recently reported by Maulide *et al.* (Scheme IV.2).[12]



Scheme IV.2. Traceless asymmetric α-allylation described by Maulide *et al.*

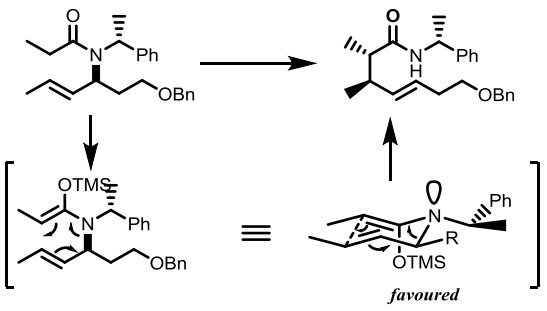
IV.1.1.3 Claisen variants

The synthetic potential of the procedure expanded the reaction to different substrates, creating a wide number of Claisen versions (Scheme IV.3). The Carroll rearrangement, described in 1940, is a thermal rearrangement of allylic β-ketoesters **IV.1** to form the corresponding α-allyl-β-ketocarboxylic acid (**IV.3**). This intermediate suffers a decarboxylation to yield γ,δ-unsaturated ketones **IV.4**.[13] Years later, in 1964, Eschenmoser reports the [3,3] rearrangement of *in situ* formed *N,O*-ketene acetals **IV.7 (**from allylic alcohol) to yield γ,δ-unsaturated amides **IV.8**.[14] The Johnson rearrangement consists of heating an allylic alcohol with an excess of ethyl orthoacetate **IV.9** to yield a γ,δ-unsaturated ester **IV.11**.[15] Few years later, Ireland and Mueller reports the rearrangement of allyl trimethylsilyl ketene acetals **IV.12**, prepared by reaction of allylic ester enolates with trimethylsilyl chloride, to yield γ,δ-unsaturated carboxylic acids **IV.14**.[16]



Scheme IV.3. [3,3]-Sigmatropic rearrangements related to the Claisen rearrangement.

Aza-Claisen rearrangement is other variant of Claisen reaction, giving access to attractive heterocyclic backbones.[17]



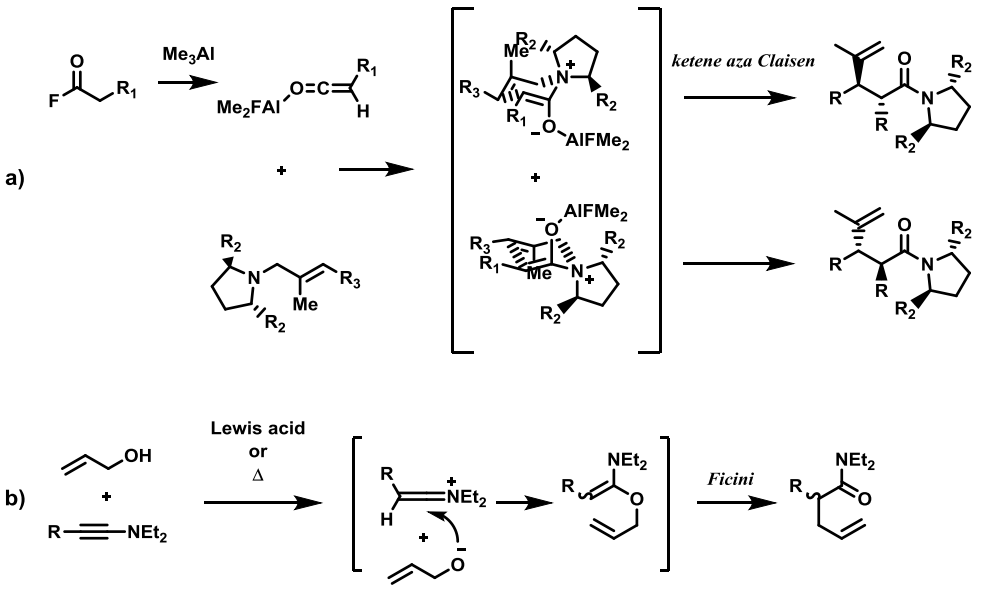
Scheme IV.4. Example of chiral auxiliaries in an asymmetric approach.[18]

The oxygen replacement by the nitrogen atom can offer some advantages. For instance, while two valences of the nitrogen are occupied by allyl and vinyl substituents (involved in the rearrangement system), the third position can be used to anchor chiral auxiliaries in asymmetric approaches (Scheme IV.4).[18]

The first successful examples of the aza-Claisen rearrangement were reported in the 1960s initially by Marcinkiewicz and later by Hill and Gilman.[19] These uncatalyzed systems were carried under harsh conditions, hindering the substrate scope and leading to undesired side products.

Thus, both aliphatic and aromatic aza-Claisen variants stood apart for a long period of time compared to the classic Claisen rearrangement. Gladly this trend has been changed, and in the last years, the introduction of Bronsted and Lewis-acids as well as transition-metals catalysis, allowed the aza-reaction to proceed under milder conditions. Moreover, as already seen for the oxygen substrates, a profusion of aza-Claisen variants were developed, making the aza-Claisen more attractive to construct complex compounds.[8a,17]

Within these variants it can be found the ketene-aza Claisen rearrangement.[20] The sigmatropic framework requires the addition of a tertiary allylamine to the carbonyl center of a ketene, generating an *N*-allyl ammonium enolate which can undergo the rearrangement. However, the key step is the formation of the ketene with a lifetime sufficient for the subsequent attack of the nucleophile, avoiding the competing [2+2] cycloadditions. Recently, Nubbemeyer and coworkers described a zwitterionic ketene aza-Claisen rearrangements of 4-arylbut-3-enoyl fluorides **7**, were chiral pyrrolidines played an important role as asymmetric auxiliaries delivering the products with high diastereoselectivities (Scheme IV.5.a).[21]

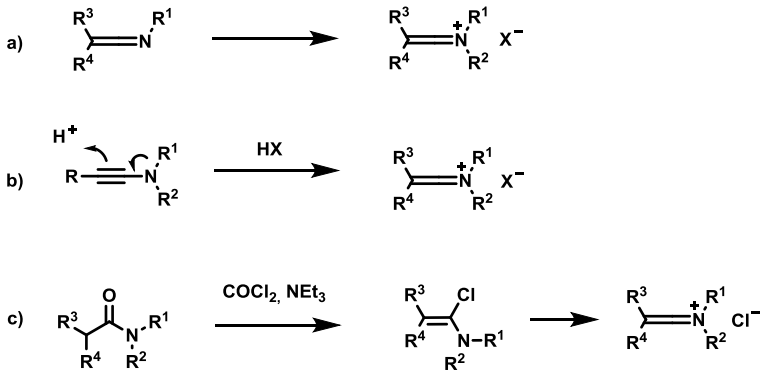


Scheme IV.5. Rearrangements involving heteroallenes: a) aza-ketene and b) Ficini-Claisen.

Other rearrangements involving heteroallenes can be pointed out (Scheme IV.5.b). An example, is the Ficini-Claisen rearrangement, an ynamine-Claisen rearrangement – where allylic alcohol reacts with an ynamine to form γ,δ-unsaturated amides.[22] Due to the instability and synthetic inaccessibility of ynamines, ynamides have also emerged as alternatives in the Ficini–Claisen reaction.[23]

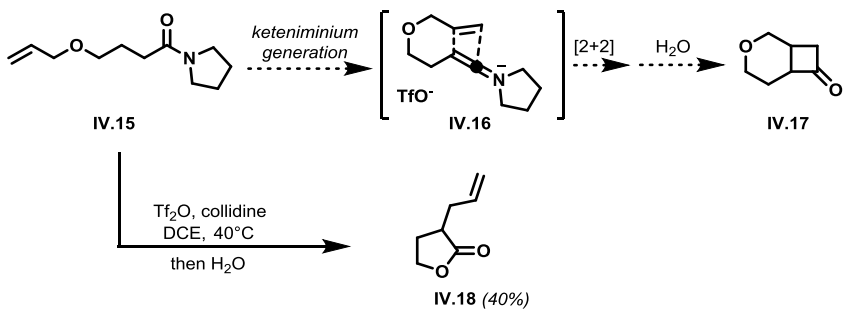
IV.1.2 Amide Electrophilic Activation

In 2010, Maulide *et al.* envisaged the use of keteniminium salts as key intermediates for the synthesis of natural products.[24] In fact, keteniminium salts are powerful electrophilic heteroallenes well-known for their selectivity and stereocontrol in [2+2] cycloadditions to olefins, carbonyls and imine derivatives. Keteniminium salts can be prepared through different approaches (Scheme IV.6).[25]



Scheme IV.6. Preparation of keteniminium salts.[25]

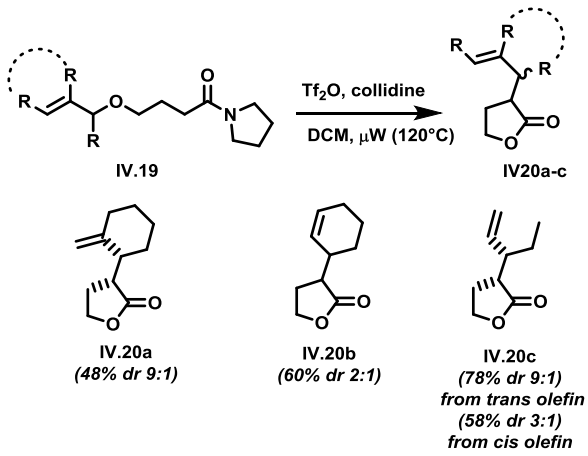
In the synthetic plan, Maulide and his coworkers envisioned the direct preparation of bicyclobutanone **IV.17** from amide **IV.15** through a [2+2] cycloaddition of the keteniminium intermediate. However, treatment of δ-allyloxyamide **IV.15** with triflic anhydride and 2,4,6-collidine in dichloroethane did not lead to the desired product **IV.17**. Instead, it was observed an unprecedented Claisen rearrangement leading to allylated lactone **IV.18** (Scheme IV.7). [24]



Scheme IV.7. Unexpected Claisen rearrangement observed by Maulide *et al.*[24]

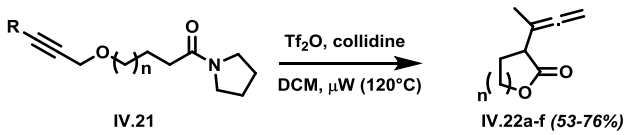
Careful optimization of reaction conditions conducted to the use collidine as base, triflic anhydride as the most suitable amide activating agent and dichloromethane as solvent. The optimal protocol also required microwave irradiation of the reaction mixture for only 5 min, followed by hydrolytic workup with aqueous bicarbonate solution.

Further investigation of substrate scope revealed a moderate to good functional-group tolerance and that lactones bearing branched allyl substitutents in the α position were generated with high diastereoselectivities (Scheme IV.8).[24]



Scheme IV.8. Investigation of substrate scope.[24]

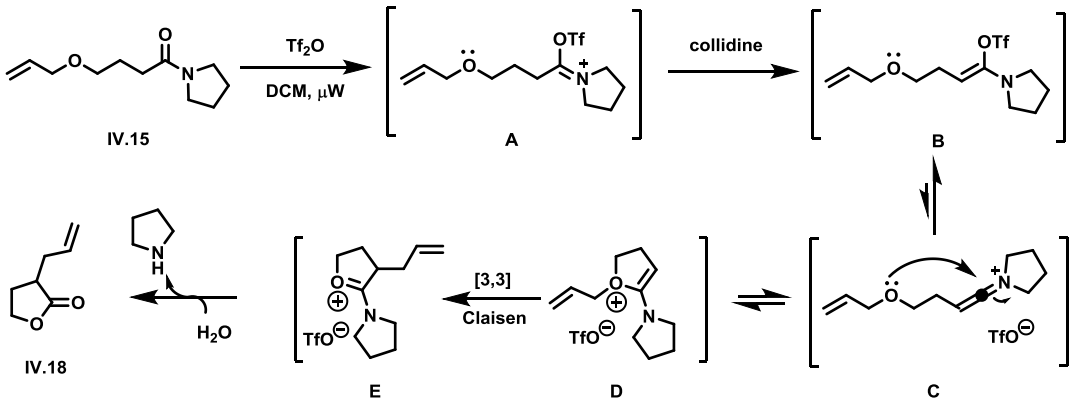
Moreover, the substrate scope was also extended to propargyl ethers. Fortunately, alkynes smoothly rearranged to α-allenyl lactone in good yields (Scheme IV.9). Neither the isomeric propargylated butyrolactone nor [2+2] cycloadducts were detected in the reaction mixture.



Scheme IV.9. Use of propargyl ethers to form the corresponding α-allenyl lactones.

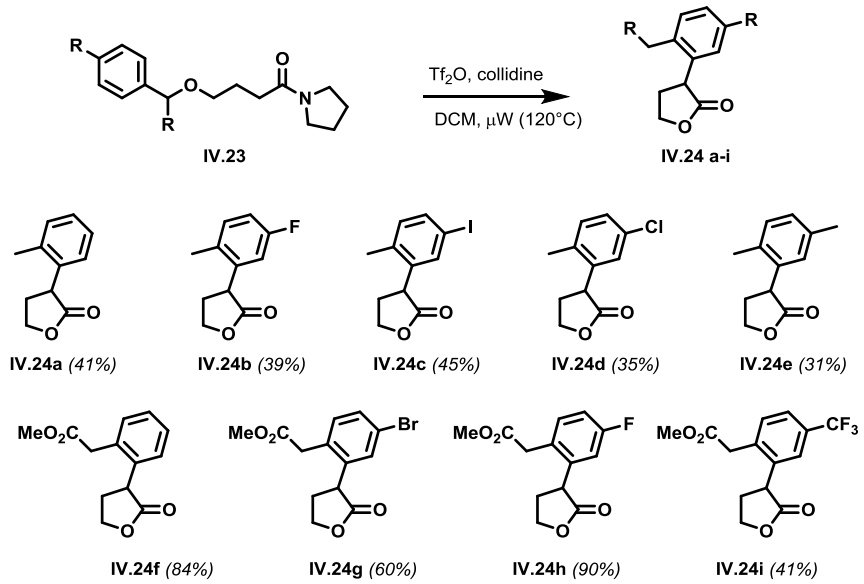
The proposed reaction mechanism is shown in Scheme IV.10. The initial activation of the amide carbonyl group by the triflic anhydride would give the iminium triflate **A** that can be converted into enamine triflate **B**. Upon microwave or thermal activation, an elimination to form keteniminium ion **C** probably takes place.

The enhanced electrophilicity of this intermediate then triggers an unusual nucleophilic attack of the ether, which may be reversible. This addition, however, generates a vinyl allyl oxonium intermediate **D**. Such a species should be ideally poised to undergo a "Claisen-like" [3,3]-sigmatropic rearrangement, leading to the stabilized carbenium ion **E**. Finally, hydrolysis of **E** leads to the formation of lactone **IV.18**.



Scheme IV.10. Proposed mechanism for the unprecedented Claisen rearrangement.

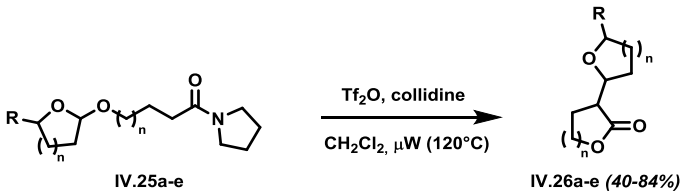
This unprecedent rearrangement of keteniminium salts – that allowed a direct and stereoselective access to challenging substituted lactones – was also extended to a "benzyl variant" procedure (Scheme IV.11).[26] In this case, the α-arylated lactones (**IV.24a-i**) were obtained, although in moderate yields. The rearrangement proved to be tolerant to the presence of several substituents on the aromatic ring. Additionally, some of the obtained products (**IV.24f-i**) contained two chemo-differentiated carboxylate moieties for further elaboration.



Scheme IV.11. Benzyl Claisen rearrangement reported by Maulide group.[26]

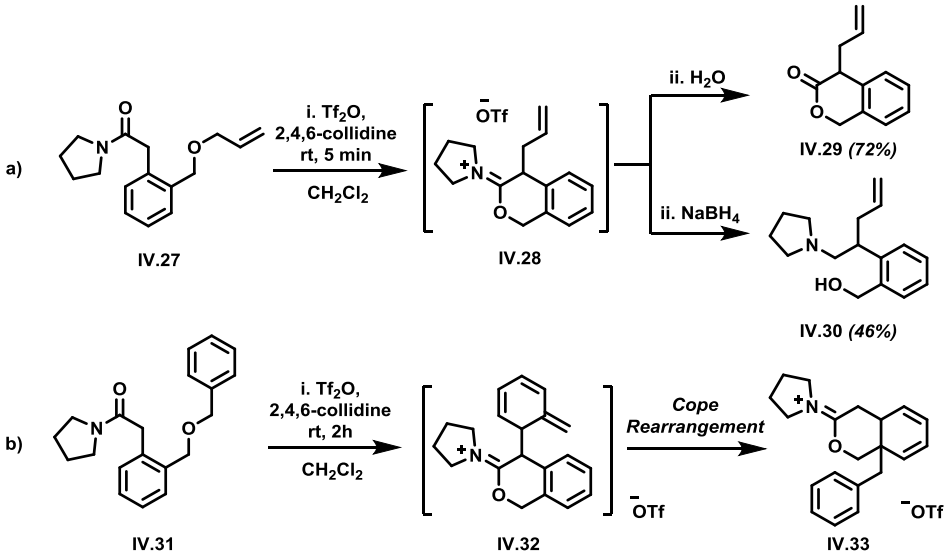
However, it was also verified along with the α-arylated lactones, the presence of the corresponding α-benzylated lactones as side products. Furthermore, substrates with R = OMe, CN and CF_3 groups, exclusively afforded the benzylic transfer adducts as the products of the reaction.[26]

The group also reported the smoothly rearrangement of the simple tetrahydrofuran and tetrahydropyran protected hydroxylamides **IV.25a-e** in the same reaction conditions, to the corresponding adducts **IV.26a–e** in moderate to good yields (Scheme IV.12).



Scheme IV.12. Tetrahydrofuran and tetrahydropiran protected hydroxylamides substrates.

More recently, the same group reported structures incorporating a phenyl group within the alkyl tether, thereby restricting the conformational flexibility in the substrate (Scheme IV.13).[27] The conditions for this type of substrates are remarkably mild proceeding at room temperature. The explanation could reside in the reduced conformational flexibility, resulting in significant pre-organization for the cyclization/Claisen rearrangement events, perhaps combined with favorable formation of a conjugated keteniminium salt intermediate. Nucleophilic capture of the rearrangement product **IV.28** gave rise to isochromanone **IV.29** or the aromatic aminoalcohol **IV.30** (Scheme IV.13. a). [27]



Scheme IV.13. Rearrangement of structures incorporating a phenyl group within the alkyl tether.[27]

Surprisingly, for the *O*-benzyl analogue it was observed that the major product of this transformation was not the expected iminium ether **IV.32** (or its rearomatized derivative), but the isomeric compound **IV.33** (Scheme IV.13. b). The formation of **IV.33** can be explained by the generation of iminium ether **IV.32** that evolves through a Cope-type sigmatropic rearrangement.

The unexpected skeletal reorganization observed in Maulide's group that gave access to useful lactones, was the driving force that encouraged us to further explore this reaction for structures incorporating a phenyl group within the alkyl tether.

The development of this novel concept will allow the preparation of aromatic heterocycles with increased structural diversity and stereoselectivity, and could be the departure point to expand the methodology to different applications.

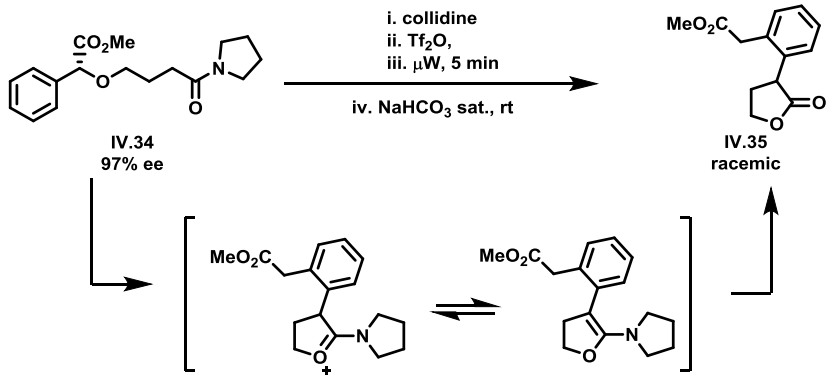
IV.2 Results and Discussion

The relevance of the chromanones and coumaranones and its nitrogenated counterparts in natural products and as medicinal agents[28] justify the synthetic efforts to create these heterocyclic structures with high levels of functionalization and sterocontrol.

Based on these objectives and using the amide electrophilic activation/Claisen rearrangement methodology developed in Maulide's group, it was proposed to perform additional studies on the substrates incorporating a phenyl ring within the alkyl tether and to expand the procedure to its aza analogs.

IV.2.1 Six membered rings

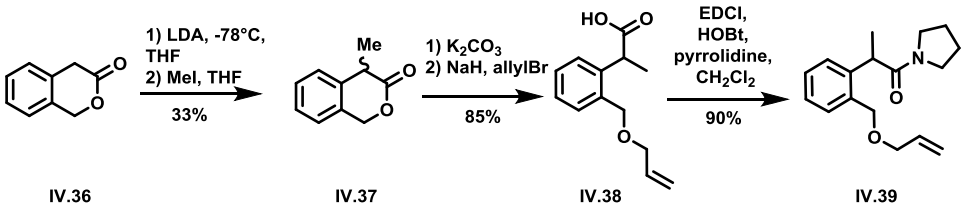
According to the preliminary studies performed in Maulide's lab, α-arylated lactones generated from derivatives bearing a benzyl moiety were obtained in the racemic form, since basic hydrolysis conditions can cause racemization of the intermediate *via* a formal "iminium"-"enamine" equilibrium (Scheme IV.14). Moreover, the extended conjugation through the aromatic substituent can also promote epimerization at the chiral center under basic conditions.



Scheme IV.14. Benzyl Claisen rearrangement of substrate **IV.34**.

In order to achieve enantioenriched compounds, it was proposed to introduce a methyl group in the α position to the carbonyl to prevent the epimerization of the iminium ether intermediate.

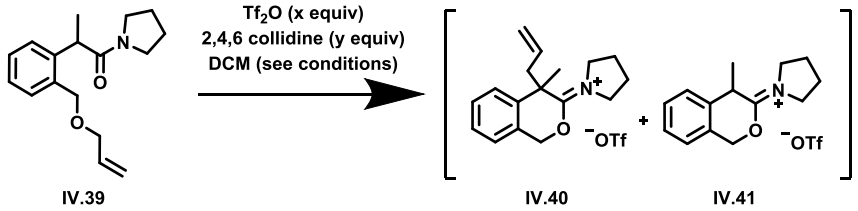
To test this hypothesis, substrate **IV.39** was synthesized in three steps using 3-isochromanone **IV.36** as starting material (Scheme IV.15). This compound was methylated by treatment of **IV.36** with LDA at -78ºC, followed by the addition of methyl iodide in THF. The ring opening product was achieved by treatment with potassium carbonate, followed by a simple *O*-allylation to yield compound **IV.38**. The corresponding amide was constructed through a carboxylic acid activation using the EDCI/HOBt system and pyrrolidine in CH_2Cl_2. Compound **IV.39** was achieved with an overall yield of 25 %.



Scheme IV.15. Synthesis of compound **IV.39** from 3-isochromanone.

The initial experiments were performed using the previously established conditions for the Claisen rearrangement (treatment of a 0.2 M solution of **IV.39** in CH_2Cl_2 with 1.20 equivalents of collidine and 1.05 equivalents of triflic anhydride, followed by microwave irradiation). NMR analysis of crude mixture showed the formation of **IV.40** along with the deallylated product **IV.41** as the major product (entry1, Table IV.2). In order to prevent the deallylation reaction, the procedure was performed at room temperature overnight (entry 2). However, the reaction did not proceed (monitored by TLC) and it was further subjected to the irradiation conditions. In this case, the amount of the desired compound **IV.40** increased, but compounds **IV.39** and **IV.41** were also identified (Figure IV.2.b).

Table IV.2. Optimization of Claisen reaction to obtain the intermediate **IV.40**.



Entry	Tf_2O (equiv)	Collidine (equiv)	Conditions	Ratio **IV.39:IV.40:IV.41**
1	1.05	1.2	μW (120°C), 5 min	0:0.3:1
2	1.05	1.2	rt, overnight → μW (120°C), 5 min	0.5:0.5:1
3	1.05	1.2	Addition at -78º C → μW (120°C), 15 min	0:0:1
4	1.5	1.2	Addition at -78º C → μW (120°C), 15 min	0:0.2:1
5	1.1	3	μW (120°C), 5 min	1:0.3:0.3
6	1.1	1.5	μW (80°C), 5 min	1:1:1

The addition of collidine and Tf_2O to **IV.39** solution at -78ºC, did not give any improvement. Moreover, irradiating the mixture for 15 min. yielded **IV.41** as the almost exclusive product (entry 3).

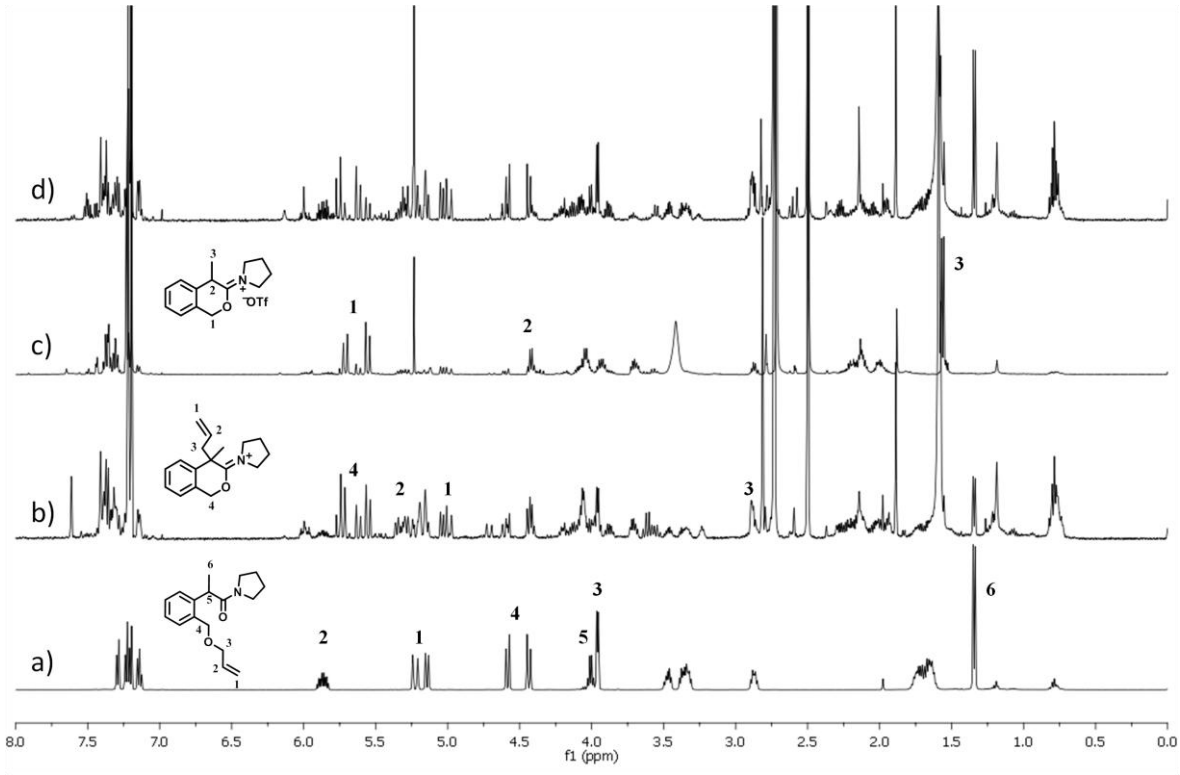
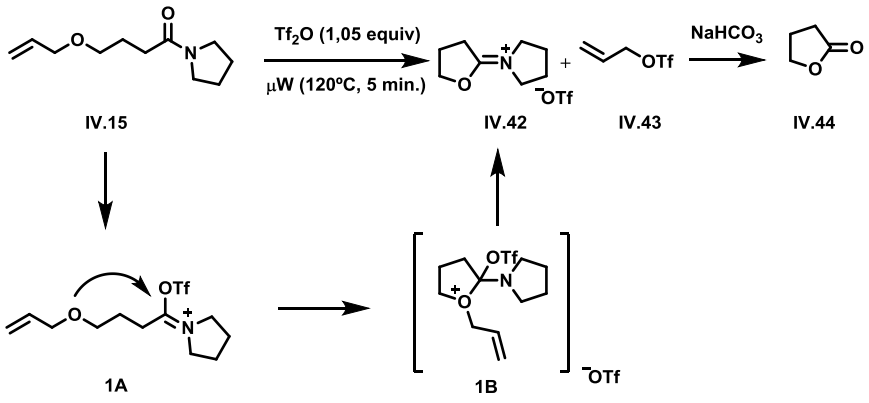


Figure IV.2. a) Compound **IV.39**; b) entry 2; c) entry 3; d) entry 5.

In fact, the formation of the deallylated lactone **IV.44** was previously reported by Dr. I. Jurberg.[29a] In his studies he confirmed the presence of the allyl triflate specie **IV.43** in the crude reaction mixture that could be implicated in the formation of the deallylated byproduct (Scheme IV.16).



Scheme IV.16. Studies described by Dr. I. Jurberg confirming the presence of the allyl triflate species.

It was proposed that deallylation might occur by a mechanism where the cyclization takes place by nucleophilic attack of the oxygen atom directly on the iminium triflate **1A (**before abstraction of the proton by the base), followed by loss of the allyl moiety by the triflate anion to form the iminium ether **IV.42** (Scheme IV.16). Experiments performed by Dr. C. Madelaine and Dr. I. Jurberg, support this pathway. When allyloxy amide **IV.15** was treated exclusively with triflic

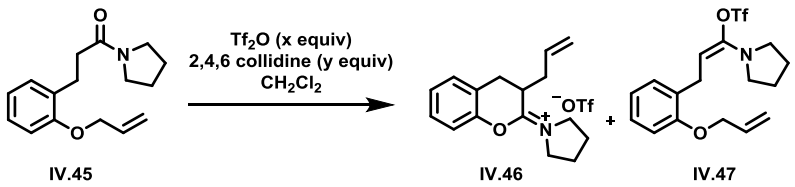
anhydride, iminium ether **IV.42** and allyl triflate **IV.43** were identified as the only products in the crude reaction mixture.[29] Additionally, Madeleine and Jurberg observed that an increased amount of collidine led to a decreased formation of deallylated lactone. Other mechanism that explains the loss of the allylic moiety was also recently proposed by the Maulide *et al*.[29b]

Their results are also in accordance with the different ratios observed in our study upon modifying the relative quantities of reagents. Increasing the activating agent to 1.5 equivalents while maintaining the collidine, did not change the reaction output (Table IV.2, entry 4). On the other hand, using 3.0 equivalents of collidine conducted to a significant decrease of **IV.41**. However, a large amount of the unreacted starting material **IV.39** was still observed (Table IV.2, entry 5). This could be explained by the presence of the methyl group that may increase the steric hindrance in the center, inhibiting the rearrangement reaction.

Decreasing the reaction temperature to 80ºC, a mixture of **IV.39, IV.40** and **IV.41** was formed in an almost equal ratio (entry 6).

Due to the failure of Claisen rearrangement for compound **IV.39** we next explored the reaction methodology with compound **IV.45**, a 2-chromanone derivative. In this case the phenyl ring within the tether is directly attached to the oxygen atom, which was expected to slightly change the electronic properties of the system and consequently the reactivity.

Table IV.3. Optimization of Claisen reaction to obtain the intermediate **IV.46**.



IV.45 IV.46 IV.47

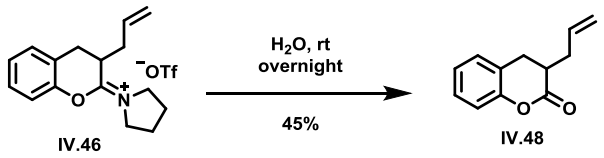
Entry	Tf_2O (equiv)	Collidine (equiv)	Conditions	Ratio **IV.45:IV.46:IV.47**
1	1,05	1,2	RT overnight → μW (120°C), 5 min	1:0:1
2	1,05	1,2	μW (120°C), 5 min	0:1:0
3	1,05	2	μW (120°C), 5 min	0:0:1

In fact, while derivative **IV.27** went through rearrangement at room temperature in 5 min. (*see* Scheme IV.13.a), reacting **IV.45** at room temperature for 18 h yielded a mixture of starting material and the suggested activated specie **IV.47** in a 1:1 ratio (Table IV.3, entry 1). The explanation could reside on the less available pair of electrons of the oxygen to undertake nucleophilic attack of the ketiminium intermediate. Moreover, this derivative does not have the

possibility of formation of a conjugated keteniminium salt intermediate, and the conformational flexibility is to some extent, higher than derivative **IV.27**.

When the standard microwave conditions (120°C, 5 min.) were applied to **IV.45**, it was obtained **IV.46** with full conversion (Table IV.3, entry 2). As seen before, raising the amount of collidine to 2 equivalents gave the activated product **IV.47** (Table IV.3, entry 3).

The water hydrolysis (room temperature, overnight) was achieved with full conversion of the **IV.46** to afford the product **IV.48** (45%) (Scheme IV.17).

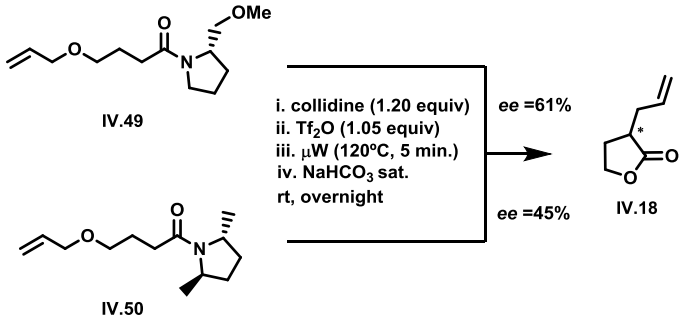


Scheme IV.17. Water hydrolysis of compound **IV.46**.

Since the reaction proceeded in quite well manner for derivative **IV.46** it was proposed to explore an asymmetric variant of this rearrangement.

Encouraged by the previous literature that report a transfer of the stereochemical information by a removable auxiliary, Maulide's group explored the use of chiral pyrrolidines as "traceless" chiral auxiliaries. These pyrrolidines would be present as the amine part of the starting amide, and cleaved (and possibly recovered) to give away the final product.

Initial studies performed by Dr. C. Madelaine, showed that enantiomeric excesses can be obtained with alkoxyamide derived from the (*S*)-methoxymethylpyrrolidine **IV.49** (61% *e.e.*), as well as with **IV.50** incorporating a C2-symmetric chiral pyrrolidine (45% *e.e.*) (Scheme IV.18).[24]

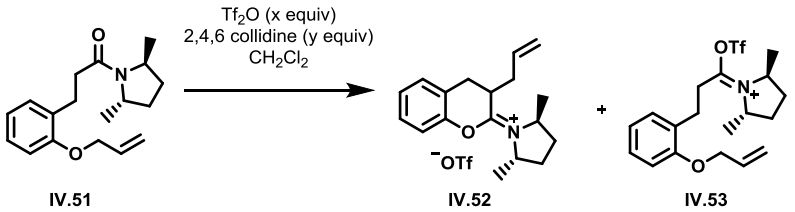


Scheme IV.18. Use of chiral pyrrolidines for asymmetric induction.[24]

Thus, derivative **IV.51** was prepared in order to perform the asymmetric studies. Unfortunately, when the standard microwave conditions were applied (120°C, 5 min.) to **IV.51**, the NMR of the crude mixture showed the presence of starting material along with other two minor products – the iminium ether **IV.52** and the presumable activated intermediate **IV.53**. The mixture was then

subjected to microwave irradiation at 120°C for 15 min., in order to push it to full conversion, but without any improvement (Table IV.4, entry 1).

Table IV.4. Optimization of Claisen reaction to obtain the intermediate **IV.52**.



IV.51 **IV.52** **IV.53**

Entry	Tf_2O (equiv)	Collidine (equiv)	Conditions	Ratio **IV.51:IV.52:IV.53**
1	1,05	1,2	μW (120°C), 5 min → μW (120°C), 15 min	1:0.5:0.5
2	1,05	1,2	rt, overnight → 50°C, 3 h	1:0.1:0.1
3	1,05	1,2	μW (80°C), 5 min	1:0.3:0.1

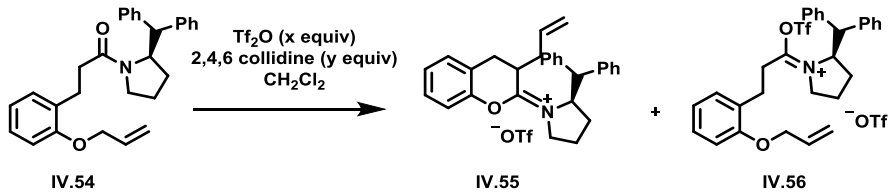
Performing the reaction at room temperature (overnight) the rearrangement did not proceed, and only small amounts of compounds **IV.52** and **IV.53** were observed. The mixture was then heated to 50°C for 3 h, but **IV.52** was not formed in higher amounts (entry 2).

The reaction was also performed under microwave conditions at 80ºC, but once more it was achieved a poor conversion of **IV.51** to the desired compound **IV.52** (entry 3).

When the same standard conditions were applied to derivative **IV.54** (at room temperature for ~18 h), it was verified that the rearrangement did not proceed, and only the starting material was observed (Table IV.5, entry 1). When the microwave conditions (120°C, 5 min.) were employed (Table IV.5, entry 2), the NMR of the crude mixture showed a different profile. Analyzing the COSY spectrum it was verified that the structure did not correspond to the expected compound **IV.55**, being most likely the activated **IV.56**. The reaction was also performed under the same microwave conditions for 1 h, however without improved results (entry 3).

It can be concluded that compound **IV.54** is poorly reactive, fact that can be attributed to the bulkiness of the two phenyl rings present in the chiral auxiliary, which can impede the correct conformation for the rearrangement to take place.

Table IV.5. Optimization of Claisen reaction to obtain the intermediate **IV.55**.



IV.54 IV.55 IV.56

Entry	Tf_2O (equiv.)	Collidine (equiv.)	Conditions	Ratio **IV.54:IV.55:IV.56**
1	1,05	1,2	rt, overnight → μW (120°C), 5 min. → μW (120°C), 1 h	1:0:0
2	1,05	1,2	μW (120°C), 5 min	0:0:1
3	1,1	1,5	μW (120°C), 1 h	0:0:1

Having proved the unsuccessful trials of the asymmetric reaction we then further explored the five membered rings.

IV.2.2 Five membered rings

2-Coumaranones (or 3-*H* 2-benzofuranones) are prominent structural motifs of some natural products or in compounds with relevant properties, and many of them feature a chiral quaternary stereocenter at the C-3 position of the heterocyclic ring (Figure IV.3).[30] Due to their relevance, the catalytic enantioselective functionalization at the C-3 position represents the most direct approach to chiral benzofuranones.[31]

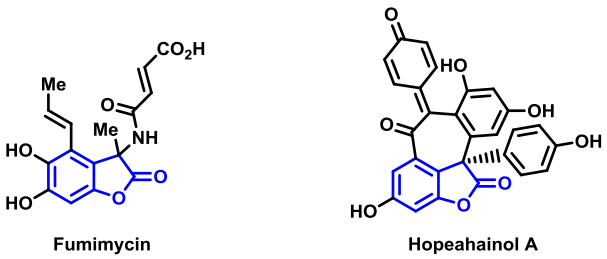
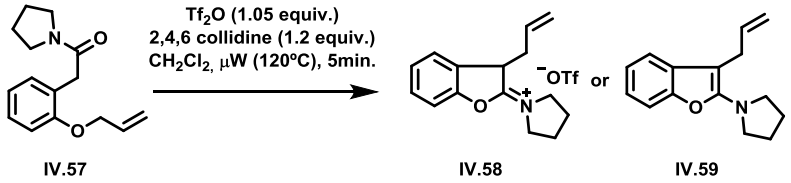


Figure IV.3. Natural products containing the coumaranone moiety.

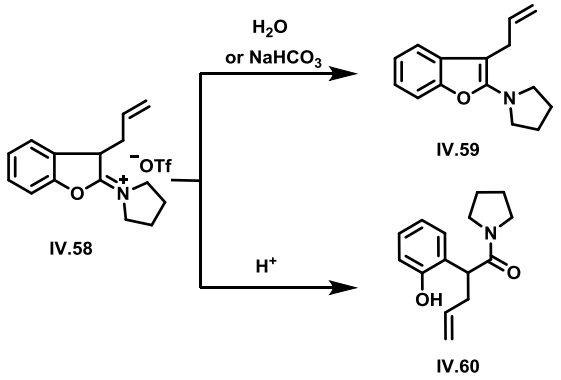
Initial studies using the five member substrate **IV.57** showed that the rearrangement did not proceed at room temperature. On the other hand, applying the usual microwave conditions (120°C, 5 min.) it was observed the full conversion of **IV.57** to an intermediate that could be either the iminium **IV.58** or the enamine **IV.59**. It was formulated that **IV.59** could be formed due to the rearomatization of the aromatic system to form the most stable compound.



Scheme IV.19. Study of Claisen reaction with derivative **IV.57**.

In order to clarify the intermediate structure, were performed 2D NMR experiments - HSQC and HMBC - of the crude mixture. The compound was identified as the structure **IV.58** due to the observed correlation between proton in position C-3 and the CH_2 of the allylic moiety.

Hydrolysis attempts showed that **IV.58** evolved to compound **IV.59** under neutral or basic conditions, while washing the crude mixture with HCl 1M or saturated NH_4Cl solutions furnished the ring open structure **IV.60** as the major product (Scheme IV.20). Any other manipulation before column gave the benzofuran **IV.59**.



Scheme IV.20. Different hydrolysis conditions used on **IV.58** evolved to compound **IV.59** or **IV.60**.

The spectra of the compounds involved in these transformations are depicted in Figure IV.4.

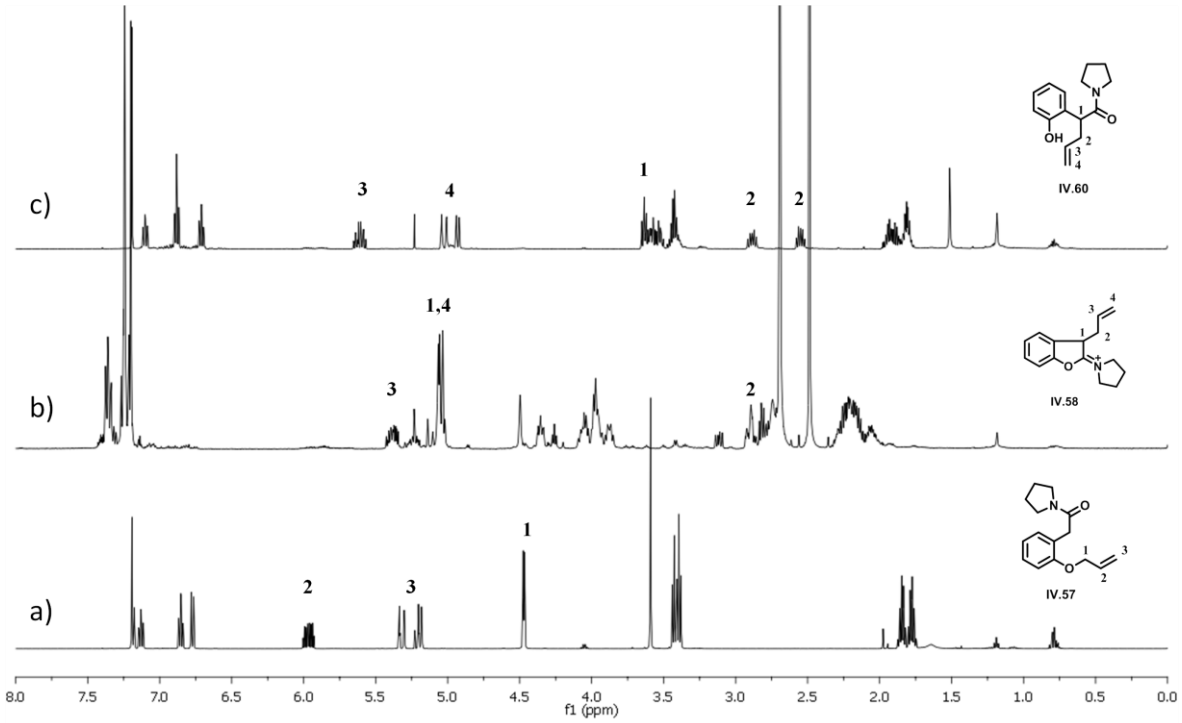
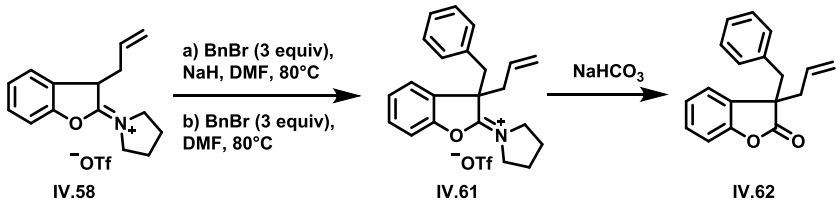


Figure IV.4. a) – Compound **IV.57**; b) – Crude mixture after standard μW conditions; c) – Compound **IV.60**.

It was also made an attempt concerning the trapping of intermediate **IV.58** with benzyl bromide with and without NaH (Scheme IV.21). Treatment with NaH led to **IV.61** (10%) that after hydrolysis with a sat. $NaHCO_3$ solution furnished the desired product **IV.62** in low yield. However, without base it was also verified by NMR the presence of trace amounts of **IV.61**.



Scheme IV.21. Trapping reaction with BnBr to obtain the intermediate **IV.62**.

IV.2.3 Aza-Claisen rearrangements

Like the *O*-counterparts (chromanones and 2-coumaranones), indolinone and dihydroquinolinone frameworks possessing a C-3 quaternary stereocenter can be observed in some natural products and in therapeutic agents.[32]

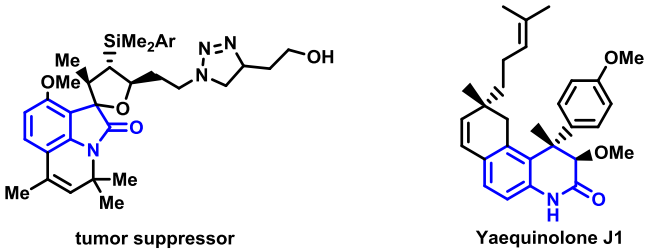
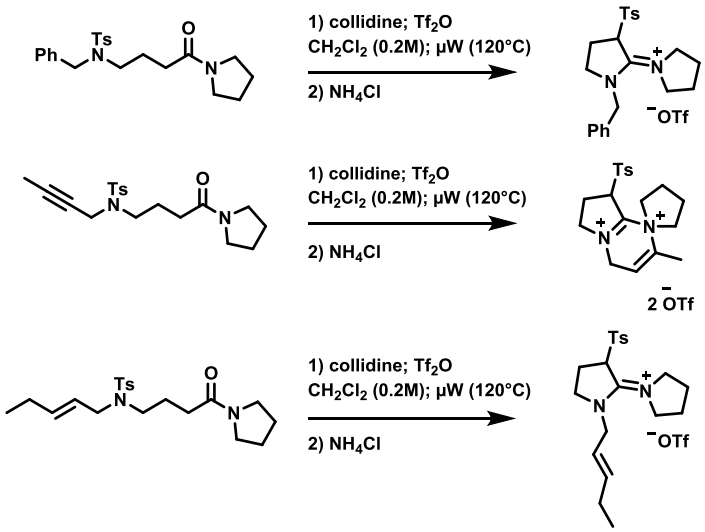


Figure IV.5. Natural products containing the oxindole moiety.

The aza-Claisen rearrangements were already explored in Maulide's group although with unsatisfactory results. The initial concern involved the substrate design, since the involvement of a nucleophilic and basic nitrogen atom implies an extra position to be protected. In addition, nitrogenated compounds could be hard to handle and sometimes difficult to purify.

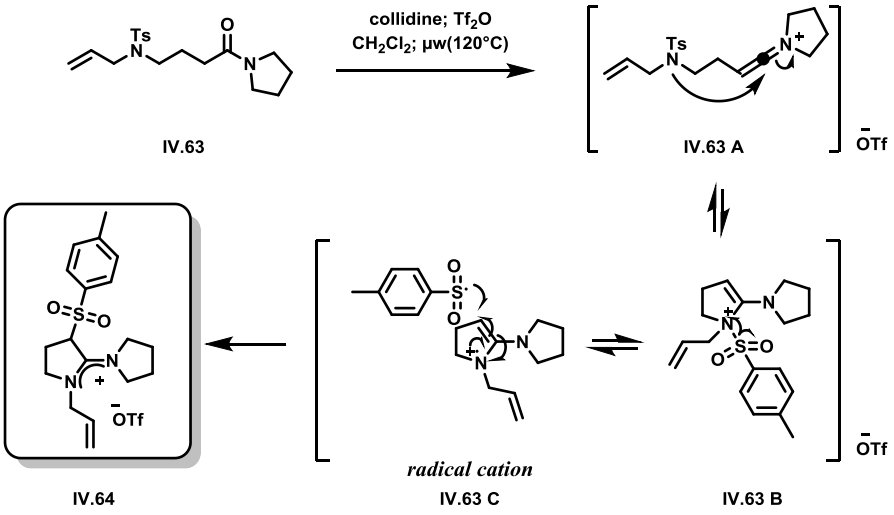
In the initial studies performed by D. Petkova, the protecting group was carefully chosen as it could influence the reactivity of the substrate under the electrophilic reaction conditions. It was anticipated that alkyl groups could be possible substituents, but would render the more basic nitrogen. On the other hand, acetyl or Boc groups would reduce the nucleophilicity of the nitrogen atom, and should compete with the amide moiety for the Tf_2O. Thus, on the first efforts to construct the nitrogen analogues of the already reported lactones,[24] tosyl group was chosen as *N*-protecting group, given that it fulfilled all the desired requirements.



Scheme IV.22. Preliminary studies on aza-Claisen rearrangements performed by Maulide's group.

It was expected that the reaction proceed through a mechanism similar to the previously reported for the oxygen derivatives. However, in all the experiments performed by D. Petkova, it was verified an unexpected tosyl migration towards position 3 instead the desired Claisen rearrangement (Scheme IV.22).

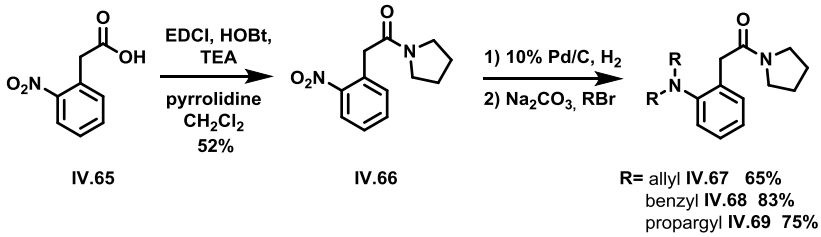
In the proposed mechanism the rearrangement proceed through the ketiminium formation followed by nucleophilic attack by the nitrogen atom. However, in this case, homolitic scission could take place to form a radical cation and a tosyl radical. This sulphur-based radical can attack the radical cation **IV.63 C** to yield the corresponding amidinium compound **IV.64** (Scheme IV.23).



Scheme IV.23. Proposed mechanism for tosyl migration.

Based on the results obtained for oxygen containing structures incorporating a phenyl group within the alkyl tether and on the preliminary studies made for *N*-tosylated derivatives, we decided to adopt a combined strategy to explore aza-Claisen rearrangement for the construction of heterocyclic compounds. Despite the initial concerns, it was chosen to perform the dialkylation of nitrogen atom, with allyl, benzyl and propargyl groups. The obtained disubstituted anilines were then subjected to rearrangement conditions in order to obtain either fused five- or six-membered ring heterocycles.

The corresponding derivatives **IV.67-IV69** were synthesized according to Scheme IV.24. Using 2-nitro phenyl acetic acid **IV.65** as starting material, the corresponding amide derivative **IV.66** was prepared using a standard carboxylic acid activation procedure. It was verified that the presence of the nitro group strongly influences this reaction, yielding the product in moderate yields (52 %). Then, it was performed a simple reduction of nitro group using 10% Pd/C in toluene and the resulting reaction mixture was filtered and evaporated, followed by the dialkylation to afford compounds **IV.67-IV69** in good yields.



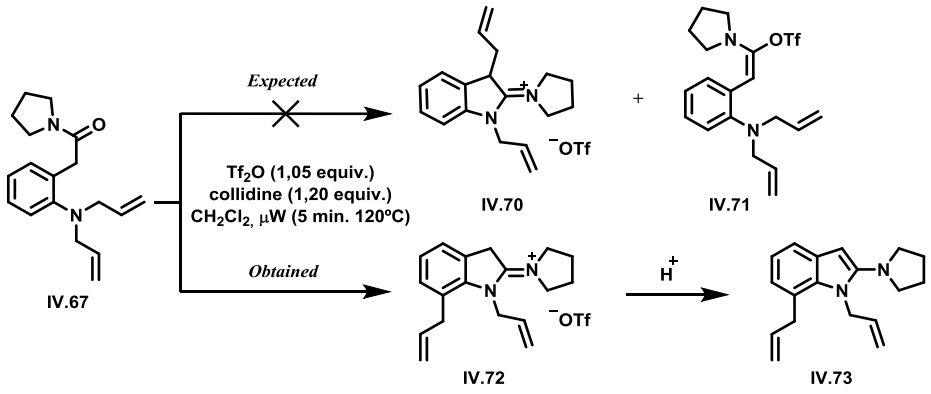
Scheme IV.24. Synthesis of allyl, benzyl and propargyl derivatives **IV.67**-**IV.69**.

The initial studies were made using the diallylated derivative **IV.67**. Upon treatment of **IV.67** with Tf_2O (1.05 equiv) and 2,4,6-collidine (1.2 equiv) in CH_2Cl_2 (0.2 M) under room temperature conditions, it was verified that the reaction was not complete being observed by NMR a mixture of an intermediate and starting material (Table IV.6).

Table IV.6. Optimization of Claisen reaction with **IV.67**.

Entry	Tf_2O (equiv)	Collidine (equiv)	Conditions	Ratio **IV.72:IV.71**
1	1.05	1.2	rt, overnight	1:0.5
2	1.05	1.2	μW (120°C), 5 min.	1:0
3	1.05	1.2	μW (150°C), 15 min.	1:0

Appling microwave conditions (120°C, 5 min.), the NMR from the crude mixture indicated that **IV.67** underwent the Claisen rearrangement leading to a mixture of two products, presumably the amidinium **IV.70** and the activated specie **IV.71** (Scheme IV.25).

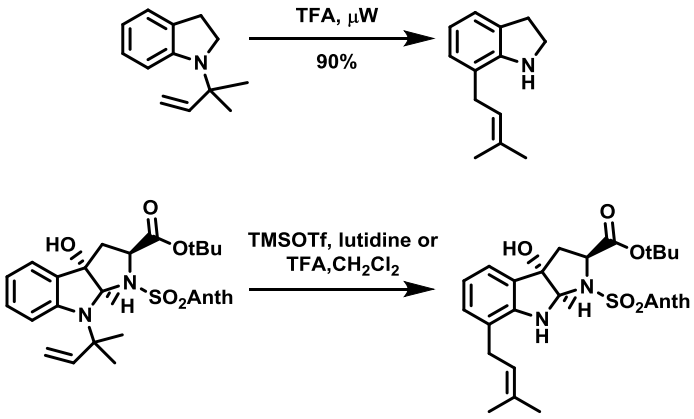


Scheme IV.25. Unexpected migration of allyl group under the usual conditions.

However, attempts to isolate **IV.70** revealed that the Claisen product was not the expected compound but the amidinium **IV.72** that after column or acidic work up (stirring in HCl 1M solution) gave the product **IV.73**.

It was also attempted to increase the reaction time (15 min.) and temperature (to 150ºC, the maximum allowed for CH_2Cl_2 under microwave conditions) in order to examine if the reaction proceed towards the desired rearrangement. However, the same NMR profile was obtained (entry 3).

This aromatic Claisen rearrangement to position 7 of indole moiety could be anticipated since some examples describing this type of transformation have been described in the literature (Scheme IV.26).[33]

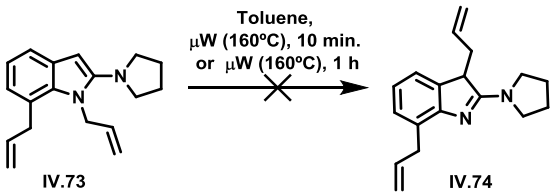


Scheme IV.26. Aromatic Claisen rearrangement to position 7 of indole-like moieties.[33]

This [3,3]-sigmatropic rearrangement of *N*-allyl-*N*-arylamines, usually requires very high temperatures, affording the corresponding anilines along undesired byproducts.[1,6] Therefore, in the last years several developments were made to turn this transformation in a milder process, such as using Lewis-acid catalysts.

Consequently, the rearrangement of **IV.67** to the derivative **IV.73** could be a step further in the development of a milder process since it evolves in a fast way under microwave conditions.

It was also considered that derivative **IV.73** could proceed *via* Claisen rearrangement to yield **IV.74** if exposed to the regular Claisen conditions (Scheme IV.27). Thus, **IV.73** was dissolved in toluene in order to reach higher temperatures under microwave conditions (in this case, 160ºC). However, it was verified by ^{1}H NMR that **IV.73** was highly stable under these reaction conditions, even when submitted to microwave conditions for 1 h.



Scheme IV.27. Attempts of Claisen rearrangement.

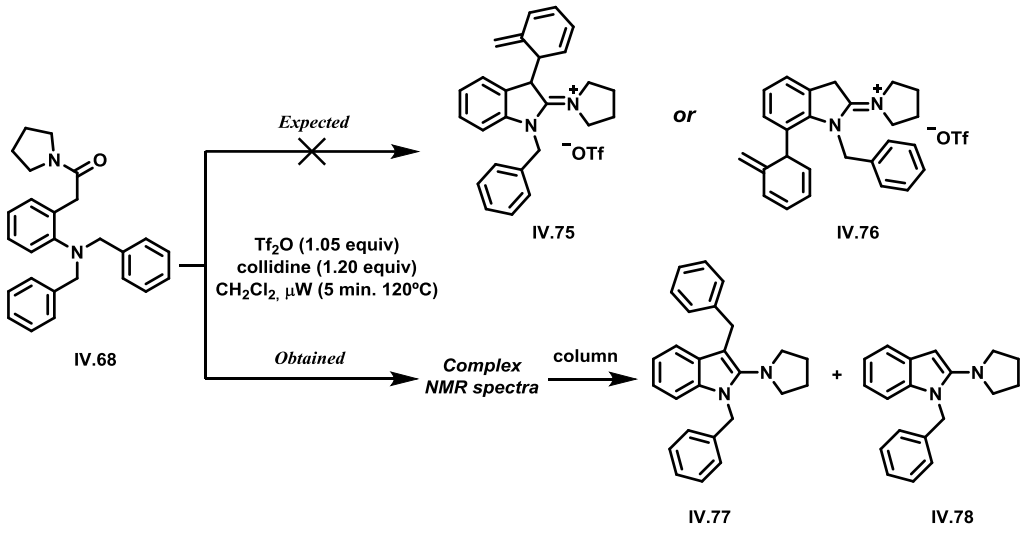
As previously observed by Petkova in the preliminary experiments (unpublished results), hydrolysis of the amidinium product (**IV.72**) to the corresponding oxindole was not possible. Compound **IV.72** couldn't be hydrolysed either with water or under basic conditions (sat. solution $NaHCO_3$).

Expecting a similar rearrangement from the benzylated and propargylated derivatives, **IV.68** and **IV.69**, respectively, the standard electrophilic activation conditions were applied either at room temperature or under the usual microwave conditions.

For compound **IV.68**, since the NMR spectra of crude mixture presented a complex profile – thus not providing the evidence (as for derivative **IV.67**) of the migration of benzilic moiety to the position 7 of aromatic ring – it was performed a simple aqueous work-up and a chromatographic column. After a careful assignment of ^1H NMR and COSY experiments, it was confirmed the presence of indoles **IV.77** and **IV.78**, isolated in 17% and 7% yield (unoptimized yields) (Scheme IV.28).

In fact, from the NMR of the crude mixture it could be anticipated the presence of the amidinium corresponding to **IV.77**, since a correlation between the CH_2 of benzylic moiety and the hydrogen of position C-3 of indole was clearly verified.

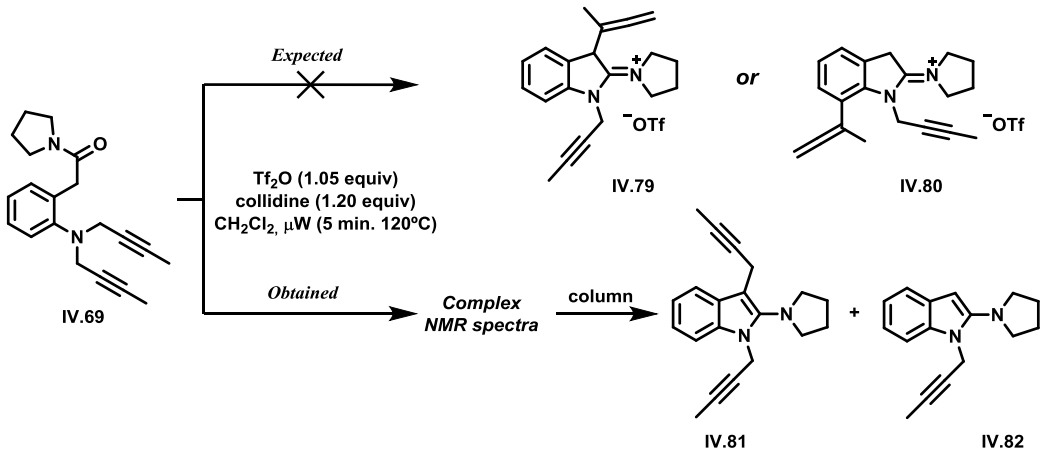
This result diverges from the result obtained for the allyllic derivative **IV.67**, where allyllic chain migrates to the aromatic moiety.



Scheme IV.28. Products obtained for benzylated derivative **IV.68** when submitted to the usual conditions.

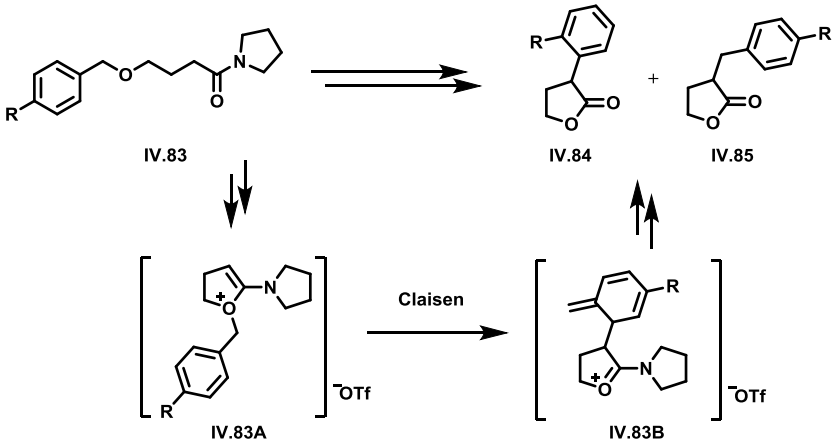
The same study was performed for propargylated derivative **IV.69** (Scheme IV.29). Once more, the NMR from the crude mixture showed a complex pattern. After chromatographic isolation the corresponding indoles **IV.81** and **IV.82** were obtained in 23% and 7% yield respectively (unoptimized yields).

It could be said that **IV.69** follows the same trend as the benzylated derivative **IV.68**, and that a migration of the benzyl and propargyl substituents is favored, while in the case of **IV.67** it is difficult to understand whether a migration of allyl chain or a rearrangement took place.



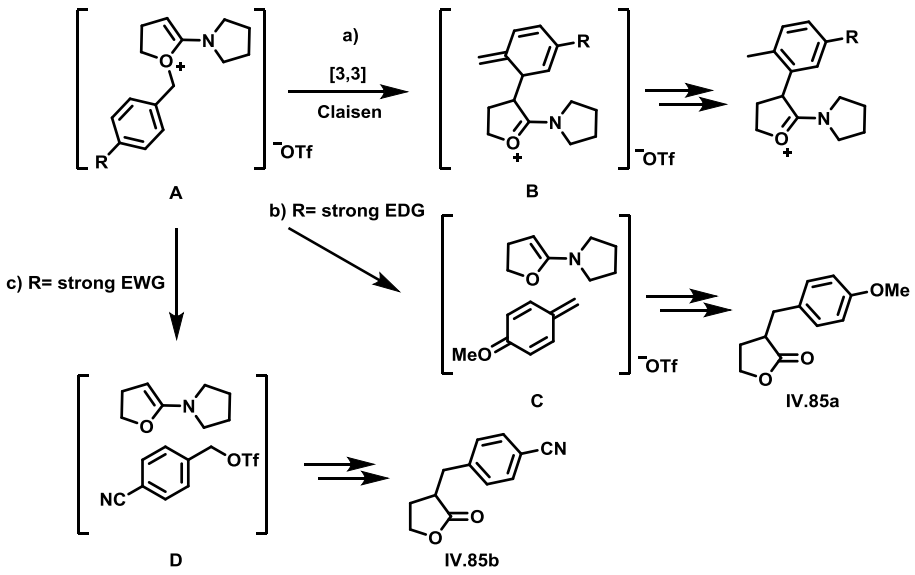
Scheme IV.29. Products obtained for propargylated derivative **IV.69** when submitted to the usual conditions.

In fact, as previously detected by V. Valerio *et al.* in the "benzyl-Claisen" studies, in addition to the expected rearranged products **IV.84**, in most cases the crude reaction mixture showed the presence of the corresponding α-benzylated γ-butyrolactones **IV.85** as side products (Scheme IV.30).[26]



Scheme IV.30. Debenzylated γ-butyrolactones obtained from the benzyl containing derivatives.

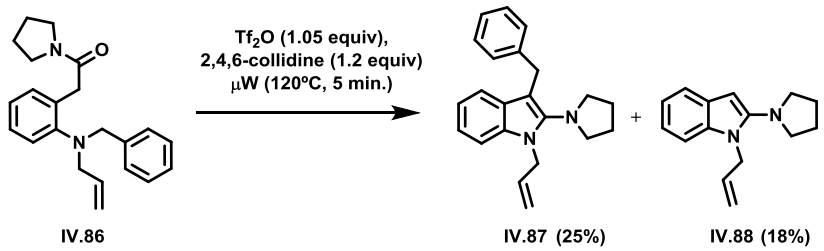
Besides the regular charge-accelerated "Claisen-like" [3,3] sigmatropic rearrangement, delivering the α-arylated lactone products *via* dearomatized species **B** (Scheme IV.31, a), some other mechanistic hypothesis were proposed.



Scheme IV.31. Mechanistic hypothesis suggested by V.Valerio *et al.*[26]

The observed formation of "benzyl-transfer" products **IV.85** could be explained by the dissociation of the benzylic moiety from the oxonium intermediate **A**, generating two fragments **C**, that could combine to yield product **IV.85a** (Scheme IV.31, b). An alternative scenario might explain the formation of such products **IV.85b** from electron-poor benzyl moieties as proceeding through an uncharged electrophile represented in **D** (Scheme IV.31, c).[26]

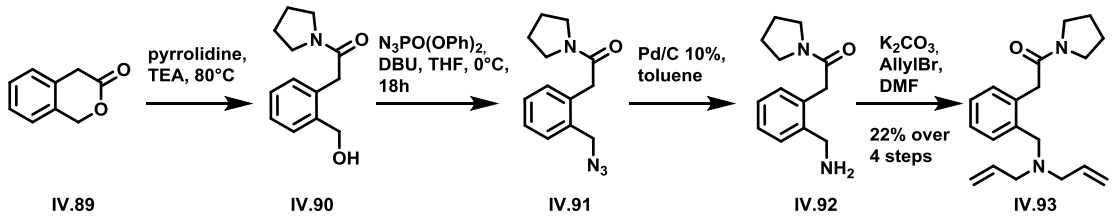
In order to understand whether a migration or a rearrangement were favoured under these conditions, and its dependence on the nature of the substituent, one experiment was carried where both benzyl and allyl groups were installed at the nitrogen atom (Scheme IV.32). Therefore, compound **IV.86** was submitted to regular rearrangement conditions being obtained a complex NMR mixture. After work-up and isolation, indolic compounds **IV.87** and **IV.88** were obtained in 25% and 18% yields, respectively (unoptimized yields).



Scheme IV.32. Products obtained for *N*-allyl-*N*-benzyl derivative **IV.86** when submitted to the usual conditions.

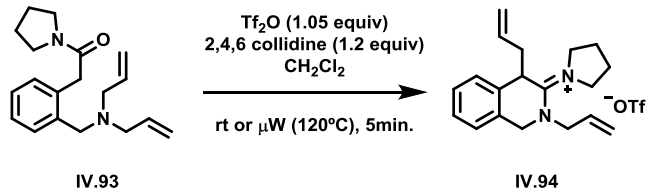
This result suggests that under these conditions, the migration of benzyl moiety is favored over the rearrangement (after cyclization to the indole unit), and that the benzyl group migrates preferentially to C-3.

The six-membered nitrogenated compound **IV.93** was prepared from the 3-isochromanone (**IV.89**). The 3-isochromanone was hydrolysed in pyrrolidine/TEA affording the compound **IV.90** that upon treatment with diphenylphosphoryl azide (DPPA) in basic conditions afforded the corresponding **IV.91** in excellent conversion, as confirmed by ^{1}H NMR. This last compound was converted to the amine **IV.92** under 10% Pd/C reductive conditions. Compound **IV.92** when subjected to treatment with K_2CO_3/allyl bromide in THF afforded the corresponding **IV.93** in 22% yield (4 steps) (Scheme IV.33).



Scheme IV.33. Synthesis of compound **IV.93**.

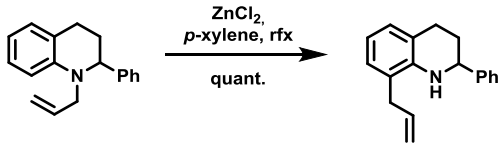
The diallylated derivative **IV.93** was then subjected to the standard activation conditions either at room temperature (5 min.) and microwave (120°C, 5 min.) conditions (Scheme IV.34). Fortunately, in both cases the product was full converted to the corresponding amidinium intermediate **IV.94**.



Scheme IV.34. Claisen reaction to obtain the six member amidinium compound **IV.94**.

Like the *O*-allyl amide counterpart, **IV.93** was quantitatively converted to iminium ether **IV.94** after five minutes at room temperature (*see* Scheme IV.13.a, page 191).[27] As already reported by Peng *et al.* the mild conditions required for this transformation can be a result of the reduced conformational flexibility due to the phenyl ring within the alkyl tether, resulting in a pre-organization for the cyclization/Claisen rearrangement events, perhaps combined with the favorable formation of a conjugated keteniminium salt intermediate.

However, if the compound was the corresponding isomer 2-oxo-1,2,3,4-tetrahydroquinoline derivative, probably the result would be a similar rearrangement observed for derivative **IV.67** (Scheme IV.25, page 204). The rearrangement to position 8 was already reported for similar compounds (Scheme IV.35).[34]

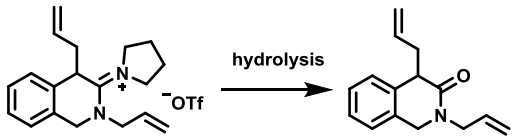


Scheme IV.35. Claisen rearrangement to obtain 8-allyl-2-phenyl-1,2,3,4-tetrahydroquinoline.[34]

It was further observed, that the amidinium derivative **IV.94** could not be hydrolyzed under the mild conditions used for the oxygenated derivatives, such as water or $NaHCO_3$ saturated solution at room temperature (Table IV.7, entries 1 and 2). Instead, strong basic were required, and although hydrolysis of **IV.94** could be attained at room temperature using a 2 M NaOH solution, the NMR showed a mixture between **IV.94** and the desired lactam **IV.95** (entry 3).

The hydrolysis with water under microwave conditions did not afford the desired product **IV.95** (entry 5). When a 1M $NaHCO_3$ solution was used, the hydrolysis product was obtained as the major product. Using a saturated solution of $NaHCO_3$, the amidinium was fully converted to **IV.95** (Table IV.7, entries 6 and 7).

Table IV.7. Hydrolysis conditions used on amidinium **IV.94** to obtain **IV.95**.[a]



IV.94 **IV.95**

Entry	Conditions	Temp.	NMR (**IV.94**:**IV.95**)	Isolated yield
1	H_2O	rt, overnight	---[b]	---
2	$NaHCO_3$ sat.	rt, overnight	1:0	---
3	NaOH 2M	rt, 5 h	0.5:1	17%
4	HCl 1M	rt, 3 days	1:0	---
5	H_2O	μW (120°C), 5 min → 15 min.	1:0	---
6	$NaHCO_3$ 1M	μW (120°C), 5 min	0.3:1	---
7	$NaHCO_3$ sat.	μW (120°C), 15 min	0:1	90%

[a] The crude mixture was evaporated to dryness and redissolved in THF (0,5 mL) and the corresponding aqueous solution (0,5 mL). [b] TLC indicated that no conversion occurred

IV.3 Conclusions

The discovery of an unprecedented Claisen rearrangement through an amide electrophilic activation of allyl amides could be a valuable methodology to the enantioselective synthesis of heterocyclic structures. Consequently, several efforts were conducted to prepare coumaranones- and chromanones-based compounds with an enantioenriched center at position C-3 (Figure IV.6). Moreover the procedure was also attempted for the preparation of some nitrogenated analogues, the indolinones and dihydroquinolinones.

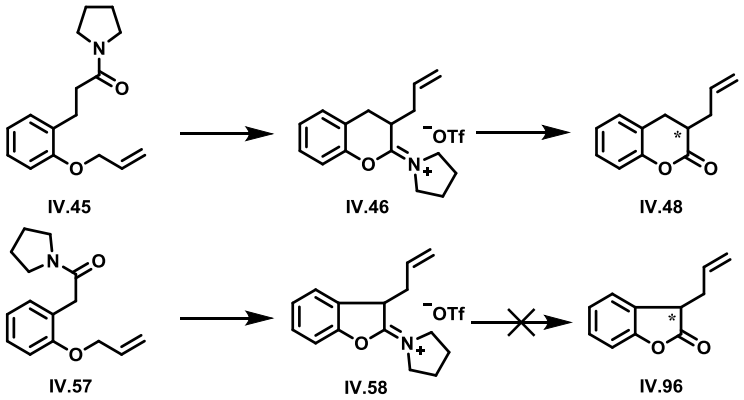


Figure IV.6. Results obtained for the δ-allyloxyamides incorporating a phenyl group within the alkyl tether.

It was verified that the conditions used for the previous reported lactones (μW 120ºC, 5 min.) were suitable for the ε- or δ-allyl amides comprising an arene within the alkyl tether (**IV.45** and **IV.57**, respectively), to obtain the corresponding amidinium intermediates (Figure IV.6). The hydrolysis step to remove the pyrrolidine moiety was easily performed for compound **IV.46** in order to attain **IV.48**; however, the amidinium **IV.58** could not be hydrolyzed even using extreme basic conditions. The harsh hydrolysis conditions needed for these derivatives could be attributed to a stable conjugation of iminium with the aromatic ring.

Due to the promising results obtained for the six membered derivative **IV.46**, it was also attempted a chiral approach using appropriate chiral auxiliaries. However, all the attempts were unsuccessful.

In the aza-Claisen approach (Figure IV.7), the *N*,*N*-diallylated derivative **IV.67** suffered a rearrangement towards the arene ring, yielding the 7-allylated indole **IV.73**. For the benzyl and propargyl derivatives, it was verified that a migration mechanism was favored over the rearrangement. Moreover, it was also verified that the hydrolysis step was an intricate task, requiring harsh hydrolysis conditions which could compromise the chiral center integrity.

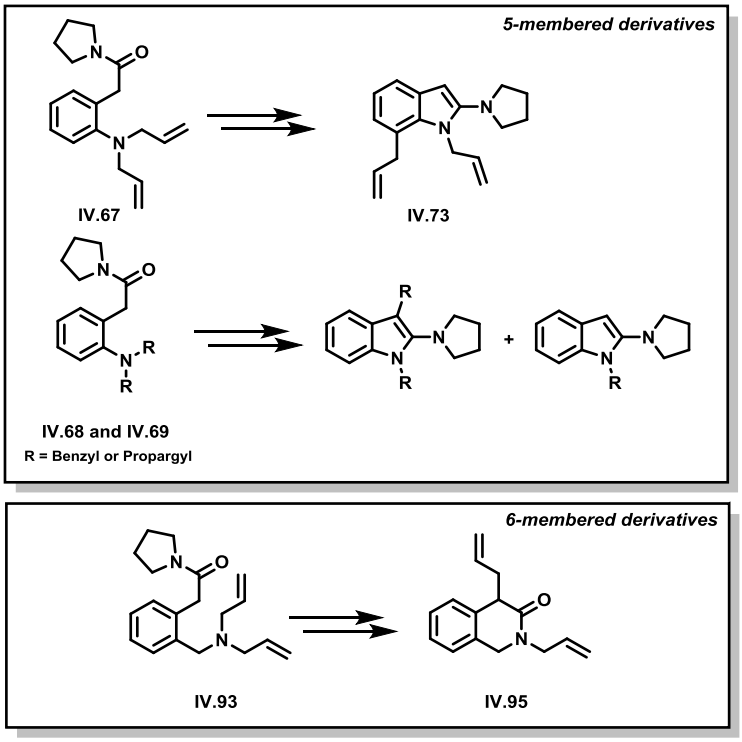


Figure IV.7. Results obtained for the nitrogenated derivatives.

IV.4 Experimental

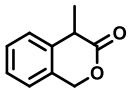
IV.4.1 Materials

All commercially obtained reagents were used without further purification unless specified. All the mentioned solvents used in the reactions were dried by usual methods.[130] Column chromatography was carried out with silica gel 60 Å (220-440 mesh) using the described eluent for each case. Analytical TLC was performed with silica gel 60 F254 aluminum plates (Merck).TLCs were visualized by fluorescence quenching with UV light at 254 nm and/or by staining with potassium permanganate or phosphomolybdic acid stains.

IR spectra were recorded using a Perkin-Elmer Spectrum 1000 FT-IR. The IR bands are classified as strong (s), medium (m), or weak (w). NMR spectra were recorded with a Brucker Avance 500 spectrometer using $CDCl_3$ as solvent using the corresponding $CHCl_3$ signal as reference. The spectra were recorded at room temperature (298 K) unless otherwise stated.

Microwave experiments were performed using a CEM Discover Microwave SP-D (CEM). High resolution mass spectra were obtained on a Bruker APEX III FT-MS (7 T magnet). All masses are given in atomic units/elementary charge (m/z) and reported in percentage relative to the basic peak.

IV.4.1.1 4-Methylisochroman-3-one (IV.37) [35]



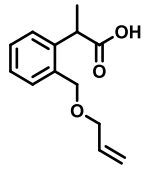
In a two necked flask was prepared LDA (14.85 mmol, 1.1 equiv) *in situ* in THF (60 mL). To the cooled mixture at -78 °C was added 3-isochromanone (2 g, 13.5 mmol) in THF (30 mL). The resulting mixture was stirred for 1 h, and MeI (0.92 mL, 14.85 mmol) was added. The mixture was allowed to reach room temperature and then stirred for 2 h. The reaction was quenched with NH_4Cl, extracted with EtOAc, dried and evaporated to dryness. Purification by flash column chromatography using hexane and ethyl acetate as eluent (gradient system from hexane to hexane: ethyl acetate 4:1) afforded the product as white solid (735 mg, 34 %). *Rf* = 0.5, hexane:EtOAc 4:1.

Mp: 49-50 °C;

^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.43 – 7.21 (m, Ar*H*, 4H), 5.29 (m, CH_2, 2H), 3.63 (q, *J* = 7.0 Hz, C*H*, 1H), 1.63 (d, *J* = 7.0 Hz, CH_3, 3H);

^{13}C NMR (126 MHz, $CDCl_3$) δ: 173.7 (*C*=O), 135.6 (*C*Ar), 132.0 (*C*Ar), 129.0 (*C*Ar), 127.2 (*C*Ar), 124.8 (*C*Ar), 124.7 (*C*Ar), 69.3 (CH_2), 39.5 (*C*H), 12.9 (CH_3).

IV.4.1.2 2-(2-((Allyloxy)methyl)phenyl)propanoic acid (IV.38)



The product (0.735 g, 4.56 mmol) was hydrolyzed in K_2CO_3 (375 mg, 2.72 mmol) in H_2O (25 mL), at reflux, overnight. The mixture was evaporated to dryness and the salt was kept under vacuum for 1 day. THF (9 mL, 0,5M) was added and the flask was placed in an ice bath. NaH (360 mg, 9.06 mmol) was added portion wise and the mixture was allowed to reach room temperature and stirred for 1.5h. Then allyl bromide (0.8 mL, 9.06 mmol) was added and the mixture was stirred at room temperature overnight. The reaction was quenched with water, extracted with EtOAc, washed with water and brine, dried over Na_2SO_4 and evaporated to dryness. Purification by flash column chromatography using hexane and ethyl acetate as eluent (gradient system from hexane to hexane: ethyl acetate 1:1) afforded the product as colourless oil (850 mg, 85 %). *Rf* = 0.4, hexane:EtOAc 4:1.

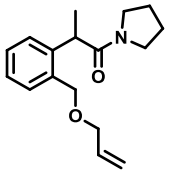
IR (cm-1): 2982 (w), 2871 (w), 1703 (s, *C=O*);

^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.45 – 7.24 (m, Ar*H*, 4H), 5.95 (ddt, *J* = 11.7, 10.8, 8.1 Hz, -CH_2CHCH_2,1H), 5.35 (ddd, *J* = 17.2, 2.9, 1.4 Hz, -CH_2CHCH_2, 1H), 5.29 – 5.25 (m, -CH_2CHCH_2, 1H), 4.69 (d, *J* = 11.3 Hz, CH_2, 1H), 4.60 (d, *J* = 11.3 Hz, CH_2, 1H), 4.10 – 4.03 (m, -CH_2CHCH_2,C*H*, 3H), 1.53 (d, *J* = 7.0 Hz, CH_3, 3H);

^{13}C NMR (126 MHz, $CDCl_3$) δ: 139.7 (*C*Ar), 135.0 (*C*Ar), 133.7 (-CH_2CHCH_2), 130.4 (*C*Ar), 129.3 (*C*Ar), 127.5 (*C*Ar), 127.4 (*C*Ar), 118.7 (-CH_2CHCH_2), 71.7 (-CH_2CHCH_2), 71.3 (CH_2), 40.8 (*C*H), 17.8 (CH_3);

HRMS: *m/z* Calculated for $C_{17}H_{23}NO_2$ $[M+Na]^+$: 243.0992, found: 243.0992.

IV.4.1.3 2-(2-((Allyloxy)methyl)phenyl)-1-(pyrrolidin-1-yl)propan-1-one (IV.39)



To 2-(2-((allyloxy)methyl)phenyl)propanoic acid (160 mg, 0.73 mmol) in DCM (5 mL), was added EDCI.HCl (139 mg, 0.73 mmol), HOBt (98 mg, 0.73 mmol), triethylamine (0.1 mL, 0.73 mmol) and pyrrolidine (60 μL, 0.73 mmol). The mixture was stirred at room temperature overnight. The mixture was evaporated to dryness and the crude product was purified by flash column chromatography using hexane and ethyl acetate as eluent (gradient system from hexane to hexane: ethyl acetate 1:1). The product was obtained as colourless oil (170 mg, 90 %). R_f = 0.4 hexane:EtOAc (1:1).

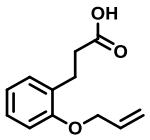
IR (cm-1): 2973 (w), 2872 (w), 1637 (s, *C=O*), 1617 (s), 1422 (s), 1059 (s), 759 (s);

^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.44 – 7.14 (m, Ar*H*, 4H), 5.94 (ddt, *J* = 16.4, 10.8, 5.6 Hz, -CH_2CHCH_2, 1H), 5.26 (ddd, *J* = 40.9, 13.8 Hz, -CH_2CHCH_2, 2H), 4.66 (d, *J* = 11.5 Hz, CH_2, 1H), 4.51 (d, *J* = 11.5 Hz, CH_2, 1H), 4.15 – 4.00 (m, -CH_2CHCH_2, C*H*, 3H), 3.61 – 3.49 (m, CH_2, 1H), 3.49 – 3.35 (m, CH_2, 2H), 3.01 – 2.89 (m, CH_2, 1H), 1.88 – 1.65 (m, CH_2, 4H), 1.42 (d, CH_3, 2H);

^{13}C NMR (126 MHz, $CDCl_3$) δ: 172.6 (C=O), 141.5 (*C*Ar), 134.6 (*C*Ar), 134.5 (-CH_2CHCH_2), 130.2 (*C*Ar), 129.2 (*C*Ar), 127.3 (*C*Ar), 126.7 (*C*Ar), 117.5 (-CH_2CHCH_2), 71.4 (-CH_2CHCH_2), 70.8 (CH_2), 46.3 (CH_2 pyrrolidine), 46.2 (CH_2 pyrrolidine), 40.8 (*C*H), 26.2 (CH_2 pyrrolidine), 24.3 (CH_2 pyrrolidine), 19.9 (CH_3);

HRMS: *m/z* Calculated for $C_{17}H_{23}NO_2$ $[M+Na]^+$: 296.1619, found: 296.1621.

IV.4.1.4 3-(2-(Allyloxy)phenyl)propanoic acid



Dihydrocoumarin (5 g, 33.75 mmol) was hydrolyzed by K_2CO_3 (3.73 g, mmol) in H_2O (150 mL) at reflux for 5 h. The mixture was evaporated to dryness and the salt was kept under vacuum overnight. Then, THF (100 mL) was added and the flask was placed in an ice bath. NaH (2.7 g, 67.5 mmol) was added portion wise and the mixture was allowed to reach room temperature and stirred for 1.5h. Then allyl bromide (5.8 mL, 67.5 mmol) was added and the mixture was stirred at room temperature overnight. The reaction was quenched with water, extracted with EtOAc, washed with water and brine, dried over Na_2SO_4 and evaporated to dryness. The crude product was purified by flash column chromatography using a gradient system from hexane to hexane:EtOAc (1:1). The product was obtained as white solid (5.5 g, 79%). R_f = 0.2 hexane:EtOAc (4:1).

Mp: 65-67°C;

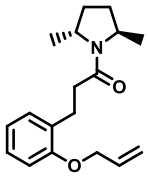
IR (cm-1): 3023 (w), 2923 (w), 1703 (s, *C=O*), 1493 (m), 1239 (m), 749 (s);

^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.22 – 7.15 (m, Ar*H*, 2H), 6.89 (td, *J* = 7.4, 0.7 Hz, Ar*H*, 1H), 6.84 (d, *J* = 8.0 Hz, Ar*H*, 1H), 6.07 (ddt, *J* = 17.2, 10.3, 5.0 Hz, -CH_2CHCH_2, 1H), 5.46 – 5.38 (m, -CH_2CHCH_2, 1H), 5.28 (dd, *J* = 10.6, 1.4 Hz, -CH_2CHCH_2, 1H), 4.56 (dd, *J* = 4.8, 1.5 Hz, -CH_2CHCH_2, 2H), 2.99 (t, *J* = 7.8 Hz, CH_2, 2H), 2.78 – 2.61 (m, CH_2, 2H);

^{13}C NMR (126 MHz, $CDCl_3$) δ: 156.6 (*C*Ar), 133.5 (-CH_2CHCH_2), 130.3 (*C*Ar), 128.9 (*C*Ar), 127.8 (*C*Ar), 120.8 (*C*Ar), 117.2 (-CH_2CHCH_2), 111.6 (*C*Ar), 68.7 (-CH_2CHCH_2), 34.1 (CH_2), 26.1 (CH_2);

HRMS: *m/z* Calculated for $C_{12}H_{14}NO_3$ $[M+Na]^+$: 229.0837, found: 229.0835.

IV.4.1.5 3-(2-(Allyloxy)phenyl)-1-((2R,5R)-2,5-dimethylpyrrolidin-1-yl)propan-1-one (IV.51)



To the substrate (200 mg, 0.97 mmol) in DCM (5 mL), was added EDCI.HCl (186 mg, 0.97 mmol), HOBt (131 mg, 0.97 mmol), triethylamine (0.27 mL, 0.97 mmol) and (2R,5R)-2,5-dimethylpyrrolidine hydrochloride (132 mg, 0.97 mmol). The mixture was stirred at room temperature overnight. The mixture was evaporated to dryness and the crude product was purified by flash column chromatography using a gradient system from hexane to hexane:EtOAc (1:1). The product was obtained as colourless oil (225 mg, 81 %). *Rf* = 0.3, 2x (hexane:EtOAc, 4:1).

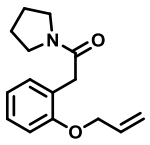
IR (cm-1): 2966 (w), 1629 (s, *C=O*), 1409 (s), 1239 (m), 750 (s);

^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.22 – 7.11 (m, Ar*H*, 2H), 6.90 – 6.82 (m, Ar*H*, 2H), 6.06 (ddt, *J* = 17.2, 10.4, 5.1 Hz, -CH_2CHCH_2, 1H), 5.46 – 5.37 (dd, -CH_2CHCH_2, 1H), 5.26 (dd, *J* = 10.5, 1.4 Hz, -CH_2CHCH_2,1H), 4.56 (dt, *J* = 5.0, 1.4 Hz, , -CH_2CHCH_2, 2H), 4.21 (m, *J* = 6.4 Hz, C*H*, 1H), 3.91 (m, *J* = 6.4 Hz, C*H*, 1H), 3.10 – 2.91 (m, CH_2, 2H), 2.66 – 2.50 (m, CH_2, 2H), 2.14 – 2.02 (m, CH_2, 2H), 1.50 (m, *J* = 21.0, 6.0 Hz, CH_2, 2H), 1.13 (d, *J* = 6.3 Hz, CH_3, 3H), 1.09 (d, *J* = 6.4 Hz, CH_3, 3H);

^{13}C NMR (126 MHz, $CDCl_3$) δ: 171.4 (*C*=O), 156.6 (*C*Ar), 133.6 (-CH_2CHCH_2), 130.7 (*C*Ar), 130.1 (*C*Ar), 127.4 (*C*Ar), 120.8 (*C*Ar), 117.2 (-CH_2CHCH_2), 111.5 (*C*Ar), 68.8 (-CH_2CHCH_2), 53.6 (*C*H), 53.0 (*C*H), 35.0 (CH_2), 30.9 (CH_2), 29.2 (CH_2), 27.4 (CH_2), 21.8 (CH_3), 19.3 (CH_3);

HRMS: *m/z* Calculated for $C_{18}H_{25}NO_2$ $[M+Na]^+$: 310.1775, found: 310.1777.

IV.4.1.6 2-(2-(Allyloxy)phenyl)-1-(pyrrolidin-1-yl)ethanone (IV.57)



2-Coumaranone (1 g, 7.5 mmol) was refluxed in pyrrolidine (1.22 mL, 14.9 mmol) and triethylamine (3.12 mL, 22.4 mmol) overnight. The mixture was evaporated to dryness and THF (20 mL) was added. The flask was placed in an ice bath. NaH (597 mg, 14.9 mmol) was added portion wise and the mixture was allowed to reach room temperature and stirred for 1.5 h. Then allyl bromide (1.3 mL, 14.9 mmol) was added and the mixture was stirred at room temperature overnight. The reaction was quenched with water, extracted with EtOAc, washed with water and brine, dried over Na_2SO_4 and evaporated to dryness. The crude product was purified by flash

column chromatography a gradient system from hexane to hexane: EtOAc (1:1). The product was obtained as orange solid (1.55 g, 85 %). R_f = 0.2, hexane:EtOAc, 3:2.

Mp: 68-69°C;

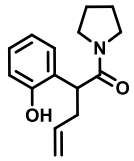
IR (cm-1): 2970 (w), 2873 (w), 1635 (s, *C=O*), 1425 (m), 1242 (m), 745 (m);

^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.28 – 7.16 (m, Ar*H*, 2H), 6.92 (td, *J* = 7.5, 0.8 Hz, Ar*H*, 1H), 6.84 (d, *J* = 8.2 Hz, Ar*H*, 1H), 6.03 (ddt, *J* = 17.2, 10.4, 5.2 Hz, -CH_2CHCH_2, 1H), 5.39 (dd, *J* = 17.3, 1.6 Hz, -CH_2CHCH_2, 1H), 5.28 – 5.25 (m, -CH_2CHCH_2, 1H), 4.54 (dt, *J* = 5.1, 1.5 Hz, -CH_2CHCH_2, 2H), 3.66 (s, CH_2, 2H), 3.51 – 3.45 (m, CH_2, 4H), 1.97 – 1.78 (m, CH_2, 4H);

^{13}C NMR (126 MHz, $CDCl_3$) δ: 170.1 (*C*=O), 156.2 (*C*Ar), 133.6 (-CH_2CHCH_2), 130.6 (*C*Ar), 128.0 (*C*Ar), 124.4 (*C*Ar), 121.0 (*C*Ar), 117.3 (-CH_2CHCH_2), 111.7 (*C*Ar), 69.0 (-CH_2CHCH_2), 46.9 ($CH_{2\ pyrr}$), 45.9 ($CH_{2\ pyrr}$), 36.1 (CH_2), 26.3 ($CH_{2\ pyrr}$), 24.6 ($CH_{2\ pyrr}$);

HRMS: *m/z* Calculated for $C_{15}H_{19}NO_2$ $[M+Na]^+$: 268.1309 found:268.1308.

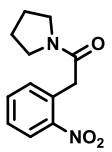
IV.4.1.7 2-(2-Hydroxyphenyl)-1-(pyrrolidin-1-yl)pent-4-en-1-one (IV.60)



^{1}H NMR (500 MHz, $CDCl_3$) δ: 10.79 (s, -OH, 1H), 7.17 (dd, *J* = 7.9, 1.6 Hz, 1H), 6.98 – 6.90 (m, 2H), 6.78 (dd, *J* = 7.4, 1.2 Hz, 1H), 5.68 (ddt, *J* = 17.1, 10.0 Hz, -CH_2CHCH_2, 1H), 5.14 – 4.95 (m, -CH_2CHCH_2, 2H), 3.74 – 3.43 (m, -CH_2, -C*H*, 5H), 2.99 – 2.90 (m, -CH_2CHCH_2, 1H), 2.66 – 2.57 (m, -CH_2CHCH_2, 1H), 2.05 – 1.80 (m, -CH_2, 4H);

^{13}C NMR (126 MHz, $CDCl_3$) δ: 173.85 (C=O), 157.27 (CAr-OH), 135.29 (-CH_2CHCH_2), 131.14 (C-Ar), 129.34 (C-Ar), 123.36, 119.64 (C-Ar), 119.19 (C-Ar), 117.51 (-CH_2CHCH_2), 51.71 (CH), 47.53 ($CH_{2\ pyrr}$), 46.46 ($CH_{2\ pyrr}$), 35.55 (-CH_2CHCH_2), 26.09 ($CH_{2\ pyrr}$), 24.46 ($CH_{2\ pyrr}$).

IV.4.1.8 2-(2-Nitrophenyl)-1-(pyrrolidin-1-yl)ethanone (IV.66) [36]



To 2-nitrophenylacetic acid (1.5 g, 8.3 mmol) in DCM (27 mL) was added dropwise DIC (1.3 mL, 9.1 mmol), and the solution turned from yellow to red. The mixture was stirred at room temperature for 30 min. Then, triethylamine (1.3 mL, 9.1 mmol) and freshly distilled pyrrolidine (0.75 mL, 9.1 mmol) were added. The mixture was stirred at room temperature overnight. The mixture was diluted with EtOAc and washed with HCl 1M solution, water and brine, dried over

Na_2SO_4 and evaporated. The crude was purified by flash column chromatography using a gradient system from hexane to hexane: EtOAc (1:1). The product was obtained as white solid (1.5 g, 77 %). *Rf* = 0.2 hexane:EtOAc (1:1).

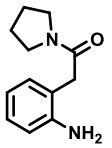
To 2-nitrophenylacetic acid (1.5 g, 8.3 mmol) was added EDCI.HCl (1.6 g 8.3 mmol), HOBt (1.12 g, 8.3 mmol), triethylamine (1.2 mL, 8.3mmol) and pyrrolidine (0.7 mL, 8.3 mmol). The mixture was stirred at room temperature until total SM consumption (TLC) *Rf* = 0.3 hexane:EtOAc (1:1). The mixture was diluted with EtOAc and washed with water and brine, dried over Na_2SO_4 and evaporated. The crude was purified by flash column chromatography using a gradient system from hexane to hexane:EtOAc (1:1). The product was obtained as white solid (1 g, 52 %). *Rf* = 0.2, hexane:EtOAc (1:1).

IR (cm-1): 2971 (w), 2876 (w), 1645 (s, *C=O*), 1522 (s), 1433 (m) 1346 (m);

^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.08 (d, *J* = 8.2, 1.1 Hz, Ar*H*, 1H), 7.57 (dd, *J* = 7.5, 1.2 Hz, Ar*H*, 1H), 7.50 – 7.41 (dd, Ar*H*, 1H), 7.36 (d, *J* = 7.6 Hz, Ar*H*, 1H), 3.99 (s, CH_2, 2H), 3.57 (t, *J* = 6.8 Hz, CH_2, 2H), 3.49 (t, *J* = 6.9 Hz, CH_2, 2H), 2.08 – 1.96 (m, CH_2, 2H), 1.90 (m, CH_2, 2H);

^{13}C NMR (126 MHz, $CDCl_3$) δ: 167.4 (*C*=O), 133.4 (*C*Ar), 133.3 (*C*Ar), 128.0 (*C*Ar), 125.03 (*C*Ar), 46.7 ($CH_{2\ pyrr}$), 45.9 ($CH_{2\ pyrr}$), 39.9 (*C*H_2), 26.1 ($CH_{2\ pyrr}$), 24.4 ($CH_{2\ pyrr}$).

IV.4.1.9 2-(2-Aminophenyl)-1-(pyrrolidin-1-yl)ethanone



2-(2-Nitrophenyl)-1-(pyrrolidin-1-yl)ethanone (250 mg, 1.07 mmol) was dissolved in toluene (10 mL) and Pd/C 10% (113 mg, 10 mol%) was added. The mixture was stirred under H_2 atmosphere until total consumption of starting material. The mixture was filtered through a celite pad and evaporated to dryness. The product was obtained as white solid (210 mg, quant.). *Rf* = 0.4, EtOAc.

Mp: 85°C;

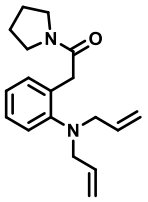
IR (cm-1): 3373 (w), 3230 (w), 2972 (w), 2882 (w), 1604 (s), 1592 (s), 1443 (s);

^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.11- 7.03 (m, Ar*H*, 2H), 6.91 – 6.73 (m, Ar*H*, 2H), 3.67 (s, CH_2, 2H), 3.60 (t, *J* = 6.9 Hz, CH_2, 2H), 3.45 (t, *J* = 6.9 Hz, CH_2, 2H), 1.96 (m, CH_2, 2H), 1.89 – 1.81 (m, CH_2, 2H);

^{13}C NMR (126 MHz, $CDCl_3$) δ: 169.91 (*C*=O), 131.2 (*C*Ar), 128.4 (*C*Ar), 119.7 (*C*Ar), 117.7 (*C*Ar), 47.3 ($CH_{2\ pyrr}$), 46.2 ($CH_{2\ pyrr}$), 39.9 (*C*H_2), 26.3 ($CH_{2\ pyrr}$), 24.4 ($CH_{2\ pyrr}$);

HRMS: *m/z* Calculated for $C_{12}H_{16}N_2O$ $[M+Na]^+$: 227.1155, found: 277.1155.

IV.4.1.10 2-(2-(Diallylamino)phenyl)-1-(pyrrolidin-1-yl)ethanone (IV.67)



2-(2-Aminophenyl)-1-(pyrrolidin-1-yl)ethanone (210 mg, 1.07 mmol) was dissolved in DMF (10 mL). Na_2CO_3 (171 mg , 3.2 mmol) and allyl bromide (0.37 mL, 3.2 mmol) were added and the mixture was stirred at 100°C overnight. The reaction was quenched with water, extracted with EtOAc, washed with water and brine, dried over Na_2SO_4 and evaporated to dryness. Purification by flash column chromatography using a gradient system from hexane to hexane: ethyl acetate (2:3), afforded the product as colourless oil (170 mg, 65%). *Rf* = 0.3, hexane:EtOAc (3:2).

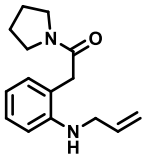
IR (cm-1): 2974 (w), 2876 (w), 1623 (s), 1420 (s);

^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.29 (d, Ar*H*, 1H), 7.18 (dd, Ar*H*, 1H), 7.08 – 7.03 (m, Ar*H*, 2H), 5.75 (ddt, *J* = 16.5, 10.2, 6.2 Hz, -CH_2CHCH_2, 2H), 5.17 – 5.08 (m, -CH_2CHCH_2, 4H), 3.75 (s, CH_2, 2H), 3.60 – 3.43 (m, -CH_2CHCH_2, CH_2, 6H), 3.29 (t, *J* = 6.5 Hz, CH_2, 2H), 1.84 (m, CH_2, 4H);

^{13}C NMR (126 MHz, $CDCl_3$) δ: 170.6 (*C*=O), 149.8 (*C*Ar), 135.1 (-CH_2CHCH_2), 131.9 (*C*Ar), 130.1 (*C*Ar), 127.1 (*C*Ar), 124.2 (*C*Ar), 122.8 (*C*Ar), 117.5 (-CH_2CHCH_2), 56.5 (-CH_2CHCH_2), 46.8 ($CH_{2\ pyrr}$), 45.9 ($CH_{2\ pyrr}$), 37.8 (CH_2), 26.2 ($CH_{2\ pyrr}$), 24.5 ($CH_{2\ pyrr}$);

HRMS: *m/z* Calculated for $C_{18}H_{24}N_2O$ $[M+Na]^+$: 307.1778, found: 307.1781.

IV.4.1.11 2-(2-(Allylamino)phenyl)-1-(pyrrolidin-1-yl)ethanone



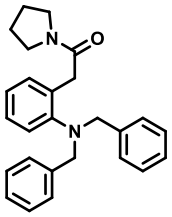
Colourless oil (20 mg, 6 %). *Rf* = 0.35, hexane:EtOAc, 3:2.

IR (cm-1): 3310 (w), 2972 (w), 2874 (w), 1692 (s), 1620 (s), 1589 (s), 1431 (s);

^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.15 (td, *J* = 8.0, 1.5 Hz, Ar*H*, 1H), 7.04 (dd, *J* = 7.6, 1.4 Hz, Ar*H*, 1H), 6.70 – 6.60 (m, Ar*H*, 2H), 5.99 (ddt, *J* = 17.2, 10.3, 5.1 Hz, -CH_2CHCH_2, 1H), 5.35 (dd, *J* = 17.2, 3.3, 1.7 Hz, -CH_2CHCH_2, 1H), 5.19 (dd, *J* = 10.4, 3.1, 1.5 Hz, -CH_2CHCH_2, 1H), 3.81 (d, *J* = 5.0, 1.5 Hz, -CH_2CHCH_2, 2H), 3.65 – 3.56 (m, CH_2, $CH_{2\ pyrr}$, 4H), 3.44 (t, *J* = 6.9 Hz, $CH_{2\ pyrr}$, 2H), 1.95 (m, *J* = 6.8 Hz, $CH_{2\ pyrr}$, 2H), 1.83 (m, *J* = 6.9 Hz, $CH_{2\ pyrr}$, 2H);

HRMS: *m/z* Calculated for $C_{15}H_{20}N_2O$ $[M+Na]^+$: 267.1465, found: 267.1468.

IV.4.1.12 2-(2-(Dibenzylamino)phenyl)-1-(pyrrolidin-1-yl)ethanone (IV.68)



2-(2-Aminophenyl)-1-(pyrrolidin-1-yl)ethanone (110 mg, 0.54 mmol) was dissolved in DMF (2.5 mL). K_2CO_3 (223 mg, 1.62 mmol) and benzyl bromide (0.19 mL, 1.62 mmol) were added and the mixture was stirred at 80°C overnight. The reaction was quenched with water, extracted with EtOAc, washed with water and brine, dried over Na_2SO_4 and evaporated to dryness. Purification by flash column chromatography using a gradient system from hexane to hexane:ethyl acetate (1:4) afforded the product as colourless oil (170 mg, 83%). *Rf* = 0.35, hexane:EtOAc (3:2). Monobenzylated: (20 mg, 13%). *Rf* = 0.4, hexane:EtOAc, 3:2.

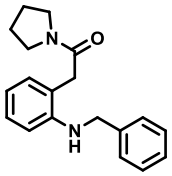
IR (cm-1): 3060 (w), 2971 (w), 2872 (w), 1638 (s, *C=O*), 1421 (s), 697 (s);

^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.28 – 7.03 (m, Ar*H*, 14 H), 4.04 (s, CH_2, 4H), 3.80 (s, CH_2, 2H), 3.46 (t, *J* = 6.7 Hz, CH_2, 2H), 3.08 – 3.01 (m, CH_2, 2H), 1.80 – 1.76 (m, CH_2, 4H).

^{13}C NMR (126 MHz, $CDCl_3$) δ: 170.5 (*C*=O), 149.7 (*C*Ar), 138.4 (*C*Ar), 131.9 (*C*Ar), 129.7 (*C*Ar), 129.1 (*C*Ar), 128.2 (*C*Ar), 127.2 (*C*Ar), 127.1 (*C*Ar), 124.6 (*C*Ar), 123.4 (*C*Ar), 57.8 (*C*H_2), 46.6 ($CH_{2\ pyrr}$), 45.9 ($CH_{2\ pyrr}$), 38.1 (*C*H_2), 26.2 ($CH_{2\ pyrr}$), 24.5 ($CH_{2\ pyrr}$);

HRMS: *m/z* Calculated for $C_{26}H_{28}N_2O$ $[M+Na]^+$: 407.2092,found 407.2094.

IV.4.1.13 2-(2-(Benzylamino)phenyl)-1-(pyrrolidin-1-yl)ethanone



2-(2-Aminophenyl)-1-(pyrrolidin-1-yl)ethanone (360 mg, 1.76 mmol) was dissolved in DMF (6 mL). K_2CO_3 (487 mg, 3.52 mmol) and benzyl bromide (0.26 mL, 2.2 mmol) were added and the mixture was stirred at room temperature overnight. The reaction was quenched with water, extracted with EtOAc, washed with water and brine, dried over Na_2SO_4 and evaporated to dryness. Purification by flash column chromatography using a gradient system from hexane to hexane:ethyl acetate (3:2) afforded the product as a white solid (370 mg, 71%). *Rf* = 0.4, hexane:EtOAc 3:2. Dibenzylated: (50 mg, 17%). *Rf* = 0.35, hexane:EtOAc 3:2.

MP: 63-64ºC;

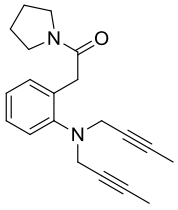
IR (cm-1): 3313 (w), 2971 (w), 2874 (w), 1621 (s), 1445 (s),1428 (s);

^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.40 (d, *J* = 7.5 Hz, Ar*H*, 2H), 7.32 (dd, *J* = 7.6 Hz, Ar*H*, 2H), 7.25 – 7.23 (m, Ar*H*, 2H), 7.11 – 7.04 (m, Ar*H*, 2H), 6.66 – 6.61 (m, Ar*H*, 2H), 4.41 (s, CH_2, 2H), 3.65 (s, CH_2, 2H), 3.61 (t, *J* = 6.8 Hz, CH_2, 2H), 3.45 (t, *J* = 6.9 Hz, CH_2, 2H), 1.95 (m, *J* = 6.8 Hz, CH_2, 2H), 1.84 (m, *J* = 6.9 Hz, CH_2, 2H);

^{13}C NMR (126 MHz, $CDCl_3$) δ: 169.8 (*C*=O), 131.0 (*C*Ar), 128.6 (*C*Ar), 128.4 (*C*Ar), 128.3 (*C*Ar), 127.4 (*C*Ar), 126.9 (*C*Ar), 116.8 (*C*Ar), 111.9 (*C*Ar), 47.3 (*C*H_2 and $CH_{2\ pyrr}$), 46.28 ($CH_{2\ pyrr}$), 40.57 (*C*H_2), 26.31 ($CH_{2\ pyrr}$), 24.42 ($CH_{2\ pyrr}$);

HRMS: *m/z* Calculated for $C_{19}H_{22}N_2O$ $[M+Na]^+$: 307.1627, found 317.1624.

IV.4.1.14 2-(2-(Di(but-2-yn-1-yl)amino)phenyl)-1-(pyrrolidin-1-yl)ethanone (IV.69)



2-(2-Aminophenyl)-1-(pyrrolidin-1-yl)ethanone (150 mg, 0.73 mmol) was dissolved in DMF (3 mL). K_2CO_3 (235 mg, 1.54 mmol) and 2-butynyl bromide (135 μL, 1.54 mmol) were added and the mixture was stirred at 80°C overnight. The reaction was quenched with water, extracted with EtOAc, washed with water and brine, dried over Na_2SO_4 and evaporated to dryness. Purification by flash column chromatography using a gradient system from hexane to hexane:ethyl acetate (2:3) afforded the product as colourless oil (210 mg, 93%). *Rf* = 0.4, EtOAc.

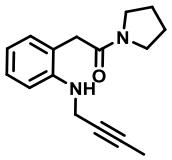
IR (cm-1): 2970 (w), 2874 (w), 1634 (s, *C=O*), 1422 (s), 725 (m);

^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.31 (m, Ar*H*, 2H), 7.24 (dd, *J* = 7.6 Hz, Ar*H*, 1H), 7.10 (dd, *J* = 7.4 Hz, Ar*H*, 1H), 3.80 – 3.77 (m, *J* = 3.4 Hz, CH_2, 6H), 3.54 – 3.40 (m, CH_2, 4H), 1.91 – 1.75 (m, CH_2, CH_3, 10H);

^{13}C NMR (126 MHz, $CDCl_3$) δ: 170.5 (*C*=O), 132.0 (*C*Ar), 130.3 (*C*Ar), 127.3 (*C*Ar), 125.2 (*C*Ar), 122.7 (*C*Ar), 46.9 ($CH_{2\ pyrr}$), 45.9 ($CH_{2\ pyrr}$), 43.0 (*C*H_2), 37.6 (*C*H_2), 26.3 ($CH_{2\ pyrr}$), 24.6 ($CH_{2\ pyrr}$), 3.7 (*C*H_3);

HRMS: *m/z* Calculated for $C_{20}H_{24}N_2O$ $[M+Na]^+$: 331.1779, found: 331.1781.

IV.4.1.15 2-(2-(But-2-yn-1-ylamino)phenyl)-1-(pyrrolidin-1-yl)ethanone



Colourless oil (12 mg, 7%). *Rf* = 0.5, EtOAc.

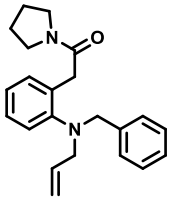
IR (cm-1): 3301 (w), 2971 (w), 2874 (w), 1620 (s, *C=O*), 1588 (m), 1429 (s), 725 (s);

^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.19 (dd, *J* = 7.7 Hz, Ar*H*, 1H), 7.04 (d, *J* = 7.3 Hz, Ar*H*, 1H), 6.79 (d, *J* = 8.1 Hz, Ar*H*, 1H), 6.70 (dd, *J* = 7.3 Hz, Ar*H*, 1H), 3.90 (s, CH_2, 2H), 3.65 – 3.55 (m, CH_2, 4H), 3.43 (t, *J* = 6.9 Hz, CH_2, 2H), 2.02 – 1.93 (m, CH_2, 2H) 1.84 – 1.72 (m, CH_2, CH_3, 5H);

^{13}C NMR (126 MHz, $CDCl_3$) δ: 169.83 (*C*=O), 147.3 (*C*Ar), 130.9 (*C*Ar), 128.4 (*C*Ar), 120.5 (*C*Ar), 117.5 (*C*Ar), 111.9 (*C*Ar), 47.3 ($CH_{2\ pyrr}$), 46.2 ($CH_{2\ pyrr}$), 40.2 (*C*H_2), 33.8 (*C*H_2), 26.3 ($CH_{2\ pyrr}$), 24.4 ($CH_{2\ pyrr}$), 3.8 (*C*H_3);

HRMS: *m/z* Calculated for $C_{16}H_{20}N_2O$ $[M+Na]^+$: 279.1466, found: 279.1468.

IV.4.1.16 2-(2-(Dibenzylamino)phenyl)-1-(pyrrolidin-1-yl)ethanone (IV.73)



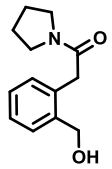
2-(2-(benzylamino)phenyl)-1-(pyrrolidin-1-yl)ethanone (150 mg, 0.51 mmol) was dissolved in DMF (1.1 mL). Cs_2CO_3 (332 mg, 1.02 mmol) and allyl bromide (0.106 mL, 1.02 mmol) were added and the mixture was stirred at 80ºC overnight. The reaction was quenched with water, extracted with EtOAc, washed with water and brine, dried over Na_2SO_4 and evaporated to dryness. Purification by flash column chromatography using a gradient system from hexane to hexane: EtOAc (1:4) afforded the product as colourless oil (140 mg, %). *Rf* = 0.5, hexane:EtOAc, 4:1.

IR (cm-1): 2972 (w), 2872 (w), 1641 (s, *C=O*), 1492 (s), 1421 (s);

^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.23 (m, Ar*H*, 7H), 7.15 – 7.03 (m, Ar*H*, 2H), 5.82 (qd, *J* = 12.0, 6.3 Hz, -CH_2C*H*CH_2, 1H), 5.16 (m, -CH_2CHCH_2, 2H), 4.11 (s, CH_2, 2H), 3.79 (s, CH_2, 2H), 3.55 – 3.46 (m, CH_2, 4H), 3.19 (s, CH_2, 2H), 1.83 (m, CH_2, 4H);

^{13}C NMR (126 MHz, $CDCl_3$) δ: 170.5 (*C*=O), 149.8 (*C*Ar), 138.6 (C-q), 134.9 (C-allyl), 131.8 (*C*Ar), 130.0 (*C*Ar), 129.0 (*C*Ar), 128.2 (*C*Ar), 127.1 (*C*Ar), 127.04 (*C*Ar), 124.4 (*C*Ar), 123.0 (*C*Ar), 117.8 (C-Allyl), 57.6 (CH_2Bn), 56.6 (C-Allyl), 46.7 ($CH_{2\ pyrr}$), 45.9 ($CH_{2\ pyrr}$), 37.9 (*C*H_2), 26.2 ($CH_{2\ pyrr}$), 24.5 ($CH_{2\ pyrr}$).

IV.4.1.17 2-(2-(Hydroxymethyl)phenyl)-1-(pyrrolidin-1-yl)ethanone (IV.90)



3-Isochromanone (0.50 g, 3.37 mmol) was refluxed in pyrrolidine (0.55 mL, 6.75 mmol) and triethylamine (1.4 mL, 10.1 mmol) until total consumption of starting material, *Rf* = 0.3 EtOAc. The mixture was evaporated to dryness and the product was used without further purification.

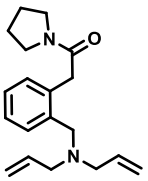
IR (cm-1): 3357 (m), 2971 (w), 2874 (w), 1614 (s, *C=O*,), 1440 (s), 1015 (m), 747 (s);

^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.41 (dd, Ar-*H*, 1H), 7.26 (m, Ar-*H*, 2H), 7.15 (dd, *J* = 6.8 Hz, Ar-*H*, 1H), 4.60 (s, C*H*$_2$, 2H), 3.77 (s, C*H*$_2$, 2H), 3.65 (t, *J* = 6.8 Hz, C*H*$_2$, 2H), 3.47 (t, *J* = 6.9 Hz, C*H*$_2$, 2H), 2.04 – 1.98 (m, C*H*$_2$, 2H), 1.91 – 1.85 (m, C*H*$_2$, 2H);

^{13}C NMR (126 MHz, $CDCl_3$) δ: 170.4 (*C*=O), 140.6 (*C*Ar), 133.9 (*C*Ar), 130.9 (*C*Ar), 130.6 (*C*Ar), 128.3 (*C*Ar), 127.8 (*C*Ar), 63.8 (*C*H$_2$), 47.5 (CH$_{2\ pyrr}$), 46.4 (CH$_{2\ pyrr}$), 39.0 (*C*H$_2$), 26.3 (CH$_{2\ pyrr}$), 24.4 (CH$_{2\ pyrr}$);

HRMS: *m/z* Calculated for $C_{13}H_{17}NO_2$ $[M+Na]^+$: 242.1152, found: 242.1151.

IV.4.1.18 2-(2-((Diallylamino)methyl)phenyl)-1-(pyrrolidin-1-yl)ethanone (IV.93)



2-(2-(Hydroxymethyl)phenyl)-1-(pyrrolidin-1-yl)ethanone (200 mg, 0.92 mmol) was dissolved in THF (6 mL) and the flask was placed in an ice bath. Diphenyl phosphoryl azide (0.25 mL, 1.19 mmol) was then added followed by DBU (0.18 mL, 1.19 mmol) and the mixture was allowed to reach room temperature and stirred overnight. The mixture was dissolved with EtOAc, washed with water and brine, dried over Na_2SO_4 and evaporated to dryness. The crude product was used without further purification. *Rf* = 0.5 EtOAc.

^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.34 – 7.19 (m, Ar-*H*, 4H), 4.39 (s, C*H*$_2$, 2H), 3.71 (s, C*H*$_2$, 2H), 3.47 (m, C*H*$_2$, 4H), 1.95 (m, C*H*$_2$, 2H), 1.85 (m, C*H*$_2$, 2H).

The crude product was dissolved in toluene (4.5 mL) and Pd/C 10% (97 mg, 10 mol%) was added. The mixture was stirred under H_2 atmosphere for 5 h until total consumption of starting material, *Rf* = 0.1 EtOAc. The mixture was filtered through a celite pad and evaporated to dryness.

The crude product was dissolved in THF (6 mL) and K_2CO_3 (633 mg, 4.58 mmol) and allyl bromide (0.2 mL, 2.29 mmol) were added and the mixture was stirred at 50°C overnight. The reaction was filtrated through a celite pad and evaporated to dryness. The crude product was

purified by flash column chromatography using a gradient system from hexane to hexane: ethyl acetate (1:5). The product was obtained as yellow oil (80 mg, 29 % over 4 steps). *Rf* = 0.2, hexane:EtOAc (1:1).

IR (cm-1): 3372 (w), 2974 (w), 2874 (w), 1639 (s, *C*=*O*), 1417 (s), 915 (s), 748 (s);

^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.21 – 7.16 (m, Ar*H*, 4H), 5.83 (ddt, *J* = 6.7 Hz, -CH_2CHCH_2, 2H), 5.14 (m, -CH_2CHCH_2, 4H), 3.85 (s, CH_2, 2H), 3.55 – 3.49 (m, -CH_2CHCH_2, 4H), 3.38 (t, *J* = 6.5 Hz, 2H), 3.03 (d, *J* = 5.8 Hz, CH_2, 4H), 1.93-1.83 (m, CH_2, 4H);

^{13}C NMR (126 MHz, $CDCl_3$) δ: 170.2 (*C*=O), 135.7 (-CH_2CHCH_2), 135.1 (*C*Ar), 130.8 (*C*Ar), 129.4 (*C*Ar), 127.5 (*C*Ar), 126.5 (*C*Ar), 126.3 (*C*Ar), 117.7 (-CH_2CHCH_2), 56.6 ($CH_{2\ pyrr}$), 46.9 ($CH_{2\ pyrr}$), 46.0 (-CH_2CHCH_2), 39.3 (CH_2), 26.3 ($CH_{2\ pyrr}$), 24.6 ($CH_{2\ pyrr}$);

HRMS: *m/z* Calculated for $C_{19}H_{26}N_2O$ $[M+Na]^+$: 321,1939 found: 321,1937.

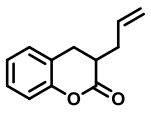
IV.4.2 General procedure

To a microwave vial charged with the allyl amide (1 equiv) in DCM (0.1 M) under argon at room temperature, was added 2,4,6-collidine (x equiv), followed by Tf_2O (y equiv) (*see* Table). The reaction was allowed to stir at room temperature overnight or placed in the microwave (*see* Tables). The reaction was concentrated under reduced pressure and the presence of intermediate was verified by ^{1}H NMR and COSY.

For hydrolysis, the reaction was concentrated under reduced pressure, then diluted in THF (0,5 mL) and in the adequate aqueous solution (0,5 mL). Then, the reaction was allowed to stir at room temperature of under microwave conditions (*see* Table).

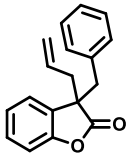
Purification by flash column chromatography afforded the corresponding products (except the indicated compounds which chemical shifts are taken from crude).

IV.4.2.1 3-Allylchroman-2-one (IV.48) [37]



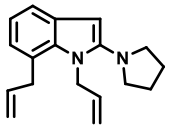
^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.28 – 7.23 (m, Ar*H*, 1H), 7.17 (d, *J* = 7.3 Hz, Ar*H*, 1H), 7.09 (td, *J* = 7.4, 1.0 Hz, Ar*H*, 1H), 7.04 (d, *J* = 8.1 Hz, Ar*H*, 1H), 5.90 – 5.78 (m, -CH_2CHCH_2, 1H), 5.13 (m, -CH_2CHCH_2, 2H), 2.99 (dd, *J* = 15.2, 5.4 Hz, CH_2, 1H), 2.86 – 2.66 (m, CH_2, -CH_2CHCH_2, 3H), 2.35 (dt, *J* = 14.3, 7.8 Hz, C*H*,1H).

IV.4.2.2 3-Allyl-3-benzylbenzofuran-2(3H)-one (IV.62)



^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.28 – 6.80 (m, Ar*H*, 9H), 5.39 (dq, *J* = 17.3, 1.6 Hz, -CH_2CHCH_2, 1H), 5.00 – 4.91 (m, -CH_2CHCH_2, 2H), 3.13 (d, *J* = 13.3 Hz, CH_2, 1H), 3.03 (d, *J* = 13.3 Hz, CH_2, 1H), 2.68 (dd, *J* = 13.6, 7.9 Hz, -CH_2CHCH_2, 1H), 2.61 (dd, *J* = 13.5, 6.8 Hz, -CH_2CHCH_2, 1H). (acc. Peaks taken from a mixture)

IV.4.2.3 1,7-Diallyl-2-(pyrrolidin-1-yl)-1H-indole (IV.73)

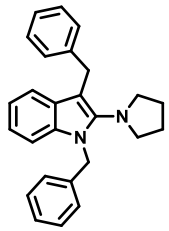


^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.28 – 7.16 (m, Ar*H*, 1H), 6.92 (dd, *J* = 7.5, 0.8 Hz, Ar*H*, 1H), 6.84 (d, *J* = 8.2 Hz, Ar*H*, 1H), 6.03 (ddt, *J* = 17.2, 10.4, 5.2 Hz, -CH_2CHCH_2, 2H), 5.91 (s, C*H*, 1H), 5.14 - 5.05 (m, -CH_2CHCH_2 , 2H), 4.91-4.78 (m, -CH_2CHCH_2, CH_2CHCH_2, 4H), 3.66 (m, -CH_2CHCH_2, 2H), 3.54 – 3.42 (m, CH_2, 4H), 1.97 – 1.78 (m, CH_2, 4H);

^{13}C NMR (126 MHz, $CDCl_3$) δ: 150.4 (*C*Ar), 138.8 (-CH_2CHCH_2), 136.7 (-CH_2CHCH_2), 133.7 (*C*Ar), 129.6 (*C*Ar), 122.6 (*C*Ar), 122.0 (*C*Ar), 119.8 (*C*Ar), 117.2 (*C*Ar), 115.5 (-CH_2CHCH_2), 115.1 (-CH_2CHCH_2), 86.4 (*C*H), 53.2 ($CH_{2\ pyrr}$), 46.5 (-CH_2CHCH_2), 36.2 (-CH_2CHCH_2), 24.8 ($CH_{2\ pyrr}$);

HRMS: *m/z* Calculated for $C_{18}H_{22}N_2$ $[M+Na]^+$: 266.1782, found: 266.1783.

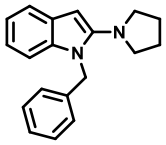
IV.4.2.4 1,3-Dibenzyl-2-(pyrrolidin-1-yl)-1H-indole (IV.77)



^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.35 – 6.95 (m, Ar*H*, 14 H), 5.30 (s, CH_2, 2H), 4.17 (s, CH_2, 2H), 3.14 (m, CH_2, 4H), 1.89 – 1.78 (m, CH_2, 4H);

^{13}C NMR (126 MHz, $CDCl_3$) δ: 141.9 (C-1), 139.2 (C-2), 134.0 (C-3), 128.7 (C-Ar^1), 128.4 (C-Ar^1), 128.3 (C-Ar^2), 127.0 (C-Ar^2), 126.4 (C-Ar^2), 125.8 (C-Ar^1), 121.0 (C-Ar-indole), 119.2 (C-Ar-indole), 118.5 (C-Ar-indole), 109.7 (C-Ar-indole), 104.3 (C-4), 52.8 (C-Pyrr), 45.9 (CH2), 30.2 (CH2), 26.2 (C-Pyrr).

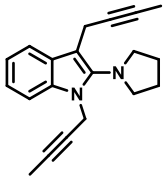
IV.4.2.5 1-Benzyl-2-(pyrrolidin-1-yl)-1H-indole (IV.78)



^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.32 – 7.00 (m, Ar*H*, 9 H), 5.91 (s, C*H*, 1H), 5.32 (s, CH_2, 2H), 3.15 – 3.14 (m, CH_2, 4H), 1.93 (m, CH_2, 4H);

^{13}C NMR (126 MHz, $CDCl_3$) δ: 150.0 (*C*N), 138.5 (*C*Ar), 135.8 (*C*Ar), 129.1 (*C*Ar), 128.7 (*C*Ar), 128.3 (*C*Ar), 127.1 (*C*Ar), 127.0 (*C*Ar), 126.1 (*C*Ar), 119.9 (*C*Ar), 119.6 (*C*Ar), 118.6 (*C*Ar), 109.3 (*C*Ar), 85.0 (*C*H), 52.9 (*C*H_2), 47.0 (*C*H_2), 25.0 (*C*H_2).

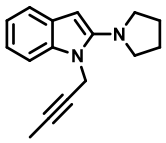
IV.4.2.6 1,3-Di(but-2-ynyl)-2-(pyrrolidin-1-yl)-1H-indole (IV.81)



^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.60 (d, *J* = 7.7 Hz, Ar*H*, 1H), 7.35 (d, *J* = 8.0 Hz, Ar*H*, 1H), 7.14 (m, Ar*H*, 2H), 4.74 (s, CH_2, 2H), 3.57 (s, CH_2, 2H), 3.38 (s, CH_2, 4H), 2.03 (s, CH_2, 4H), 1.76 (s, CH_3, 6H);

^{13}C NMR (126 MHz, $CDCl_3$) δ: 133.0 (*C*Ar), 127.2 (*C*Ar), 121.1 (*C*Ar), 119.4 (*C*Ar), 118.1 (*C*Ar), 109.6 (*C*Ar), 78.8 (C-prop), 74.8 (C-prop), 52.6 (*C*H_2), 31.7 (*C*H_2), 26.2 (*C*H_2), 14.2 (*C*H_2), 3.8 (*C*H_3), 3.7 (*C*H_3).

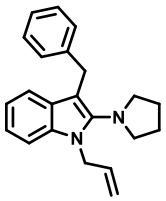
IV.4.2.7 1-(But-2-ynyl)-2-(pyrrolidin-1-yl)-1H-indole (IV.82)



^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.41 (d, *J* = 7.5 Hz, Ar*H*, 1H), 7.32 (m, Ar*H*, 1H), 7.08 (m, Ar-H, 2H), 5.78 (s, H-3, 1H), 4.72 (s, CH2, 2H), 3.26 (s, CH2, 4H), 1.98 (s, CH2, 4H), 1.80 (s, CH3, 3H);

^{13}C NMR (126 MHz, $CDCl_3$) δ: 149.0 (C-2), 135.0 (*C*Ar), 128.5 (*C*Ar), 120.0 (*C*Ar), 119.6 (*C*Ar), 118.7 (*C*Ar), 108.9 (*C*Ar), 84.7 (*C*H), 79.5 (C-prop), 75.0 (C-prop), 52.8 (*C*H_2), 33.4 (*C*H_2), 24.9 (*C*H_2), 3.7 (*C*H_3).

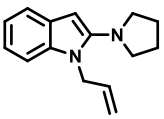
IV.4.2.8 1-Allyl-3-benzyl-2-(pyrrolidin-1-yl)-1H-indole (IV.87)



^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.38 – 7.05 (m, Ar*H*, 9H), 6.09 – 5.94 (m, -CH_2CHCH_2, 1H), 5.18 (d, *J* = 10.2 Hz, -CH_2CHCH_2, 1H), 5.05 (d, *J* = 17.3 Hz, -CH_2CHCH_2, 1H), 4.75 – 4.74 (m, -CH_2CHCH_2, 2H), 4.20 (s, CH_2, 2H), 3.27 – 3.17 (m, CH_2, 4H), 1.94 – 1.89 (m, CH_2, 4H);

^{13}C NMR (101 MHz, $CDCl_3$) δ: 142.9 (-CH_2CHCH_2), 141.9 (*C*N), 134.6 (-CH_2CHCH_2), 133.6 (*C*Ar), 130.3 (*C*Ar), 128.8 (*C*Ar), 128.5 (*C*Ar), 128.3 (*C*Ar), 128.2 (*C*Ar), 125.6 (*C*Ar), 120.7 (*C*Ar), 118.9 (*C*Ar), 118.2 (*C*Ar), 115.7 (-CH_2CHCH_2), 109.4 (*C*Ar), 103.7 (*C*Ar), 52.6 (CH_{2pyrr}), 44.6 (-CH_2CHCH_2), 30.0 ($PhCH_2$), 26.1 ($CH_{2\ pyrr}$).

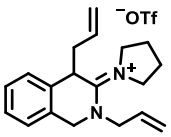
IV.4.2.9 1-(Allyl)-2-(pyrrolidin-1-yl)-1H-indole (IV.88)



^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.44 (m, Ar*H*, 1H), 7.17 – 7.11 (m, Ar*H*, 1H), 7.07 – 7.00 (m, Ar*H*, 2H), 6.12 – 5.96 (m, -CH_2CHCH_2, 1H), 5.81 (s, C*H*, 1H), 5.14 (dd, *J* = 44.8, 13.7 Hz, -CH_2CHCH_2, 2H), 4.66 (m, -CH_2CHCH_2, 2H), 3.20 (m, CH_2, 4H), 1.96 (m, CH_2, 4H);

^{13}C NMR (126 MHz, $CDCl_3$) δ: 149.8 (*C*N), 135.6 (-CH_2CHCH_2), 134.1 (*C*Ar), 128.5 (*C*Ar), 119.8 (*C*Ar), 119.4 (*C*Ar), 118.5 (*C*Ar), 116.2 (-CH_2CHCH_2), 109.1 (*C*Ar), 84.6 (*C*H), 52.8 ($CH_{2\ pyrr}$), 46.0 (-CH_2CHCH_2), 25.0 ($CH_{2\ pyrr}$).

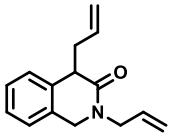
IV.4.2.10 1-(2,4-Diallyl-1,2-dihydroisoquinolin-3(4H)-ylidene)pyrrolidin-1-ium (IV.94)



^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.28 – 7.16 (m, Ar*H*, 1H), 6.92 (d, *J* = 7.5, 0.8 Hz, 1H), 6.84 (d, *J* = 8.2 Hz, 1H), 6.03 (ddt, *J* = 17.2, 10.4, 5.2 Hz, 1H), 5.39 (dq, *J* = 17.3, 1.6 Hz, 1H), 5.31 – 5.23 (m, 1H), 4.54 (dt, *J* = 5.1, 1.5 Hz, 1H), 3.66 (s, 1H), 3.54 – 3.42 (m, CH_2, 2H), 1.97 – 1.78 (m, CH_2, 2H);

^{13}C NMR (126 MHz, $CDCl_3$) δ: 159.5 (*C*=N), 153.1, 132.5 (*C*Ar), 131.7 (-CH_2CHCH_2), 130.1 (*C*Ar), 128.7 (*C*Ar), 128.3 (*C*Ar), 125.8 (*C*Ar), 120.2 (-CH_2CHCH_2), 119.7 (-CH_2CHCH_2), 55.7 (-CH_2CHCH_2), 53.3 (CH_2), 45.9 (*C*H), 35.1 (-CH_2CHCH_2), 22.3 (CH_2), 19.5 (CH_2). (acc. Peaks taken from a mixture)

IV.4.2.11 2,4-Diallyl-1,2-dihydroisoquinolin-3(4H)-one (IV.95)



IR (cm-1): 2972 (w), 2921 (w), 1638 (s), 915 (m), 751 (m);

^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.34 – 7.20 (m, Ar*H*, 2H), 7.15 (d, *J* = 7.5 Hz, Ar*H*, 2H), 5.81 – 5.67 (m, -CH_2CHCH_2, 1H), 5.22 – 5.19 (m, -CH_2CHCH_2, 1H), 5.03 – 4.99 (m, -CH_2CHCH_2, 2H), 4.59 (d, *J* = 15.8 Hz, CH_2, 1H), 4.28 – 4.08 (m, CH_2, -CH_2CHCH_2, 3H), 3.65 (t, *J* = 6.5 Hz, C*H*, 1H), 2.63 (m, -CH_2CHCH_2, 2H);

^{13}C NMR (126 MHz, $CDCl_3$) δ: 171.1 (*C*=O), 135.9 (*C*Ar), 134.4 (-CH_2CHCH_2), 132.8 (-CH_2CHCH_2), 131.2 (*C*Ar), 127.8 (*C*Ar), 127.6 (*C*Ar), 126.7 (*C*Ar), 125.3 (*C*Ar), 118.0 (-CH_2CHCH_2), 117.9 (-CH_2CHCH_2), 49.9 (-CH_2CHCH_2), 49.5 (CH_2), 47.4 (*C*H), 38.6 (-CH_2CHCH_2);

HRMS: *m/z* Calculated for $C_{15}H_{17}NO$ $[M+Na]^+$: 250.1203, found: 250.1202.

IV.5 Bibliography

(1) A. M. Martín Castro. "Claisen Rearrangement over the Past Nine Decades." *Chem. Rev.* **2004**, *104*, 2939-3002.

(2) L. Claisen. "Über Umlagerung von Phenol-allyläthern in C-Allyl-phenole." *Ber. Dtsch. Chem. Ges.* **1912**, *45*, 3157-3166.

(3) F. E. Ziegler. "The Thermal, Aliphatic Claisen Rearrangement." *Chem. Rev.* **1988**, *88*, 1423-1452.

(4) F. E. Ziegler. "Stereo- and regiochemistry of the Claisen rearrangement: applications to natural products synthesis." *Acc. Chem. Res.* **1977**, *10*, 227-232.

(5) A. C. Cope, E. M. Hardy. "The Introduction of Substituted Vinyl Groups. V. A Rearrangement Involving the Migration of an Allyl Group in a Three-Carbon System1." *J. Am. Chem. Soc.* **1940**, *62*, 441-444.

(6) R. P. Lutz. "Catalysis of the Cope and Claisen rearrangements." *Chem. Rev.* **1984**, *84*, 205-247.

(7) a) R. J. Giguere, T. L. Bray, S. M. Duncan, G. Majetich. "Application of Commercial Microwave-Ovens to Organic-Synthesis." *Tetrahedron Lett.* **1986**, *27*, 4945-4948; b) C. O. Kappe, B. Pieber, D. Dallinger. "Microwave Effects in Organic Synthesis: Myth or Reality?" *Angew. Chem. Int. Ed.* **2013**, *52*, 1088-1094.

(8) a) U. Nubbemeyer. "Recent advances in charge-accelerated aza-Claisen rearrangements." *Top. Curr. Chem.* **2005**, *244*, 149-213; b) K. C. Majumdar, S. Alam, B. Chattopadhyay. "Catalysis of the Claisen rearrangement." *Tetrahedron* **2008**, *64*, 597-643.

(9) a) B. D. Sherry, F. D. Toste. "Gold(l)-catalyzed propargyl Claisen rearrangement." *J. Am. Chem. Soc.* **2004**, *126*, 15978-15979; b) T. Cao, E. C. Linton, J. Deitch, S. Berritt, M. C. Kozlowski. "Copper(II)- and Palladium(II)-Catalyzed Enantioselective Claisen Rearrangement of Allyloxy- and Propargyloxy-Indoles to Quaternary Oxindoles and Spirocyclic Lactones." *J. Org. Chem.* **2012**, *77*, 11034-11055.

(10) J. Yang: *Six-Membered Transition States in Organic Synthesis*; Wiley, 2008.

(11) H. Ito, T. Taguchi. "Asymmetric claisen rearrangement." *Chem. Soc. Rev.* **1999**, *28*, 43-50.

(12) B. Peng, D. Geerdink, N. Maulide. "Electrophilic Rearrangements of Chiral Amides: A Traceless Asymmetric alpha-Allylation." *J. Am. Chem. Soc.* **2013**, *135*, 14968-14971.

(13) a) M. F. Carroll. "Addition of beta gamma-unsaturated alcohols to the active methylene group Part III Scope and mechanism of the reaction." *J. Chem. Soc.* **1941**, 507-511; b) M. F. Carroll. "Addition of alpha beta-unsaturated alcohols to the active methylene group Part I The action of ethyl acetoacetate on linalool and geraniol." *J. Chem. Soc.* **1940**, 704-706; c) M. F. Carroll. "Addition of beta gamma-unsaturated alcohols to the active methylene group Part II The

action of ethyl acetoacetate on cinnamyl alcohol and phenylvinylcarbinol." *J. Chem. Soc.* **1940**, 1266-1268.

(14) a) D. Felix, Gschwend.K, A. E. Wick, A. Eschenmoser. "Claisen Rearrangement of Allyl and Benzyl Alcohols with 1-Dimethylamino-1-Methoxyethene." *Helv. Chim. Acta* **1969**, *52*, 1030-1042; b) A. E. Wick, K. Steen, D. Felix, A. Eschenmoser. "Claisensche Umlagerungen Bei Allyl-Benzylalkoholen Mit Hilfe Von Acetalen Des N,N-Dimethylacetamids." *Helv. Chim. Acta* **1964**, *47*, 2425-2429.

(15) W. S. Johnson, Werthema.L, W. R. Bartlett, T. J. Brocksom, T. T. Li, D. J. Faulkner, M. R. Petersen. "A Simple Stereoselective Version of Claisen Rearrangement Leading to Trans-Trisubstituted Olefinic Bonds . Synthesis of Squalene." *J. Am. Chem. Soc.* **1970**, *92*, 741-743.

(16) a) R. E. Ireland, R. H. Mueller. "Claisen Rearrangement of Allyl Esters." *J. Am. Chem. Soc.* **1972**, *94*, 5897-5898; b) R. E. Ireland, R. H. Mueller, A. K. Willard. "Ester Enolate Claisen Rearrangement - Stereochemical Control through Stereoselective Enolate Formation." *J. Am. Chem. Soc.* **1976**, *98*, 2868-2877.

(17) K. C. Majumdar, T. Bhattacharyya, B. Chattopadhyay, B. Sinha. "Recent Advances in the Aza-Claisen Rearrangement." *Synthesis* **2009**, 2117-2142.

(18) S. G. Davies, A. C. Garner, R. L. Nicholson, J. Osborne, P. M. Roberts, E. D. Savory, A. D. Smith, J. E. Thomson. "Doubly diastereoselective [3,3]-sigmatropic aza-Claisen rearrangements." *Org. Biomol. Chem.* **2009**, *7*, 2604-2611.

(19) R. K. Hill, N. W. Gilman. "A nitrogen analog of the Claisen rearrangement." *Tetrahedron Lett.* **1967**, *8*, 1421-1423.

(20) a) M. Diederich, U. Nubbemeyer. "Diastereoselective Zwitterionic Aza-Claisen Rearrangement: Synthesis of Nine-Membered Ring Lactams and Transannular Ring Contraction." *Chem. Eur. J.* **1996**, *2*, 894-900; b) U. Nubbemeyer. "1,2-Asymmetric Induction in the Zwitterionic Claisen Rearrangement of Allylamines." *J. Org. Chem.* **1995**, *60*, 3773-3780.

(21) N. M. Friedemann, A. Harter, S. Brandes, S. Gross, D. Gerlach, W. Munch, D. Schollmeyer, U. Nubbemeyer. "Zwitterionic Aza-Claisen Rearrangements Controlled by Pyrrolidine Auxiliaries - Useful Key Steps in Convergent Enantioselective Syntheses." *Eur. J. Org. Chem.* **2012**, 2346-2358.

(22) a) J. Ficini, Lumbroso.N, Poulique.J. "Synthesis of Beta-Allenic Amides by Transposition of Adducts Formed during Reaction of Propargyl Alcohols on Ynamines." *Tetrahedron Lett.* **1968**, 4139-&; b) J. Ficini, C. Barbara. "Rearrangement De Claisen Et Elimination Au Cours De Laddition Des Ynamines Sur Les Alcools Et Les Amines Allyliques." *Tetrahedron Lett.* **1966**, 6425-&.

(23) a) J. A. Mulder, R. P. Hsung, M. O. Frederick, M. R. Tracey, C. A. Zificsak. "The first stereoselective Ficini-Claisen rearrangement using chiral ynamides." *Org. Lett.* **2002**, *4*, 1383-1386; b) K. A. DeKorver, H. Li, A. G. Lohse, R. Hayashi, Z. Lu, Y. Zhang, R. P. Hsung.

"Ynamides: A Modern Functional Group for the New Millennium." *Chem. Rev.* **2010**, *110*, 5064-5106.

(24) C. Madelaine, V. Valerio, N. Maulide. "Unexpected electrophilic rearrangements of amides: a stereoselective entry to challenging substituted lactones." *Angew. Chem. Int. Ed.* **2010**, *49*, 1583-1586.

(25) C. Madelaine, V. Valerio, N. Maulide. "Revisiting keteniminium salts: more than the nitrogen analogs of ketenes." *Chem. Asian J.* **2011**, *6*, 2224-2239.

(26) V. Valerio, C. Madelaine, N. Maulide. "Steering reaction pathways: from benzyl Claisen rearrangements to powerful ionic shifts." *Chem. Eur. J.* **2011**, *17*, 4742-4745.

(27) B. Peng, D. H. O'Donovan, I. D. Jurberg, N. Maulide. "Dual nucleophilic/electrophilic capture of in situ generated iminium ethers: towards the synthesis of functionalized amide building blocks." *Chem. Eur.J.* **2012**, *18*, 16292-16296.

(28) R. Bai, X. Huang, X. Yang, W. Hong, Y. Tang, H. Yao, J. Jiang, J. Liu, M. Shen, X. Wu, J. Xu. "Novel hybrids of natural isochroman-4-one bearing N-substituted isopropanolamine as potential antihypertensive candidates." *Bioorg. Med. Chem.* **2013**, *21*, 2495-2502.

(29) a) V. Valerio. "Cascade Electrophilic Activation of Amides: Stereoselective Synthesis of Substituted Lactones." Fakultät für Chemie und Biochemie der Ruhr-Universität Bochum, **2013**; b) V.Valerio, D. Petkova, C. Madelaine, N. Maulide "Direct Room-Temperature Lactonisation of Alcohols and Ethers onto Amides: An "Amide Strategy" for Synthesis" *Chem. Eur. J.* **2013**, *19*, 2606-2610.

(30) a) H. M. Ge, C. H. Zhu, D. H. Shi, L. D. Zhang, D. Q. Xie, J. Yang, S. W. Ng, R. X. Tan "Hopeahainol A: An Acetylcholinesterase Inhibitor from Hopea hainanensis." *Chem. Eur. J.* **2008**, *14*, 376-381; b) Y.-J. Kwon, M.-J. Sohn, C.-J. Zheng, W.-G. Kim. "Fumimycin: A Peptide Deformylase Inhibitor with an Unusual Skeleton Produced by Aspergillus fumisynnematus." *Org. Lett.* **2007**, *9*, 2449-2451.

(31) C. Zhu, L. Yang, J. Nie, Y. Zheng, J. Ma. "Organocatalytic Asymmetric Branching Sequence of MBH Carbonates: Access to Chiral Benzofuran-2(3H)-one Derivatives with Three Stereocenters." *Chin. J. Chem.* **2012**, *30*, 2693-2702.

(32) B. Li, Y. Park, S. Chang. "Regiodivergent access to five- and six-membered benzo-fused lactams: Ru-catalyzed olefin hydrocarbamoylation." *J. Am. Chem. Soc.* **2014**, *136*, 1125-1131.

(33) a) J. M. Roe, R. A. B. Webster, A. Ganesan. "Total Synthesis of (+)-Okaramine J Featuring an Exceptionally Facile N-Reverse-prenyl to C-Prenyl Aza-Claisen Rearrangement." *Org. Lett.* **2003**, *5*, 2825-2827; b) X. Xiong, M. C. Pirrung. "Practical Synthesis of 7-Prenylindole." *J. Org. Chem.* **2007**, *72*, 5832-5834.

(34) O. Benali, M. A. Miranda, R. Tormos, S. Gil. "Entropy-Controlled Diastereoselectivity in the Photocyclization of Rigid Derivatives of o-Allylaniline." *J. Org. Chem.* **2002**, *67*, 7915-7918.

(35) H. Wang, B. Dong, Y. Wang, J. Li, Y. Shi *Org. Lett.*, **2014**, *16*, 186-189.

(36) D. H. Huh, J. S. Jeong, H. B. Lee, H. Ryu, Y. G. Kim *Tetrahedron*, **2002**, *58*, 9925-9932.

(37) A. Patra and S. K. Misra, *Magn. Reson. Chem*., **1991**, *29*, 749-752.

V. Sulfonylation studies

V.1Background

The sulfonamide moiety has a great importance in medicinal chemistry, being a pharmacophore present in a large number of pharmaceutical agents, such as antimicrobial, anti-inflammatory or anti-hypertensive drugs (Figure V.1).[1] Its incorporation in therapeutically active molecules was first reported eighty years ago, when a German group discovered that red dye Prontosil was as an effective antibacterial agent.[2] In fact, Prontosil acts as a pro-drug, which was converted in the body into its therapeutically active metabolite, sulfanilamide.

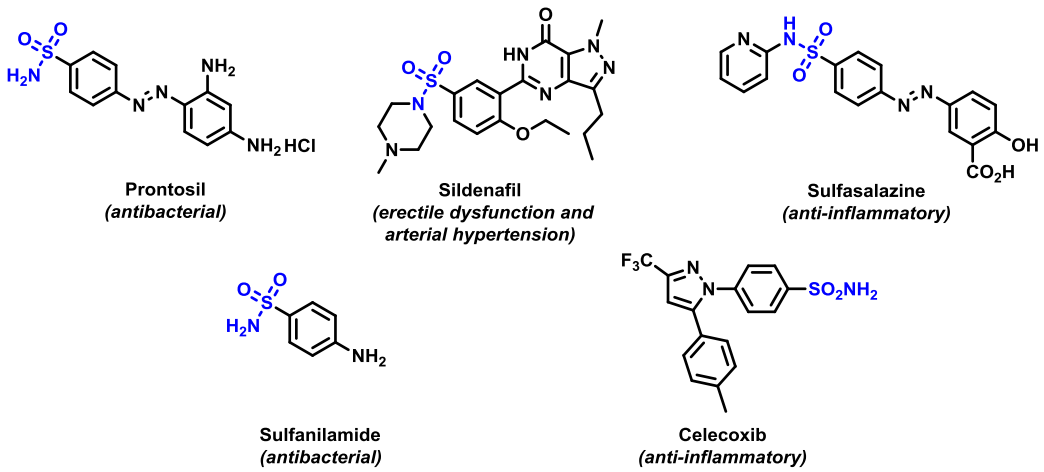
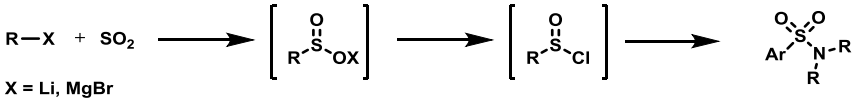


Figure V.1. Pharmaceutical agents containing sulfonamide moiety.

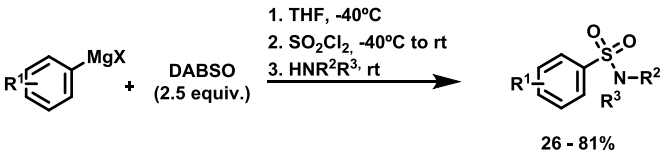
The most usual way to assemble sulfonamides is by treatment of sulfonyl chlorides with an amine or ammonia. However, the main difficulty associated with arylsulfonamides synthesis is the preparation of the corresponding arylsulfonyl chlorides. Furthermore, sulfonyl chlorides are not easily handled and are unstable when stored for long periods of time. These intermediates are usually prepared by electrophilic aromatic substitution with chlorosulfonic acid,[3] or from the respective sulfonic acid or its sodium salt using chlorinating agents such as PCl_5, *N*-chlorosuccinimide (NCS) or $SOCl_2$.[4] Other methods include oxidative chlorination of organosulfur compounds.[5] However, these approaches suffer from limitations. Sulfonamide synthesis from sulfonic acids is restricted to the commercially available starting materials. In electrophilic aromatic substitution the regioselectivity is driven by the arene substituents, limiting the desired substitution patterns. Moreover, some of these methods require acidic conditions, restricting the substrate scope. Other methods were developed, although requiring the use of toxic SO_2 gas and sensitive organometallics (Scheme V.1).[6]



Scheme V.1. Other methods to attain sulfonamides involving SO_2 gas.

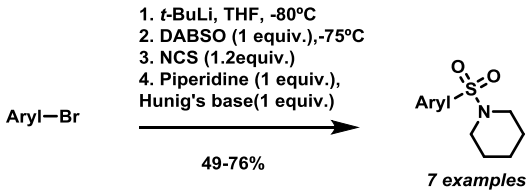
The reported issues regarding sulfonyl chlorides conducted to the development of numerous synthetic routes to prepare sulfonamides, such the use of sulfinic acids salts,[7] although these methods are also limited to the commercially available sulfinic acid salts.

More recently, some procedures were reported employing DABSO, a charge transfer complex generated from the combination of 1,4-diazabicyclo[2.2.2]octane and two equivalents of sulfur dioxide (DABCO·$(SO_2)_2$), as the source of SO_2.[8] In 2011, Willis group explored the one-pot preparation of sulfonamides from aryl Grignard reagents in the presence of this reagent, attaining the desired compounds in good yields (Scheme V.2).[9]



Scheme V.2. One-Pot preparation of sulfonamides from Grignard reagents using DABSO.[9]

More recently, Kopka *et al.* described the one-pot synthesis of aryl sulfonamides where the key step is a bromine-lithium exchange reaction followed by SO_2 insertion using DABSO to attain the sulfonyl chlorides intermediates (Scheme V.3). It was also observed that the exact amount of *t*-BuLi was sufficient for the exchange reaction in order to reach the desired sulfonamides.[10]



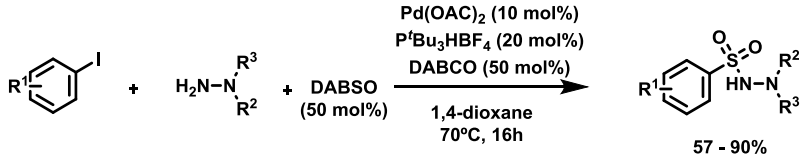
Scheme V.3 Bromine-Lithium Exchange in arylsulfonamide synthesis. [10]

V.1.1 Pd-catalyzed aminosulfonylation

The Pd-catalyzed aminosulfonylation processes, although scarcely developed, can solve the problems of classical sulfonamide synthesis allowing variation of both sulfonyl (R^1–SO_2–R^2) substituents.[11]

The first Pd-catalyzed aminosulfonylation process was reported by Willis group in 2010.[12] Inspired by his previous work on palladium-catalyzed aminocarbonylation reactions of aryl halides, Willis explored the catalytic process using SO_2 in place of CO. In fact, it is documented that the coordination behavior between sulfur dioxide and metal centers is similar to carbon monoxide, suggesting that SO_2 can perform viable insertions into metal-carbon bonds.[12]

Despite the unsuccessful trials using gaseous SO_2, the reaction evolved using DABSO. Although the reaction was only possible using *N*,*N*-dialkylhydrazines as nucleophiles, aryl *N*-aminosulfonamides were successfully prepared from aryl iodides in good to excellent yields (Scheme V.4).[12]

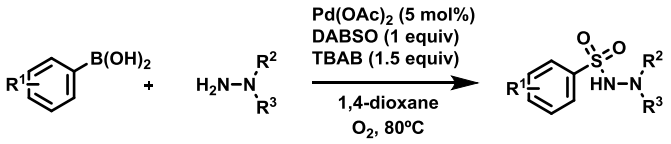


Scheme V.4. Pd-Catalyzed aminosulfonylation of aryl halides.[12]

In additional studies, Willis group investigated the scope of this aminosulfonylation reaction. Several aryl-, alkenyl- and heteroaryl iodides were tested employing different *N*,*N*-dialkylhydrazines to provide *N*-aminosulfonamides in good to excellent yields in the presence of an excess of DABSO.[13] One example using *p*-bromotoluene in the same conditions yielded the corresponding sulfonamide in 64% yield, while increasing the temperature from 70ºC to 90ºC rose the yield to 93%.

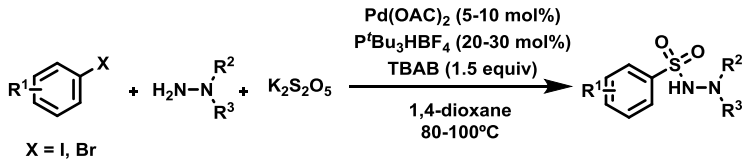
This Pd-catalyzed aminosulfonylation method was also used by Willis as the key-step in a one-pot, three-component sulfone synthesis.[14]

In 2012, Wu *et al.* described an efficient palladium catalyzed three-component route to aryl *N*-aminosulfonamides that relied on the utilization of DABSO and arylboronic acids instead of aryl iodides (Scheme V.5).[15] As previously stated by Willis only hydrazines worked as efficient coupling partners in this procedure.



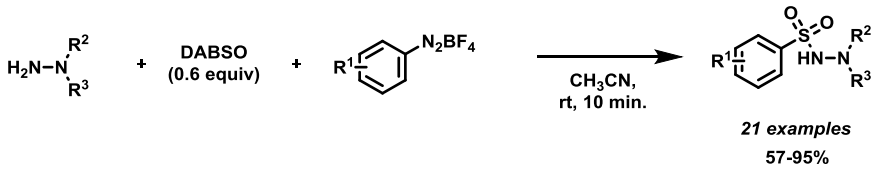
Scheme V.5. Pd-catalyzed aminosulfonylation of arylboronic acids.[15]

Interestingly, Wu group also described a successful Pd-catalyzed aminosulfonylation of aryl halides using potassium metabisulfite as the SO_2 source (Scheme V.6).[16] Despite the wide range of ligands tested, it was verified that P^{t}Bu$_3$HBF$_4$ was the best ligand for this three-component reaction. Moreover, it was also verified that TBAB was fundamental since in its absence the reaction did not proceed. As previously observed by Willis, to ensure the same level of reactivity for the bromo containing substrates, the reaction temperature was raised from 80 °C to 100 °C using the same Pd/ligand conditions.



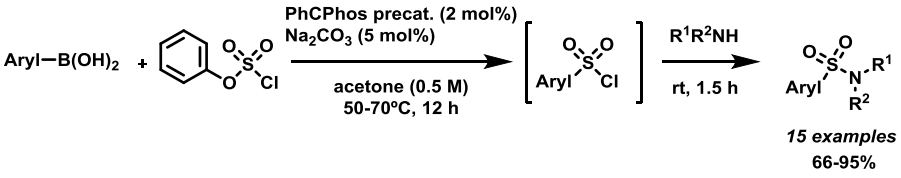
Scheme V.6. Pd-catalyzed aminosulfonylation of aryl halides using potassium metabisulfite.[16]

Very recently, Wu *et al.* described a metal-free coupling of aryldiazonium tetrafluoroborates, hydrazines and DABSO to prepare aryl *N*-aminosulfonamides (Scheme V.7). The reaction proceeded in a very fast and smooth manner at room temperature also showing broad functional-group tolerance. Several mechanistic studies were conducted suggesting that an aryl radical is involved as intermediate, which was presumably produced from the aryldiazonium salt.[17]



Scheme V.7. Metal-free aminosulfonylation of aryldiazonium tetrafluoroborates.[17]

In order to overcome the amine nucleophiles restrictions imposed by the previous described coupling methods, Buchwald envisaged the oxidative addition of LPd(0) to an electrophile of the type ArO−SO_2−Cl, possessing two leaving groups of different reactivity. Subsequent coupling with arylboronic acids afforded the corresponding sulfonyl chlorides, which were further reacted with several primary and secondary amines to achieve the sulfonamide products (Scheme V.8).[18]



Scheme V.8. Metal-free aminosulfonylation of aryldiazonium tetrafluoroborates.[18]

Despite the efforts developed by several groups, a general, cheap and versatile method is not yet reported, to prepare sulfonamides (from alkyl and arylamines). The described methods still possess limited scope and require special ligands/additives. Thus a general method to prepare sulfonylated compounds is still essential.

V.2Results and Discussion

Following the previous studies developed in our group towards new methodologies for the synthesis of sulfonyl-containing compounds, it was proposed to develop a cheap, efficient and broad arylsulfonylation process using a safe and reliable SO_2 surrogate.

The preliminary studies, conducted in our group by Mónica Estevão,[19] consisted on the investigation of a versatile intermediate to be used under mild conditions. Thus, several studies were conducted to attain a viable SO_2 source, such as a Bt-SO_2-Cl intermediate or a carbene·SO_2 adduct (result from SO_2 capture by the NHC with the aim of developing a solid-phase catalytic system) for a subsequent transfer of this moiety to an aromatic unit. Unfortunately, the attempts to attain these intermediates failed (Figure V.2).[19]

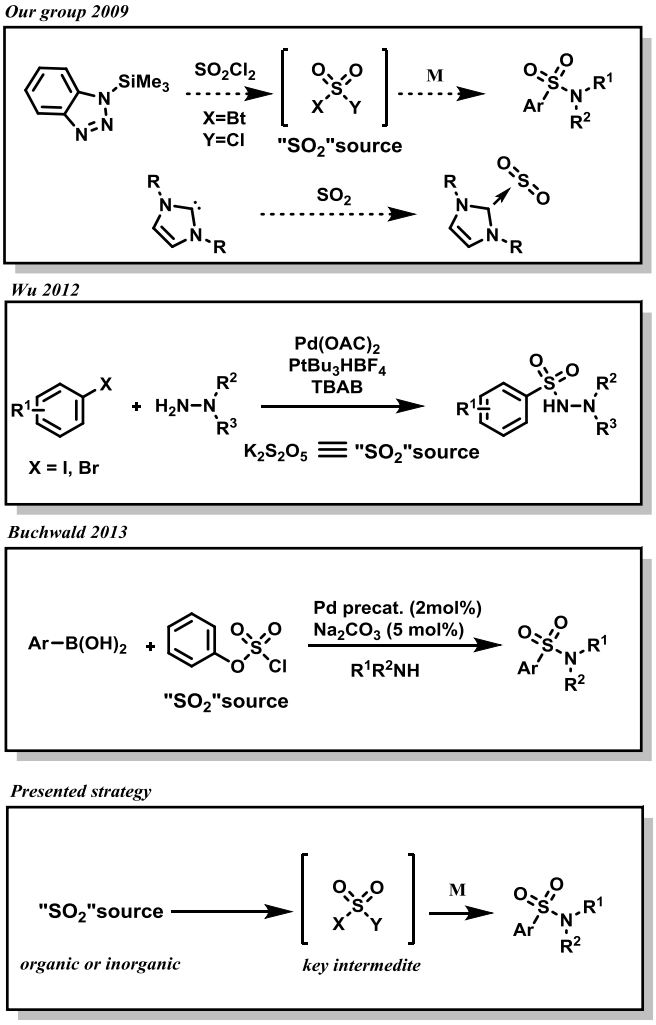


Figure V.2. Previous described sulfonylation methods and presented strategy.

Inspired by Wu work,[16] we firstly directed our efforts to the screening of several copper salts using sodium metabisulfite as SO_2 source. Due to the high costs associated with palladium based catalyst systems, their substitution by copper catalysts appear as an attractive alternative. To date

several methods are described for copper-catalyzed coupling of sulfinic acid salts with aryl and alkyl halides in order to achieve a variety of alkyl−aryl, diaryl, and alkyl−heteroaryl sulfones.[20] However, the use of sulfinic acids is also limited to the commercial available ones (tolyl, methyl, phenyl). Moreover, a similar procedure was carried using silver salts, as well as a combination of Pd/Ag salts, in order to extend the reaction scope.

In our subsequent studies it was envisaged the use of a versatile intermediate of general formula X-SO_2-Y, to be used as SO_2 surrogate. The different reactivity between X and Y could firstly aid the cross coupling reaction followed by the amine nucleophilic attack. During the course of this work, Buchwald reported a very similar work using an electrophile of the type ArO−SO_2−Cl, using arylboronic acids as coupling partners.[18] However, the reported work still present limitations, such as the reliance on exotic catalytic systems.

V.2.1 Cu-catalyzed sulfonylation of iodo-toluene with potassium metabisulfite

The examination of copper-catalyzed aminosulfonylation using sodium metabisulfite as SO_2 source is described in Table V.1. The initial experiments were performed using CuI. Generally, copper-catalyzed cross-coupling reactions are not too sensitive to the choice of the copper source [usually copper(I)], but can highly depend on other parameters, such as substrate, ligand, base or solvent.[21]

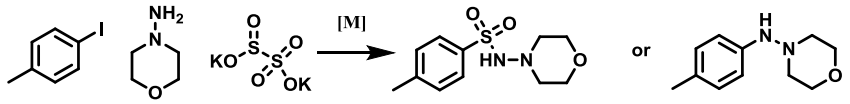
As in Wu work, TBAB was employed as an additive. The role of TBAB is not quite clear and it is suggested to act as a base or to stabilize the catalyst.[16,22]

Initially several ligands were tested, such as L-proline, neocuproine and DMEDA (entries 1-8). The system CuI/L-proline was described in the coupling reaction of aryl iodides with aliphatic and aromatic amines or hydrazines.[23] However, for this type of reaction the system failed to give the desired product, being observed trace amounts of corresponding arylated compound **V.4** (entry 1). The observation of **V.4** is an indication that oxidative addition is taking place followed by coordination of amine to palladium, and that the insertion of sulfur dioxide is not occurring.

1,10-Phenanthroline and its derivatives, such as neocuproine are already described as efficient ligands in copper catalyzed arylations.[24] In our studies it failed to give any product either in the presence of TBAB or a strong base as Na^{t}OBu (entries 2 and 3).

The next ligand tested was *N*,*N'*-dimethylethylenediamine (DMEDA) a widely used copper ligand.[25] Using an organic base, such as pyridine, in the presence of TBAB the reaction yielded an unidentified compound (entry 4). The NMR characterization can suggest the existence of an adduct or complex between hydrazine and SO_2 or Cu, respectively, since only delocalized signals corresponding to hydrazine are observed. Using Cs_2CO_3, the arylated product **V.4** was obtained (entry 5).

Table V.1. Studies on Cu-catalyzed sulfonylation.[a]



V.1 V.2 V.3 V.4

Entry	Catalytic system	Ligand	Additive	Observation
1[b]	CuI (10 mol%)	L-Proline Na salt (10mol%)	--	**V.4**
2[b]	CuI (10 mol%)	Neocuproine (15 mol%)	--	--
3[b,d]	CuI (10 mol%)	Neocuproine (15 mol%)	Na^{t}OBu (1.5 equiv)	--
4	CuI (10 mol%)	DMEDA (20 mol%)	Pyridine (4 equiv)	Unknown
5[d]	CuI (10 mol%)	DMEDA (20 mol%)	Cs_2CO_3 (4 equiv)	**V.4**
6[c,d]	CuI (10 mol%)	DMEDA (20 mol%)	Cs_2CO_3 (4 equiv)	Unknown
7	CuI (20 mol%)	DMEDA (40 mol%)	--	--
8	CuI (1 equiv.)	DMEDA (40 mol%)	--	--
9	CuCl (20 mol%)	DMEDA (40 mol%)	--	**V.4**
10	$Cu(OAc)_2$ (20 mol%)	DMEDA (40 mol%)	--	**V.4**
11[b]	$Cu(OAc)_2$ (5 mol%)	--	--	**V.4**
12	$Cu(OAc)_2$ (1 equiv.)	DMEDA (40 mol%)	--	Mixture
13	Pd_2dba_3 (5 mol%)	XPhos (10 mol%)	--	Mixture
14	$Pd(OAc)_2$(5 mol%) /CuI(20 mol%)	BINAP (10 mol%)	--	Mixture

[a] iodo-toluene (1 equiv), $K_2S_2O_5$ (1 equiv), TBAB (1.5 equiv), DMF, 100 ºC, overnight; [b] dioxane, 100ºC, overnight; [c] 1) CuI, DMEDA and $K_2S_2O_3$ at 100ºC overnight, 2) aminomorpholine and Cs_2CO_3; [d] without TBAB.

However, pre-heating the CuI, ligand and potassium metabisulfite overnight with subsequent addition of aminomorpholine and Cs_2CO_3 gave the same unknown product (entry 6). Increasing the amount of CuI and DMEDA in the presence of TBAB, no products were obtained (entries 7 and 8).

Other copper salts were also tested (entries 9-12). Using CuCl (20 mol %) in the presence of TBAB and DMEDA (40 mol %) gave **V.4**. Using a copper (II) salt such as $Cu(OAc)_2$ (20 mol%) gave the same result. Increasing the amount of $Cu(OAc)_2$ (1 equiv.) gave a complex mixture, while decreasing $Cu(OAc)_2$ (5 mol%) in the absence of DMEDA gave the same arylated product **V.4**.

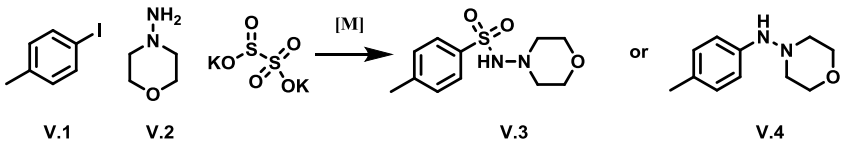
Two trials using Pd(0) and a combination of Pd(II)/Cu were also conducted (entries 13 and 14). However, in both experiments complex mixtures of products were obtained.

The current study using several copper salts under different several conditions, gave unsatisfactory results. Thus it was envisaged to explore silver salts or a combination of silver with Pd or Cu salts.

V.2.2 Ag-catalyzed sulfonylation of iodo-toluene with potassium metabisulfite

Silver salts have been reported as efficient metal catalysts and co-catalysts in cross coupling reactions, despite the exact role of Ag salts is not clearly understood.[22,26] Silver salts have been used to sequester the halides in direct arylation reactions or, when combined with Pd salts, used as an oxidizing agent to regenerate the Pd catalyst.[26-27] Very recently, Chakaburty *et al*. reported the use of Ag(I) salts and DMEDA as ligand as efficient systems in C-N cross-coupling reactions.[28]

Table V.2 Studies on Cu-catalyzed sulfonylation.[a]



Entry	Catalytic system	Ligand	Additive	Observation
1	CuI (20 mol%)/ $AgNO_3$(20 mol%)	DMEDA (40 mol%)	--	Unknown
2	CuI (1 equiv)/ $AgBF_4$ (10 mol%)	DMEDA (40 mol%)	--	**V.4**
3	$AgNO_3$ (20 mol%)	--	--	--
4	$AgBF_4$ (50 mol%)	--	---	Mixture
5	$Pd(OAc)_2$ (5 mol%)/ $AgBF_4$ (1 equiv)	---	AcOH (1.5 equiv)	Mixture
6[b]	$Pd(OAc)_2$ (5 mol%)/ $AgBF_4$ (1 equiv)	P^tBu_3 (10 mol%)	AcOH (1.5 equiv)	Mixture
7	$Pd(OAc)_2$ (5 mol%)/ Ag_2CO_3 (1 equiv)	P^tBu_3 (10 mol%)	--	Mixture
8[c]	$Pd(OAc)_2$ (5 mol%)/ Ag_2CO_3 (1 equiv)	P^tBu_3 (10 mol%)	--	Mixture
9	$Pd(OAc)_2$ (5 mol%)/ Ag_2CO_3 (1 equiv)	P^tBu_3 (10 mol%)	Cs_2CO_3 (2 equiv)	Mixture
10	$Pd(OAc)_2$ (5 mol%)/ Ag_2O (1 equiv)	P^tBu_3 (10 mol%)	AcOH (1.5 equiv)	Mixture

[a] iodo toluene (1 equiv), amino morpholine (1.2 equiv), $K_2S_2O_5$ (1 equiv), TBAB (1.5 equiv), dioxane, 100ºC, overnight; [b] addition order inverted; [c] toluene, 100ºC, overnight;

The same approach was attempted using silver salts in the presence of potassium metabisulfite and TBAB as reported in Table V.2. Further screening of the reaction conditions using $Pd(OAc)_2$ and several Ag salts were also made.

The first trials comprised the use of CuI/Ag systems. Using $AgNO_3$ (20 mol%) in the presence of DMEDA gave trace amounts of the previously observed unidentified compound, while using $AgBF_4$ yielded the arylated specie **V.4** (entries 1 and 2). Performing the same reactions with

$AgNO_3$ and $AgBF_4$ without using the copper source, as well as the corresponding ligand yielded no products or a product mixture, respectively (entries 3 and 4).

The introduction of combined Pd/Ag systems could improve the reaction scope, since the reported procedure was only efficient for hydrazines. However, all the employed systems were unsuccessful. $Pd(OAc)_2$ (5mol%)/Ag_2BF_4 (1 equiv) and $Pd(OAc)_2$ (5mol%)/Ag_2CO_3 (1 equiv) systems revealed complex mixtures (entries 6-10) while using $Pd(OAc)_2$ (5mol%)/Ag_2O (1 equiv) did not improve the reaction outcome (entry 11). The poor results can be attributed to the lack of a specific ligand, since all the reported methodologies make use of uncommon metal/ligand systems. Other reason can be the use of stoichiometric amount of Ag salts. Despite the commonly use in Pd catalyzed reactions, is known that it can lead to the formation of side products.

Since Wu does not report any mechanism involving the potassium metabisulfite,[16] one can suggest that palladium might be a determinant mediator in the reaction.

V.2.3 Synthesis of Aryl Sulfonamides via Palladium-Catalyzed Chlorosulfonylation

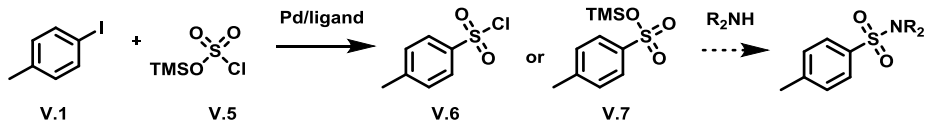
Following the work developed on the group to attain a safe and efficient SO_2 source, in 2013 it was decided to use the commercially available trimethylsilyl chlorosulfonate (TMSO−SO_2−Cl), as a SO_2 surrogate.

In our strategy, Pd should suffer oxidative addition to this electrophile entity, which provides two different leaving groups of different reactivity. Additionally, it was envisioned the use aryl iodides (instead of the reported arylboronic acids) as coupling partners, to attain the corresponding sulfonyl chloride (**V.6**). Moreover, the optimizations studies were generally performed using $Pd(OAc)_2$ as the Pd source and several Buchwald ligands. All the conditions and corresponding results are described on Table V.3.

Cross-coupling reactions are usually conducted in toluene. In our case this solvent could not be used since trimethylsilyl chlorosulfonate can act as a sulfonating agent, performing electrophilic substitutions with arene moieties.[29] Ether like solvents, such as THF or dioxane were also not suitable with this reagent due to its ability to form sulfur trioxide adducts.[30]

Thus, the initial attempts were performed in chloroform at reflux (entries 1-4). Using $Pd(OAc)_2$/BrettPhos system did not afford any product. The same outcome was obtained using other catalytic systems such as $Pd(OAc)_2$/JohnPhos (entry 3) or BrettPhos precatalyst system (entry 4).

Table V.3 Synthesis of Aryl Sulfonamides via Palladium-Catalyzed Chlorosulfonylation.[a]



V.1 V.5 V.6 V.7

Entry	[Pd]	Ligand	Solvent	Additive	Obs.
1	$Pd(OAc)_2$	BrettPhos	$CHCl_3$	Na_2CO_3 (50 mol%)	-
2	$Pd(OAc)_2$	BrettPhos	$CHCl_3$	-	-
3	$Pd(OAc)_2$	JohnPhos	$CHCl_3$	-	-
4	BrettPhosPd	BrettPhos	$CHCl_3$	-	-
5	$Pd(OAc)_2$	BrettPhos	CH_3CN	Na_2CO_3 (50 mol%)	-
6	$Pd(OAc)_2$	BrettPhos	CH_3CN	-	**V.6** Trace
7	$Pd(OAc)_2$	BrettPhos	DCM	Na_2CO_3 (5 mol%)	**V.6** Trace
8	$Pd(OAc)_2$	BrettPhos	DCM	-	**V.6** Trace
9	$Pd(OAc)_2$	BrettPhos	DCM	TMSOTf (10 mol%)	**V.6** Trace
10	$Pd(OAc)_2$	JohnPhos	DCM	-	**V.6** Trace
11	BrettPhosPd	BrettPhos	DCM	-	**V.6** Trace
12	$Pd(OAc)_2$	XPhos	DCM	-	**V.6** Trace
13	$Pd(OAc)_2$	RuPhos	DCM	-	**V.6** Trace
14[b]	$Pd(OAc)_2$	BINAP	DCM	-	-
15[d]	$Pd(OAc)_2$	BrettPhos	DMSO	-	?
16[c,d]	$Pd(OAc)_2$/CuI	BrettPhos	DMSO	-	-
17[c]	$Pd(OAc)_2$/CuI	BrettPhos	CH_3CN	-	-
18	Pd_2dba_3	BrettPhos	DCM	-	-

[a] [Pd] (4 mol%), ligand (8 mol%), trimethylsilylchlorosulfonate (1 equiv), 4-iodo toluene (1.5 equiv), reflux, overnight; [b][Pd] (20 mol%), ligand (50 mol%); [c] [Pd] (15 mol%), CuI (1.5 equiv); [d] 80ºC

The unsuccessful results of these initial tests could be attributed to the low temperature used. Consequently, acetonitrile was chosen due to its higher boiling point. Fortunately, when $Pd(OAc)_2$/BrettPhos system was employed in the absence Na_2CO_3, trace amounts of the corresponding sulfonyl chloride (**V.6**) were obtained (entry 6). The reaction did not undergo using 50 mol% of base (entry 5). This observation is according to the results previous reported by Buchwald.[18]

Interestingly, when the reaction was carried in dichloromethane using $Pd(OAc)_2$ and BrettPhos ligand, trace amounts of the corresponding sulfonyl chloride (**V.6**) were obtained (entries 7-9). Moreover, when the amount of Na_2CO_3 was decreased to 5 mol%, **V.6** was also detected (entry 7).

Despite the trace amounts detected, these results compelled one to explore new catalytic systems in order to attain the optimal conditions to obtain **V.6**. Thus, several systems were employed such as $Pd(OAc)_2$/JohnPhos (entry 10), BrettPhos precatalyst system (entry 11) $Pd(OAc)_2$/XPhos (entry 12), $Pd(OAc)_2$/RuPhos (entry 13). Unfortunately, all presented only trace amounts of **V.6**. On the other hand, **V.6** was not detected when BINAP was employed as ligand (entry 14).

When DMSO was used as solvent no reaction occurred (entry 15-16). In fact, other minor product was obtained. NMR interpretation suggests the presence of an adduct of DMSO with SO_2. Combined systems like $Pd(OAc)_2$/CuI were also not suitable (entries 16 and 17).

Using a Pd(II) source, such as Pd_2dba_3, with BrettPhos in DCM did not give any product (entry 18).

V.3 Conclusions

The pursuit of an efficient arylsulfonylation process for a wide substrate scope has conducted several groups to the development of sulfonylation agents that could act as SO_2 surrogates. However, to the best of our knowledge, this aim was not yet achieved since the majority of the developed processes are only functional for a small number of substrates. Unfortunately, our efforts to attain a cheap and versatile arylsulfonylation method also failed.

The experiments performed with potassium metabisulfite in the presence of copper or silver salts were all unsuccessful. Since the mechanism by which the reaction proceeds in unknown, it is suggested that the reaction requires specific catalytic systems, mostly based on palladium metal. The addition of specific additives, such as TBAB, can also highly influence this approach.

The experiments conducted with the commercially available trimethylsilyl chlorosulfonate as a SO_2 surrogate, were also not effective. Despite the disappointing results obtained for the tested systems, the observation of trace amounts of **V.6** can indicate that the reaction proceeds, although in poor way. Consequently, the optimal reaction conditions still need to be investigated. Simultaneously to our work, Buchwald group reported a palladium catalyzed sulfonylation.[18] In his work, Buchwald included specific palladacycle precatalysts, which allowed the reaction to undergo at mild temperatures (in acetone). This fact can suggest that this type of reaction is highly dependent on the catalytic system, which can hinder a cheaper and broader methodology.

V.4 Experimental

V.4.1 Cu-catalyzed sulfonylation of iodo-toluene with potassium metabisulfite

To a Schlenk-tube equipped with a magnetic stir bar, was added the Cu salt (5-100 mol %), $K_2S_2O_5$ (1 equiv), ligand (10-40 mol %) and the iodo toluene (1 equiv) (*see* Table V.1, page 235). In the experiments having a solid additive, like TBAB (1.5 equiv) or a base it was added in this step. The tube was sealed with a suba-seal, evacuated and backfilled with argon. The solvent was added (0.25 M) and several cycles vacuum/argon were performed. Then amino morpholine (1.2 equiv.) was then added *via* syringe. The reaction mixture was heated at 100ºC overnight. The solution was allowed to cool to room temperature, quenched by the addition of water and diluted with EtOAc and water. After extracting with 3 portions of EtOAc, the combined organic layers were washed with water, brine, dried over Na_2SO_4, filtered and concentrated in vacuo.

V.4.2 Ag-catalyzed sulfonylation of iodo-toluene with potassium metabisulfite

To a Schlenk-tube equipped with a magnetic stir bar, was added the Ag salt (10-100%), $K_2S_2O_5$ (1 equiv), TBAB (1.5 equiv) and the iodo toluene (1 equiv). In the experiments having an additive, like a Cu or Pd salt (5-100 mol%) and the corresponding ligands (10-40 mol %) it was added in this step. The tube was sealed with a suba-seal, evacuated and backfilled with argon. The solvent was added (0.25 M) and several cycles vacuum/argon were performed. Then amino morpholine (1.2 equiv) was then added *via* syringe. The reaction mixture was heated at 100ºC overnight. The solution was allowed to cool to room temperature, quenched by the addition of water and diluted with EtOAc and water. After extracting with 3 portions of EtOAc, the combined organic layers were washed with water, brine, dried over Na_2SO_4, filtered and concentrated in vacuo.

V.4.3 Synthesis of Aryl Sulfonamides via Palladium-Catalyzed Chlorosulfonylation

To a Schlenk-tube equipped with a magnetic stir bar, was added the Pd salt (2 mol%), ligand (4 mol%) the 4-iodo toluene (1.5 equiv). The tube was sealed with a suba-seal, evacuated and backfilled with argon. The solvent was added (0.5 M) and several cycles vacuum/argon were performed. Then trimethylsilyl chlorosulfonate (1 equiv) was then added *via* syringe. The reaction mixture was heated at desired temperature overnight. The solution was allowed to cool to room temperature, quenched by the addition of water and diluted with EtOAc and water. After extracting with 3 portions of EtOAc, the combined organic layers were washed with water, brine, dried over Na_2SO_4, filtered and concentrated in vacuo.

V.5 Bibliography

(1) S. S. Shah, G. Rivera, M. Ashfaq. "Recent advances in medicinal chemistry of sulfonamides. Rational design as anti-tumoral, anti-bacterial and anti-inflammatory agents." *Mini. Rev. Med. Chem.* **2013**, *13*, 70-86.

(2) M. Wainwright, J. E. Kristiansen. "On the 75th anniversary of Prontosil." *Dyes and Pigments* **2011**, *88*, 231-234.

(3) R. J. Cremlyn: *Chlorosulfonic Acid - A Versatile Reagent*; Royal Society Chemistry, 2002.

(4) L. De Luca, G. Giacomelli. "An Easy Microwave-Assisted Synthesis of Sulfonamides Directly from Sulfonic Acids." *J. Org. Chem.* **2008**, *73*, 3967-3969.

(5) a) S. W. Wright, K. N. Hallstrom. "A Convenient Preparation of Heteroaryl Sulfonamides and Sulfonyl Fluorides from Heteroaryl Thiols." *J. Org. Chem.* **2006**, *71*, 1080-1084; b) K. Bahrami, M. M. Khodaei, D. Khaledian. "Synthesis of sulfonyl chlorides and thiosulfonates from H2O2–TiCl4." *Tetrahedron Lett.* **2012**, *53*, 354-358.

(6) a) A. R. Katritzky, V. Rodriguez-Garcia, S. K. Nair. "A General and Efficient Synthesis of Sulfonylbenzotriazoles from N-Chlorobenzotriazole and Sulfinic Acid Salts." *J. Org. Chem.* **2004**, *69*, 1849-1852; b) R. Pandya, T. Murashima, L. Tedeschi, A. G. M. Barrett. "Facile One-Pot Synthesis of Aromatic and Heteroaromatic Sulfonamides." *J. Org. Chem.* **2003**, *68*, 8274-8276.

(7) X. Tang, L. Huang, C. Qi, X. Wu, W. Wu, H. Jiang. "Copper-catalyzed sulfonamides formation from sodium sulfinates and amines." *Chem. Commun.* **2013**, *49*, 6102-6104.

(8) P. S. Santos, M. T. S. Mello. "The Raman-Spectra of Some Molecular-Complexes of 1-Azabicyclo[2.2.2]Octane and 1,4-Diazabicyclo[2.2.2]Octane." *J. Mol. Struct.* **1988**, *178*, 121-133.

(9) H. Woolven, C. Gonzalez-Rodriguez, I. Marco, A. L. Thompson, M. C. Willis. "DABCO-bis(sulfur dioxide), DABSO, as a convenient source of sulfur dioxide for organic synthesis: utility in sulfonamide and sulfamide preparation." *Org. Lett.* **2011**, *13*, 4876-4878.

(10) C. Waldmann, O. Schober, G. Haufe, K. Kopka. "A Closer Look at the Bromine-Lithium Exchange with tert-Butyllithium in an Aryl Sulfonamide Synthesis." *Org. Lett.* **2013**, *15*, 2954-2957.

(11) P. Bisseret, N. Blanchard. "Taming sulfur dioxide: a breakthrough for its wide utilization in chemistry and biology." *Org. Biomol. Chem.* **2013**, *11*, 5393-5398.

(12) B. Nguyen, E. J. Emmett, M. C. Willis. "Palladium-catalyzed aminosulfonylation of aryl halides." *J. Am. Chem. Soc.* **2010**, *132*, 16372-16373.

(13) E. J. Emmett, C. S. Richards-Taylor, B. Nguyen, A. Garcia-Rubia, B. R. Hayter, M. C. Willis. "Palladium-catalysed aminosulfonylation of aryl-, alkenyl- and heteroaryl halides: scope of the three-component synthesis of N-aminosulfonamides." *Org. Biomol. Chem.* **2012**, *10*, 4007-4014.

(14) a) C. S. Richards-Taylor, D. C. Blakemore, M. C. Willis. "One-pot three-component sulfone synthesis exploiting palladium-catalysed aryl halide aminosulfonylation." *Chem. Sci.* **2014**, *5*, 222-228; b) E. J. Emmett, B. R. Hayter, M. C. Willis. "Palladium-Catalyzed Three-Component Diaryl Sulfone Synthesis Exploiting the Sulfur Dioxide Surrogate DABSO." *Angew. Chem. Int. Ed.* **2013**, *52*, 12679-12683.

(15) S. Ye, J. Wu. "A palladium-catalyzed three-component coupling of arylboronic acids, sulfur dioxide and hydrazines." *Chem. Commun.* **2012**, *48*, 7753-7755.

(16) S. Ye, J. Wu. "A palladium-catalyzed reaction of aryl halides, potassium metabisulfite, and hydrazines." *Chem. Commun.* **2012**, *48*, 10037-10039.

(17) D. Zheng, Y. An, Z. Li, J. Wu. "Metal-Free Aminosulfonylation of Aryldiazonium Tetrafluoroborates with DABCO(SO2)2 and Hydrazines." *Angew. Chem. Int. Ed.* **2014**, *53*, 2451-2454.

(18) J. R. DeBergh, N. Niljianskul, S. L. Buchwald. "Synthesis of Aryl Sulfonamides via Palladium-Catalyzed Chlorosulfonylation of Arylboronic Acids." *J. Am. Chem. Soc.* **2013**, *135*, 10638-10641.

(19) M. Estevão, **2014**. "Applications of the Indole Scaffold in Medicinal Chemistry: Development of New Antioxidants, COX Inhibitors and Antitubercular Agents." Doctoral dissertation, FCT-UNL.

(20) a) J. M. Baskin, Z. Wang. "An Efficient Copper Catalyst for the Formation of Sulfones from Sulfinic Acid Salts and Aryl Iodides." *Org. Lett.* **2002**, *4*, 4423-4425; b) W. Zhu, D. Ma. "Synthesis of Aryl Sulfones via l-Proline-Promoted CuI-Catalyzed Coupling Reaction of Aryl Halides with Sulfinic Acid Salts." *J. Org. Chem.* **2005**, *70*, 2696-2700.

(21) G. Evano, N. Blanchard, M. Toumi. "Copper-mediated coupling reactions and their applications in natural products and designed biomolecules synthesis." *Chem. Rev.* **2008**, *108*, 3054-3131.

(22) X. Gong, G. Y. Song, H. Zhang, X. W. Li. "Palladium-Catalyzed Oxidative Cross-Coupling between Pyridine N-Oxides and Indoles." *Org. Lett.* **2011**, *13*, 1766-1769.

(23) a) L. Jiang, X. Lu, H. Zhang, Y. Jiang, D. Ma. "CuI/4-hydro-L-proline as a more effective catalytic system for coupling of aryl bromides with N-boc hydrazine and aqueous ammonia." *J. Org. Chem.* **2009**, *74*, 4542-4546; b) X. D. Xiong, Y. W. Jiang, D. W. Ma. "Assembly of N,N-Disubstituted Hydrazines and 1-Aryl-1H-indazoles via Copper-Catalyzed Coupling Reactions." *Org. Lett.* **2012**, *14*, 2552-2555.

(24) a) R. K. Gujadhur, C. G. Bates, D. Venkataraman. "Formation of aryl-nitrogen, aryl-oxygen, and aryl-carbon bonds using well-defined copper(I)-based catalysts." *Org. Lett.* **2001**, *3*, 4315-4317; b) M. Wolter, A. Klapars, S. L. Buchwald. "Synthesis of N-aryl hydrazides by copper-catalyzed coupling of hydrazides with aryl iodides." *Org. Lett.* **2001**, *3*, 3803-3805.

(25) Y. Liu, Y. Bai, J. Zhang, Y. Li, J. Jiao, X. Qi. "Optimization of the conditions for copper-mediated N-arylation of heteroarylamines." *Eur. J. Org. Chem.* **2007**, 6084-6088.

(26) C. S. Bryan, M. Lautens. "Silver-Promoted Domino Pd-Catalyzed Amination/Direct Arylation: Access to Polycyclic Heteroaromatics." *Org. Lett.* **2008**, *10*, 4633-4636.

(27) *Silver in Organic Chemistry*; John Wiley & Sons, Inc., 2010.

(28) R. Das, M. Mandal, D. Chakraborty. "Silver-Nitrate-Catalyzed N-Arylation of Amines and O-Arylations of Phenols and Alcohols." *Asian J. Org. Chem.* **2013**, *2*, 579-585.

(29) P Genova-Dimitrova, B Baradie, D Foscallo, C. Poinsignon, J. Y. Sanchez. "Ionomeric membranes for proton exchange membrane fuel cell (PEMFC): sulfonated polysulfone associated with phosphatoantimonic acid." *J. Membr. Sci.* **2001**, *185*, 59-71.

(30) A. R. Bassindale, I. Katampe, P. G. Taylor. "Improved simple synthesis of cyclic sulfates from trimethylsilyl chlorosulfonate." *Can. J. Chem.* **2000**, *78*, 1479-1483.

VI. General Conclusions and Future Perspectives

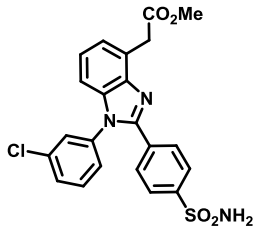
In the last years benzimidazole scaffold gained relevance in drug discovery. Present in a wide number of pharmaceutical compounds, until the date (to the best of our knowledge), was not reported the existence of a commercially available anti-inflammatory drug containing this motif. Thus, considering its unique properties, it was proposed to develop a novel anti-inflammatory drug devoid of adverse side effects.

It was successfully demonstrated that the rational drug design strategy is an important method to attain promising drugs in a direct and efficient way. It was also verified that although presenting several limitations, the docking evaluation was a reasonable tool to predict the most promising compounds from a wide number of available structures.

The proposed benzimidazole-based library was successfully synthesized from 7-bromo isatin, taking advantage of this heterocyclic structure to achieve the desired final substitution pattern. After a careful optimization of the synthetic strategy, the compounds were attained in seven to eight synthetic steps, in good yields.

Eager to find potent and selective COX-2 inhibitors, all the compounds were biologically evaluated. It was verified that the acid derivatives were very poor COX inhibitors, while the ester demonstrated an elevated inhibitory activity towards COX-1 and COX-2, though showing a poor selectivity for COX-2. These results are in opposition to the docking predictions which anticipated an enhanced potency and COX-2 selectivity for all the synthesized compounds. However, the balanced COX-1/COX-2 inhibition observed for the ester derivatives can indicate that these compounds are excellent anti-inflammatory candidates with minimized adverse side effects.

Compound **II.77d** was the most promising structure, showing 60 ± 8% of COX-2 inhibition at 0.5 μM, almost similar to the inhibition percentage found for celecoxib at higher concentration (72 ± 10 %, at 5 μM). Moreover, compound **II.77d** was found to inhibit COX-1 in 41 ± 13%, at 0.625 μM. It was also observed that the compound containing the chlorine atom in *meta* was the most active, which is in accordance with the docking results.



II.77d
COX-2 inhibition percentage: 60% +/- 8% (0.5 μM)

The STD-NMR experiments performed with the acid derivatives (**II.78a-e**) and with the ester **II.77a** showed that all the studied compounds interact with COX-2. Moreover, the competitive STD-NMR studies conducted with ibuprofen, naproxen and diclofenac, unveiled some of the binding mechanisms of these compounds within the protein. The results obtained for acid **II.78a** suggest that these type of compounds bind to a third binding site that do not contribute for COX

inhibition. This observation supports the biological evaluation results, which revealed that the acid derivatives do not possess relevant COX inhibitory activity. Moreover, the ester derivative **II.77d** seems to compete with naproxen for E_{allo}. This can indicate that the esters inhibit COX-2 similarly to naproxen.

The studies conducted on Pd-catalyzed arylamination on PEG, demonstrated that PEG 2000 can efficiently act as a soluble polymeric support for aryl halides involved in C-N cross-coupling reactions with anilines. Moreover, it was also demonstrated that the cross-coupling reaction could be undertaken in solventless conditions, proving that PEG could as polymeric support and solvent. The reaction monitoring was performed by the usual ^{1}H NMR without cleaving the compounds from the resin. It was proved that this methodology is highly valuable to prepare heterocyclic structures in a fast and efficient way, taking advantage of a solid-phase synthesis approach.

The studies conducted on amide electrophilic activation/Claisen rearrangement methodology developed in Maulide's group, demonstrated that despite the promising results achieved for the δ or ε-allyl amides comprising an arene within the alkyl tether, the chiral approach using appropriate auxiliaries was not successful. Moreover, the hydrolysis of the pyrrolidine auxiliary was highly difficult, fact that could compromise the chiral centre. For the aza analogs, the *N*,*N*-diallylated five member derivative suffered a rearrangement towards the arene ring, yielding the 7-allylated indole. For the benzylated and propargylated derivatives was verified that a migration was favored over rearrangement. It was also verified that hydrolysis of the pyrrolidine moiety was an intricate task.

All the attempts to attain a cheap, efficient and broad arylsulfonylation process using a safe and reliable SO_2 surrogate, were unsuccessful.

The results obtained in this thesis contributed to the development of novel anti-inflammatory drugs, which demonstrates a balanced inhibitory activity against COX-1/COX-2. These results indicate that the prepared benzimidazole library can potentially have reduced side effects. Thus, more studies would still be required in order to confirm this hypothesis. Moreover, despite the insights obtained with the NMR studies concerning the binding mechanism within COX-2, complementary x-ray crystallography studies can unravel the orientation of the molecules inside COX active site.

VII.Appendix

VII.1 STD-NMR spectra

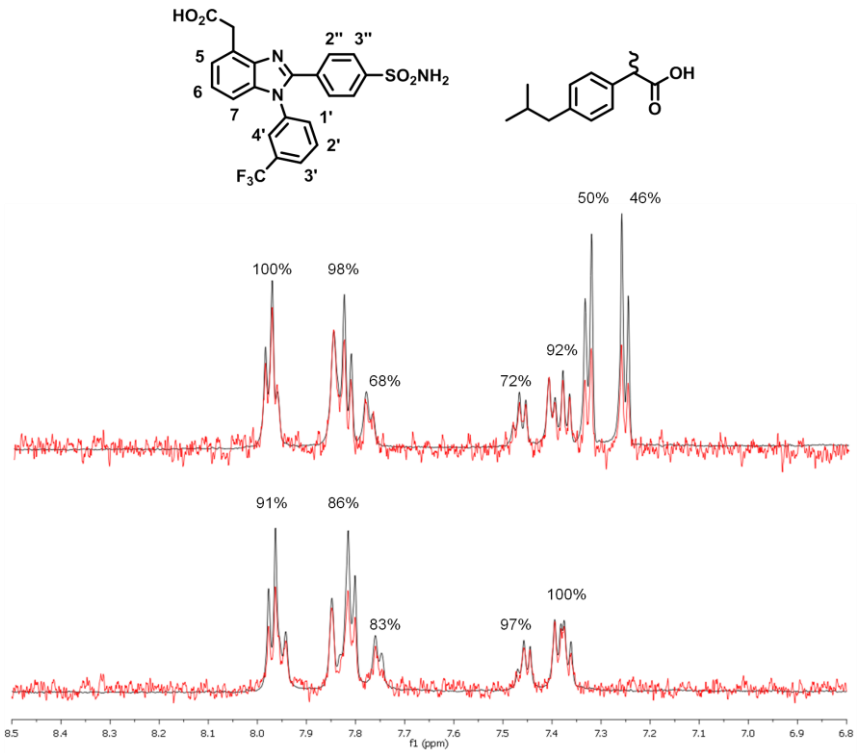


Figure VII.1 Expansion of the aromatic region of the reference (black) and ^{1}H STD-NMR (red) spectra of: a) **80b** (300 μM) with b) ibuprofen (300 μM) in the presence of oCOX-2 (3 μM), at 600 MHz and 37 °C.

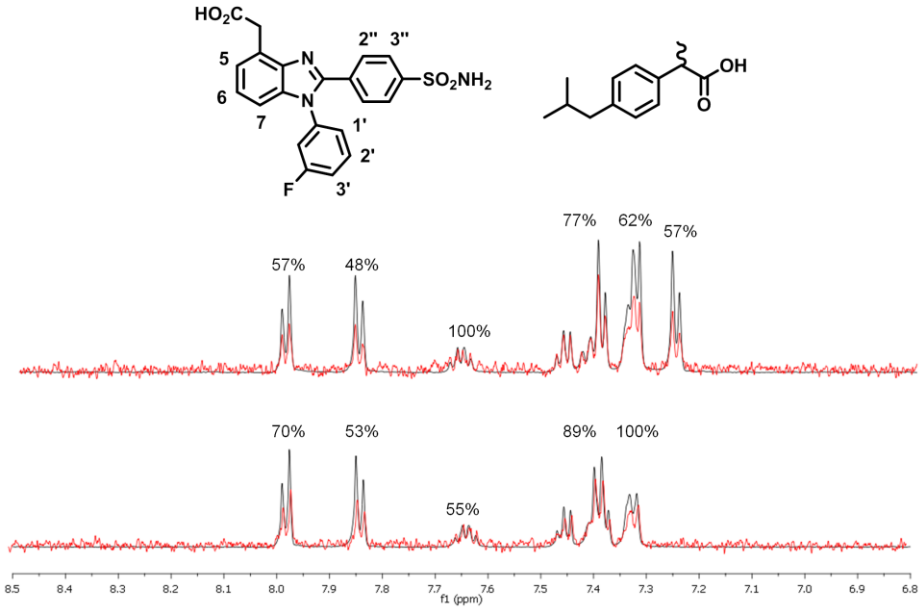


Figure VII.2 Expansion of the aromatic region of the reference (black) and ^{1}H STD-NMR (red) spectra of: a) **80c** (300 μM) with b) ibuprofen (300 μM) in the presence of oCOX-2 (3 μM), at 600 MHz and 37 °C.

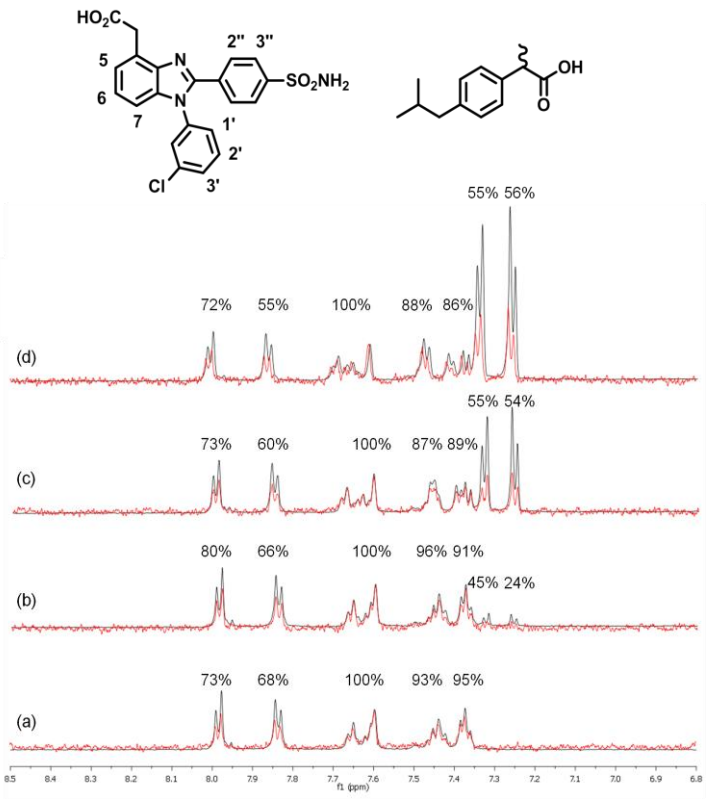


Figure VII.3 Expansion of the aromatic region of the reference (black) and ^{1}H STD-NMR (red) spectra of: a) **80d** (300 μM) and with ibuprofen: b) 150 μM; c) 300 μM; d) 600 μM in the presence of oCOX-2 (3 μM), at 600 MHz and 37 °C.

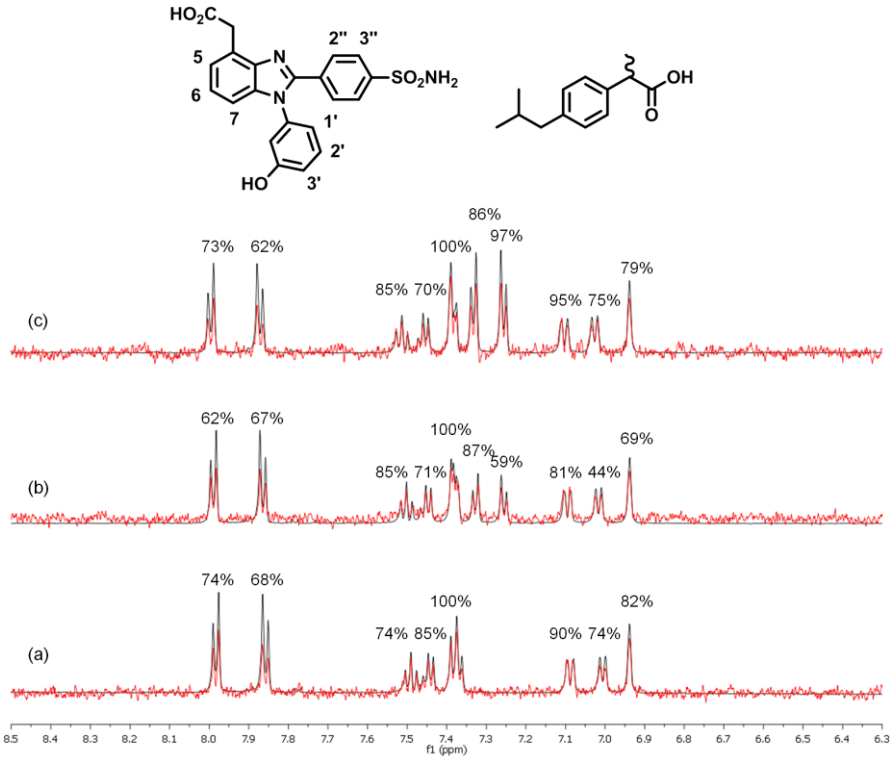


Figure VII.4 Expansion of the aromatic region of the reference (black) and ^{1}H STD-NMR (red) spectra of: a) **80e** (300 μM) and with ibuprofen: b) 150 μM; c) 300 μM in the presence of oCOX-2 (3 μM), at 600 MHz and 37 °C.

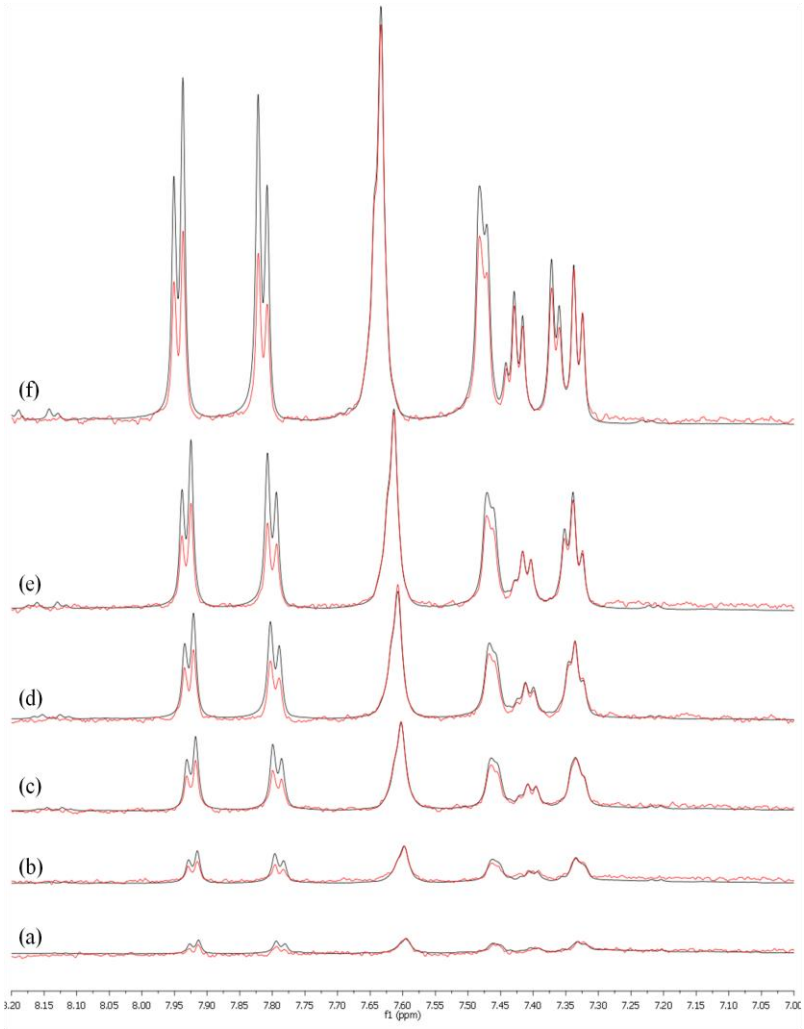


Figure VII.5 Expansion of the aromatic region of the reference (black) and ^{1}H STD-NMR (red) spectra of: **80a** at a) 32.6 μM; b) 81.5 μM; c) 163 μM; d) 245 μM; e) 326 μM and f) 652 μM in the presence of oCOX-2 (3.26 μM), at 600 MHz and 37 °C.

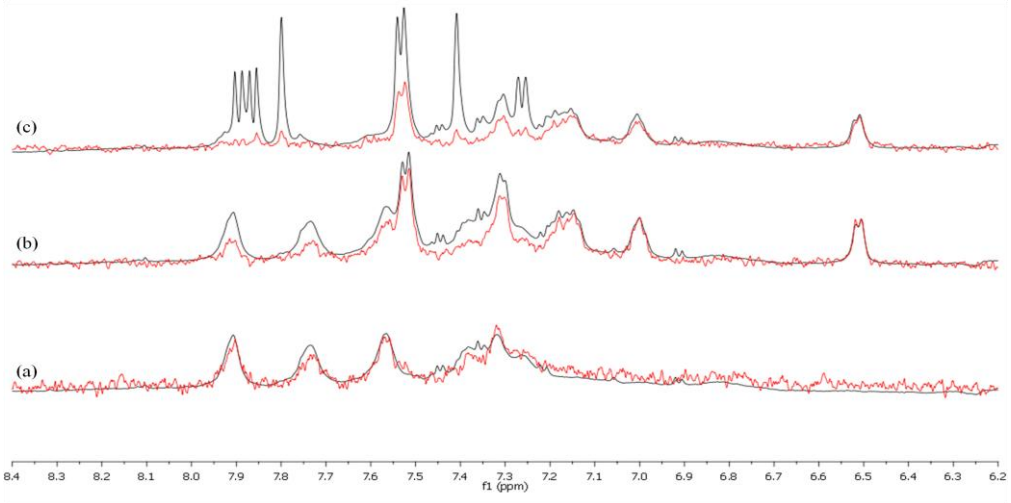


Figure VII.6 Expansion of the aromatic region of the reference (black) and ^{1}H STD-NMR (red) spectra of **79a** (163 μM) with: b) diclofenac (163 μM), c) diclofenac and naproxen (163 μM) in the presence of oCOX-2 (3.26 μM), at 600 MHz and 37 °C.

Scientific articles:

1. Luísa C. R. Carvalho, Eduarda Fernandes, M. Manuel B. Marques, "Developments Towards Regioselective Synthesis of 1,2-Disubstituted Benzimidazoles*" Chem. Eur. J.*, **2011**, *17*, 12544 –12555. DOI: 10.1002/chem.201101508
   http://onlinelibrary.wiley.com/doi/10.1002/chem.201101508/abstract

2. Luísa C. R. Carvalho, Marina J. Dias Pires, Eduarda Fernandes, M. Manuel B. Marques, "Pd-catalysed amination on a soluble polymer support: arylation of anilines with PEG-supported aryl halides" *RSC Adv.*, **2013**, *3*, 25711-25715.
   DOI: 10.1039/C3RA45177A
   http://pubs.rsc.org/en/content/articlelanding/2013/ra/c3ra45177a#!divAbstract

Communications:

1. Luísa C. R. Carvalho, Eduarda Fernandes, M. Manuel B. Marques
   "Towards new benzimidazole based COX inhibitors"
   ESOC – European Symposium on Organic Chemistry 10 - 15 July **2011** – Crete, Greece (Poster communication)

2. Luísa C. R. Carvalho, Eduarda Fernandes, M. Manuel B. Marques
   "New benzimidazole based COX inhibitors"
   BOSS XIII – 13th Belgian Organic Synthesis Symposium, 15 - 20 July **2012** – Leuven, Belgium (Poster communication)

3. Luísa C. R. Carvalho, Eduarda Fernandes, M. Manuel B. Marques
   "New Benzimidazole based COX inhibitors – a drug design approach" – **Best Poster award for NMR techniques**
   10º ENQO – Encontro Nacional de Química Orgânica 4 - 6 September **2013** – Lisboa (Poster communication)

4. M. Manuel B. Marques, Luísa C. R. Carvalho, Eduarda Fernandes
   "Pd-catalysed amination on a soluble polymer support: a sustainable version of homogeneous C-N cross-coupling reaction"
   10º Encontro Nacional de Química Orgânica – 10º ENQO, 4 - 6 September **2013** – Lisboa (Oral communication)

5. Luísa C. R. Carvalho, Daniela Ribeiro, Artur Silva, Eduarda Fernandes, M. Manuel B. Marques "Rational Design of Novel Anti-inflammatory drugs: is COX-2 selectivity an advantage" 4th Portuguese Young Chemists Meeting – 4 Pychem, 29 Abril - 01 May **2014** – Coimbra (Oral communication)