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Applications of the Indole Scaffold in Medicinal Chemistry: Development of New Antioxidants, COX Inhibitors and Antitubercular Agents

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"The important thing is not to stop questioning.
Curiosity has its own reason for existing."

Albert Einstein

Ao meu avô Quim e avó Caetana.

Aos meus pais.

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Resumo

Esta dissertação baseia-se na síntese de novos compostos derivados do núcleo indólico e a sua avaliação biológica, de modo a procurar novos candidatos a fármacos, incluindo, antioxidantes, inibidores selectivos da ciclooxigenase-2 (COX-2) e antituberculares.

Uma biblioteca de derivados do triptofano e da triptamina foi sintetizada, particularmente composta por compostos indólicos prenilados, e foi investigada a sua capacidade de captar espécies reactivas de oxigénio (ERO) e espécies reactivas de azoto (ERA). O padrão de substituição da biblioteca incluiu várias cadeias alquílicas nas posições N-1 e C-2 do núcleo de indole, assim como diferentes grupos na cadeia lateral (em C-3) que permitiram a investigação de uma possível estabilização de radicais. Os resultados obtidos mostraram que o triptofano (**8**), a triptamina (**9**), a *N*-ftaloil triptamina (**5**) e o *N*-prenil triptofano **13**, foram os mais activos contra o radical peróxilo ($ROO^{\cdot}$), com actividades superiores à do trolox, que foi usado como controlo.

A biblioteca de compostos foi também avaliada quanto à capacidade de captar o ácido hipocloroso (HOCl), tendo-se obtidos os valores de IC_{50} de 3,50 ± 0,40 e 6,00 ± 0,60 μM para o triptofano (**8**) e a triptamina (**9**), respectivamente. Os estudos foram alargados às ERA e os melhores resultados foram obtidos contra o anião peroxinitrito ($ONOO^{-}$) na presença de $NaHCO_3$. O derivado de triptofano **18** apresentou uma actividade elevada com um IC_{50} de 14,0 ± 6,8 μM.

Os resultados obtidos neste estudo demonstraram que os compostos testados são captadores eficientes de ERO e ERA, e sugeriram que a estabilização radicalar é fortemente dependente do tipo de substituintes na unidade indólica assim como a sua posição relativa. Concluiu-se que a dissipação radicalar dentro do sistema indólico é obrigatória para a actividade antioxidante observada. Estudos de voltametria cíclica revelaram que todos os compostos apresentavam um pico de potencial de oxidação inferior ao observado para o indole (Epox = 1,035 V), mas superior ao descrito para o antioxidante, melatonina (Epox = 0,715 V).

Na procura de potenciais candidatos a fármacos anti-inflamatórios, foi ainda utilizado o núcleo indólico, que foi funcionalizado de modo favorecer a inibição selectiva da COX-2. Uma pequena biblioteca de novos compostos indólicos, envolvendo dois padrões de substituição diferentes, foi sintetizada de modo a estabelecer uma relação entre a distribuição espacial de grupos funcionais conhecidos com a capacidade de inibição da COX-2. Os dois padrões de substituição investigados envolveram substituintes nas posições N-1, C-3 e C-5, e nas posições C-2, C-3 e C-5 do núcleo indólico. As posições C-5, C-3 e N-1 foram substituídas com um grupo sulfonamida ou metilsulfona em C-5, um grupo *p*-halo benzílico em C-3, e uma cadeia alquílica com um grupo trifluorometílico em N-1. Alternativamente, um grupo *p*-halo benzílico foi introduzido em C-2, deixando o átomo de azoto indólico livre. Foram realizados estudos de inibição e os resultados obtidos relativamente às isoformas da COX (COX-1 e COX-2) foram racionalizados, com base em estudos de *docking* e de RMN. Os estudos de *docking* demostraram que a dialquilação em C-2 e C-3 favorece a ligação à COX-2, com uma

orientação semelhante à do inibidor selectivo conhecido, SC-558. Verificou-se que este padrão de substituição está correlacionado com a actividade de inibição e selectividade mais elevada: 67 ± 5% de inibição da COX-2 e uma fraca inibição da COX-1 (18 ± 9%). Adicionalmente, os estudos de diferença de transferência de saturação (STD)-RMN revelaram padrões de interacção diferentes com ambas a isoformas da COX, os quais podem estar relacionados com as diferentes orientações do grupo sulfonamida no centro activo.

Dada a importância do grupo sulfonamida nos compostos testados e em fármacos, foram realizados diversos ensaios com o objectivo de sintetizar um reagente de sulfonilação novo e versátil para a preparação de sulfonamidas. Os ensaios realizados consistiram sobretudo em sintetizar sulfinatos de sódio derivados de benzotriazole/benzimidazole, e em investigar o uso de carbenos *N*-heterocíclicos (NHC) na formação de aductos de carbeno·SO_2. Ambas as aproximações revelaram que os intermediários usados, ou gerados *in situ*, ou não se formaram ou não tinham estabilidade suficiente que permitisse o isolamento e caracterização dos compostos formados.

Por último, cinco derivados de indole, direccionados por modelos computacionais, foram sintetizados e submetidos para avaliação como potenciais agentes antituberculares. Estes compostos foram divididos em três padrões de substituição diferentes: padrão A – substituição nas posições C-5 e N-1; padrão B – substituição em C-5 e C-2; e padrão C – substituição em C-5 e C-3 do núcleo indólico. Várias aproximações sintéticas e diferentes metodologias foram aplicadas na síntese destes compostos envolvendo, por exemplo, métodos de indolização tais como a indolização de Fischer. Os compostos sintetizados foram submetidos para avaliação biológica.

Os resultados obtidos nesta dissertação permitiram o estudo e preparação de novos derivados indólicos e a sua aplicação em diversos alvos biológicos importantes. Este trabalho permitiu a elucidação dos mecanismos envolvidos, do reconhecimento molecular, e de interações importantes entre os compostos preparados e diversos alvos biológicos estudados. Desta forma este trabalho contribuiu para o *design* de novos fármacos antioxidantes, anti-inflamatórios e antituberculares mais potentes e selectivos.

Termos chave: Síntese de indole, química medicinal, antioxidantes, inibidores da COX, agentes antituberculares

Abstract

This dissertation is based on the synthesis of novel compounds with an indole scaffold and its biological evaluation, on the search for potential drug candidates, including, antioxidants, cyclooxygenase-2 (COX-2) selective inhibitors and antitubercular agents.

A library of tryptophan and tryptamine derivatives was prepared, in particular prenylated indole compounds, and their scavenging activity for reactive oxygen species (ROS) and reactive nitrogen species (RNS) was investigated. The library substitution pattern included several alkyl chains at positions N-1, C-2 of the indole nucleus, including prenyl and isopentyl chain, as well as different groups at the side chain (C-3), which allowed the investigation of a possible radical stabilization. The obtained results showed that tryptophan (**8**), tryptamine (**9**), *N*-phthaloyl tryptamine (**5**) and *N*-prenyl tryptophan **13** were the most active against peroxyl radical ($ROO^{\cdot}$) with higher activities than trolox, which was used as control. The scavenging of hypochlorous acid (HOCl) was also evaluated and tryptophan (**8**) and tryptamine (**9**) showed IC_{50} of 3.50 ± 0.40 and 6.00 ± 0.60 μM, respectively.

The studies were extended to RNS and best results were obtained against peroxynitrite anion ($ONOO^-$) in the presence of $NaHCO_3$. The tryptophan derivative **18** showed a high activity with an IC_{50} of 14.0 ± 6.8 μM. The results show that the tested compounds are effective scavengers of ROS and RNS, and suggest that the radical stabilization is strongly dependent on the type of substituents on the indolic moiety and on their relative positions. In addition, the radical dissipation inside the indolic system is mandatory for the observed antioxidant activity. The cyclic voltammetry study undertaken showed that all the compounds presented an oxidation potential peak lower than that observed for indole (Epox = 1.035 V), but higher than that described for the antioxidant melatonin (Epox = 0.715 V).

The indole scaffold was further investigated on the search for COX-2 selective inhibitors, as potential anti-inflammatory drugs. Thus a small library of new indolic compounds involving two different substitutions patterns at the indole scaffold was synthesized in order to establish a relationship between the spatial distributions of known functional groups related with COX-2 inhibitory activity. The two substitution patterns explored involved substitution at positions N-1, C-3, C-5, and at positions C-2, C-3 and C5 of the indole scaffold. Accordingly, indole positions C-5, C-3 and N-1 were substituted with a sulfonamide or methylsulfone group at C-5, a *p*-halo-benzyl group at C-3, and an alkyl chain with a trifluoromethyl group at N-1. Alternatively, a *p*-halo-benzyl group was introduced at C-2, leaving the indolic nitrogen free. Inhibitory studies were performed and the activity results obtained against both COXs isoforms (COX-1 and COX-2) were rationalized based on docking and NMR studies. Docking studies showed that dialkyation at C-2 and C-3 favors binding, with an orientation similar to that of the known selective inhibitor, SC-558. It was verified that this substitution pattern is correlated with the highest inhibitory activity and selectivity found, 67 ± 5% COX-2 inhibition at 50 μM, and low COX-1 inhibition (18 ± 9%).

Saturation transfer difference (STD) NMR experiments reveal different interaction patterns with both COXs isoforms that may be related with different orientations of the sulfonamide group in the binding pocket.

Due to the high relevance of the sulfonamide group in the tested compounds as well as in several known drugs, several attempts were made to synthetize a new and versatile sulfonylation reagent for the synthesis of sulfonamides. The experiments performed relied mainly on two approaches, consisting on the synthesis of sodium benzotriazole/benzimidazole sulfinates, and on the use of N-heterocyclic carbenes (NHC) for the formation of carbene·SO_2 adduct. Both approaches revealed that the intermediates used, or generated in situ, either did not form or did not have enough stability to allow isolation and characterization of the formed compounds.

The indole scaffold was also investigated on the search for novel and potent antitubercular agents. Thus, five indole derivatives, directed by computational models, were synthesized and were submitted to evaluation as antitubercular agents. These compounds were divided in three different substitution patterns: pattern A –substitution at C-5 and N-1 position; pattern B – substitution at C-5 and C-2 and; pattern C – substitution at C-5 and C-3 of the indole scaffold. Several synthetic approaches and methodologies were applied for the synthesis of these compounds involving, for example, indolization methods such as the Fischer indolization reaction. The synthetized compounds were submitted to biological evaluation.

The results obtained in this work allowed the study and preparation of novel indole derivatives, and its application in several biologically important targets. This work contributed to the understanding of the molecular recognition, mechanisms involved and key interactions of the compounds prepared with the targets, and thus to the design of novel antioxidant, anti-inflammatory and antitubercular drugs with higher potency and selectivity.

Keywords: Indole synthesis, medicinal chemistry, antioxidants, COX inhibitors, antitubercular agents.

Contents

Index of figures

Index of schemes

Index of tables

Abbreviations and symbols

AA	arachidonic acid
AAPH	α,α'-azodiisobutyramidine dihydrochloride
ABTS	2,2'-azino-*bis*(3-ethylbenzothiazoline-6-sulphonic acid)
Ac	acetyl
aq	aqueous
B^-	base
Boc	*tert*-butyloxycarbonyl
Bt	benzotriazole
Bu	butyl
cat.	catalyst
CDP	chlorodiphenylphosphine
CMMP	cyanomethylenetrimethyl phosphorane
COX	cyclooxygenase
d	doublet
DABCO	1,4-diazabicyclo[2.2.2]octane
DABSO	DABCO-*bis*(sulfur dioxide)
DAF-2	4,5-diaminofluorescein diacetate
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCH	1,3-dichloro-5,5-dimethylhydantoin
DCM	dichloromethane
DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
DEAD	diethyl azodicarboxylate
DFT	density functional theory
DHR	dihydrorhodamine 123
DIPEA	*N*,*N*-diisopropylethylamine
DMAP	4-(dimethylamino)pyridine
DMEDA	*N*,*N'*-dimethylethylenediamine
DMF	*N*,*N*-dimethylformamide
dmmp	1,1-*bis*(diphenylphosphino)methane
DMSO	dimethyl sulfoxide
DTPA	diethylenetriaminepentaacetic acid
e.g.	*exempli gratia* (for example)
E^+	electrophile
EI	electron ionization
EMA	European Medicines Agency

equiv.	equivalent(s)
ESI	electrospray ionization
Et	ethyl
EWG	electron withdrawing group
FDA	Food and Drug Administration
FGI	functional group interconversion
FI	field ionization
GC	gas chromatography
hal	halogen
HIV	human immunodeficiency virus
HMDS	hexamethyldisilazane
HPLC	high-performance liquid chromatography
HRMS	high resolution mass spectrometry
HTS	high throughput screening
i.e.	*id est* (that is)
IC_{50}	half maximal inhibitory concentration
IIA	indole 3-acetic acid
IND	Investigational New Drug Application
^{i}Pr	*iso*-propyl
IR	infrared
J	constant coupling
Lit.	literature
m	multiplet
m.p.	melting point
M.tb	*Mycobacterium tuberculosis*
MDR	multi-drug resistant
Me	methyl
MIC	minimum inhibitory concentration
Ms	methanesulfonyl
MS	mass spectrometry
MW	molecular weight
NADH	β-nicotinamide adenine dinucleotide
NBS	*N*-bromosuccinimide
NBT	nitroblue tetrazolium chloride
NDA	New Drug Application
NHC	*N*-heterocyclic carbene

NMP	*N*-methyl-2-pyrrolidone
NMR	nuclear magnetic resonance
NOC-5	3-(aminopropyl)-1-hydroxy-3-isopropyl-2-oxo-1-triazene
NSAID	non-steroidal anti-inflammatory drug
Nu	nucleophile
ORAC	oxygen radical absorbance capacity
OTf	trifluoromethanesulfonate
ox	oxidation
PG	prostaglandin
Ph	phenyl
Phth	phthalimide
PMS	phenazine methosulfate
PPA	polyphosphoric acid
PTLC	preparative thin layer chromatography
q	quartet
QSAR	quantitative structure-activity relationship
red	reduction
rfx	reflux
RNS	reactive nitrogen species
ROS	reactive oxygen species
rt	room temperature
s	singlet
SAR	structure-activity relationship
S_N2	bimolecular nucleophilic substitution
S_NAr	nucleophilic aromatic substitution
SSA	silica-gel-supported sulfuric acid
STD	saturation-transfer difference
t	triplet
TB	tuberculosis
TBAI	tetrabutylammonium iodide
^{t}Bu	*tert*-butyl
TEA	triethylamine
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography
TMS	tetramethylsilane

Ts	transition state
Ts	4-toluenesulfonyl
TX	thromboxane
vs.	versus
WHO	World Health Organization
XDR	extensively drug resistant
δ	chemical shift

Chapter I Introduction

I.1 Drug development

The process of drug development is challenging, expensive and time consuming. Developing a new drug from an original idea to the launch of a finished product is a complex process which can take 12–15 years and cost in excess of €1 billion (figure I.1).

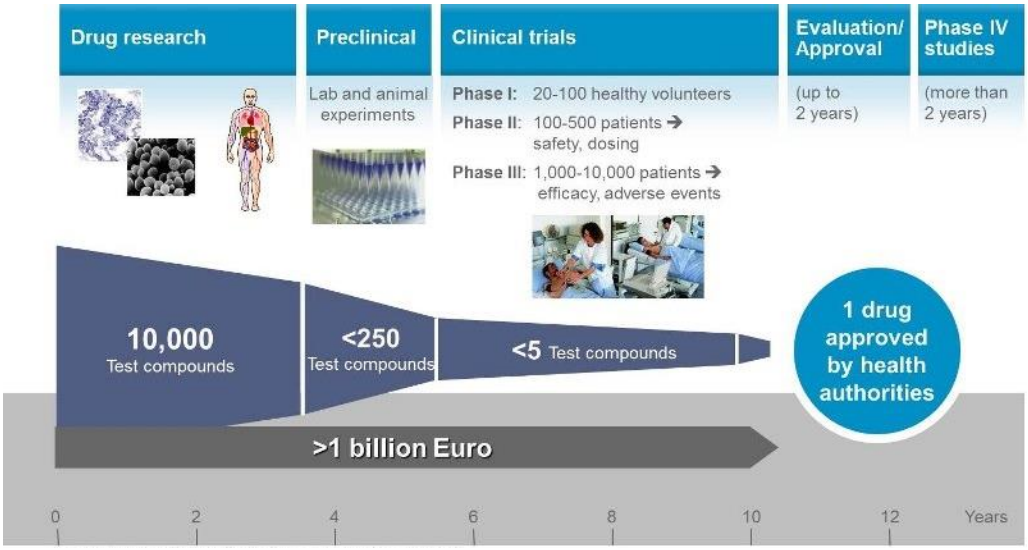


Figure I.1 – Drug development process (adapted from Bayer).[1]

The drug discovery process initiates with the identification of a medical need, including a judgment on the adequacy of existing therapies (if there are any). From this analysis, together with an evaluation of the current knowledge about the target disease, will come hypotheses on how to possibly improve therapy. Key subsequent steps in the process include detecting relevant biological activity (a 'hit') for a structurally novel compound *in vitro*, then finding a related compound with *in vivo* activity in an appropriate animal model, followed by maximizing this activity through the preparation of analogous structures, and finally selecting one compound as the drug development candidate. This drug candidate then undergoes toxicological testing in animals, as required by law. If the compound passes all these tests, all the collected research data are assembled and submitted as an Investigational New Drug Application (IND) to the European Medicines Agency (EMA) in European Union or to the Food and Drug Administration (FDA) in the United States before clinical trials are initiated. In the clinic tests, there is sequential evaluation in normal human volunteers of toleration (Phase I), efficacy and dose range in patients (Phase II), followed by widespread trials in thousands of appropriate patients to develop a broad database of efficacy and safety. For the few (4–7%) drug candidates that survive this series of development trials, a New Drug Application (NDA) that contains all the gathered research data is filed for thorough review by the experts at the EMA or FDA. Phase IV consists on collecting the data (e.g. side effects) of the drug that has already been allowed onto the market.[2]

Drugs fail in the clinical studies due to two main reasons: either they are ineffective or are not safe. Thus, one of the most important steps in drug development is target identification and validation. A drug target is a biomolecule which is involved in signaling or metabolic pathways that are specific to a disease process which may include proteins, genes and RNA, for example. The ideal target has to be efficacious, safe, meet clinical and commercial needs and, most of all, has to be accessible to the alleged drug molecule, causing a biological response that can be measured *in vitro* and *in vivo*. Once identified, the target must be validated. Validation techniques range from *in vitro* tools through the use of animal models in order to modulate the desired target in human patients. After the validation target process, it is during the lead discovery phase that screening assays are developed.[2]

Several approaches can be taken to identify a lead. The first requirement for all of the approaches is to have a resource to assay compounds for a particular biological activity, to know when a compound is active. A bioassay (or screen) is a mean of determining in a biological system, relative to a control compound, if a compound has the desired activity (biological or pharmacological effect), and what the relative potency (strength of that effect) of the compound is.[3] Table I.1 summarizes some screening strategies used in lead discovery.

Table I.1 – Screening strategies.[2]

Screen	Description
High throughput screening (HTS)	Very rapid and sensitive assay which can be carried out robotically in 1536- or 3456-well titer plates on submicrogram scale of compound
Focused screen	Compounds previously identified as hitting specific classes of targets (*e.g.* kinases) and compounds with similar structures
Fragment screen	Identification of small chemical fragments, which may bind only weakly to the biological target, and then growing them or combining them to produce a lead with a higher affinity
Structural aided drug design	Use of crystal structures to help design molecules
Virtual screen	Prediction of the binding orientation of small molecule drug candidates to their protein targets in order to in turn predict the affinity and activity of the small molecule (docking models)
Physiological screen	A tissue-based approach used to determinate the effects of a drug at the tissue rather than the cellular or subcellular level, *e.g.* muscle contractility
NMR screen	Exploits changes in either relaxation rates or diffusion rates of small molecules when they bind to a macromolecule. It also can be used to screen mixtures of compounds to determine the ones that bind best.

After the screen is developed, several approaches can be taken to obtain a lead compound. The typical lead compound for a receptor or enzyme is the natural ligand for the receptor or substrate for the enzyme. Another good source of lead compounds is marketed drugs. In the absence of known drugs and other compounds with desired activity, a random screening is a valuable approach. Random screening involves no rationalization. All compounds are tested in the bioassay without regard to their structures. This usually is the chosen method when nothing is known about the receptor target. However, none of these method approaches involves a rational element.

The first step for a rational drug design is the identification of the disease state, since several diseases are caused from an imbalance of particular chemicals in the body, from the invasion of a foreign organism, or from aberrant cell growth.[3,4] Therefore, it is necessary to perform a meticulous analysis of the structural and chemical features of the target binding site (*i.e.*, amino acid residues of the protein pocket: tautomerism, protonation, ionization). Protein structures (apo, ligand-free; or holo, ligand-bound) are experimentally determined by X-ray crystallography and NMR. Alternatively, protein structure homology models can be a valuable alternative.[5-7] Several *in silico* methods can be used in combination with experimental evidences to extract and organize the molecular information in order to assist the understanding of the structural and chemical basis involved in receptor-ligand binding affinity and biological activity (pharmacodynamics).[8]

There are other features to lead modification that are as important to increase the binding to the target receptor, such as pharmacokinetics (absorption, distribution, metabolism, and excretion or ADME). The identification of the pharmacophoric and auxophoric groups of the lead compound, and of the auxophoric groups, which are interfering with lead compound binding and which are not detrimental to binding, is crucial to know which groups must be removed and which can be kept or modified.

The knowledge generated (chemical and biological) from these approaches is a key component in medicinal chemistry, and can be used in the iterative design of new ligands with improved properties characteristics. For this purpose, 3D quantitative structure-activity relationships (3D QSAR) methods are among the most important strategies that can be applied for the successful optimization of leads. In this context, 3D QSAR models are generated to explain the relationships between the intermolecular interactions related to the 3D conformations of a set of structurally related molecules and their experimental activity (*e.g.*, IC_{50}, K_i), therefore, providing a rational basis for the development of new promising compounds (figure I.2).[9-11]

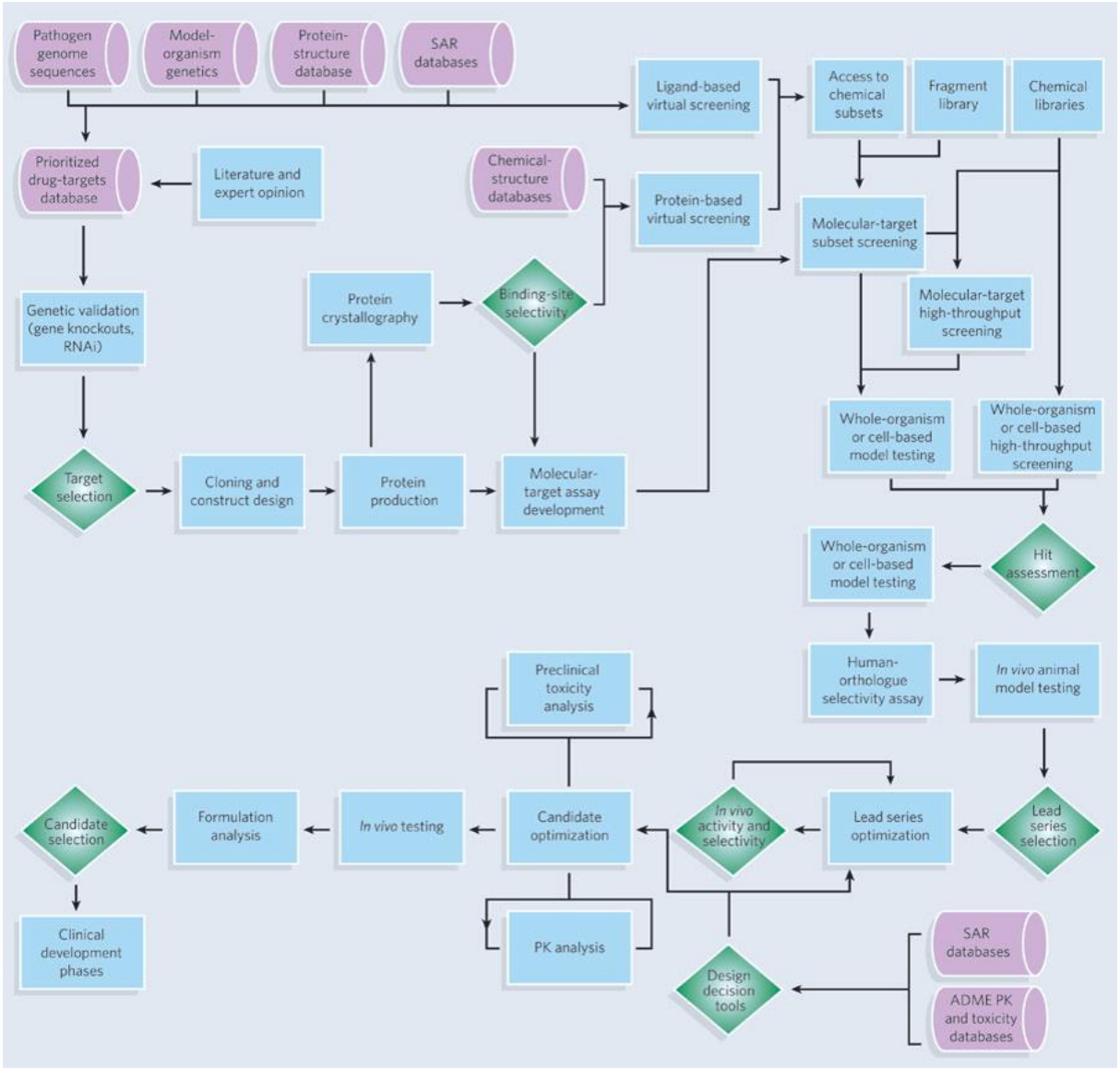


Figure I.2 - Iterative process used in rational drug discovery.[12]

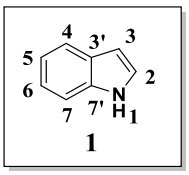
Rational approaches have increasingly demonstrated their value in drug design. The impact of these technologies on early discovery and lead optimization is significant. The ability to make knowledge-based decisions during the early phases of drug discovery is the key to decreasing hit-to-lead and lead optimization cycle times. The significant advances in structural capabilities (e.g., protein generation and purification techniques, high throughput crystallography, virtual screening, SAR by NMR) combined with robust and more efficient computational tools (faster and cheaper) have improved the drug discovery and development.

I.2 The indole scaffold

I.2.1 Indole: a "privileged structure"

The term "privileged structure" was introduced by Evans *et al.*, in 1988, to define scaffolds that *"are capable of providing useful ligands for more than one receptor and that judicious modification of such structures could be a viable alternative in the search for new receptor agonists and antagonists"*.[13] In 1999, IUPAC defined this concept in its Glossary of Terms Used in Combinatorial Chemistry (Technical Report) as *"substructural feature which confers desirable (often drug-like) properties on compounds containing that feature. Often consists of a semi-rigid scaffold which is able to present multiple hydrophobic residues without undergoing hydrophobic collapse"*.[14]

Indole (**1**), or benzo[*b*]pyrrole (figure I.3), is a benzopyrrole in which the benzene and pyrrole rings are fused through the 2- and 3-positions of the pyrrole nucleus. It is a privileged motif that enjoys widespread inclusion in molecules, both naturally occurring and designed, which find applications in pharmaceutical, agrochemical and materials industries.[15]



1

Figure I.3 – Chemical structure of the indole (1) scaffold.

The indole (**1**) scaffold probably represents one of the most important structural subunits in drug discovery.[16] Tryptophan (**2**) is one of the naturally-occurring amino acids and plays critical roles in protein-protein and protein-ligand recognition and binding as well as in human nutrition, as biosynthetic precursor of serotonin [5-hydroxytryptamine (**3**)]. This is a key neurotransmitter in central nervous system, regulates smooth muscle function in the cardiovascular and gastrointestinal systems, and regulates platelet function.[17] Indole-3-acetic acid (**4**) is the most common, naturally-occurring, plant hormone of the auxin class. Figure I.4 depicts some examples of, both natural and synthetic, indole-containing structures.

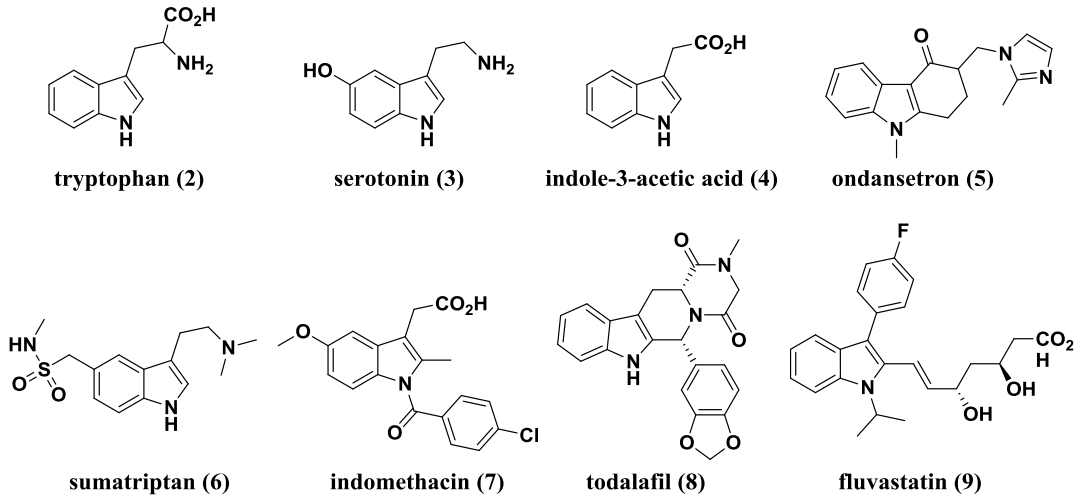


Figure I.4 – Examples of indole-containing structures.

Thus, it is not surprising that there are several classes of active compounds comprising a central indole scaffold with elaborate and specific functionality. Indeed, several marketed drugs contain the indole nucleous: ondansetron (**5**) (a $5HT_3$ receptor antagonist) is a potent anti-emetic; sumatriptan (**6**) (a $5HT_{1D}$ receptor agonist) is used for the treatment of migraine; indomethacin (**7**) (cyclooxygenase antagonists) is a non-steroidal anti-inflammatory drug; tadalafil (**8**) (a PDE-5 selective inhibitor) is used for treating erectile dysfunction and pulmonary arterial hypertension; and fluvastatin (**9**) (a HMG-CoA reductase inhibitor) is administrated to treat hypercholesterolemia and to prevent cardiovascular disease.[15,16,18] In addition to this, molecules containing the indole scaffold are aromatase (CYP19) ihnibitors,[19] HIV-1 attachment inhibitors,[20] selective cathepsin K inhibitors,[21] and agonists of the CB1 receptor,[22] to name just a few.

As a result of the overall drug-like character of these compounds, combined with their chemical tractability in both solution- and solid-phase synthesis, the preparation of indole-based collections seems an ideal approach to targeted library construction. Based on the privileged structure hypothesis, it should be possible to modify the indole core with diverse functionality to tune it for different biological activities.

I.2.2 Synthesis and Functionalization of indoles

Since the discovery of indole, by Adolf von Baeyer in 1869,[23] synthesis[24-26] and direct functionalization[27-29] of this heteroaromatic scaffold has been a major area of research for synthetic organic chemists, and numerous methods have been developed.

There are several routes for the indole synthesis and, very recently, Taber and Tarunahari[30] proposed a system for classifying indole synthesis that could be universally understood. For the classification of the methods of synthesis, the authors have focused on the last bond formed, from the

four bond in the five-membered indole ring (Type 1 to Type 6) (figure I.5). They also differentiated between forming a bond to a functionalized aromatic carbon, and forming a bond to an aromatic carbon H-substituted (Type 1 *vs.* Type 2 and Type 3 *vs.* Type 4).

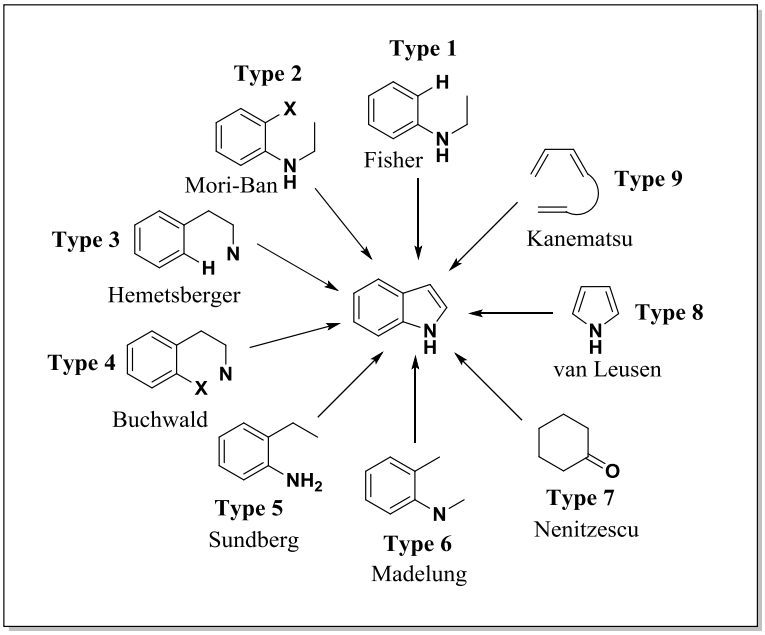
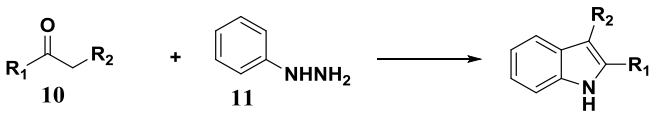


Figure I.5 – The nine types of indole synthesis and some associated name reaction, proposed by Taber and Tirunahari.[30]

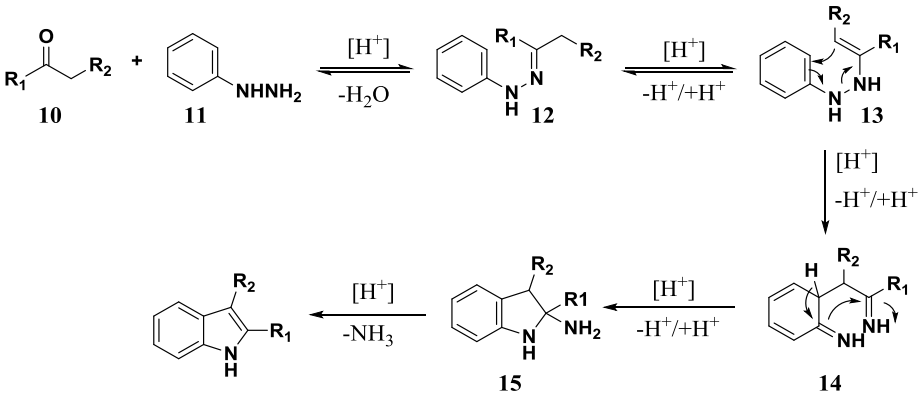
The last step of the Type 5 is the C-N bond formation, while in Type 6 the last step is a C-C bond formation. In Type 7, the benzene ring has been derived an existing cyclohexane, and in Type 8, the benzene ring has been built onto an existing pyrrole, Finally, in Type 9, both rings have been constructed.

Despite the existence of several name reactions associated with indole synthesis, the classical Fischer indole synthesis is reported as one of the first choice route to prepare this scaffold (figure I.5, Type 1). The first indolization of an arylhydrazone was reported in 1883 by Fischer and Jourdan,[31] and for over a century, the Fischer indole reaction has remained an extremely useful and important method for the synthesis of a variety of indole intermediates and biologically active compounds. This reaction often provides a simple, efficient method for the transformation of enolizable *N*-phenylhydrazones into indoles. In many cases, the indolization reaction is carried out by simply heating the ketone or aldehyde **10** and the phenylhydrazine **11** with the appropriate acid or acid catalyst without isolation of the hydrazone intermediate (scheme I.1).



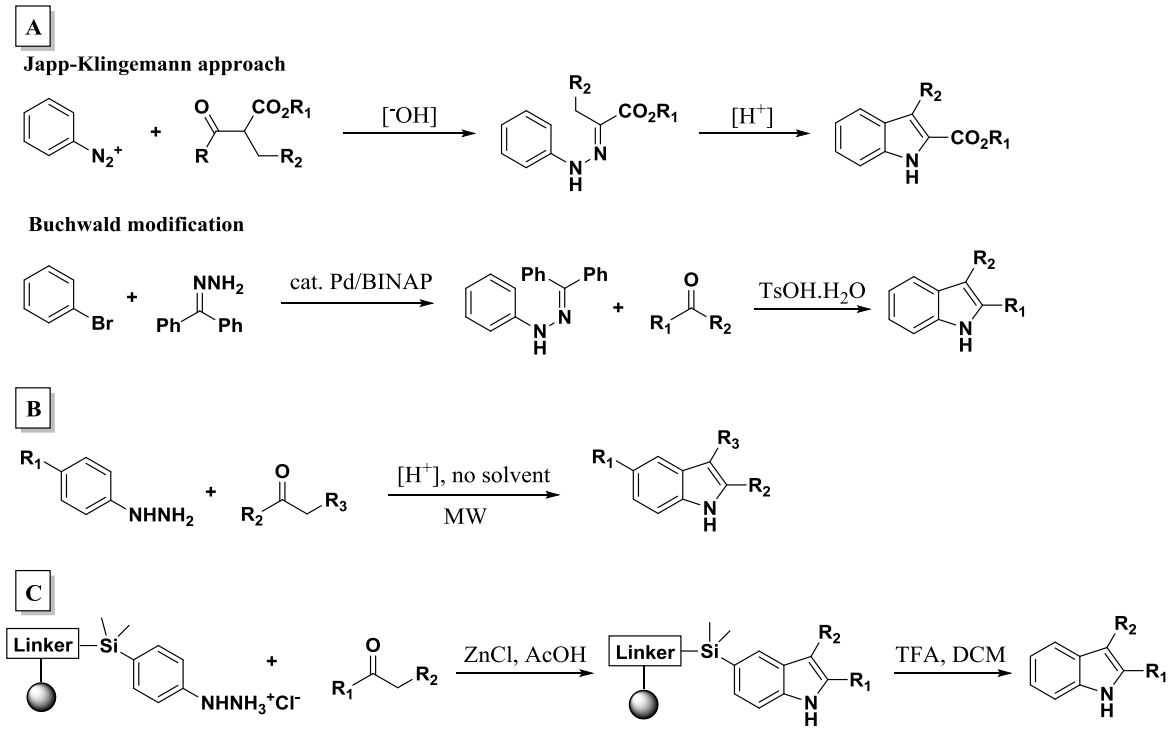
Scheme I.1 – Fischer indole synthesis.

The mechanism of the Fischer indole cyclization involves the formation of a phenylhydrazone **12** which isomerizes to the respective enamine **13**. After protonation, a cyclic [3,3]-sigmatropic rearrangement occurs producing an imine **14**. The resulting imine forms a cyclic aminoacetal **15**, which, under acid catalysis, eliminates NH_3, resulting in the thermodynamic favorable aromatic indole (scheme I.2).



Scheme I.2 – Mechanism of the Fischer indole reaction.

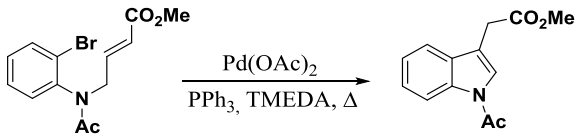
This reaction has been extensively modified to various conditions, such as alternative routes for the phenylhydrazones synthesis[32,33] (scheme I.3A), microwave-assisted reaction[34] (scheme I.3B) and solid-phase synthesis[35] (scheme I.3C).



Scheme I.3 – Some modifications to the Fischer indole reaction.

The Mori-Ban approach also allows an easy access to the indole nucleus (figure I.5, Type 2). In 1977, Mori and Ban[36] discovered that palladium catalyzes the intramolecular reaction of *o*-halo-*N*-allylanilines to indoles under Heck reaction conditions [$Pd(OAc)_2$, PPh_3 and tetramethylethylenediamine (TMEDA)] (scheme I.4).

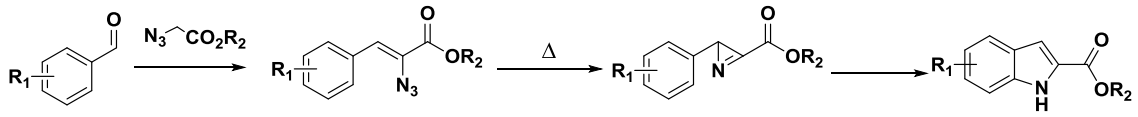
It was the first intramolecular Heck reaction applied for the synthesis of heterocyclic compounds. In the past three decades, the Mori-Ban reaction has been improved and applied to a variety of organic compound synthesis.[37-41]



Scheme I.4 – The Mori-Ban indole synthesis.

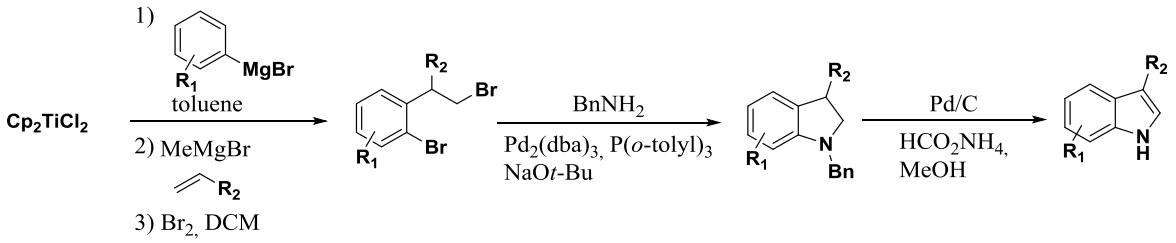
Unlike the Fischer indole synthesis, the C3–C3' bond is already present in the precursor for the Hemetsberger reaction (figure I.5, Type 3).[42] It is thus particularly suited to the regiospecific synthesis of 4- or 6-substituted indoles from *ortho*- or *para*-substituted benzaldehydes (scheme I.5). Since the reaction is effected simply by heating, it can be used in combination with other thermal processes such as the Claisen rearrangement.[43] Despite the potential hazards associated with the use of azides, the

Hemetsberger reaction is amenable to scale up and has been carried on 90 g scale in Cook's synthesis of roeharmine.[44]



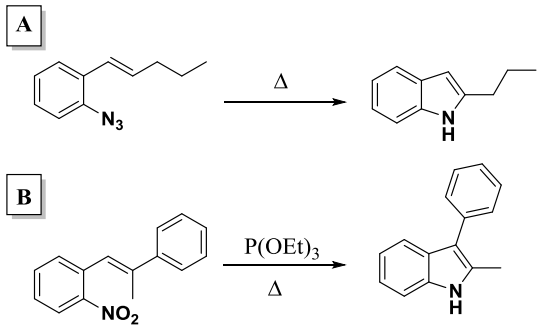
Scheme I.5 – The Hemetsberger indole synthesis.

In 1998, Buchwald *et al.*[45] described a new approach for the construction of indoles, employing the air- and moisture-stable reagent Cp_2TiCl_2 (figure I.5, Type 4). This procedure involves two key steps: the intermolecular insertion reactions of an olefin and a titanocene-stabilized benzyne complex; and the Pd-catalyzed aryl amination reaction followed by oxidation of the resulting indoline to the corresponding indole (scheme I.6). This approach has been modified over the past years and applied in several organic synthesis reactions.[46-48]



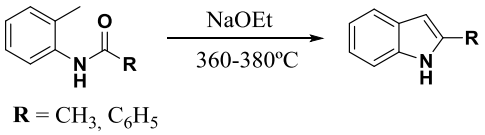
Scheme I.6 – The Buchwald indole synthesis.

In 1969, Sundberg *et al.*[49] reported an indole synthesis (figure I.5, Type 5) consisting on the thermolysis of *o*-azidostyrenes to give indoles *via* a nitrene intermediate (scheme I.7A). The heating of *o*-nitrostyrenes in the presence of triethyl phosphite also affords the indole ring (scheme I.7B).[50]



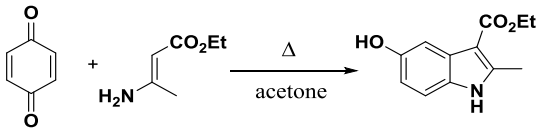
Scheme I.7 – The Sundberg indole synthesis.

In 1912, Madelung[51] (figure I.5, Type 6) reported that *o*-acetotoluidine and *o*-benzotoluidine provided the corresponding 2-methylindole and 2-phenylindole, respectively, when heated to 360-380°C with 2 equivalents of sodium ethoxide (scheme I.8). The Madelung reaction could be performed at lower temperature when *n*-BuLi or lithium diisopropylamide (LDA) are employed as bases.[52] Also, the introduction of an electron withdrawing group (EWG) at the benzylic carbon atom of the *N*-acylated-*o*-alkylanilines affords the indole moiety effectively.[53-55]



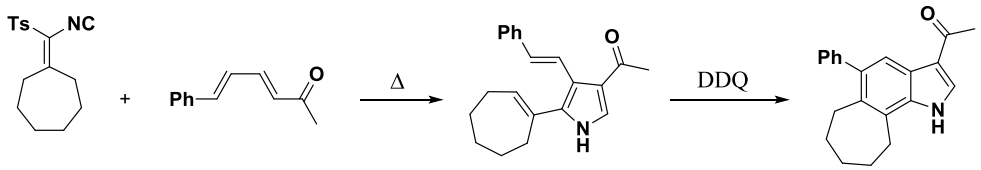
Scheme I.8 – The Madelung indole synthesis.

In 1929, Nenitzescu[56] (figure I.5, Type 7) reported that *p*-benzoquinone treated with ethyl 3-aminocrotonate, in boiling acetone, yielded ethyl 5-hydroxy-2-methylindole-3-carboxylate (scheme I.9). The procedure was largely ignored until the 1950s when interest in melanin-related substances and recognition of serotonin as a 5-hydroxy indole derivative stimulated the exploration of the scope of the reaction. Nowadays, the Nenitzescu reaction is one of the most efficient processes for the preparation of 5-hydroxyindoles.[57-59]



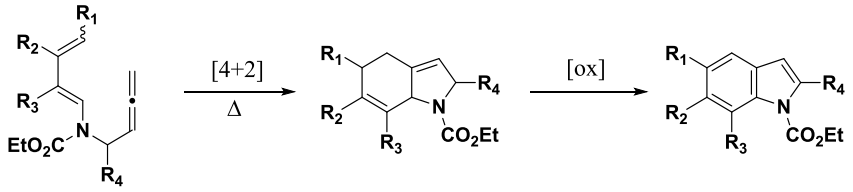
Scheme I.9 – The Nenitzescu indole synthesis.

In 1986, van Leusen *et al.*[60] (figure I.5, Type 8) established a route to highly substituted indoles, based on the condensation of isonitriles with unsaturated ketones to give the 2,3-bisalkenylpyrrole. Heating followed by aromatization with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) completed the synthesis of the indole nucleus (scheme I.10). Over the past decades this approach has been modified and applied on several indole derivatives synthesis reactions.[61-65]



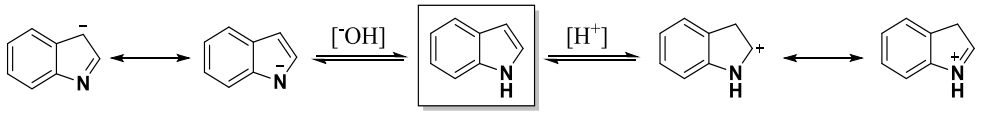
Scheme I.10 – The van Leusen indole synthesis.

Kanematsu *et al.*[66] described a pioneer route for the synthesis of indoles, in 1986 (figure I.5, Type 9). The key step of this approach is an intramolecular Diels-Alder reaction to simultaneous construction of both rings of the indole nucleus (scheme I.11).Three more related approaches were reported since that time.[67-69]



Scheme I.11 – The Kanematsu indole synthesis.

Indole is a π-excessive aromatic heterocycle and is highly reactive towards classical electrophilic substitution reactions such as protonation, halogenation, alkylation and acylation. Thus, electrophilic substitution in the indole ring has been extensively studied.[70] In these reactions, the indole moiety can undergo attack under neutral, positively and also negatively charged particles (scheme I.12).



Scheme I.12 – Resonance structures of indole negatively and positively charged, respectively.

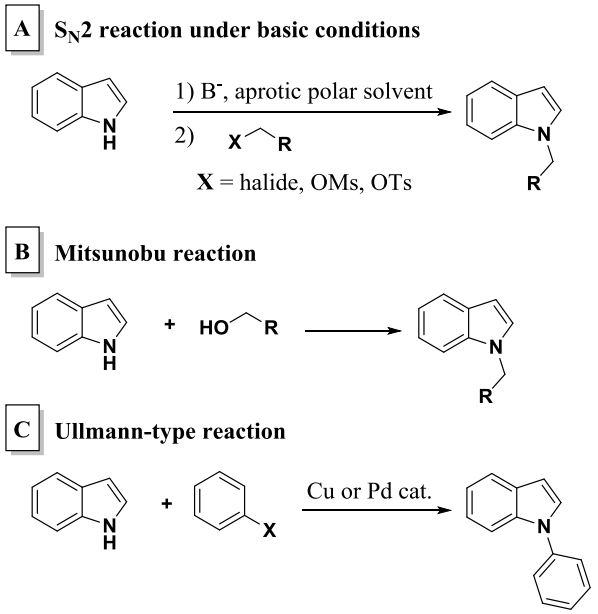
Indole is a weak acid (pKa 16.7 and 21.0, in water[71] and DMSO,[72] respectively), a weak base (pKb -3.5)[73] and only forms ions in strongly acid or basic media. As a consequence of the non-uniform distribution of the electron density in the neutral form of indole, the most nucleophilic position is C-3. The nitrogen atom can be made the most reactive nucleophilic site by deprotonation, so procedures for N-1 substitution normally involve base-catalysed nucleophilic substitution or conjugate addition reactions. The most versatile methods for C-2 substitution involve organometallic intermediates obtained by C-2 lithiation.

I.2.2.1 Functionalization at N-1 position

Despite the neutral indole ring is not very nucleophilic at nitrogen, the corresponding anion is a good nucleophile (scheme I.12). Therefore, procedures for *N*-alkylation typically are done under conditions where the nitrogen is deprotonated, using a strong base in a polar aprotic solvent. The alkylating reagents must be able to undergo through a bimolecular nucleophilic substitution (S_N2) reaction. Primary alkyl, benzyl and allyl halides as well as sulfonates are usually excellent electrophiles

(scheme I.13A).[25] Indoles can also be *N*-alkylated using alcohols, *via* Mitsunobu reaction (scheme I.13B).[74-76]

Concerning the *N*-arylation of indole, nucleophilic aromatic substitution (S_NAr) reactions allow access to these compounds. However, they are limited to strongly electron-deficient arenes, or by Ullmann-type reactions (scheme I.13C).[77] Since the pioneering work by Buchwald *et al*[78] alternative reaction conditions for the *N*-arylation reaction have been investigated with improvements in terms of mildness, functional group tolerance, and eventual omission of the base and ligand.[29]



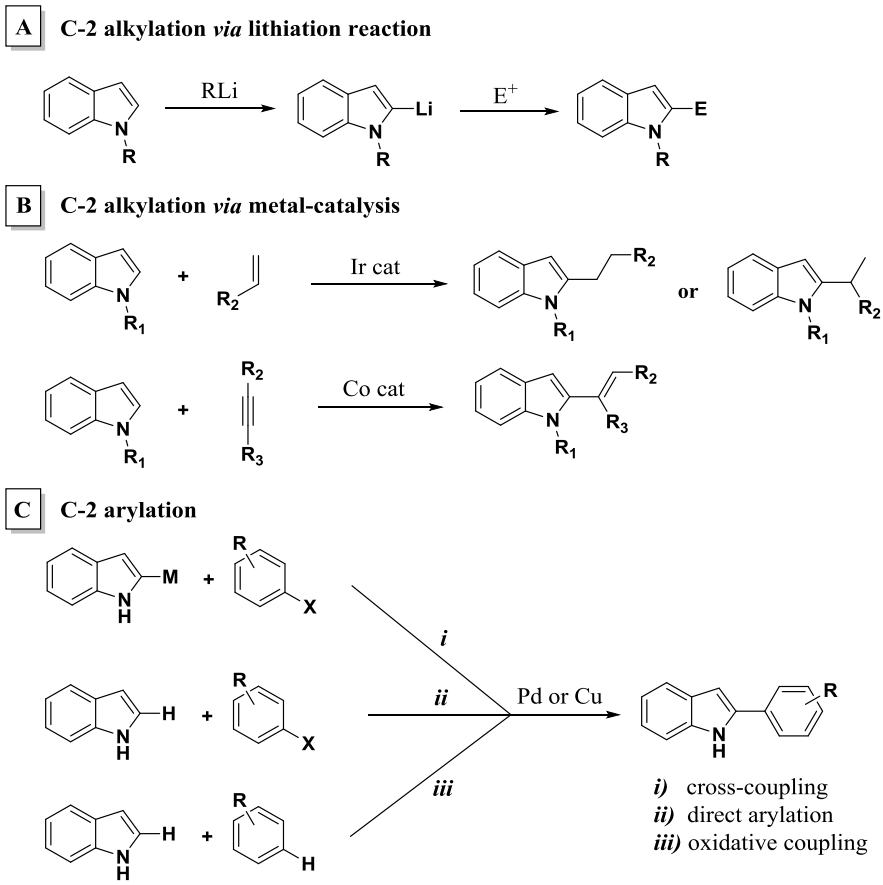
Scheme I.13 – Strategies for N-1 functionalization of indole compounds.

I.2.2.2 Functionalization at C-2 position

Lithiation of *N*-substituted indoles is one of the premiere methods for preparing 2-substituted indoles. 1-Alkylindoles, such as 1-methylindole, are readily lithiated and undergo typical reactions, such as *n*-BuLi at low temperature, with electrophiles. Thus, to synthesize *N*-unsubstituted indoles as final products, the use of a protecting group is required (scheme I.14A).[79-81] More recently, examples of direct C-2 alkylation *via* transition-metal catalyzed C−H activation were reported (scheme I.14B). The iridium-catalyzed C-2 alkylation reaction of *N*-substituted indole derivatives with several alkenes afforded linear or branched 2-alkylindoles in high to excellent selectivity.[82] Likewise, the direct alkenylation of the C-2 position, of indoles bearing an easily removable *N*-pyrimidyl group with alkynes, has been achieved by using a cobalt catalyst in a highly efficient and regioselective manner.[83]

To date, three main strategies have been adopted for the C-2 arylation of indoles. All these approaches rely on the use of a transition metal catalyst, and vary depending on the level of pre-

functionalization on the substrate starting materials: *i)* traditional cross-coupling reaction between two pre-functionalized substrates (for example, Stille and Suzuki couplings); *ii)* direct arylation, where only the arene coupling partner is pre-functionalized; and *iii)* oxidative couplings, where neither the indole nor the arene coupling partner are pre-functionalized (scheme I.6C).[84]

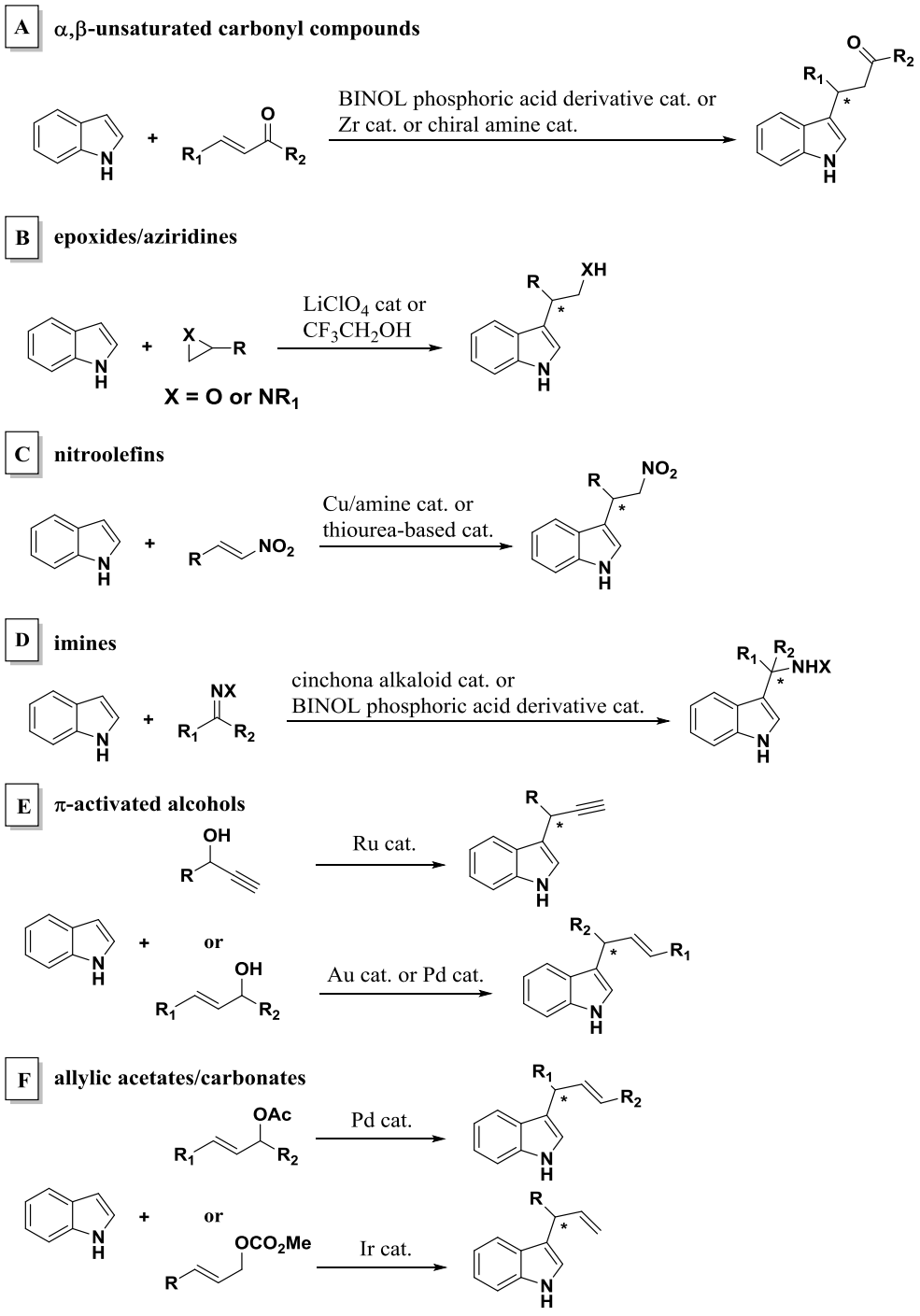


Scheme I.14 – Strategies for C-2 functionalization of indole compounds.

I.2.2.3 Functionalization at C-3 position

C-3 position is the most reactive position on indole over electrophilic aromatic substitution reactions (scheme I.12), and it is 10^{13} times more reactive than benzene positions.[25] The Friedel–Crafts alkylation is one of the archetypical acid catalyzed C–C bond-forming reactions to introduce alkylic chains onto aromatic compounds *via* electrophilic reagents.[85] Many electrophiles can be employed for the Friedel–Crafts alkylation of indoles, such as: α,β-unsaturated carbonyl compounds *via* Michael addition (scheme I.15A);[86-88] epoxides/aziridines (scheme I.15B);[89,90] nitroolefins (scheme I.15C);[91,92] imines (scheme I.15D);[93-95] π-activated alcohols (scheme I.15E);[96-98] and allylic acetates/carbonates (scheme I.17F).[99,100]

Concerning to C-3 arylation, the same strategies used for C-2 arylation can be applied.[29]



Scheme I.15 – Strategies for C-3 functionalization of indole compounds *via* Friedel–Crafts alkylation.

Several other methodologies for the synthesis/functionalization of the indole ring have been described and reviewed.[15] Nevertheless, due to its great importance and presence in diverse fields, from materials to medicine, the indole chemistry is still a growing research area.

I.3 Objectives

The aim of this study was to explore the indole scaffold as a privileged structure, in order to find new compounds with relevant biological activities. Therefore, novel synthetic strategies were developed to achieve a proper substitution pattern in this moiety, attaining new indole-based libraries with antioxidant, anti-inflammatory and antitubercular activities.

Thus, this work had the following objectives:

- Identification of new compounds with radical (ROS and RNS) scavenging activity, and consequently potential drug candidates to the oxidative/nitrosative stress related diseases;
- Establishment of a set of indolic compounds with different substitutions that would improve the radical scavenging activity and perform an SAR study;
- To explore the mechanisms involved in their scavenging activity in order to establish a new platform for future drug development;
- Design, synthesis and biological evaluation of a new indole-based selective COX-2 inhibitors, concerning the development of novel anti-inflammatory drugs candidates;
- Investigation of different substitution patterns of the indole scaffold in order to explore the different interactions with key residues of COX-1 *vs.* COX-2;
- Use of different tools, such as NMR and docking, to rationalize the obtained results in the biological evaluation;
- Validate and integrate a medicinal chemistry approach to find selective COX-2 inhibitors and understanding the mode of action of the new compounds;
- Develop the synthesis of new indolic compounds in which the functionalization has been directed by computational models and evaluate their activity as antitubercular agents.

These objectives intent to contribute to the development of new, better and safer antioxidant, anti-inflammatory and antitubercular drugs.

I.4 Thesis structure

This thesis is the result of the work developed from 2009 to 2013 and originated four publications in international and peer-reviewed journals.

This work is divided in three main sections. The first describes the synthetic approaches used to assemble a trytamine/tryptophan-based library as well as the evaluation of its radical scavenging potency, for ROS and RNS and also the assessment of its electrochemical behavior, by cyclic voltammetry.

This work is published in:

Estevão, M. S.; Carvalho, L. C.; Ribeiro, D.; Couto, D.; Freitas, M.; Gomes, A.; Ferreira, L. M.; Fernandes, E.; Marques, M. M. B., Antioxidant activity of unexplored indole derivatives: Synthesis and screening *Eur. J. Med. Chem.* **2010**, *45*, 4869;

Estevão, M. S.; Carvalho, L. C.; Ferreira, L. M.; Fernandes, E.; Marques, M. M. B., Analysis of the antioxidant activity of an indole library: cyclic voltammetry versus ROS scavenging activity *Tetrahedron Lett.* **2011**, *52*, 101.

Also contributes for the work developed in:

Carvalho, L. C.; Estevão, M. S.; Ferreira, L. M.; Fernandes, E.; Marques, M. M. B., A new insight on the hypochlorous acid scavenging mechanism of tryptamine and tryptophan derivatives *Bioorg. Med. Chem. Lett.* **2010**, *20*, 6475.

The second section refers to the search for novel selective COX-2 inhibitors, including the establishment of the substitution patterns, the synthetic approach and protocol development to attain the desired indole-based library. Several synthetic routes were explored and, in some cases, synthetic steps were optimized based on the reported literature as well as some mechanistic studies were developed. This section also describes the tools used to evaluate the inhibitory capacity of the synthesized compounds and its rationalization using NMR and *in silico* tool, as well as the efforts carried towards a new methodology for the synthesis of sulfonyl-containing compounds.

This work is published in:

Estevão, M. S.; Carvalho, L. C. R.; Freitas, M.; Gomes, A.; Viegas, A.; Manso, J.; Erhardt, S.; Fernandes, E.; Cabrita, E. J.; Marques, M. M. B., Indole based cyclooxygenase inhibitors: Synthesis, biological evaluation, docking and NMR screening *Eur. J. Med. Chem.* **2012**, *54*, 823.

Estevão, M. S.; Duarte, F. J. S.; Fernandes, E.; Santos, A. G.; Marques, M. M. B., Unexpected reactivity of trifluoromethylated olefins with indole: a mechanistic investigation *Tetrahedron Lett.* **2012**, *53*, 2132.

The last section involves the description of the synthetic strategy developed to prepare indole-based compound as potential antitubercular agents.

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Chapter II New indole derivatives as antioxidants agents

My contribution for this work was the preparation of all synthetic compounds, as well as the $ROO^{\cdot}$ scavenging and the cyclic voltammetry assays.

II.1 Introduction

Aerobic organisms are normally under a dynamic equilibrium between free radical generation and quenching. Over the past decades, these species and other reactive small molecules have emerged as important regulators of many physiological and pathological processes.

Currently, it is well established that - at physiological low levels - reactive oxygen species (ROS) and reactive nitrogen species (RNS) regulate growth, apoptosis and other signaling, at the cellular level. At the systems level, ROS and RNS contribute to complex functions such as blood pressure regulation, cognitive function and immune function. These reactive species enable the response to growth factor stimulation and the generation of the inflammatory response. Also, they have a vital role in the immune system, where they are capable to directly kill pathogens.[1,2] However, it is also known that at high levels, these short-lived reactive molecules can cause harmful effects by disturbing the cellular reduction-oxidation (redox) balance. This perturbation leads to an imbalance between the relative rates of production and degradation of ROS/RNS, called oxidative or nitrosative stress, respectively. The excess of these reactive species can damage cellular lipids, proteins or DNA, inhibiting their normal function, and are associated with a wide range of pathologies and diseases such as cancer, ischemic/reperfusion injury, cardiovascular disease, rheumatoid arthritis, diabetes and neurological disorders, such as Alzheimer and Parkinson disease (figure II.1).[2]

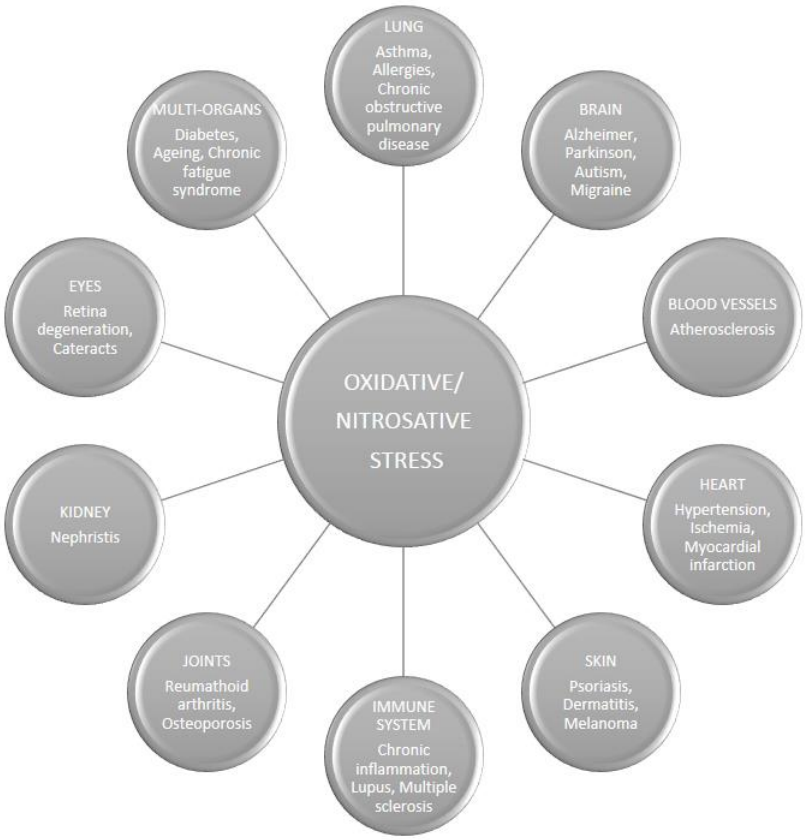


Figure II.1 - Examples of oxidative/nitrosative stress related diseases.

The "primary" ROS is the superoxide anion radical ($O_2^{\cdot -}$) arising of metabolic processes or from oxygen "activation" by physical irradiation. This reactive specie can further interact with other molecules, directly or through enzyme- or metal-catalyzed processes, generating "secondary" ROS, such as hydroxyl radical ($^{\cdot}OH$), hydrogen peroxide (H_2O_2), peroxyl radical ($ROO^{\cdot}$), hypochlorous acid (HOCl). Molecular oxygen can be itself a ROS because it can exist in a singlet state ($^1O_2$) for short periods of time, alongside with its more stable triplet state ($^3O_2$). RNS include mainly nitric oxide radical ($^{\cdot}NO$) and peroxynitrite anion ($ONOO^-$).[2,3]

Despite being typically formed as a result of normal cellular metabolism, studies have shown that the concentration of free radical in the organism increases when cells are exposed to harmful environmental influences such as pollutants, radiation, stress, smoking, and nutrition.[4] It is often assumed that mitochondria (mainly complex I and III, but also monoamino oxidase, α-ketoglutarate dehydrogenase, glycerol phosphate dehydrogenase, $p66^{shc}$) is the primary endogenous source of oxidative stress in mammalian cells.[5] However, there is no convincing experimental evidence to support this postulate.[6] Another major source of ROS is the endoplasmic reticulum (mostly cytohrome *P*-450 and *b*5 enzymes, diamine oxidase, Ero1).[7] Peroxissomes (acid fatty acid oxidation, D-amino acid oxidase, L-2-hydroxyacid oxidase and urate oxidase),[8] cytosol (NO synthases, lipoxygenases and PGH

synthase),[9] plasma membrane (NADPH oxidases, lipoxygenase),[10] and extracellular space (xanthine oxidase)[11] are other cellular sources of ROS (figure II.2).

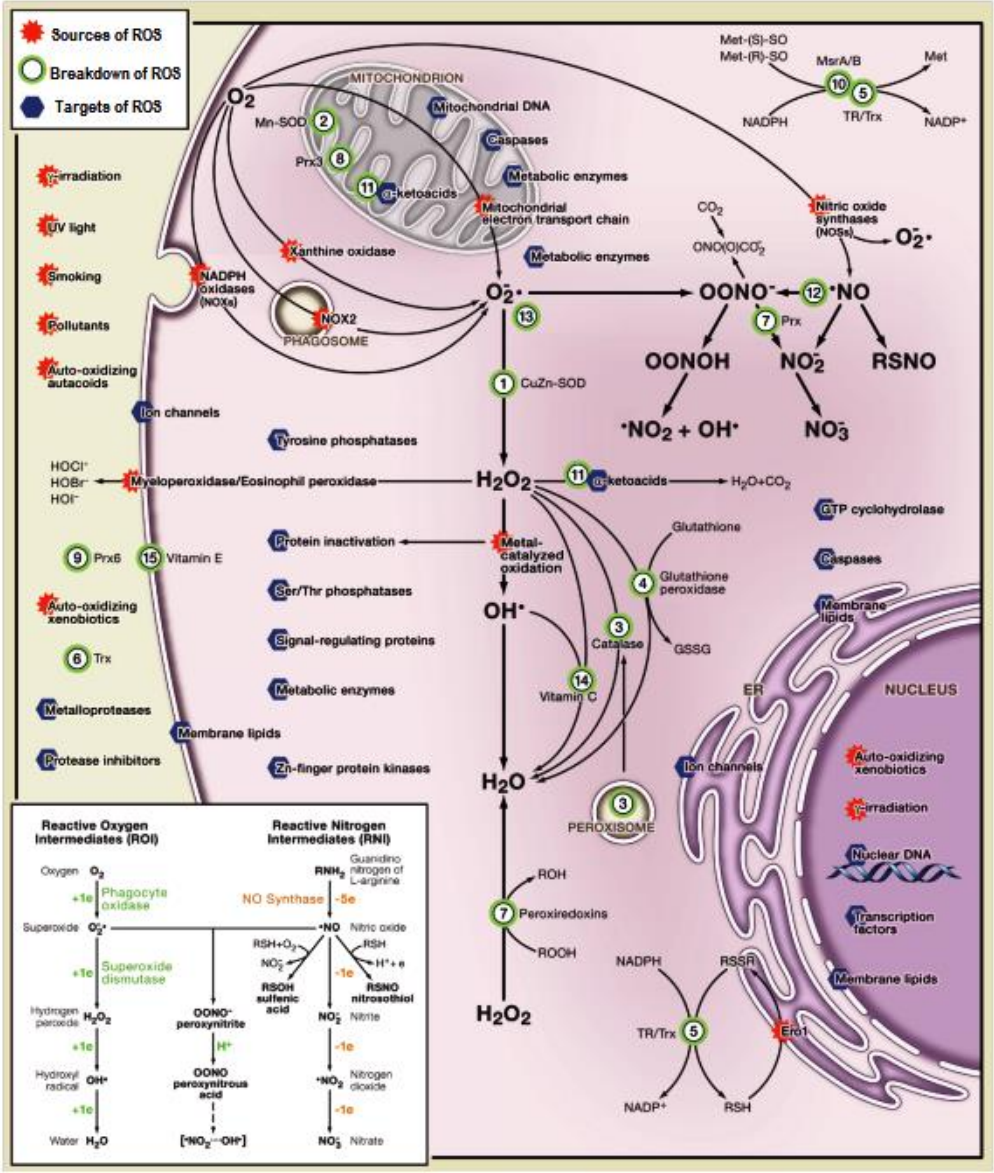


Figure II.2 - Sources of ROS (adapted from Ding 2010).[12]

Due to the constant exposure to free radicals, organisms developed different mechanisms to prevent or repair free radical-induce damages, and as well as physical and antioxidant defenses. These mechanisms include non-enzymatic proteins (transferrin, ferritin, ceruleoplasmin), enzymes (such as superoxide dismutases, catalase, glutathione peroxidase), oxidizable molecules (glutathione, vitamins E, A, C, carotenoids, flavonoids), and trace elements (copper, zinc, selenium).[13,14] These different kinds of defense systems restrain the damage induced by ROS. However, these mechanisms are not always sufficient during pathologic conditions, so the development of antioxidant compounds that are able to scavenge ROS and RNS, and restore the homeostasis, is essential.

Several indole derivatives have been reported for their antioxidant proprieties and for their free radical scavenger capacities. One of the most recognized natural antioxidants is the indole hormone melatonin (figure II.3). Melatonin is produced in the body and acts as receptor-independent free-radical scavenger, and has a broad-spectrum of antioxidant effects.[15,16] With reference to ROS, melatonin has a well-known potent scavenging effect against $^1O_2$, $O_2^{\cdot-}$, H_2O_2, $^{\cdot}OH$, and $ROO^{\cdot}$.[17] The high reactivity of melatonin with pro-oxidant reactive species is probably due to the electron-rich aromatic ring system, allowing the indoleamine to easily function as an electron donor to form the melatoninyl cation radical, or through an electrophilic radical addition at the C-3 position of indole.[16] This type of reactivity with ROS is extended to other indole derivatives, namely tryptophan, serotonin, 5-methoxytryptamine, 6-chloromelatonin and related indoles,[18-21] indole 3-acetic acid (IAA) and other plant-derived indoles,[21,22] the well-known non-steroidal anti-inflammatory drug (NSAID) indomethacin and its derivatives,[23,24] indole derivatives containing an pyrimidine and fused pyrimidine systems[25], indole amino acids derivatives,[26] 2- phenylindole derivatives,[27] indolin-2-one and indoline-2-thione derivatives,[28] and stobadine.[29] Some of these representative examples of indole derivatives showing antioxidant activity are displayed in figure II.3.

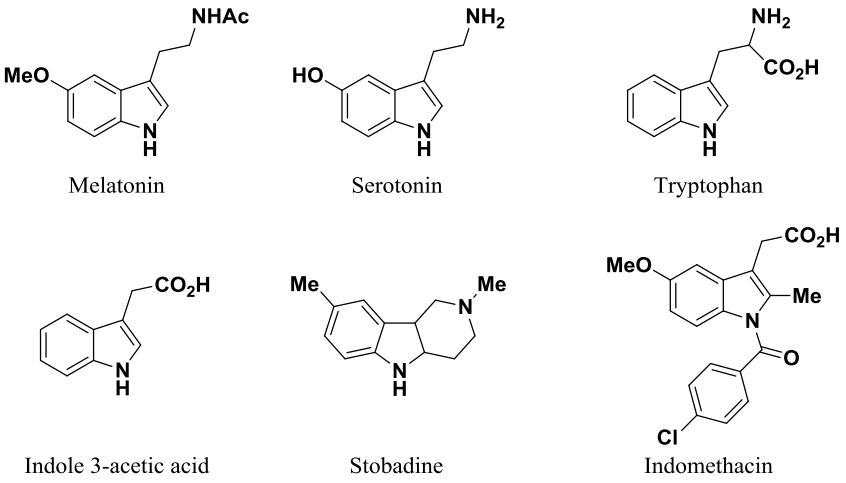


Figure II.3 - Some examples of indole derivatives that show antioxidant activity.

Moreover, HOCl scavenging activity has been investigated for the marketed indole derived NSAIDs, but no activity was found against this ROS.[23]

Thus, a small library of novel synthetic tryptophan and tryptamine derivatives was prepared to explore their radical scavenging potency, by evaluation of their *in vitro* scavenging activity for ROS ($O_2^{\cdot-}$, H_2O_2, HOCl, $ROO^{\cdot}$, $^1O_2$) and RNS ($^{\cdot}NO$ and $ONOO^-$), as well as their electrochemical behavior, by cyclic voltammetry. Due to the similarity between electrochemical and biological reactions, it can be assumed that the oxidation mechanisms taking place at the electrode and in the body share similar principles.[30] Therefore, the redox proprieties of drugs and biomolecules might be extremely relevant to

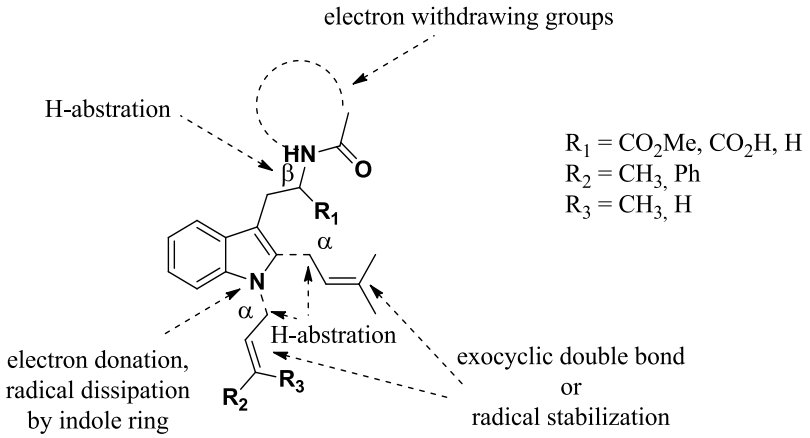
understand their *in vivo* redox behavior. In fact, this technique has been used to investigate how melatonin[31] and other indole derivatives[32,33] scavenge a variety of free radicals.

II.2 Results and discussion

The development of novel indole derivatives with ROS and RNS scavenging activities constitutes a step forward in this area in view of the myriad of different biological activities displayed by compounds belonging to this family. The discovery of new derivatives with high antioxidant potential, but without other biological effects, may be important to avert adverse reactions that may arise within a clinical setting.

The strategy followed envisaged the insertion of several groups at the indole moiety to facilitate either a radical scavenging mechanism *via* electron donation/H-abstraction or as traps for reactive species that react *via* an ionic mechanism.

The prenyl chain at N-1 or C-2 was chosen on the basis of a possible H-abstraction from the α position, allowing a radical stabilization by the allylic double bond as well as by dissipation into the indole ring. Some reactive species are known to react with double bonds by either radical or ionic mechanism, such as the $^1O_2$ and the $^{\cdot}NO$. It is worth noting that natural prenylated indolic compounds belong to a large class of alkaloids isolated from fungi, possessing a tryptophan moiety substituted with isoprenic units.[34] The presence of an exocyclic double bond would enhance the scavenging activity with these species by both mechanisms. Scheme II.1 shows the proposed plan for substitution pattern of the indole library.



Scheme II.1 – Proposed substitution pattern for the indole library.

The influence of the position of the prenyl chain was also evaluated, and consequently the importance of a free indolic nitrogen.

Moreover, the substitution of the alkyl chain at C-3 was further explored. Different electron withdrawing groups were used at the nitrogen atom from the side chain, such as the acetyl group and the phthaloyl group. These groups were selected due to their easy preparation, stability and most important the radical stabilization whether H-abstraction occurs at the β position.

II.2.1 Synthesis of the indole-based library

In order to conduct a structure-activity relationship (SAR) study, the first step consisted on the synthesis of an indole-based library. This was done by conducting the synthesis of a library of fifteen compounds starting from L-tryptophan methyl ester (**10**) and tryptamine (**9**) (figure II.4).

The synthesis of compounds **1-7**, **11** and **12** was previously described[35] and these compounds were prepared according to following procedures: protection of the nitrogen atom of the side chain, of compounds **9** and **10**, with phthalimide and acetyl groups (compounds **5**, **11** and **12**); subsequent *N*-prenylation using either 3,3-dimethylallyl bromide or 3-bromo-1-phenyl-1-propene afforded compounds **3**, **4**, **6** and **7**; removal of the phthalimide group from **4** provided compound **2**; treatment of **6** with $BF_3.OEt_2$ afforded **1**, according to the Lobo-Prabhakar sigmatropic rearrangement conditions of *N*-prenylindole derivatives.[36]

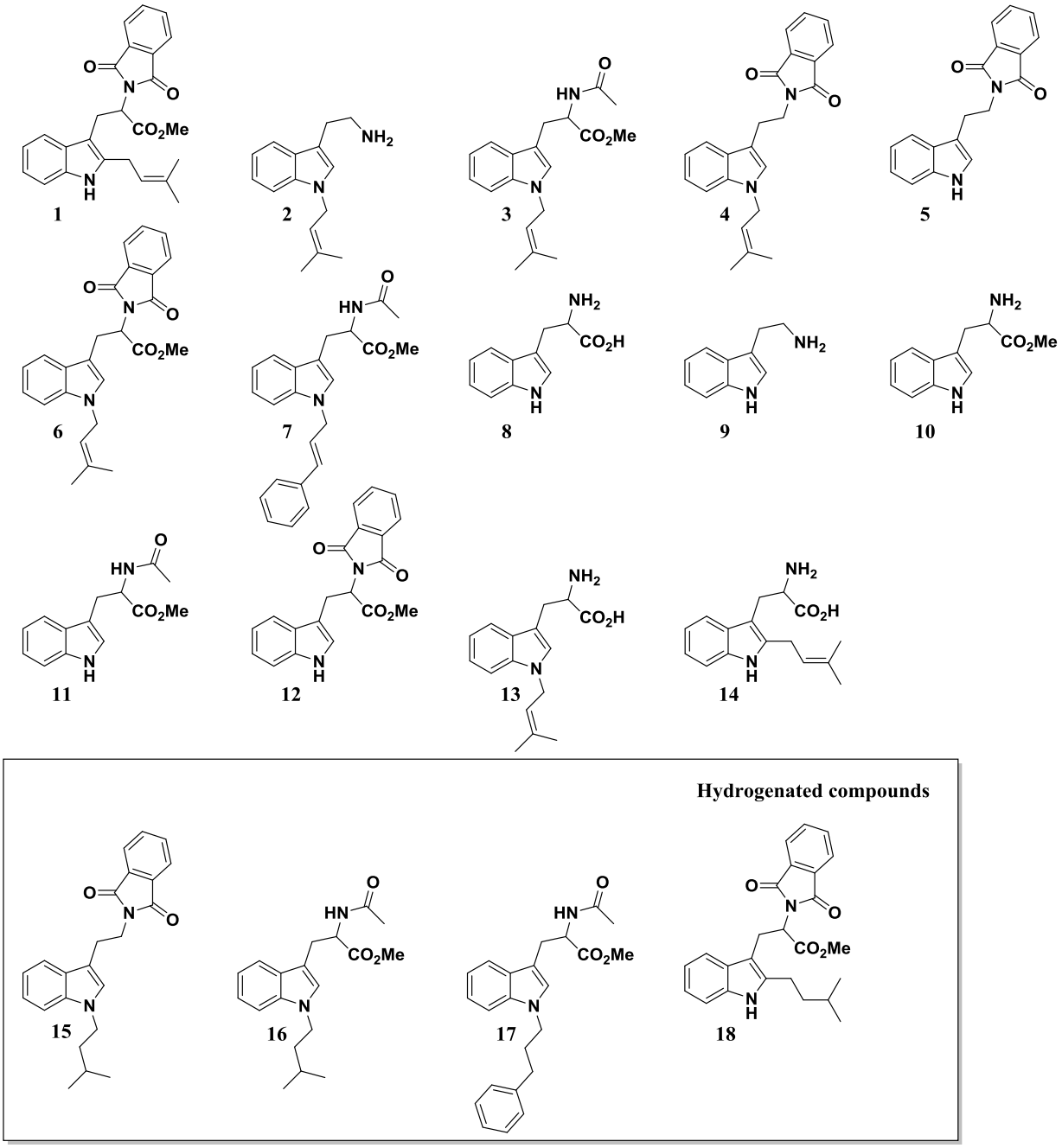


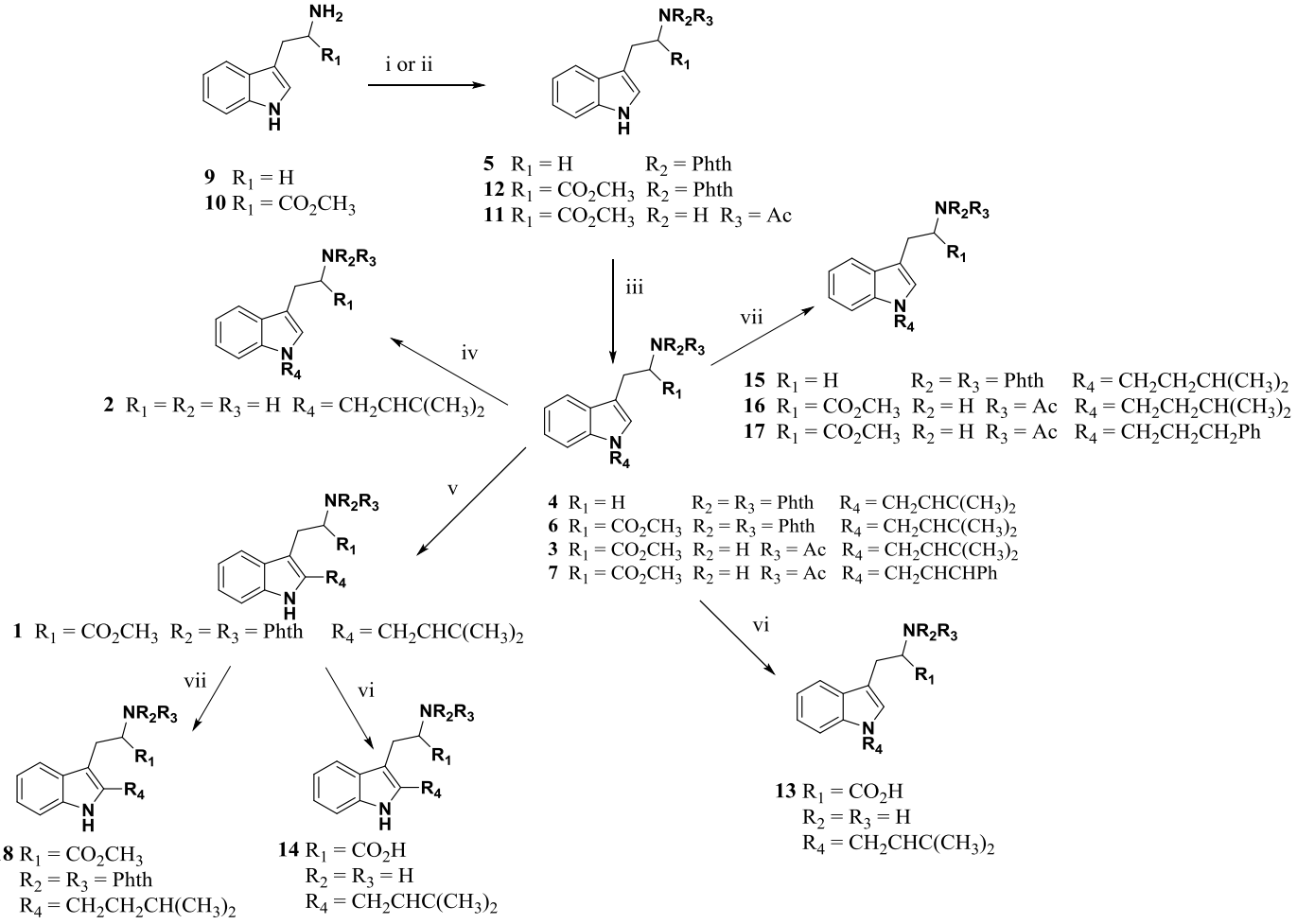
Figure II.4 - Synthesized indole based library (compounds 1-7 and 11-18), trypthopan (8), tryptamine (9) and trypthophan methyl ester (10).

Compounds **13** and **14** were synthesized from the corresponding prenylated precursors **6** and **1**, respectively, by treatment with hydrazine hydrate, to remove the phthalimide group, followed by alkaline hydrolysis, affording the desired products. Thus, the influence of the free amino acid group could be evaluated.

In order to study the effect of the exocyclic double bond on the reactivity, some hydrogenated compounds were prepared. Therefore, compounds **15**, **16**, **17** and **18** were obtained by hydrogenation of

the corresponding prenylated precursors (**4**, **3**, **7** and **1**, respectively) under Pd/C at room temperature and atmospheric pressure.

Scheme II.2 depicts the synthetic routes adopted for the synthesis of the indole based library.



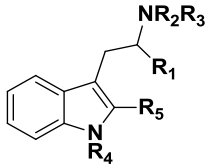
i) phthalic anhydride, TEA, toluene, rfx; **ii)** Ac_2O, TEA, EtOAc, DMAP, rt; **iii)** NaH, DMF, alkyl bromide, 0ºC; **iv)** $NH_2NH_2.H_2O$, EtOH, rfx; **v)** $BF_3.OEt_2$, DCM, -14ºC; **vi)** 1) $NH_2NH_2.H_2O$, DCM/MeOH, rt; 2) 1 M NaOH, MeOH, rt; **vii)** 10% Pd/C, MeOH, H_2, rt

Scheme II.2 - Synthetic routes adopted for the synthesis of the indole based library.

II.2.2 Radical scavenging assays

The radical scavenging activity of the synthesized library was evaluated by *in vitro* assays with different methodologies. Table II.1 shows the results obtained for the screening assays against ROS, namely $ROO^{\cdot}$, HOCl and $^1O_2$.

Table II.1 - ROS scavenging activities of the synthesized indole library (IC_{50} in µM; Mean±SD, n = 3-4)



Compound	R_1	R_2	R_3	R_4	R_5	ROO·	HOCl	$^1O_2$
						ORAC[a]	IC_{50} (µM)	
1	CO_2Me	Phth	Phth	H	$CH_2CHC(Me)_2$	0.45 ± 0.05	7.18 ± 0.41	2307 ± 447
2	H	H	H	$CH_2CHC(Me)_2$	H	0.96 ± 0.07	26.2 ± 9.55	618 ± 94
3	CO_2Me	H	Ac	$CH_2CHC(Me)_2$	H	0.21 ± 0.03	32.8 ± 6.82	777 ± 155
4	H	Phth	Phth	$CH_2CHC(Me)_2$	H	---	6.34 ± 1.54	---
5	H	Phth	Phth	H	H	1.46 ± 0.08	11.1 ± 0.78	---
6	CO_2Me	Phth	Phth	$CH_2CHC(Me)_2$	H	0.36 ± 0.01	34.3 ± 6.26	2859 ± 499
7	CO_2Me	H	Ac	$CH_2CHCHPh$	H	0.44 ± 0.01	67.7 ± 9.24	971 ± 69
8[b]	CO_2^-	H	H	H	H	2.74 ± 0.07	3.50 ± 0.40	992 ± 220
9	H	H	H	H	H	1.86 ± 0.03	6.00 ± 0.60	682 ± 102
10	CO_2Me	H	H	H	H	2.60 ± 0.16	9.60 ± 0.76	1043 ± 320
11	CO_2Me	H	Ac	H	H	1.21 ± 0.06	≈ 50	1559 ± 271
12	CO_2Me	Phth	Phth	H	H	1.09 ± 0.13	27.3 ± 4.84	943 ± 168
13[b]	CO_2^-	H	H	$CH_2CHC(Me)_2$	H	1.05 ± 0.05	4.13 ± 0.17	841 ± 59
14[b]	CO_2^-	H	H	H	$CH_2CHC(Me)_2$	0.48 ± 0.04	4.56 ± 0.48	*
15	H	Phth	Phth	$CH_2CH_2CH(Me)_2$	H	---	10.6 ± 4.80	1029 ± 216
16	CO_2Me	H	Ac	$CH_2CH_2CH(Me)_2$	H	0.28 ± 0.02	8.35 ± 1.08	718 ± 128
17	CO_2Me	H	Ac	$CH_2CH_2CH_2Ph$	H	0.44 ± 0.07	14.2 ± 2.95	506 ± 44
18	CO_2Me	Phth	Phth	H	$CH_2CH_2CH(Me)_2$	0.97 ± 0.09	3.75 ± 0.52	750 ± 128

*Compound not tested; --- not active

[a] Trolox was used as control (ORAC = 1)

[b] Under physiological conditions this compound exists in the zwitterionic form

All compounds except **4** and **15** were shown to be effective scavengers of the ROO˙. Indeed some were more active than trolox (used as control), and also more active than some NSAIDs possessing the indole ring, like indomethacin, etodolac and acemetacin.[23] The scavenging activity order found for compounds **1** to **14** was: **8** > **10** > **9** > **5** > **11** ≈ **12** ≈ **13** ≈ **2** > **14** ≈ **1** ≈ **7** > **6** > **3**. According to the obtained results, the compounds' library can be divided in two sets: the non-alkylated compounds (without the prenyl chain) and the alkylated (prenylated) ones. Figure II.5 shows the obtained results in the ROO˙-scavenging assay for compounds **5**, **8**, **11** and **14**.

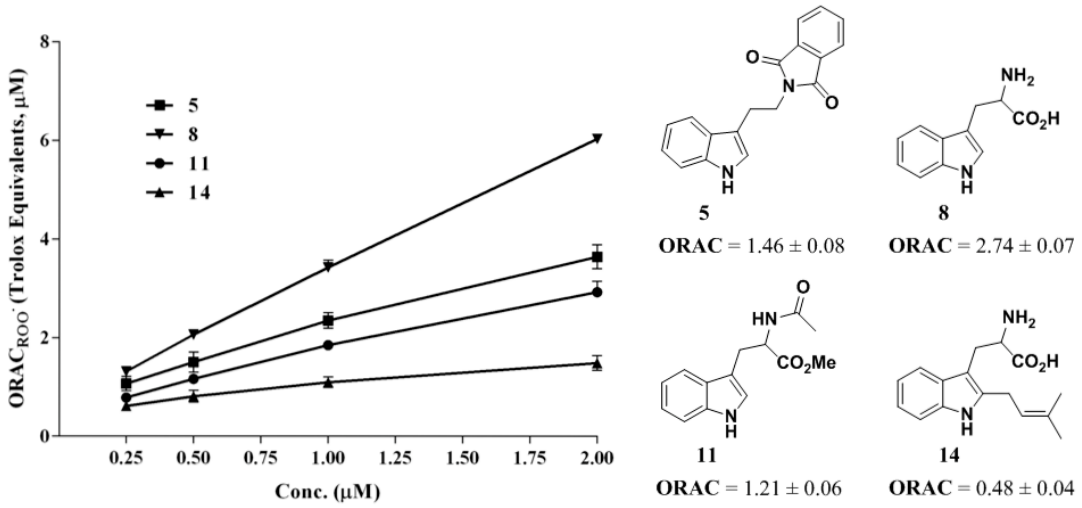


Figure II.5 - ROO˙-scavenging activity of compounds 5, 8, 11 and 14. Each point represents the values obtained from four experiments performed in triplicate (mean ± SEM).

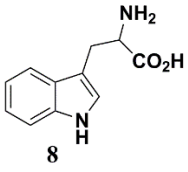
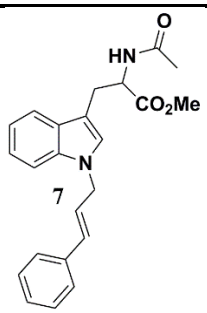
The presence of a free amine in the side chain, as well as free indolic nitrogen is important for scavenging activity. Under these conditions (physiological pH), compounds **14** and **13** exist in solution in the zwitterionic form. The non-alkylated compounds revealed to be the best scavengers of ROO˙. Indeed the protection of both nitrogen atoms (from the side chain C-3 and the indolic nitrogen N-1), combined with the lack of the carbomethoxy group at the side chain (C-3) seems to be detrimental for activity (compounds **4** and **15**).

In order to investigate the relevance of the unsaturated chain at the C-2 and N-1 position of indole, several hydrogenated compounds were prepared (compounds **15** to **18**, figure II.4) and tested. The total order of reactivity found was **8** > **10** > **9** > **5** > **11** ≈ **12** ≈ **13** ≈ **18** ≈ **2** > **14** ≈ **1** ≈ **17** ≈ **7** > **6** > **16** ≈ **3**. The hydrogenated compounds **16**, **17**, **18** were shown to be effective scavengers of the ROO˙, and some with higher activities than the corresponding unsaturated compounds (compounds **18** and **1**). These results indicate that the external double bond is not crucial for measured activity, and suggest that for the scavenging of ROO˙ the indole nitrogen is the active redox center of indoles. The indole nucleus

is already reported as the reactive center towards radical species, and this reactivity has been attributed to its high resonance stability and very low energy barrier towards radical reactions.[37] Nevertheless, the evaluated prenylated compounds possessing a free amine at the side chain (e.g. **13** and **2**) have a potency close to that observed for Trolox, and most of the tested compounds are even more active than indomethacin (ORAC reported 0.17).[23] Furthermore, the presence of a prenyl chain makes these compounds more lipophilic. Lipophilicity is a major determinant for the disposition and biological action of drugs, making them suitable for membranes protection against peroxidation.

The same library was screened as scavenger of HOCl (table II.1). Scavengers of HOCl are of reassured relevance since most NSAIDs (indole derived) are not able to scavenge this ROS.[23] All the tested compounds were found to be active against HOCl, and all hydrogenated compounds (except **15**) were more active than the corresponding unsaturated compounds. Table II.2 shows the chemical structures of the most and less active compounds against HOCl.

Table II.2 - Chemical structures of the most and less active compounds against HOCl.

Most active	Less active
 **8**	 **7**
IC_{50} = 3.50 ± 0.40 µM	IC_{50} = 67.7 ± 9.24 µM

The order of potencies found was **8** ≈ **18** > **13** ≈ **14** > **9** ≈ **4** > **1** > **16** ≈ **10** ≈ **15** ≈ **5** > **17** > **2** ≈ **12** > **3** ≈ **6** >> **11** > **7**. Considering that the reaction of amines with HOCl is well known,[38] it was anticipated that compounds possessing a free amine on the side chain would show higher activity. Indeed compounds **8**, **13**, **9**, **10** and **2** revealed to be potent scavengers of HOCl. The carboxylic acid at the side chain (C-3) (**8** and **13**), revealed to be important for activity since when the carboxylic acid is methylated (**8** vs. **10**) or is absent (**13** vs. **2**) its potency decreases.

The influence of the side chains of the indole nucleus on the $HO^{\cdot}$ scavenging ability by comparing melatonin with several analogs was previously evaluated. It was found that the 5-methoxy group as well the *N*-acetyl group at the side chain (C-3), were essential for melatonin to display potent $HO^{\cdot}$ scavenging activity.[15] However, in the present study, the *N*-acetyl group, by itself, was detrimental for HOCl scavenging activity, as may be ascertained by comparing compound **10** with *N*-acetyl-

methyltryptophan (**11**), and also for the lower activity found for compounds **3** and **7**, suggesting that a different reaction mechanism is involved.

Nevertheless, these results are in accordance to those of Poeggeler *et al.*,[18] who demonstrated that tryptamine is a better $HO^{\cdot}$ scavenger in the 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) competition assay than *N*-acetyltryptamine and 5-methoxytryptamine. On the other hand, the *N*-acetyl group may be physiologically important due to its preventing effect on the degradation of these compounds by monoamine oxidase. In that aspect, the replacement of the secondary amine by the *N*-phthaloyl group may achieve the same protective effect, while maintaining an effective scavenging activity, as shown for compounds **1**, **4**, **5**, **6**, **12**, **15** and specially **18**. It was not possible to conclude the effect of the loss of saturationat the exocyclic double bond in the scavenging activity of this family of compounds. Compound **18** showed a high potency when compared to the corresponding unsaturated compound **1**. The same was observed for compounds **16** and **3**, and for compounds **17** and **7**. However, compounds **4** and **15** showed inverted reactivity. The obtained results suggest that the observed reactivity with HOCl results from a conjugation of different functions within the tested substitution pattern and not from an isolated functionalization.

This indole-based library was also evaluated as scavenger of $^1O_2$. All the assayed compounds, except **4** and **5**, were shown to be effective scavengers for this ROS, in a concentration dependent manner, and the sequence of activity found was **17** ≈ **2** ≈ **9** ≈ **16** ≈ **18** ≈ **3** > **13** ≈ **12** ≈ **7** > **8** ≈ **15** ≈ **10** > **11** > **1** > **6**.

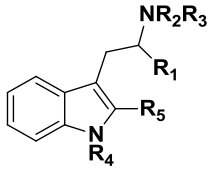
Since reactions with $^1O_2$ could take place at the exocyclic double bond, the reactivity of hydrogenated compounds was investigated. Compounds **17**, **16** and **18** were more active than the corresponding unsaturated compounds. From the activity results obtained, it is not possible to understand which structural motifs control the reactivity of the tested compounds with singlet oxygen.

None of the tested compounds was able to scavenge $O_2^{\cdot -}$ and in what concerns to H_2O_2 only a residual activity was found for some of the compounds in the highest tested concentration (2 mM).

Next the synthesized library was evaluated against RNS.

Table II.3 shows the results obtained for the scavenging activity of the synthesized library against RNS, in particular the scavenging effect of $^{\cdot}NO$ and $ONOO^-$.

Table II.3 – RNS scavenging activities of the synthesized indole library (IC_{50} in µM; Mean±SD, n=3-4).



Compound	R_1	R_2	R_3	R_4	R_5	·NO	$ONOO^-$ without $NaHCO_3$	$ONOO^-$ with $NaHCO_3$
						IC_{50} (µM)		
1	CO_2Me	Phth	Phth	H	$CH_2CHC(Me)_2$	239 ± 7	---	28 ± 10
2	H	H	H	$CH_2CHC(Me)_2$	H	1430 ± 108	905 ± 66	234 ± 46
3	CO_2Me	H	Ac	$CH_2CHC(Me)_2$	H	980 ± 142	545 ± 118	162 ± 76
4	H	Phth	Phth	$CH_2CHC(Me)_2$	H	---	---	192 ± 31
5	H	Phth	Phth	H	H	---	93 ± 36	41 ± 10
6	CO_2Me	Phth	Phth	$CH_2CHC(Me)_2$	H	---	---	241 ± 93
7	CO_2Me	H	Ac	$CH_2CHCHPh$	H	---	218 ± 26	36 ± 3
8[a]	CO_2^-	H	H	H	H	1151 ± 203	32 ± 10	19 ± 2
9	H	H	H	H	H	672 ± 105	32 ± 10	10 ± 3
10	CO_2Me	H	H	H	H	---	48 ± 7	13 ± 6
11	CO_2Me	H	Ac	H	H	---	223 ± 128	23 ± 9
12	CO_2Me	Phth	Phth	H	H	---	208 ± 49	30 ± 4
13[a]	CO_2^-	H	H	$CH_2CHC(Me)_2$	H	208 ± 26	95 ± 40	42 ± 23
14[a]	CO_2^-	H	H	H	$CH_2CHC(Me)_2$	*	*	*
15	H	Phth	Phth	$CH_2CH_2CH(Me)_2$	H	1526 ± 62	---	237 ± 55
16	CO_2Me	H	Ac	$CH_2CH_2CH(Me)_2$	H	---	548 ± 154	315 ± 83
17	CO_2Me	H	Ac	$CH_2CH_2CH_2Ph$	H	---	---	142 ± 24
18	CO_2Me	Phth	Phth	H	$CH_2CH_2CH(Me)_2$	---	---	14 ± 7

*Compound not tested; --- not active

[a] Under physiological conditions this compound exists in the zwitterionic form

From the studied compounds, only **1**, **2**, **3**, **8**, **9**, **13** and **15** were able to scavenge ˙NO achieving the IC_{50} in the tested range, and the order of reactivity found was **13** ≈ **1** > **9** > **3** > **8** > **2** > **15**. The presence of a prenyl chain is important for the activity against this RNS, on position N-1 as well as at position C-2, since compounds **13** and **1** are the most actives. The *N*-alkylated compounds are more active against ˙NO than those possessing indolic free nitrogen, with exception of compound **9**, which has a free amine at the side chain. In addition, considering the IC_{50}, the hydrogenated compound **15** is the less active, supporting that the loss of unsaturation is detrimental for the activity with this species. Nitric oxide is known as a nitrating agent in many processes and it has been used in the nitration of olefins as an economic and straightforwardly available source of nitrogen.[39,40] This may explain the scavenging activity found for the prenylated compounds of this library.

Concerning $ONOO^-$, scavenging activity was observed for all the studied compounds when the study was carried in the presence of $NaHCO_3$, plus under these conditions the activity increased. The aim of performing scavenging studies of $ONOO^-$, in the presence of $NaHCO_3$ is to simulate the physiological concentrations of CO_2 due to its fast reaction *in vivo* with $ONOO^-$ with formation of $CO_3^{\cdot-}$ and $^{\cdot}NO_2$ leading to oxidation and nitration, respectively.[41] The fact that a scavenging of $^{\cdot}NO_2$ and $CO_3^{\cdot-}$ can enhance the compounds' efficiency[42] may explain why the library compounds were more active in the presence of $NaHCO_3$. Figure II.6 depicts the obtained results in the $ONOO^-$-scavenging assay (in the presence of $NaHCO_3$) for compounds **5**, **7**, **8** and **13**.

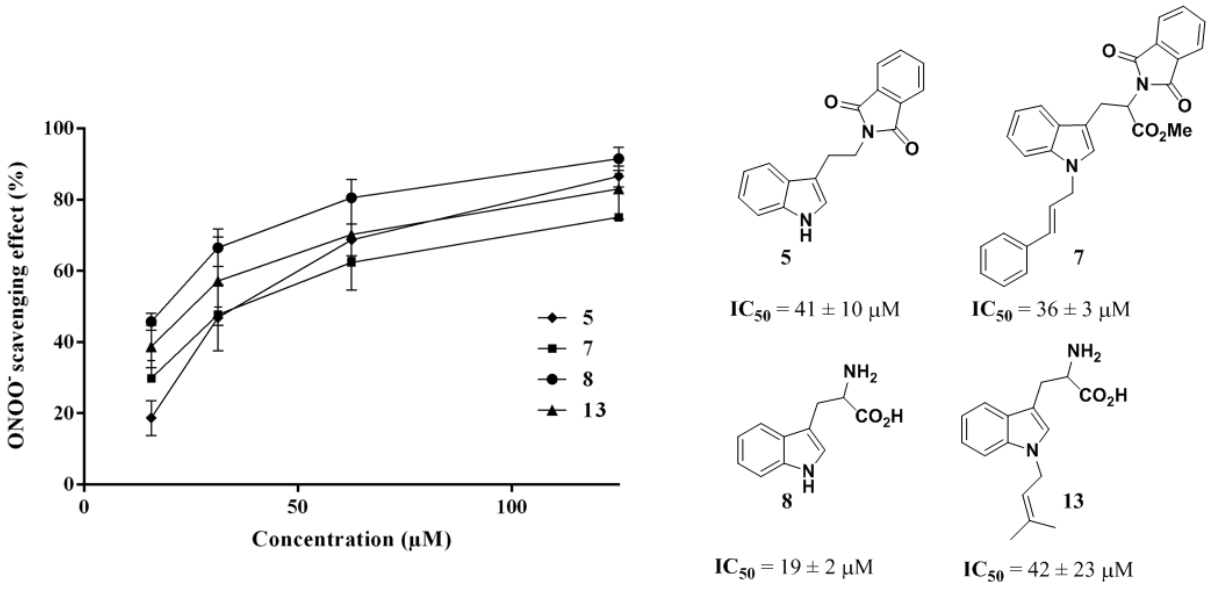


Figure II.6 - $ONOO^-$ scavenging activity (in the presence of $NaHCO_3$) of compounds 5, 7, 8 and 13. Each point represents the values obtained from four experiments performed in triplicate (mean ± SEM).

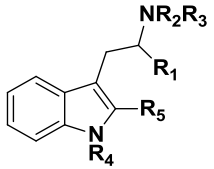
The order of reactivity found was **9** ≈ **10** ≈ **18** ≈ **8** ≈ **11** ≈ **1** ≈ **12** ≈ **7** ≈ **5** ≈ **13** >> **17** ≈ **3** ≈ **4** > **2** ≈ **15** ≈ **6** > **16**. The results obtained for this library show that the presence of a free amine at the side chain, as well as a free indolic nitrogen atom is important for activity. For the scavenging of $ONOO^-$, alkylation at N-1 is detrimental. However when alkylation occurs at C-2 (compounds **18** and **1**), leaving the N-1 position free, the reactivity of the indolic moiety is increased, since compound **12** is less active. The fact that the prenylated and hydrogenated compounds' activities were slightly different (**4**/**15**, **18**/**1** and **16**/**3**) indicates that the reaction with this radical has a different mechanism than with $^{\cdot}NO$, and that the indolic moiety is the crucial reaction center.

II.2.3 Cyclic voltammetry assays

The cyclic voltammetry methodology has been found to be an important tool for understanding the electrochemical behavior of compounds.

In order to investigate how these compounds scavenge the chosen ROS, and explore their scavenging mechanisms, a cyclic voltammetry study was undertaken. Table II.4 shows the oxidation potentials obtained from the cyclic voltammograms of the screened indole library.

Table II.4 – Chemical structures and oxidation potentials of the indole library.



Compound	R_1	R_2	R_3	R_4	R_5	Ep_{ox} (V) vs. Ag/AgCl	
						1st peak	2nd peak
1	CO_2Me	Phth	Phth	H	$CH_2CHC(Me)_2$	0.930	---
2	H	H	H	$CH_2CHC(Me)_2$	H	0.852	1.084
3	CO_2Me	H	Ac	$CH_2CHC(Me)_2$	H	0.959	1.152
4	H	Phth	Phth	$CH_2CHC(Me)_2$	H	0.986	---
5	H	Phth	Phth	H	H	1.028	---
6	CO_2Me	Phth	Phth	$CH_2CHC(Me)_2$	H	1.016	---
7	CO_2Me	H	Ac	$CH_2CHCHPh$	H	1.011	---
8	CO_2H	H	H	H	H	0.996	---
9	H	H	H	H	H	0.767	1.199
10	CO_2Me	H	H	H	H	1.055	---
11	CO_2Me	H	Ac	H	H	0.967	---
12	CO_2Me	Phth	Phth	H	H	0.999	---
13	CO_2H	H	H	$CH_2CHC(Me)_2$	H	1.008	---
14	CO_2H	H	H	H	$CH_2CHC(Me)_2$	1.002	---
15	H	Phth	Phth	$CH_2CH_2CH(Me)_2$	H	0.979	---
16	CO_2Me	H	Ac	$CH_2CH_2CH(Me)_2$	H	0.984	1.177
17	CO_2Me	H	Ac	$CH_2CH_2CH_2Ph$	H	0.993	---
18	CO_2Me	Phth	Phth	H	$CH_2CH_2CH(Me)_2$	0.968	---

All the compounds showed an oxidation potential peak lower than that observed for indole (Ep_{ox} = 1.035 V), except for compound **10**. The relative order found for the oxidation potential of the studied indoles was: **9** < **2** < **1** < **3** < **11** < **18** < **15** < **16** < **4** < **17** < **8** < **12** < **14** < **13** < **7** < **6** < **5** < **10**. Tryptamine (**9**) and its derivative **2** show two peaks in the voltammograms, while its *N*-phthaloyl protected derivatives **4**, **5** and **15** show only one peak (figure II.7). It suggests that the first oxidation peak is due to the nitrogen from the side chain and the second oxidation peak is due to the indolic nitrogen.

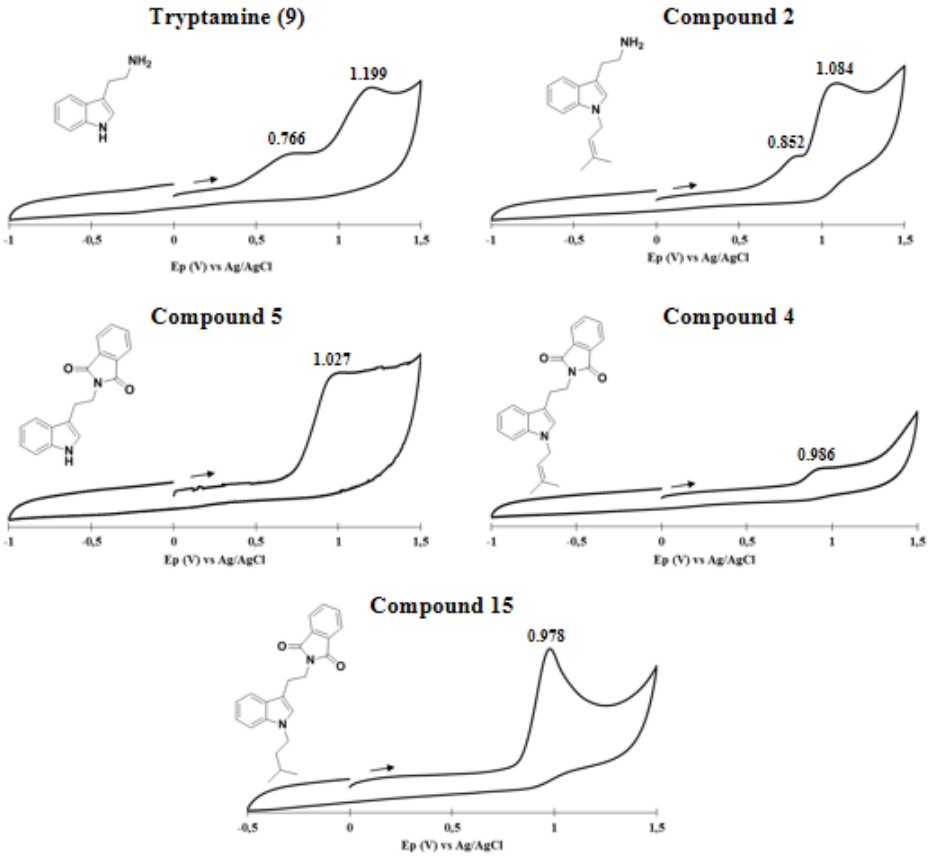


Figure II.7 – Voltammograms of tryptamine (9) and its derivatives (compounds 2, 5, 4 and 15).

For the tryptamine derivatives, it was observed that the presence of an alkyl substituent at the indolic nitrogen results in a lower oxidation potential (compounds **4** and **15** vs. compound **5**), turning these compounds into better reducing agents. When the nitrogen atom of the side chain is protected with an electron withdrawing group, like phthaloyl, the first oxidation peak disappears and a decrease of the second oxidation potential is observed (compounds **2** vs. **4** and compounds **9** vs. **5**). The same effect is observed for tryptophan derivatives. For these compounds that have no electron withdrawing groups at the side chain, it was observed that alkylation of the indole ring, either on N-1 or C-2 (compound **8** vs. compounds **13** and **14**), did not significantly influence the oxidation potential (figure II.8).

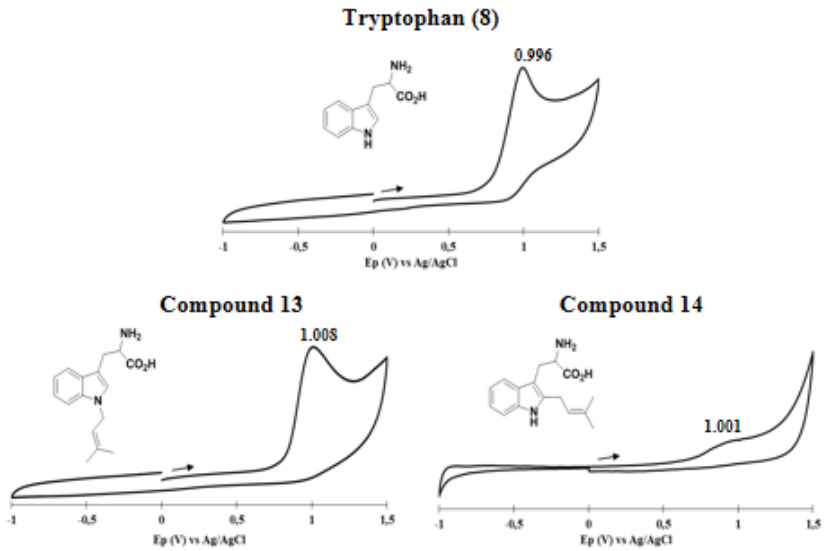


Figure II.8 - Voltammograms of tryptophan (8) and its derivatives (compounds 13 and 14).

However, when both the nitrogen and the carboxylic acid of the side chain are protected [compound **12** and **11** *vs*. tryptophan methyl ester (**10**)] a decrease in the oxidation potential was observed (figure II.9).

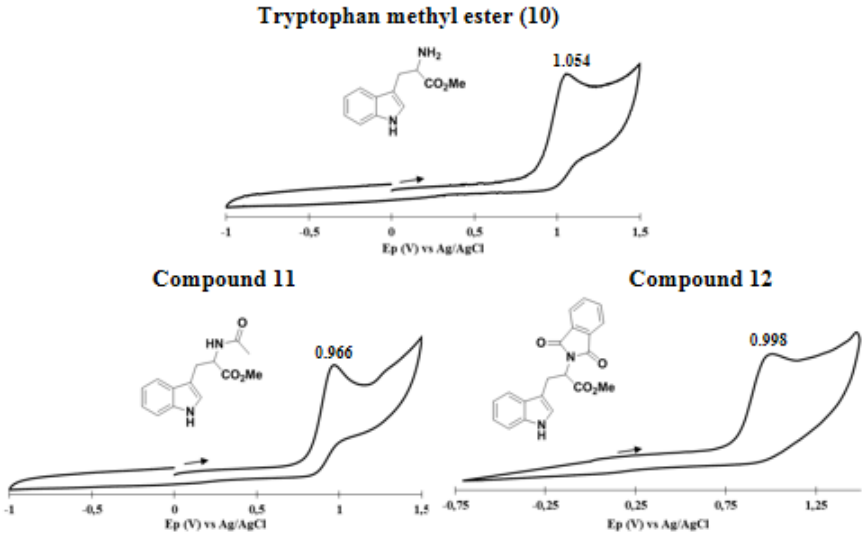


Figure II.9 – Voltammograms of tryptophan methyl ester (10) and its *N*-protected derivatives (compounds 11 and 12).

This effect is more evident when alkylation occurs at C-2 position rather than at N-1 (compound **6** vs. compound **1**). Indeed, the *N*-alkylated and non-alkylated *N*-phthaloyl derivatives (compounds **6** and **12**) showed similar potentials. These results suggest that when the nitrogen at the side chain is protected with an electron withdrawing group the indolic ring becomes the redox center, responsible for

the electron donation of these compounds. Reduction of the double bond at C-2 (compound **18**) led to a potential close to that observed for compounds **6** and **12** (figure II.10).

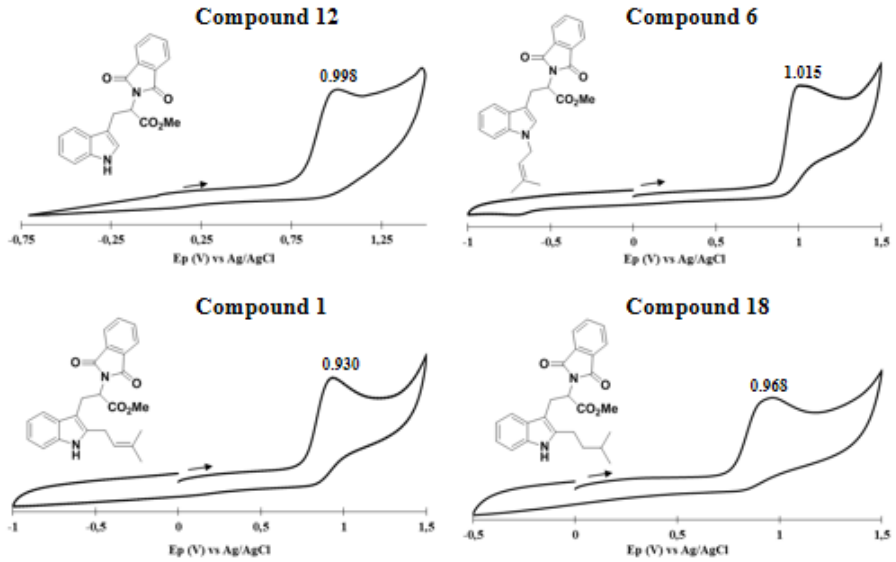


Figure II.10 – *N*-phathaloyl methyl ester tryptophan (12) and its derivatives (compounds 6, 1 and 18).

The only tryptophan derivatives showing two peaks on the voltammograms were compound **3** and the corresponding hydrogenated compound (**16**). The substitution of the alkylic chain from 3,3-dimethylallyl to phenylallyl leads to the increase of the oxidation potential (figure II.11).

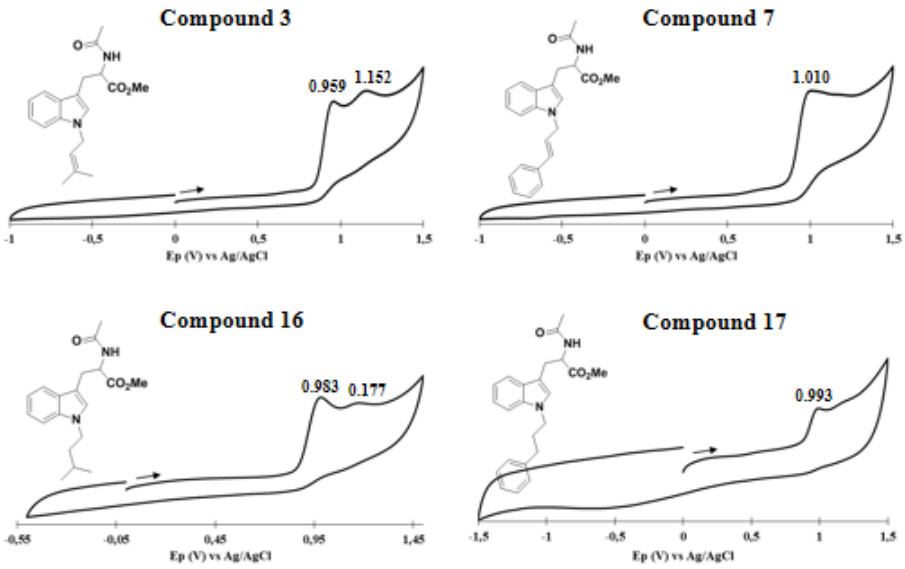


Figure II.11 – *N*-acetyl methyl ester trypthopan derivatives (compounds 3, 7, 16 and 17).

The cyclic voltammograms of the indole library demonstrated that the oxidation potential peak values were close to those of the non-substituted indole nucleus, but higher than those described for the antioxidant melatonin (Ep_{ox} = 0.715 V). In addition, no reversibility was observed in the obtained voltammograms. The lack of reversibility is an advantage, meaning that once oxidized these species do not tend to receive electrons.[31]

II.3 Conclusions

A library of tryptamine and tryptophan derivatives was prepared and evaluated for the scavenging activity against ROS and RNS. The tested indole derivatives share a heteroaromatic ring system, differing among them by the presence of diverse side chains with different functionalization (figure II.12). The results obtained in the present study reveal a strong scavenging effect of tryptophan and tryptamine for the tested ROS and RNS, which activity varies in different extensions, depending on the substituents. Best results of HOCl scavenging were obtained for the tryptophan (**8**) and tryptophan derivative **18** with IC_{50s} of 3.50 ± 0.4 and 3.75 ± 0.52 µM, respectively.

Furthermore, the prenylated compounds **13** and **14** also demonstrated to be potent scavengers with IC_{50} of 4.13 ± 0.17 and 4.56 ± 0.48 µM, respectively. Although the position of the prenyl chain is not relevant for this ROS, these are relevant results since the presence of a prenyl chain turns these compounds more lipophilic. For the $ROO^{\cdot}$ some compounds were more potent than trolox (control). The library was also evaluated against RNS and the best result was obtained for the scavenging of $ONOO^{-}$, in the presence of $NaHCO_3$, by the *N*-alkylated tryptophan (**18**), with an IC_{50} of 14 ± 6.8 µM, which demonstrated that the double bond is not crucial for the reactivity with this species.

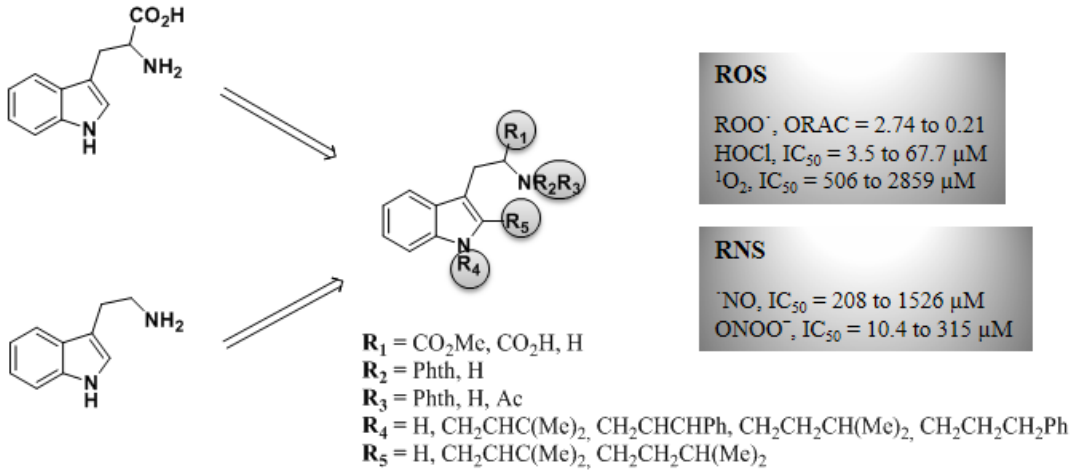


Figure II.12 – Indole-based library tested against ROS and RNS.

Unquestionably, the potent free radical scavenging capacity found for the tested compounds relies mainly on their electron-rich indole moieties, which hold high resonance stability. On the other hand, the substituents at the indole backbone substantially change the reactivity, potency and efficiency of radical scavenging. The effect of the prenyl chain was sometimes favorable and others unfavorable, depending on the reactive species.

The cyclic voltammetry showed that all the compounds have an oxidation potential peak lower than that observed for indole (Ep_{ox} = 1.035 V), except for compound **10** (Ep_{ox} = 1.054 V), but higher that those described for melatonin (Ep_{ox} = 0.715 V). Tryptamine (**9**) and its derivative (**2**) presents two

oxidation peaks, as well as tryptophan derivatives **3** and **16**, meaning that these compounds can undergo two oxidation processes. No reversibility was observed in neither of the evaluated compounds. The lack of reversibility is an advantage, since once oxidized these species do not tend to receive electrons.

The indole derivatives synthesized and tested in the present study were shown to be of reassuring importance for the development of new antioxidant drugs.

II.4 Experimental

II.4.1 General

Melting points were determined on a Reichert Thermovar apparatus and are uncorrected. ^{1}H- and ^{13}C-NMR spectra were recorded in $CDCl_3$ or $DMSO$-d_6 on a Bruker ARX 400 spectrometer at 400 and 100.62 MHz respectively. Chemical shifts are expressed in parts per million (ppm) relative to tetramethylsilane (TMS). The coupling constants (*J*) are reported in Hertz (Hz). High resolution mass spectra were recorded on an AutoSpecQ spectrometer. IR spectra were run on an FT PerkinElmer 683 instrument, with absorption frequencies expressed in reciprocal centimeters. The progress of all reaction was monitored by thin-layer chromatography, which was performed on Merck silica gel 60 F254 plates. Flash column chromatography was carried out on Merck silica gel 60 (230-400 mesh).

A microplate reader (Synergy HT, BIO-TEK), with spectrophotometric, fluorimetric, and chemiluminometric detection, plus temperature control capacity, was used for the ROS and RNS scavenging assays. All the chemicals and reagents were of analytical grade. Dihydrorhodamine 123 (DHR), 4,5-diaminofluorescein (DAF-2), 30% hydrogen peroxide, sodium hypochlorite solution, with 4% available chlorine, diethylenetriaminepentaacetic acid (DTPA), 3-(aminopropyl)-1-hydroxy-3-isopropyl-2-oxo-1-triazene (NOC-5), β-nicotinamide adenine dinucleotide (NADH), phenazine methosulfate (PMS), nitroblue tetrazolium chloride (NBT) and lucigenin were obtained from Sigma-Aldrich. α,α'-Azodiisobutyramidine dihydrochloride (AAPH), histidine and trolox were obtained from Fluka Chemie GmbH. Fluorescein sodium salt and quercetin were obtained from Aldrich.

Electrochemical experiments were conducted in a conventional three-electrode cell under argon atmosphere at 21°C, performed using a computer controlled potentiostat AUTOLAB (Eco-Chemie). The working electrode was a glassy carbon disk (BAS) polished with 0.05 μm alumina (Metkron) before each run, for linear cyclic voltammetry measurements. The auxiliary electrode was a platinum wire and the reference electrode was Ag/AgCl (BAS).

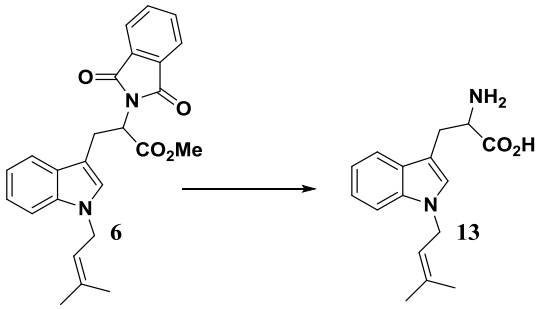
Compounds **1-7**, **11** and **12** were synthesized according to procedures described in the previous work,[35] except compounds **8**, **9** and **10** that were purchased from Sigma-Aldrich.

II.4.2 General procedure for the deprotection of amino and carboxylic acid groups

To a solution of N^b-phthaloyl-tryptophan methyl ester derivative, in 3:1 MeOH/DCM was added hydrazine hydrate (3.5 equiv.). The flask was capped and the solution stirred at room temperature for 24 h, during which time a white precipitate formed. The solution was poured into water (73 mL) and extracted with DCM (4 x 15 mL). The combined organic extracts were dried over Na_2SO_4, filtered and concentrated. The resulting crude was purified by flash column chromatography (DCM/MeOH 10:1).[43]

The product previously prepared was dissolved in 1 M NaOH aqueous solution (1 equiv.) and MeOH (0.6 M). The resulting mixture was stirred at room temperature overnight. The mixture was concentrated and acidified with 1 M HCl to pH 7-8 at 0ºC. The precipitate which formed was filtrated, washed with cold water and dried.[44]

*II.4.2.1 Synthesis of N-(3,3-dimethylallyl)-tryptophan (**13**)*



Compound **13** was isolated as light beige solid in 73% yield.

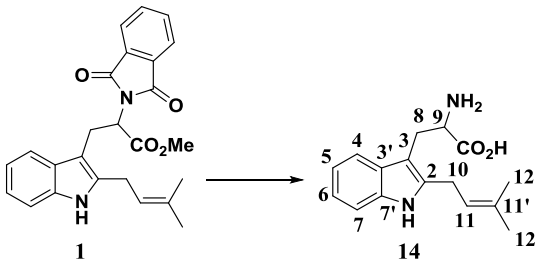
m.p. 203-204ºC (MeOH) [lit. 201-202ºC (MeOH)];[45]

^{1}H NMR (400 MHz, *DMSO-d_6*) δ 7.58 (d, *J* = 7.5 Hz, 1H), 7.35 (d, *J* = 8.0 Hz, 1H), 7.18 (s, 1H), 7.11 (t, *J* = 8.0 Hz, 1H), 7.00 (t, *J* = 7.5 Hz, 1H), 5.36-5.26 (m, 1H), 4.69 (d, *J* = 6.1 Hz, 2H), 3.30 (2H, *under the water peak*), 2.99-2.88 (m, 1H), 1.81 (s, 3H), 1.71 (s, 3H);

IR (KBr) 3448, 1720 cm^{-1};

Spectral data were in accordance with the literature.[45]

*II.4.2.2 Synthesis of 2-(3,3-dimethylallyl)-tryptophan (**14**)*



Compound **14** was isolated as light beige solid in 87% yield.

m.p. > 300ºC (decomp.);

^{1}H NMR (400 MHz, *DMSO-d_6*) δ 10.72 (s, 1H; NH), 7.50 (d, *J* = 7.6 Hz, 1H, H4), 7.25 (d, *J* = 8.0 Hz, 1H, H7), 6.98-6.88 (m, 2H, H5 and H6), 5.29 (m, 1H, H11), 4.03-3.98 (m, 1H, H9), 3.30 (2H, *under water peak*), 2.89-2.83 (m, 2H, H8), 1.70 (s, 3H, H12), 1.66 (s, 3H, H12);

^{13}C NMR (101 MHz, *DMSO-d_6*) δ 170.8 (C=O), 136.8 (C2), 135.5 (C7'), 131.7 (C11'), 128.1 (C3'), 121.7 (C6), 120.0 (C11), 118.1 (C5), 117.7 (C4), 110.6 (C7), 105.0 (C3), 55.6 (C9), 26.5 (C8), 25.5 (C10), 24.7 (C12), 17.8 (C12);

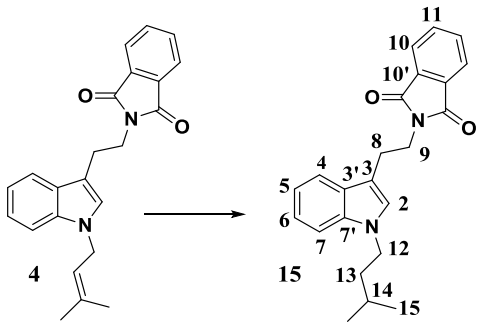
IR (KBr) 1748 cm^{-1};

HRMS (ESI-TOF) m/z 272.1528 $[M+H]^+$ (calcd for $C_{16}H_{20}N_2O_2$ 272.1525)

II.4.3 General procedure for hydrogenation

To a solution of tryptophan or tryptamine derivatives in MeOH (55 mM) was added 10% Pd/C (ca. 61 mg per mmol). The mixture was stirred at room temperature under H_2 atmosphere. After starting material consumption, the reaction mixture was filtered and concentrated under reduce pressure.

*II.4.3.1 Synthesis of N^a-(isopentyl)-N^b-phthaloyl-tryptamine (**15**)*



Purified by flash chromatography (*n*-hexane/Et_2O, 3:2) affording compound **15** with 88% yield as yellow oil.

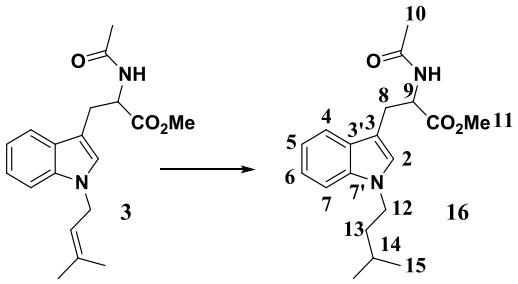
^{1}H-NMR (400 MHz, *$CDCl_3$*) δ 7.84-7.77 (m, 2H, H10), 7.72 (d, *J* = 7.5 Hz, 1H, H4), 7.69-7.64 (m, 2H, H11), 7.28 (d, *J* = 7.8 Hz, 1H, H7), 7.19 (t, *J* = 7.8 Hz, 1H, H6), 7.10 (t, *J* = 7.5 Hz, 1H, H5), 6.98 (s, 1H, H2), 4.05 (t, *J* = 7.3 Hz, 2H, H12), 3.98 (t, *J* = 7.8 Hz, 2H, H9), 3.13 (t, *J* = 7.8 Hz, 2H, H8), 1.69-1.63 (m, 2H, H13), 1.58-1.49 (m, 1H, H14), 0.92 (d, *J* = 6.6 Hz, 6H, H15);

^{13}C-NMR (101 MHz, *$CDCl_3$*) δ 168.3 (C=O), 136.2 (C7'), 133.8 (C11), 132.2 (C10'), 127.8 (C3'), 125.6 (C2), 123.1 (C10), 121.5 (C6), 119.1 (C4), 118.8 (C5), 110.7 (C3), 109.3 (C7), 44.3 (C12), 39.0 (C13), 38.6 (C9), 25.6 (C14), 24.4 (C8), 22.4 (C15);

IR (NaCl) 2955, 1770, 1714, 1396 cm^{-1};

HRMS (EI) m/z 360.1836 $[M]^+$ (calcd for $C_{23}H_{24}N_2O_2$ 360.1838).

*II.4.3.2 Synthesis of N^a-(isopentyl)-N^b-acetyl-tryptophan methyl ester (**16**)*



Purified by flash chromatography (Et_2O) affording compound **16** with 85% yield as a colorless oil.

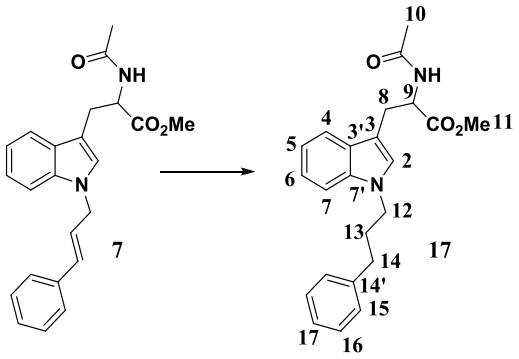
^{1}H-NMR (400 MHz, *$CDCl_3$*) δ 7.52 (d, *J* = 7.9 Hz, 1H, H4), 7.31 (d, *J* = 8.2 Hz, 1H, H7), 7.20 (t, *J* = 7.5 Hz, 1H, H6), 7.10 (t, *J* = 7.4 Hz, 1H, H5), 6.88 (s, 1H, H2), 6.15 (d, *J* = 7.5 Hz, 1H, NH), 4.96-4.91 (m, 1H, H9)), 4.08 (t, *J* = 7.3 Hz, 2H, H12), 3.69 (s, 3H, H11), 3.37-3.23 (m, 2H, H8), 1.95 (s, 3H, H10), 1.72-1.67 (m, 2H, H13), 1.61-1.51 (m, 1H, 14), 0.96 (d, *J* = 6.6 Hz, 6H, C15);

^{13}C-NMR (101 MHz, *$CDCl_3$*) δ 172.4 (C=O ester), 169.6 (C=O acetyl), 136.1 (C7'), 128.3 (C3'), 126.2 (C2), 121.6 (C6), 119.1 (C5), 118.7 (C4), 109.5 (C7), 108.4 (C3), 53.1 (C9), 52.2 (C11), 44.4 (C12), 38.9 (C13), 27.5 (C8), 25.6 (C14), 23.2 (C10), 22.4 (C15);

IR (NaCl) 3286, 2955, 1745, 1656, 1469 cm^{-1};

HRMS (EI) m/z 330.1941 $[M]^+$ (calcd for $C_{19}H_{26}N_2O_3$ 330.1943).

*II.4.3.3 Synthesis of N^a-(3-phenylpropyl)-N^b-acetyl-tryptophan methyl ester (**17**)*



Purified by flash chromatography (Et_2O) affording compound **17** as a pale yellow oil with 88% yield.

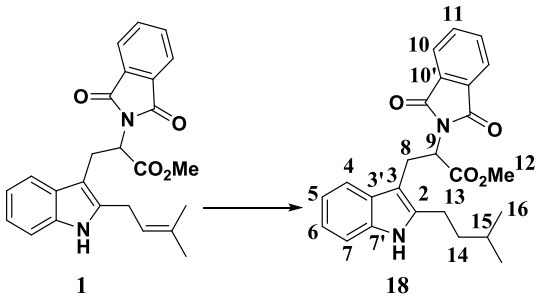
^{1}H-NMR (400 MHz, *$CDCl_3$*) δ 7.52 (d, *J* = 7.8 Hz, 1H, H4), 7.32-7.09 (m, 8H, H5, H6, H7, H15, H16 and H17), 6.87 (s, 1H, H2), 5.99 (d, *J* = 7.3 Hz, 1H, N*H*), 4.98-4.93 (m, 1H, H9), 4.10 (t, *J* = 6.9 Hz, 2H, H12), 3.70 (s, 3H, H11), 3.37-3.26 (m, 2H, H8), 2.61 (t, *J* = 7.5 Hz, 2H, H14), 2.19-2.12 (m, 2H, H13), 1.96 (s, 3H, H10);

^{13}C-NMR (101 MHz, *$CDCl_3$*) δ 172.4 (C=O ester), 169.6 (acetyl), 140.8 (C14'), 136.2 (C7'), 128.5 (C16), 128.4 (C3'), 128.3 (C15), 126.3 (C2), 126.1 (C17), 121.7 (C6), 119.2 (C5), 118.8 (C4), 109.5 (C7), 108.6 (C3), 53.2 (C9), 52.3 (C11), 45.5 (C12), 32.9 (C14), 31.5 (C13), 27.6 (C8), 23.2 (C10);

IR (NaCl) 3392, 2926, 1744, 1656, 1469 cm^{-1};

HRMS (EI) m/z 378.1948 $[M]^+$ (calcd for $C_{23}H_{26}N_2O_3$ 378.1943).

*II.4.3.4 Synthesis of 2-(isopentyl)-N^b-phthaloyl-tryptophan methyl ester (**18**)*



Purified by column chromatography (*n*-hexane/Et_2O, 1:1) affording compound **18** with 83% yield as yellow solid.

m.p. 51-53ºC;

^{1}H-NMR (400 MHz, *$CDCl_3$*) δ 7.75-7.72 (m, 3H, NH and H10), 7.65-7.63 (m, 2H, H11), 7.48 (d, *J* = 7.6 Hz, 1H, H4), 7.16 (d, *J* = 7.8 Hz, 1H, H7), 7.03-6.94 (m, 2H, H5 and H6), 5.21 (dd, *J* = 5.5,

10.0 Hz, 1H, H9), 3.79 (s, 3H, H12), 3.68-3.64 (m, 2H, H8), 2.74-2.57 (m, 2H, H13), 1.56-1.44 (m, 1H, H15), 1.38-1.30 (m, 2H, H14), 0.90 (d, *J* = 6.4 Hz, 3H, H16), 0.87 (d, *J* = 6.4 Hz, 3H, H16);

^{13}C-NMR (101 MHz, *$CDCl_3$*) δ 169.7 (C=O ester), 167.4 (C=O phth), 137.3 (C7'), 135.1 (C2), 133.9 (C11), 131.7 (C10'), 128.5 (C3'), 123.3 (C10), 121.0 (C6), 119.3 (C5), 117.8 (C4), 110.2 (C7), 106.0 (C3), 52.7 (C9), 52.5 (C12), 38.6 (C14), 27.9 (C13), 24.0 (C8), 23.8 (C13), 22.3 (C16);

IR (NaCl) 3401, 2955, 1715 cm^{-1};

HRMS (EI) m/z 418.1894 $[M]^+$ (calcd for $C_{25}H_{26}N_2O_4$ 418.1893).

II.4.4 ROS and RNS scavenging assays

II.4.4.1 Peroxyl radical scavenging assay

The ROO˙ scavenging activity was measured by monitoring the effect of the tested compounds on the fluorescence decay resulting from ROO˙-induced oxidation of fluorescein and expressed as the "Oxygen Radical Absorbance Capacity" (ORAC), as previously described.[46] ROO˙ was generated by thermodecomposition of AAPH. Reaction mixtures in the sample wells contained the following reagents at the indicated final concentrations (in a final volume of 200 µL): fluorescein (61 nM), the tested compounds (0.25-2.00 µM), dissolved in acetone and subsequently diluted in 75 mM phosphate buffer, pH 7.4, and AAPH (19 mM). The mixture was preincubated in the microplate reader for 15 min at 37ºC. The fluorescence signal was then monitored every minute at the emission wavelength 528 ± 20 nm with excitation at 485 ± 20 nm until the total decay of fluorescence. Trolox (0.25-2.00 µM) was used as a control standard in each assay. Each study corresponds to four experiments, performed in triplicate.

ORAC values were calculated according to a previous paper.[47] The net protection provided by a putative antioxidant sample was calculated using the difference between the area under the fluorescence decay curve in the presence of the sample (AUC_{sample}) and in its absence (AUC_{blank}). Regression equations between net AUC and the concentration of the sample were calculated for all the compounds. ORAC values were calculated by using the standard curve of each assay. Final results were expressed in micromole of Trolox equivalents/µmol of compound.

II.4.4.2 Hypochlorous acid scavenging assay

The HOCl scavenging activity was measured by monitoring the effect of the tested compounds on HOCl-induced oxidation of DHR to rhodamine 123, as previously described.[46] HOCl was prepared by adjusting the pH of a 1% solution of NaOCl to 6.2 with dropwise addition of 10% H_2SO_4. Lipoic acid was used as positive control. The inhibition (in percentage) of HOCl-induced oxidation of DHR by each compound was calculated. The results were expressed by the IC_{50} values. Each study corresponds to four experiments, performed in triplicate.

II.4.4.3 Singlet oxygen scavenging assay

The $^1O_2$ scavenging activity was measured by monitoring the effect of the tested compounds on the oxidation of non-fluorescent DHR to fluorescent rhodamine 123 by this ROS, as previously described.[46] Ascorbic acid was used as positive control. The inhibition (in percentage) of $^1O_2$-induced oxidation of DHR by each compound was calculated. The results were expressed by the IC_{50} values. Each study corresponds to four experiments, performed in triplicate.

II.4.4.4 Superoxide radical scavenging assay

The $O_2^{\cdot-}$ was generated by the NADH/PMS system and the $O_2^{\cdot-}$ scavenging activity was determined by monitoring the effect of the tested compounds on the $O_2^{\cdot-}$-induced reduction of NBT at 560 nm as previously described.[46] The antioxidant tiron was used as positive control. The inhibition (in percentage) of the NBT reduction to diformazan by each compound was calculated. The results were expressed by the IC_{50} values. Each study corresponds to four experiments, performed in triplicate. None of the tested compounds was able to scavenge this ROS.

II.4.4.5 Hydrogen peroxide scavenging assay

The H_2O_2 scavenging activity was measured by monitoring the effect of the tested compounds on the H_2O_2-induced oxidation of lucigenin as previously described.[46] The antioxidant ascorbic acid was used as positive control. The inhibition (in percentage) of the H_2O_2-induced oxidation of lucigenin by each compound was calculated. The results were expressed by the IC_{50} values. Each study corresponds to four experiments, performed in triplicate. Only a residual activity was found for some of the compounds in the highest tested concentration (2 mM).

II.4.4.6 Nitric oxide scavenging assay

The $^{\cdot}NO$ scavenging activity was measured by monitoring the effect of the tested compounds on $^{\cdot}NO$-induced oxidation of non-fluorescent DAF-2 to the fluorescent triazolo fluorescein (DAF-2T), as previously described.[46] Rutin was used as positive control. The inhibition (in percentage) of $^{\cdot}NO$ induced oxidation of DAF-2 by each compound was calculated. The results were expressed by the IC_{50} values. Each study corresponds to four experiments, performed in triplicate.

II.4.4.7 Peroxynitrite scavenging assay

The $ONOO^-$ scavenging activity was measured by monitoring the effect of the tested compounds on $ONOO^-$-induced oxidation of non-fluorescent DHR to fluorescent rhodamine 123, as previously described.[46] Ebselen was used as positive control. In a parallel set of experiments, the assays were performed in the presence of 25 mM $NaHCO_3$ in order to simulate the physiological CO_2 concentrations. The inhibition (in percentage) of $ONOO^-$-induced oxidation of DHR by each compound was calculated. The results were expressed by the IC_{50} values. Each study corresponds to four experiments, performed in triplicate.

II.4.5 Cyclic voltammetry

Tryptophan and tryptamine derivatives were dissolved in acetone and further diluted in a supporting electrolyte, consisting of a $LiClO_4$, reaching a final concentration of 1 mM. Deoxygenation of the solutions at the cell was achieved by bubbling argon for at least 5 min.

Cyclic voltammograms were obtained by a single cycle performed at a scan rate of 100 mV $s^{-}1$. For the scan rate studies, the scan rate was varied from 10 to 150 mV $s^{-}1$. Voltammetric scans were carried out in the potential interval of -0.1 to +1.5 V versus Ag/AgCl.

II.5 References

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Chapter III New indole derivatives as cyclooxygenase inhibitors

My contribution for this work was the preparation of all synthetic compounds.

III.1 Introduction

III.1.1 Cyclooxygenase and non-steroidal anti-inflammatory drugs

Inflammation is the immune system's response to infection and injury and has been implicated in the pathogeneses of arthritis, cancer, and stroke, as well as in neurodegenerative and cardiovascular diseases.[1] Inflammation is an intrinsically beneficial event that leads to removal of pernicious factors and renewal of tissue structure and physiological function. It is characterized by five signs: pain, redness, immobility (loss of function), swelling and heat. These symptoms are caused by the rapid influx of blood granulocytes, typically neutrophils, followed by monocytes, which mature into inflammatory macrophages that subsequently proliferate and thereby affect the functions of resident tissue macrophages.[1]

Prostaglandins (PGs) play a key role in the generation of the inflammatory response. Their biosynthesis is significantly increased in inflamed tissue, and they contribute to the development of the cardinal signs of acute inflammation.[2] The initial step in biosynthesis of prostanoids (subclass of eicosanoids consisting in PGs and thromboxane A_2 (TXA_2)) is the liberation of arachidonic acid (AA), a 20-carbon unsaturated fatty acid, from the phospholipids of the cell membrane catalyzed by phospholipase A_2. The subsequent and key step is the biotransformation of AA by cyclooxygenase (COX), also known as prostangladin endoperoxide synthase. AA is first oxidized to the unstable PGG_2, at the COX active site, that diffuses to the peroxidase (POX) active site in which is reduced to PGH_2 (figure III.1).[3,4]

There are four principal bioactive PGs generated in vivo: prostaglandin E_2 (PGE_2), prostacyclin (PGI_2), prostaglandin D_2 (PGD_2), and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). They are ubiquitously produced and act as autocrine and paracrine lipid mediators to maintain local homeostasis in the body.[5] The presence of the different prostaglandin synthases varies from tissue to tissue. For example, lung and spleen tissues are able to synthesize the whole range unlike other tissues. Platelets synthesized mainly TXA_2, while blood vessel walls primarily produce PGI_2. PGE_2 is an important mediator in the kidney and in inflammation, whereas PGD_2 is produce in mast cells and in the brain.[6]

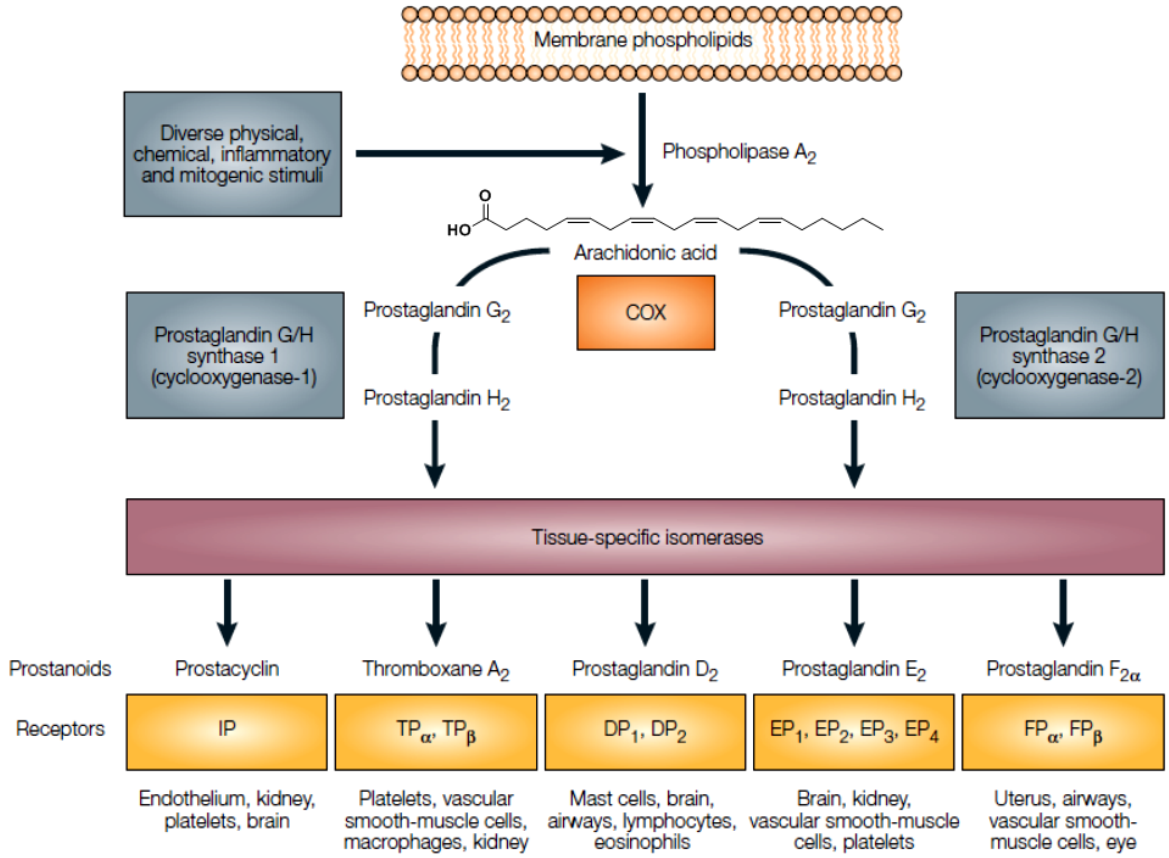


Figure III.1 – Prostaglandin biosynthetic cascade (adapted from FitzGerald 2003).[7]

In 1971, Vane demonstrated that the mechanism of action of aspirin and related non-steroidal anti-inflammatory drugs (NSAIDs) is through COX blockade, hence inhibition of prostanoid synthesis.[8] After these findings, a new course has been given to the field of inflammation research with the discovery that two isoforms of COX, a constitutive form (COX-1) and an inducible form (COX-2), exist.[4] The constitutive isoform, COX-1, is expressed in nearly all tissues including the kidney, spleen, stomach, liver, lung, heart and brain, and has clear physiological functions. For example, in the kidney and in the stomach, prostanoids synthesized by COX-1 acts as vasodilators. COX-1 performs the "housekeeping" function by synthesizing prostaglandins that regulate normal cell activity. Its activity leads, for instance, to the production of PGI_2 which, when released by the endothelium, is anti-thrombogenic[9] and when released by the gastric mucosa, it is cytoprotective.[10] Additionally, COX-1 in platelets leads to TXA_2 production, causing aggregation of the platelets to prevent inappropriate bleeding.[11] Contrariwise, COX-2 is induced by inflammatory stimuli, hormones, and growth factors, and is the most important source of prostanoid formation in inflammation and in proliferative diseases, such as cancer.[12] Up-regulation of COX-2 expression has also been implicated with a number of other pathophysiological conditions such as schizophrenia and major depression,[13] ischemic brain injury,[14] and diabetic peripheral nephropathy.[15] COX-2 has been identified as therapeutic target by researchers

to intervene with several disease conditions, demonstrating that the future of selective COX-2 inhibitors is not limited to management of inflammation and pain.

COX-1 and COX-2 are isoenzymes, therefore they are genetically independent proteins. The genes in humans for the two enzymes are located on different chromosomes (chromosome 9 and 1, for COX-1 and COX-2, respectively) and show different properties.[16,17] Both isoforms are dimeric and each monomer has a molecular weight of 71 kDa. COX-1 and COX-2 are almost identical in length, with just over 600 amino acids, of which 63% are in identical sequence.[18] Twenty-four residues line the COXs active site with only one difference between both isoforms, an isoleucine (Ile) at position 523 in COX-1 and a valine (Val) at the same position in COX-2. This difference has consequences for the size and shape of NSAID binding site. The COX-2 structure revealed a second internal pocket extending off the binding site. In this isoform, the volume of primary inhibitor binding site and the side pocket is calculated to be 394Å, whereas the volume of NSAID binding site of COX-1 is 316Å. The side pocket contributes significantly to the larger volume of this site in COX-2, although the central channel is also larger, 371Å, a 17% increase compared to COX-1 (figure III.2).[19]

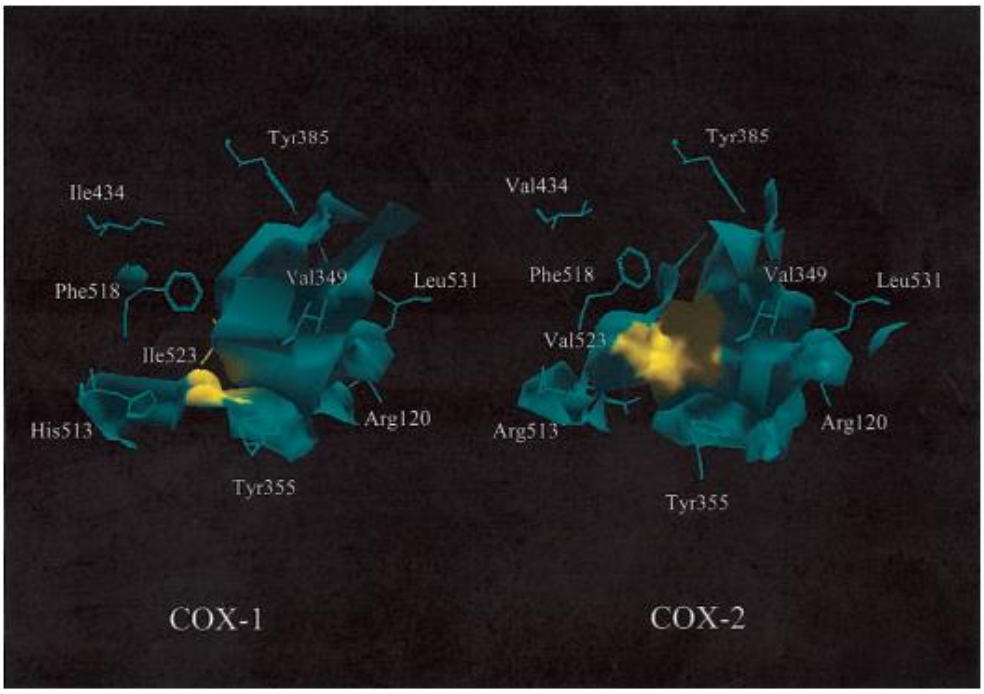


Figure III.2 – Contour of COX-1 and COX-2 cyclooxygenase active sites. The solvent-accessible surfaces of the COX-1 and COX-2 active sites, with important surrounding amino acid residues, are shown. Highlighted in yellow on the left is the effect of Ile523 on COX-1 and the effect of Val523 in COX-2, on the right side.[2]

The extensive structural analysis of the two COX isoforms allowed the design of drugs that reduce inflammation without removing the protective PGs in the stomach and kidney produced by COX-1.

Kurumbail *et al.*[20] established a classification of four classes of COX inhibitors, based on their interaction with the protein: *i)* irreversible inhibitors of COX-1 or COX-2, such as aspirin. Aspirin acetylates irreversibly the enzyme, blocking its activity; *ii)* reversible, competitive inhibitors of COX-1 and COX-2, such as indomethacin, naproxen, ibuprofen, diclofenac. These drugs compete with AA for binding in the COX active site; *iii)* slow time-dependent, reversible inhibitors of COX-1 and COX-2, such as indomethacin and flurbiprofen. These seem to act by ionic interactions between their acid carboxylic group and the arginine residue of the enzyme; *iv)* slow, time-dependent irreversible inhibitors of COX-2. This group includes the coxibs (selective inhibitors of COX-2), such as, celecoxib, rofecoxib, valdecoxib, etoricoxib and the coxib-like structure SC-558 (figure III.3).[3,21]

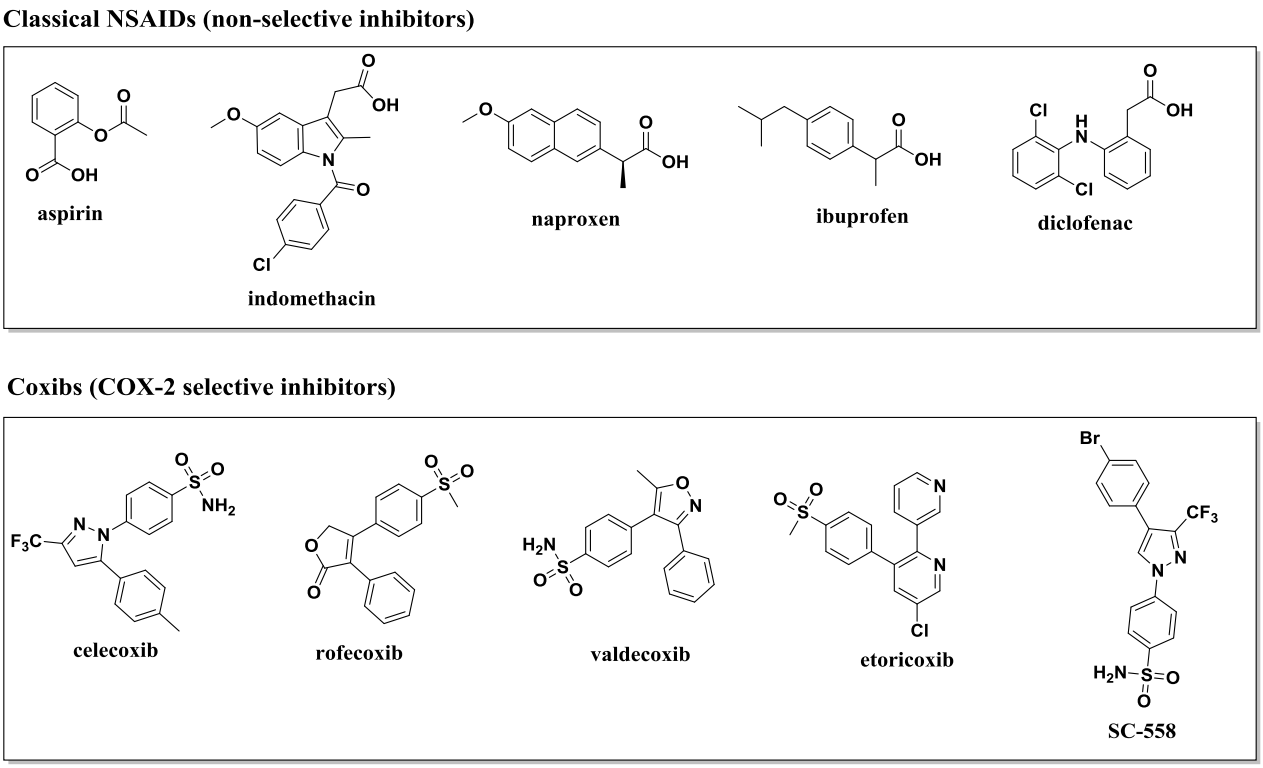


Figure III.3 - Chemical structures of some classical NSAIDs and coxibs.

For a long time the presence of a carboxyl group was thought to be a pre-requisite for the classical NSAIDs. Today it is known that this functional group accounts for the formation of a salt bridge between the carboxylic group of the drug and Arg120 at the bottom of the active site of the enzyme thus, generating COX-1 inhibition.[22] Therefore, in place of the carboxyl group of the classical NSAIDs, the structure of the COX-2 selective inhibitors contain a sulfonamide group (celecoxib, valdecoxib, SC-558) or a methylsulfone (rofecoxib, etoricoxib). The sulfur-containing substituent in the phenyl rings of these drugs binds into the side pocket of the catalytic site of COX-2 but interact weakly

with the active site of COX-1.[20] Thus, they are potent inhibitors of COX-2 and weak inhibitors of COX-1.

Figure III.4 depicts a schematic representation of SC-558 (A) and indomethacin (B) binding to COX-2.

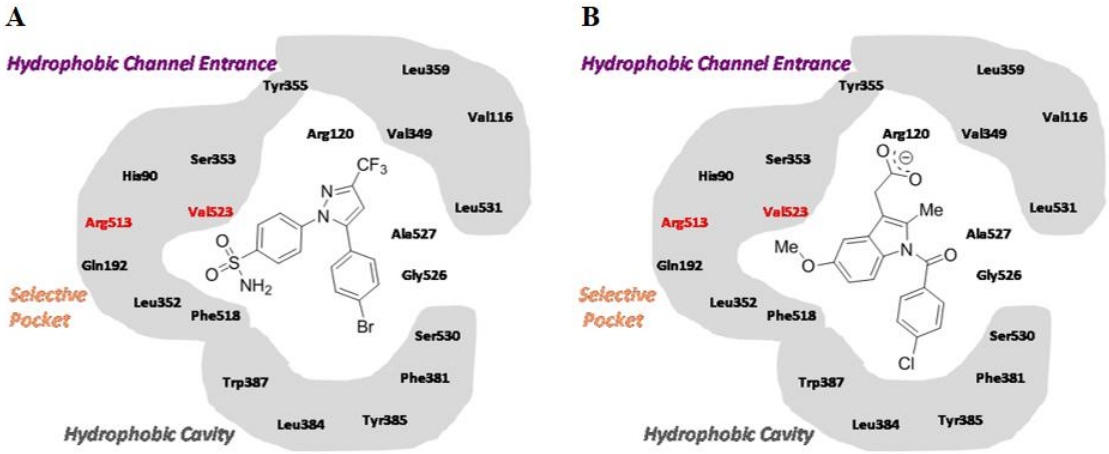


Figure III.4 - Schematic representation of SC-558 (A) and indomethacin (B) binding to COX-2.[23]

Administration of NSAIDs, for example to treat inflammatory diseases such as osteoarthritis or rheumatoid arthritis, unavoidably leads to a lack of the prostaglandins required for the physiological functions. Therapeutic effects and side-effects of this class of anti-inflammatory drugs are closely related to their biochemical mechanism of action. Long-term NSAIDs users suffer from a high incidence of gastrointestinal tract irritation that may lead to ulceration and bleeding.[24] Due to a reduced production of PGs, such as PGI2, PGE2 and PGD2, in the regulation of renal blood circulation, the rate of glomeruleric filtration is reduced. In patients with reduced renal function, this leads to retention of water, hypertension and, in some cases, to renal failure.[25,26]

Although coxib compounds benefit from the lack of gastrointestinal toxicity, this risk/benefit balance was recently considered negative as a result of the increased incidence of cardiovascular events. Consequently, rofecoxib and valdecoxib were removed from the market. The mechanism underlying the adverse cardiovascular effects is due to an imbalance between COX-1 thrombotic TXA_2 in platelets and COX-2 derived vasoprotective PGI_2 in endothelium.[27]

Therefore, the discovery of new COX-2 selective inhibitors, without cardiac effects, are crucial in the future of anti-inflammatory therapies.

For the development of a new class of inhibitors the choice of a scaffold and of its substitution pattern are of almost importance. When considering the vast amount of work published concerning specific COX-2 inhibitors, several heterocyclic scaffolds have been investigated, such as pyrrole,[28] imidazole,[29] thiazolidine,[30] pyrazole (celecoxib analogs)[31] and indole (indomethacin analogs)[32] (figure III.5).

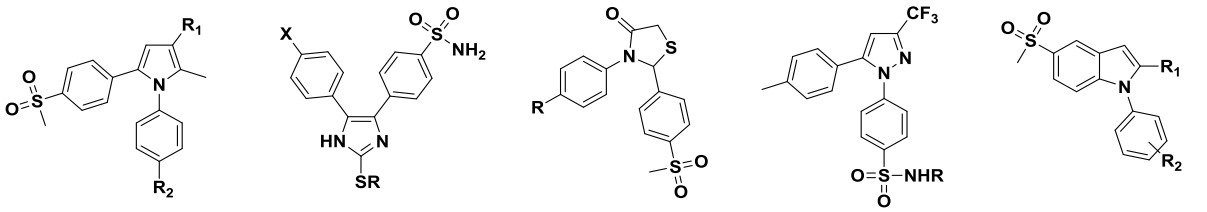


Figure III.5 – Chemical structures of several heterocyclic scaffolds investigated as selective COX-2 inhibitors.

From these, the indole ring is considered a privileged structure and an attractive scaffold for drug discovery. Structural diversity can easily be achieved *via* ring substitution and consequently diverse biological activities are associated with indole-derived drug-like molecules, including COX-1 and COX-2 inhibition.

Thus, further investigation should be carried on indole-based compounds as COX-2 selective inhibitors. One of the aims of this thesis was the development of novel and selective COX-2 inhibitors and indole was selected as scaffold for that purpose.

III.1.2 Fluorine in medicinal chemistry

Organic fluorinated compounds have received great attention in all fields of science. Currently approximately 30% of all agrochemicals and 20% of pharmaceuticals contain fluorine. Incorporation of fluorine has also been applied in material sciences such as the useful polymer polytetrafluoroethylene (Teflon®) that is perfluorinated and exemplifies the strength of the C-F bond.[33]

Flourine is a small atom (van der Waals radius of 1.47Å) and the most highly electronegative element in the Periodic Table (3.98 Pauling scale). Covalently bound fluorine occupies a smaller volume than a methyl, amino, or hydroxyl group, but is larger than a hydrogen atom (van der Waals radius of 1.2Å).

Traditional medicinal chemistry was based on the use of natural compounds or closely related derivatives, where there is a low abundance of compounds containing fluorine, thus fluorinated compounds were rare in medicinal chemistry until the 1970s.[34] Currently, many fluorinated compounds are synthesized routinely in pharmaceutical research and are widely used in treatment of diseases, such as anti-cancer agents,[35] antidepressants, anti-inflammatory agents, anesthetics and central nervous system drugs.[36] Figure III.6 shows some representative examples of drugs containing fluorine.

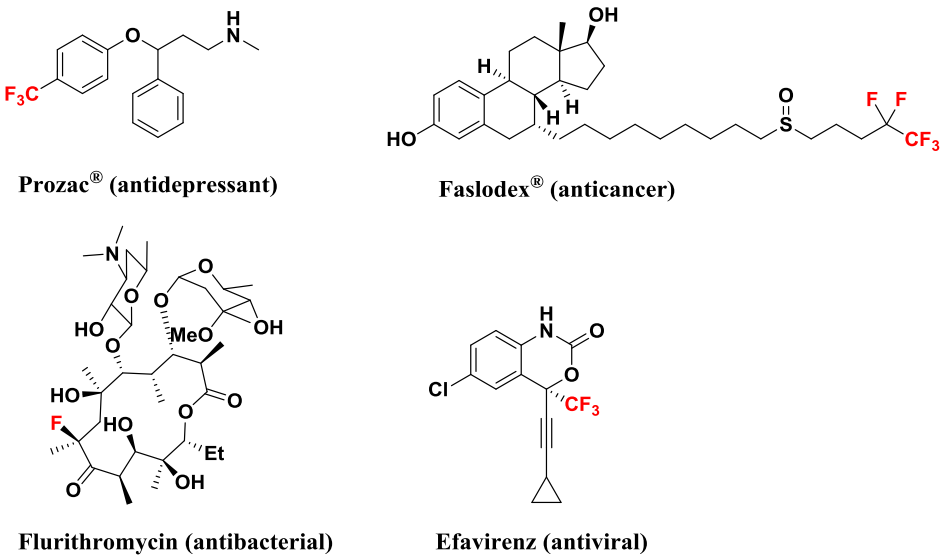


Figure III.6 – Some marketed drugs containing fluorine.

The fast progress in this field is promoted by the development of new fluorinated reagents and fluorination processes increasing the range of synthetic fluorinated building blocks.[37]

Despite the fact that fluorine is larger than hydrogen, several studies have demonstrated that it is a reasonable hydrogen mimic and is expected to cause minimal steric perturbations with respect to the mode of binding of the compound to a receptor or enzyme.[38]

Introduction of fluorine atoms into molecules improves metabolic stability, bioavailability and protein-ligand interactions.[39] One example of the importance of fluorine in determining metabolic stability is demonstrated in the development of the COX-2 selective inhibitor, celecoxib. Penning *et al.*[40] observed that a substitution of the fluorine on the benzene ring for a methyl group reduced the plasma half-life in rat from 221 to 3.5 hours (figure III.7).

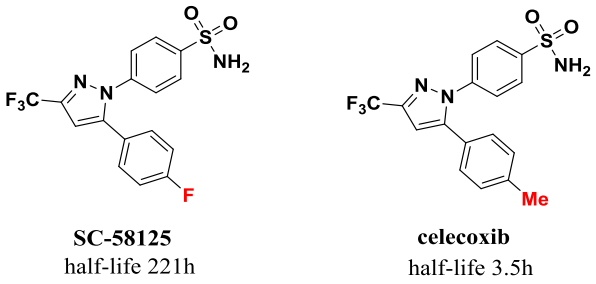


Figure III.7 – Chemical structures of SC-58125 and celecoxib.

Taking in consideration the fact that the selective COX-2 inhibitor (celecoxib) possess a CF_3 group and due to the important role of fluorine in drug discovery, it was envisaged to include a trifluoromethylated chain at the nitrogen atom ring.

III.1.3 The sulfonyl group in medicinal chemistry and synthetic approaches towards sulfonylation

Compounds containing the sulfonyl group, which mainly include sulfones and sulfonamides, have been studied for decades due to their significant role in developing therapeutics for a number of diseases.

Since the introduction in the 1930s of the so-called "sulfa-drugs" such as sulfanilamide and Prontosil (figure III.8), sulfonyl-containing compounds have found widespread use in the pharmaceutical industry and the motif is still considered valuable and safe for drug development.[41]

Sulfonyl group has been used as a biologically active moiety in the development of drugs for the treatment of bacterial infections,[42] in the design of inhibitors of carbonic anhydrases[43] (that are useful as diuretics, or in the treatment and prevention of a variety of diseases such as glaucoma, epilepsy, congestive heart failure, mountain sickness, gastric and duodenal ulcers, neurological disorders, and osteoporosis, among others),[44] and inhibitors of protein phosphatase methylesterase-1, implicated in a wide range of pathologic processes, including tumerigenicity.[45] Recent studies also suggested that this class of compounds represents promising antitumor agents.[46,47] Further, some studies demonstrated that sulfonyl is a key functional group for the various ligands binding at human 5-HT6 serotonin receptors, which could be involved in central neural systems pathologies related to this receptor, for example, schizophrenia, convulsive disorders, memory, cognition, sensory processing, circadian rhythm, brain development, and cardiovascular function.[48,49] Moreover, sulfonyl-containing compounds have been found in the design of numerous anti-Alzheimer[50] and anti-HIV[51,52] agents.

Figure III.8 depicts some available marketed drugs containing the sulfonyl group.

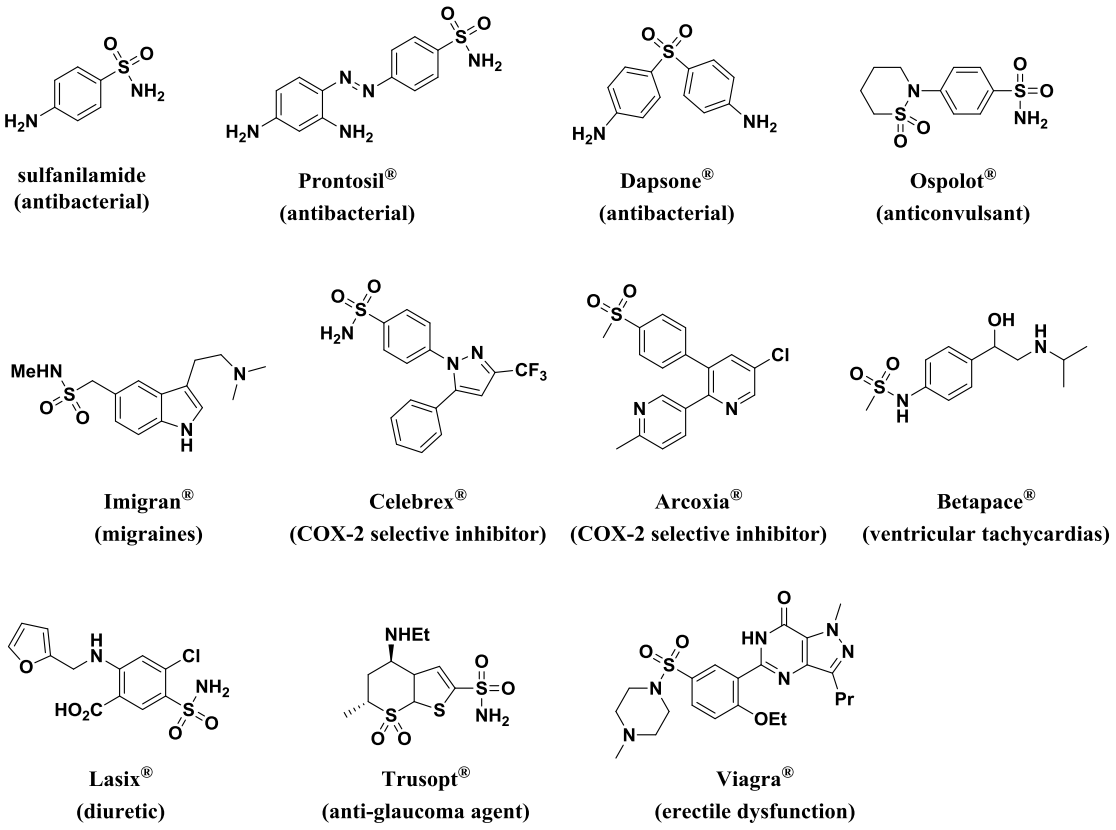


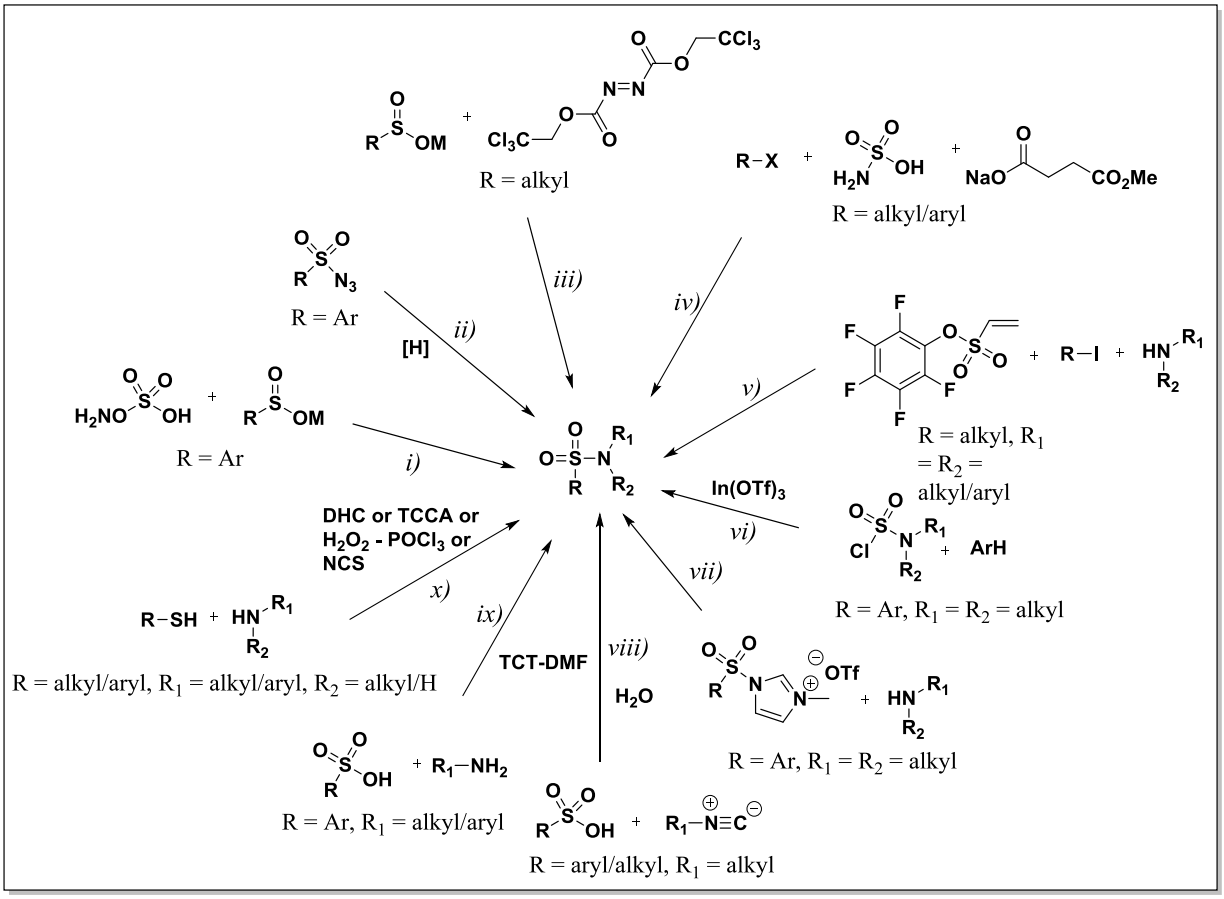
Figure III.8 - Some marketed drugs containing the sulfonyl group.

Despite the great importance of sulfonyl-containing compounds, a general and versatile method for their preparation does not exist.

The most common route for the synthesis of sulfonamides involves the reaction of sulfonyl chlorides with ammonia, followed by reaction with primary or secondary amines. This approach requires the availability of the sulfonyl chloride, some of which can be hard to prepare and difficult to store or handle. The frequently used aryl sulfonyl chlorides can be prepared *via* electrophilic aromatic substitution using $[HSO_3]^+$ or $[ClSO_2]^+$ synthons.[53] However, this is generally limited to electron-rich aromatic substrates and those that can tolerate the harsh acidic conditions. Side reactions are also possible due to the presence of the base or the liberated chlorine nucleophile. Moreover, the corresponding disulfonimide is stated to be a byproduct in reactions of sulfonyl chlorides with primary amines or ammonia.[54] Sulfuryl chlorides can also be prepared by oxidative chlorination of sulfur compounds, such as thiols, sulfides, thioacetates and thiocarbamates.[55-57]

These concerns have led to the development of several synthetic routes to prepare sulfonamides (Scheme III.1). Thus, sulfonamides are formed: *i)* by reaction of sulfinic acid salts with hydroxylamine-*O*-sulfonic acid;[58] *ii)* by synthesis[59] and reduction of arylsulfonyl azides;[60] *iii)* from aromatic and aliphatic sulfinic acid salts using bis(2,2,2-trichloroethyl)azodicarboxylate as an electrophilic nitrogen source;[61] *iv)* from alkyl or aryl halides by means of sodium 3-methoxy-3-oxopropane-1-sulfinate transfer

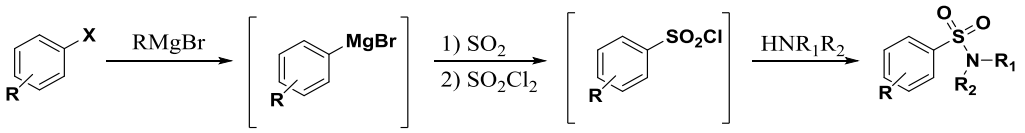
reagent;[62] *v)* by the radical addition of organo halides to pentafluorophenyl vinylsulfonate followed by substitution of the pentafluorophenyl moiety by amines;[63] *vi)* by sulfamoylation of aromatics using sulfamoyl chloride;[64] *vii)* using alkyl/arylsulfonylimidazoles as sulfonyl transfer reagents;[65] viii) from the reaction of sulfonic acids with isocyanides, in the presence of water;[66] *ix)* from the reaction of sulfonic acids and amines using cyanuric chloride (TCT)-DMF adduct;[67] *x)* from one-pot reaction from thiols using 1,3-dichloro-5,5-dimethylhydantoin (DCH),[68] trichloroisocyanuric acid (TCCA),[69] H_2O_2-$POCl_3$ system,[70] or *N*-chlorosuccinimide (NCS).[71]



Scheme III.1 – Several routes of sulfonamide synthesis (adapted from Katritzky 2004).[72]

Although these additional synthetic routes have proven to be of great utility for specific substrate classes, sulfonic acids and sulfonyl chlorides are rarely introduced into an advanced intermediate *via* C-S bond formation. Therefore, the diversity of sulfonamide functionality in pharmaceutical discovery is actually limited and cannot be readily varied at both nitrogen and sulfur in the final stage of a library synthesis.

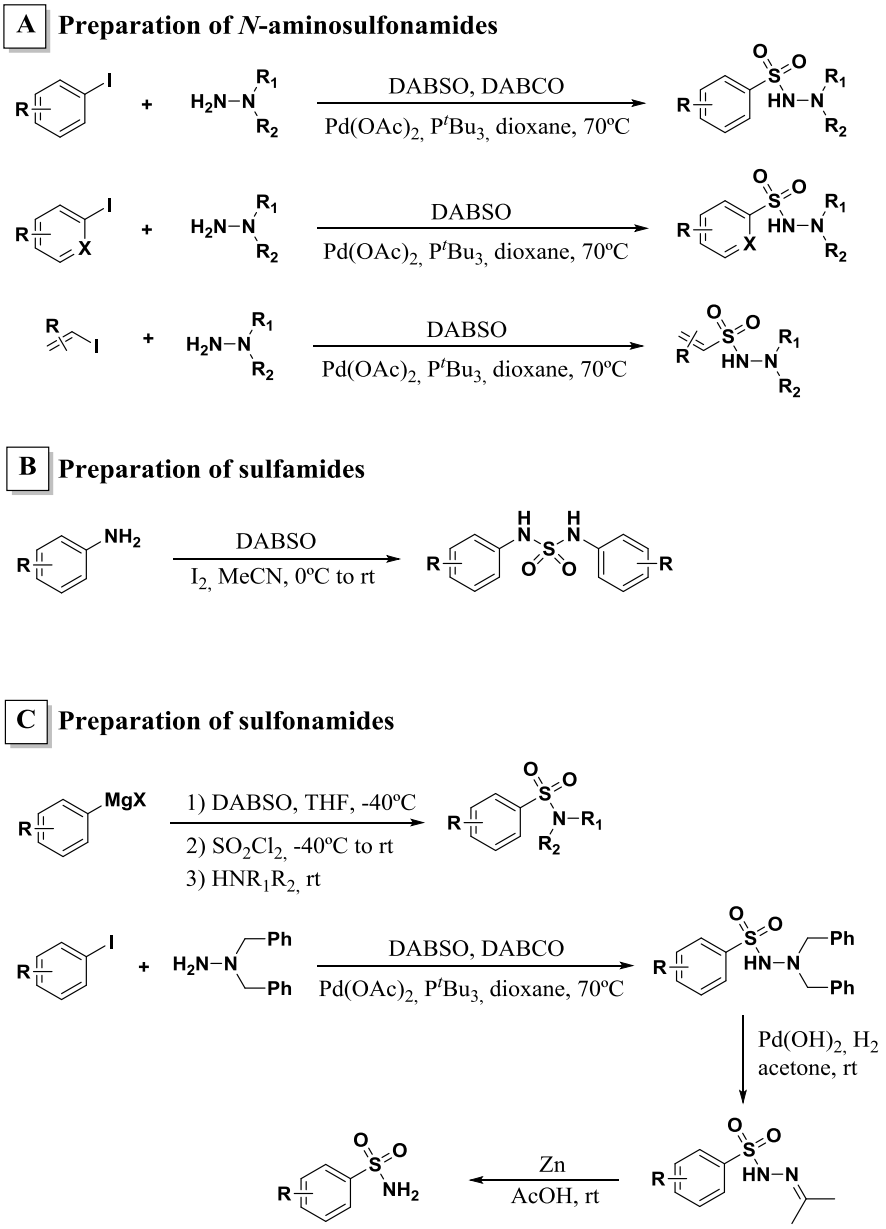
Barrett *et al.*[73] reported a one-pot sulfonamide formation from aryl or heteroaryl halides, which involves the use of Grignard reagents with sulfur dioxide, sulfuryl chloride and secondary amines (scheme III.2).



Scheme III.2 – One-pot sulfonamide synthesis reported by Barrett *et al.*[73]

However, this method requires the use of sensitive organometallics, which can be incompatible with polar functionalities, and the use of hazardous and difficult to handle sulfur dioxide gas.[74]

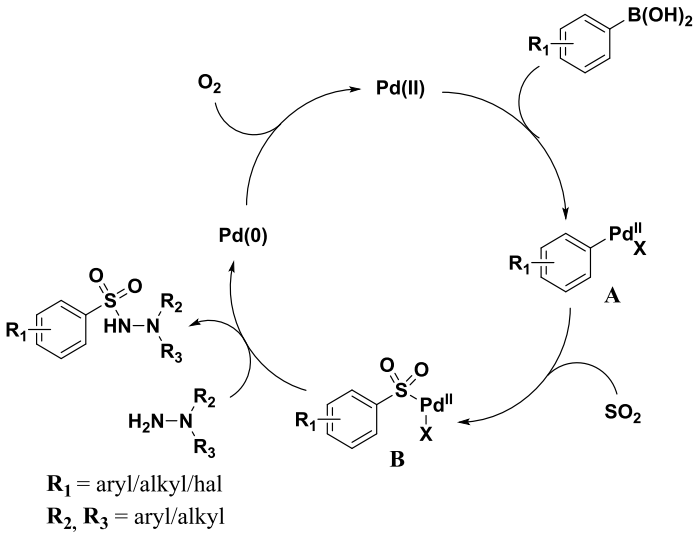
During the course of this work, Willis *et al.*[75-77] described an interesting procedure using DABCO-bis(sulfur dioxide), DABSO, as the sulfur dioxide source in several organic transformations (scheme III.3). Since the limitation of sulfur dioxide application in organic synthesis is mainly due to the difficulties associated with the handling and use of this toxic gaseous reagent, the use of DABSO, a bench-stable solid, constituted a breakthrough in the introduction of sulfur dioxide into simple organic molecules.



Scheme III.3 – Some applications of DABSO in organic synthesis, reported by Willis *et al.*[75-77]

Although DABSO constitutes a major advance in the synthesis of sulfur-containg compounds, this reagent presents an important limitation since it does not work with anilines or aliphatic amines. Thus, the synthesis of sulfonamides is only possible *via* a sulfonyl chloride intermediate or *via* an *N*-aminosulfonamide deprotection sequence (scheme III.3C).

Wu *et al.*[78] also applied DABSO for the synthesis of *N*-aminosulfonamides *via* a palladium-catalyzed three-component coupling of arylboronic acids, sulfur dioxide and hydrazines in the presence of a balloon of dioxygen. The authors proposed a mechanism for this reaction represented in scheme III.4.



Scheme III.4 – Mechanism for the palladium-catalyzed reaction of arylboronic acids, DABSO and hydrazines, proposed by Wu *et al.*[78]

A transmetallation of Pd(II) with arylboronic acid occurs first generating a Pd(II) species **A** (sheme III.4). Formation of the intermediate **B** results from the coordination and insertion of sulfur dioxide. Then a nucleophilic attack of hydrazine takes place affording the coupling product and Pd(0). The latter is oxidized in the presence of dioxygen to afford Pd(II), which re-enters the catalytic cycle.

The same authors also published a similar methodology for the synthesis of *N*-aminosulfonamides but instead of using DABSO they used potassium metabisulfite as the sulfur dioxide source.[79] However, this reaction presents the same limitation as with DABSO, since it only affords *N*-aminosulfonamides.

In this thesis studies were carried towards the synthesis of a versatile intermediate that would allow the preparation of several sulfonyl-containing compounds under mild conditions.

III.2 Results and discussion

This subchapter includes the discussion of the results obtained on the study for the development of new COX-2 selective inhibitors. This included the library synthesis, the biological evaluation and rationalization of the obtained data. During this study several challenges emerged, leading to the investigation of a reaction mechanism as well as the exploration of new synthetic routes.

Thus, this subchapter is divided in three main subjects: *i)* synthesis, biological evaluation, docking and STD-NMR studies of an indole based library as COX-2 selective inhibitors; *ii)* mechanistic investigation of the reaction of indole with trifluoromethylated olefins and; *iii)* studies towards a new methodology for the synthesis of sulfonyl-containing compounds.

III.2.1 Synthesis, biological evaluation, docking and STD-NMR studies of an indole based library as COX-2 selective inhibitors

The initial strategy envisaged the preparation of an indole-based library involving substitution of the ring in order to generate a "Y shape" structure similar to selective COX-2 inhibitor SC-558. Two substitution patterns were considered (A and B), concerning the inclusion of three substituents to afford three different interaction regions and relying on modification at Region III (figure III.9).

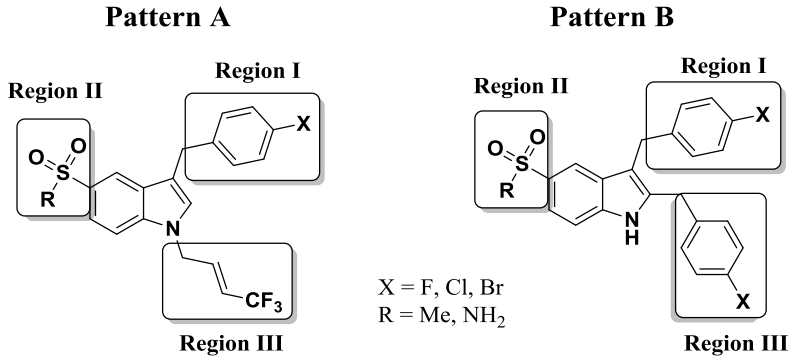


Figure III.9 – Proposed substitution pattern for the indole library.

Following detailed analysis of the reported inhibitor structures and previous theoretical studies (data not shown), the pattern included substitutions at positions: C-3 (Region I) by a *p*-halobenzyl group in order to fill the hydrophobic channel while exploring other possible hydrophobic interactions with residues at this cavity; C-5 position of the indole ring (Region II). According to previous reports, a methylsulfone[80] or a sulfonamide seem to be crucial for selectivity,[81-83] and comparing to indomethacin (methoxy group at C-5) would allow an extension of this ring, long enough to fill properly the selective pocket and allowing interaction with key residues (His90, Gln192 and Arg513). Additionally, the

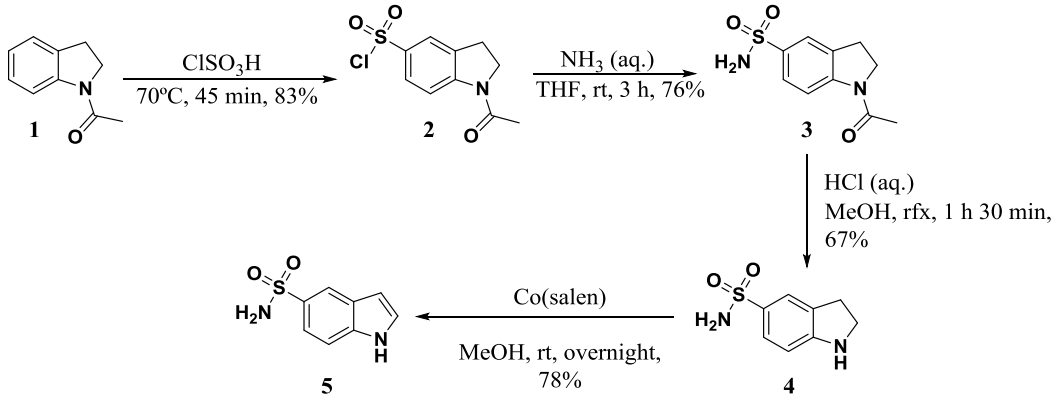
modification on the nitrogen atom (Region III, pattern A) by an allyl chain carrying a trifluoromethyl group at the end of the chain, was expected to give higher mobility and chain extension. The N-substitution intended to increase the selectivity for COX-2 by establishing unfavourable interactions with Arg120 (important for COX-1 inhibition), while enhancing the steric interaction with Ile523 (COX-1). Finally, C-2 substitution was also considered (Region III, pattern B), since a *p*-halobenzyl chain at C-2 would have the ideal shape for filling the hydrophobic pocket (figure III.4A).

In order to establish a relation between the spatial distribution of the previously described functional groups, related with COX inhibition, activity studies were performed against both COXs isoforms and the results were rationalized based on docking and NMR studies.

III.2.1.1 Synthesis of the indole-based library

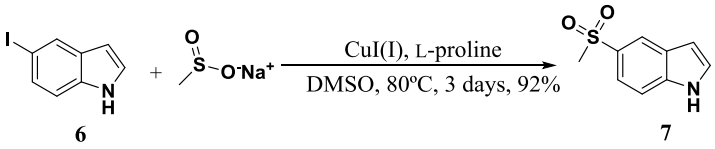
The first library (pattern A, figure III.9) was divided in two groups, those carrying a methylsulfone at C-5 and those with a sulfonamide group at the same position. The library synthesis involved three main steps: the introduction of a sulfonamide/methylsulfone at C-5, for which no direct sulfonylation has yet been reported for the indole nucleus; regioselective alkylation at C-3 and alkylation of N-1. The second library (pattern B, Figure III.9) relied on the exploration of substitution at C-2 position of indole which was achieved *via* dialkyaltion of compounds **5** and **7**.

At the time the synthesis was performed no direct method was been reported for the insertion of the groups $-SO_2NH_2$ and $-SO_2Me$ at position C-5 of the indole ring. Thus, the 1*H*-indole-5-sulfonamide (**5**) was prepared according to a reported procedure,[84] consisting on the chlorosulfonation of position C-5 of 1-acetylindoline (**1**), followed by treatment with a solution of ammonium hydroxide, amide hydrolysis and oxidation of the indoline to the indole derivative **5** in 33% yield (overall yield) (scheme III.5)



Scheme III.5 – Synthetic scheme followed for the synthesis of 1*H*-indole-5-sulfonamide (5).

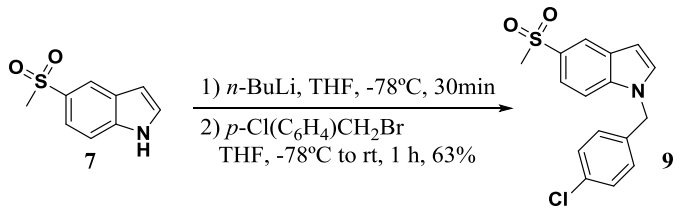
For the synthesis of 5-(methylsulfonyl)-1*H*-indole (**2**) it was adopted a method reported by Ma *et al.*[85] that consist in a C-S coupling between aryl halides and sulfinic acid salts *via* L-proline-promoted CuI-catalyzed reaction. The reaction of 5-iodoindole (**6**) with sodium methanesulfinate afforded 5-(methylsulfonyl)-1*H*-indole (**7**) in 92% yield (scheme III.6).



Scheme III.6 – Synthesis of 5-(methylsulfonyl)-1*H*-indole (7).

The synthetic procedures for alkylation of C-3 usually involve magnesium salts prepared by reaction of the indole with a Grignard reagent.[86] The magnesium salts tend to give C-3 substitution, rather than N-substitution, presumably because of the tight coordination of magnesium at nitrogen that reduces its nucleophilicity. 1-Lithioindoles are equally useful but, the position of attack depends on both solvent and the nature of the electrophile. C-Alkylation is favored in the order $Mg^{2+} > Li^{+} > Na^{+} > K^{+}$.[86]

The first approach for the synthesis of indole derivatives substituted at C-3 position consisted of the reaction of 5-(methylsulfonyl)-1*H*-indole (**7**) with *n*-BuLi, in dry THF, at -78ºC for 30 min. After that time, a solution of *p*-chlorobenzyl bromide was added and the mixture was warmed up to room temperature. This reaction afforded the *N*-substituted derivative (scheme III.7) instead of the desired product. The product, 1-(4-chlorobenzyl)-5-(methylsulfonyl)-1*H*-indole (**9**) was obtained in 63% yield as a white solid.



Scheme III.7 – Reaction of 5-(methylsulfonyl)-1*H*-indole (7) with *n*-BuLi and *p*-chlorobenzyl bromide.

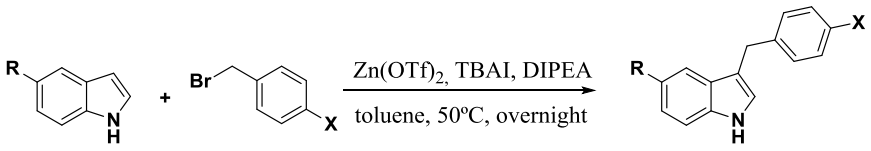
Since the reaction with the indole lithium salt afforded exclusively the *N*-alkylated product, the next choice relied on the formation of indole magnesium salt. Therefore, 5-(methylsulfonyl)-1*H*-indole (**7**) was reacted with PhMgBr in dry THF, at -78ºC, for 30 min. Then, a solution of *p*-chlorobenzyl bromide, in dry THF, was added and the mixture was warmed up to room temperature for 24 h. No product formation was observed after TLC control. The next attempt consisted of changing the Grignard reagent. Thus, MeMgI was used instead of PhMgBr. The same conditions of the previously experiment were used. After stirring for 3 h at room temperature no product formation was observed by TLC,

therefore, the reaction was heated to 50ºC and was let at that temperature overnight. Even so no product formation was observed.

After searching in the literature for alternative methods a report was found concerning to the regioselective synthesis of 3-alkylindoles mediated by zinc triflate.[87] The authors have developed a simple one-pot procedure for the direct formation of 3-alkylindoles from indole and alkyl halides that can react by a S_N1-like pathway. The indole nitrogen does not require prior protection and the avoidance of strong bases for deprotonation permits compatibility with a wide range of functional groups.

Mechanistically, the authors proposed that zinc activates the halide and also coordinates to the indole nitrogen. They observed that zinc species are serving as a Lewis acid in activating the halide, by ^{1}H-NMR experiments. When zinc triflate (1 equiv.) is added, the proton spectrum of prenyl bromide immediately shows additional sets of signals. Also, in the case of the less acidic zinc acetate, the same changes only began to appear after 2 h. On the other hand, no changes in ^{1}H-NMR spectrum were observed with zinc triflate and an unactivated halide such as isoamyl bromide. Zinc triflate also plays an important role in the formation indolylzinc species, fact that it is evidenced by the poorer yield of the reaction with *N*-methylindole, which cannot form an indolylmetal salt. Furthermore, several metals that form stronger Lewis acidic triflates than zinc were poorer reagents.[87]

Therefore, this procedure mediated by zinc triflate, in the presence of Hünig's base and tetrabutylammonium iodide, was applied for the regioselective alkylation of indoles derivatives **5** and **7** (scheme III.8).



5 R = SO_2NH_2
7 R = SO_2CH_3
10 R = Br

X = F
X = Cl
X = Br
X = SO_2NH_2

11a	R = SO_2CH_3	X = F	η = 47%
11b	R = SO_2CH_3	X = Cl	η = 41%
11c	R = SO_2CH_3	X = Br	η = 50%
11d	R = SO_2NH_2	X = F	η = 39%
11e	R = SO_2NH_2	X = Cl	η = 46%
11f	R = SO_2NH_2	X = Br	η = 42%
11g*	R = Br	X = SO_2NH_2	η = 79%

* reaction was performed at rt

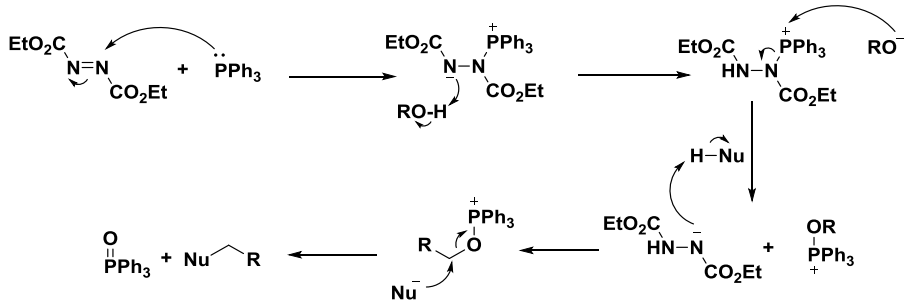
Scheme III.8 – Synthesis of 3-alkylindole derivatives 11a-g.

Several attempts were made to improve the yield of these compounds, such as reaction temperature and solvent, since compounds **5** and **7** are not soluble in toluene. At room temperature, no reaction occurred. At higher temperatures (> 50ºC), as well as in more polar solvents (DMF, THF), compounds **11a-f** were obtained with lower yields, since that in these reaction conditions the formation of the dialkylated side-product (positions C-3 and C-2) is favored. Reaction of 5-bromoindole (**10**) with

4-(bromomethyl)benzenesulfonamide was performed at room temperature since this indole is soluble in toluene, also no formation of the dialkylated side-product was observed.

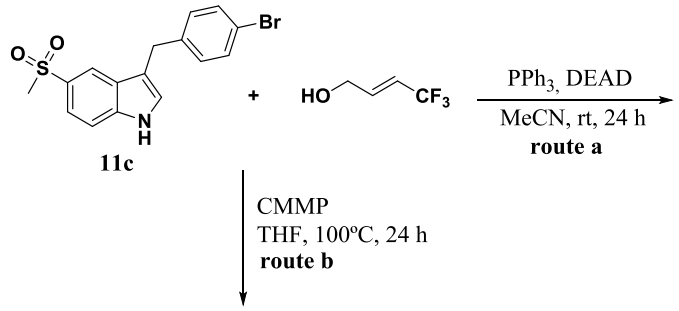
In order to proceed to *N*-alkylation, the sulfonamide derivatives would require additional protection-deprotection steps of the amine group. Celecoxib and valdecoxib possess a sulfonamide group while rofecoxib and etericoxib have a methylsulfone (figure III.3). Thus, at this stage it was decided to focus on the methylsulfones derivatives for further functionalization at N-1. To perform the *N*-alkylation of compounds **11a-c**, different approaches were tested such as the Mitsunobu reaction.

The Mitsunobu reaction consists in the formation of C-O, C-S, C-N, or C-C bond by the condensation of an acidic component with a primary or a secondary alcohol in the presence of triphenylphosphine (or another suitable phosphine) and diethyl azodicarboxylate (DEAD) or diisopropyl azodicarboxylate (DIAD).[88] Despite the fact that the Mitsunobu reaction is widely used in synthetic organic chemistry, the mechanistic details, particularly at the intermediate stages, are still a subject of debate and intensive studies.[89-91] A possible pathway is shown in scheme III.9.



Scheme III.9 - A possible mechanism of the Mitsunobu reaction.

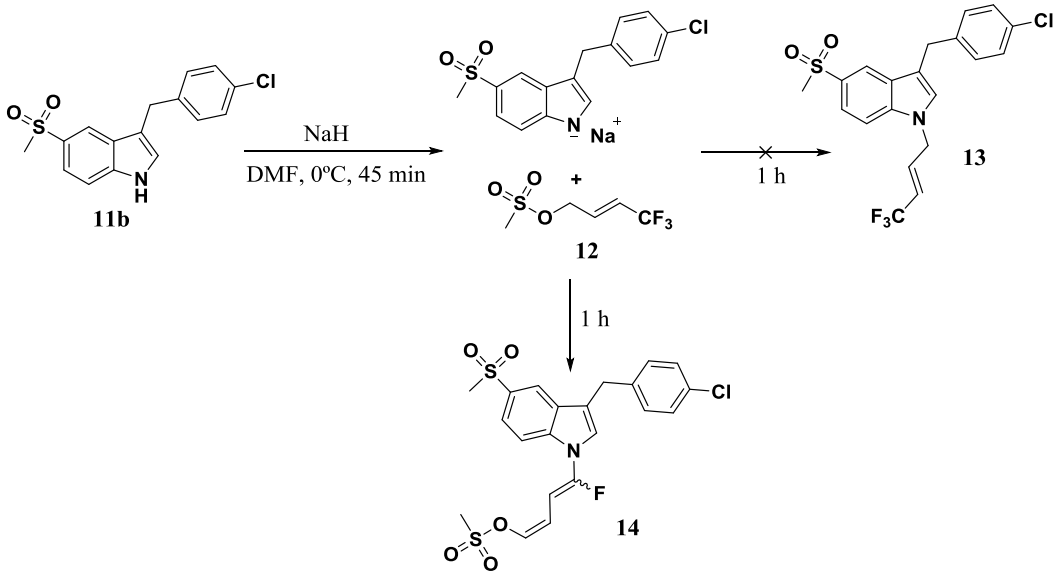
Therefore, the Mitsunobu reaction was performed for the *N*-alkylation of indole derivatives following two different reaction conditions. These consisted on the Mitsunobu reaction under the classical reaction conditions (DEAD/PPh_3) (scheme III.10, route a);[92] and a modified Mitsunobu reaction [cyanomethylenetrimethyl phosphorane (CMMP) (generated *in situ*)] (scheme III.10, route b)[93]. In both approaches 3-(4-bromobenzyl)-5-(methylsulfonyl)-1*H*-indole (**11c**) was reacted with the commercial available alcohol (*E*)-4,4,4-trifluorobut-2-en-1-ol. However, these conditions were not successful since no product formation was observed by TLC after 24 h of reaction.



Scheme III.10 – Reaction of 3-(4-bromobenzyl)-5-(methylsulfonyl)-1*H*-indole (11c) with (*E*)-4,4,4-trifluorobut-2-en-1-ol under classical (route a) and modified (route b) Mitsunobu conditions.

The next approach consisted on a bimolecular nucleophilic substitution (S_N2) reaction. To perform this reaction was necessary to form an indole metal salt and to transform the hydroxyl group of the (*E*)-4,4,4-trifluorobut-2-en-1-ol into a good leaving group. The methanesulfonyl (mesyl, $-SO_2CH_3$) group was chosen to protect the hydroxyl group due to its electronegativity and also because is a relatively small group.

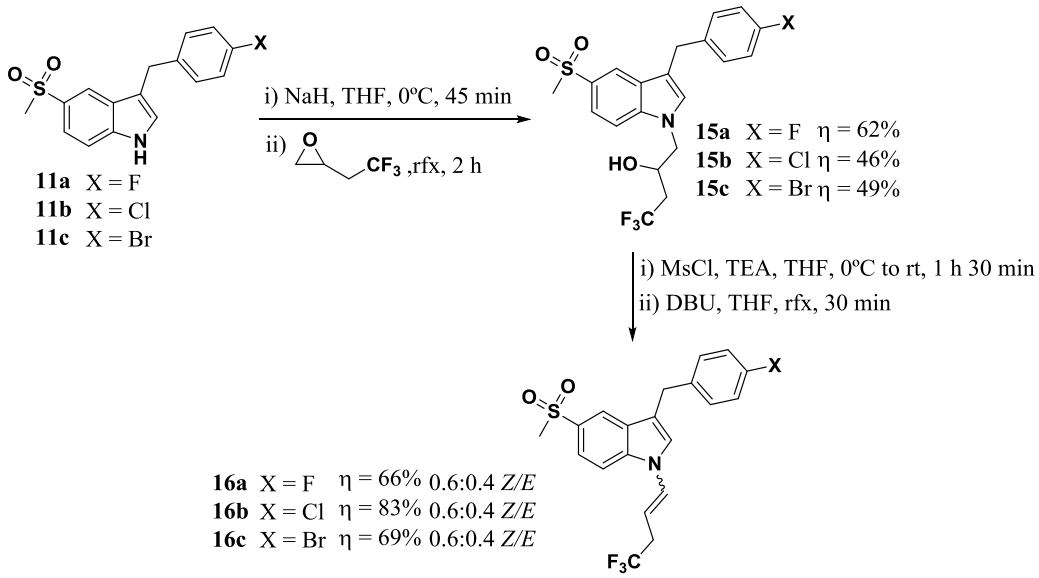
Thus, 3-(4-chlorobenzyl)-5-(methylsulfonyl)-1*H*-indole (**11b**) was treated with sodium hydride, affording the indole sodium salt derivative and then reacted with the previously prepared (*E*)-4,4,4-trifluorobut-2-en-1-yl methanesulfonate (**12**). Surprisingly, product **14** was isolated as a mixture of 1:0.5 of *Z/Z* and *Z/E* isomers in 27% yield, instead of the expected S_N2 product (**13**) (scheme III.11).



Scheme III.11 – Reaction of 3-(4-chlorobenzyl)-5-(methylsulfonyl)-1*H*-indole (11b) with (*E*)-4,4,4-trifluorobut-2-en-1-yl methanesulfonate (12).

The mechanism of this reaction will be described in detail in section III.2.2.

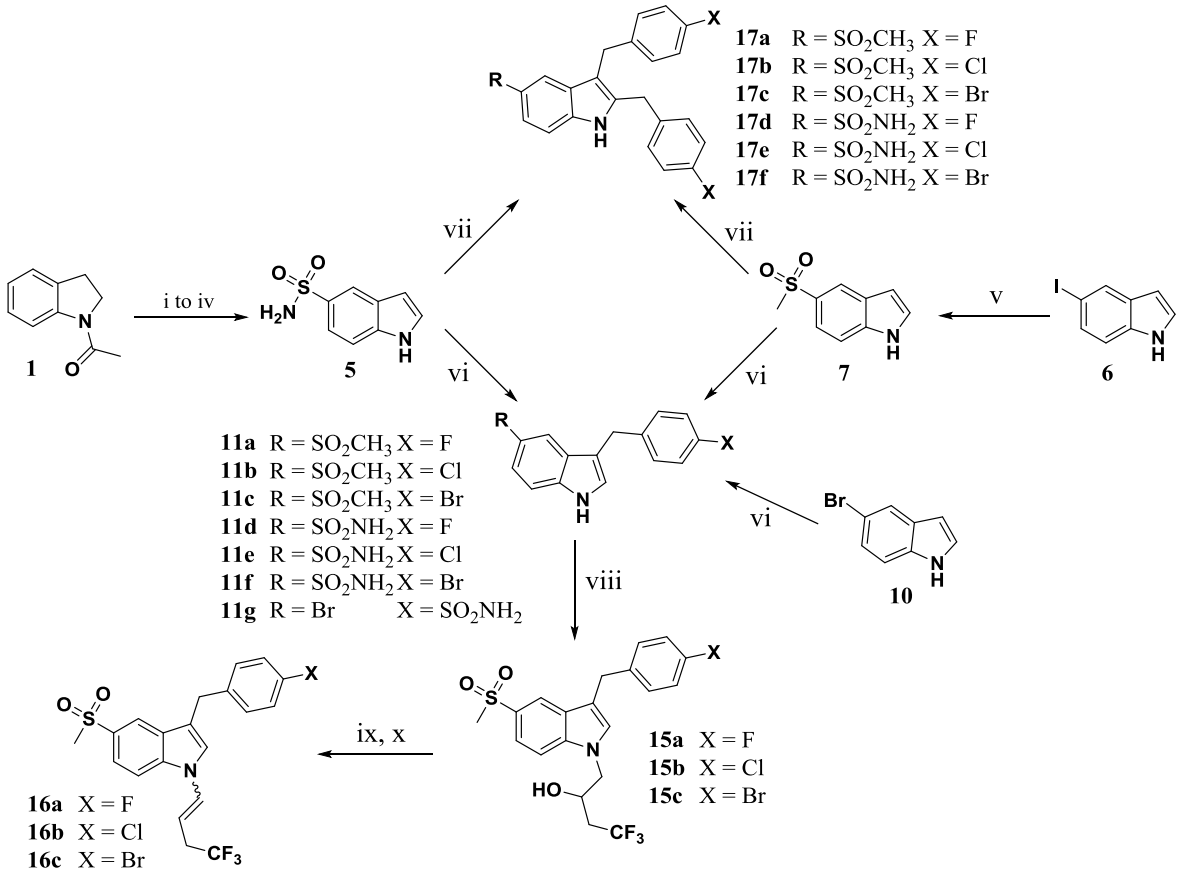
Since this approach failed, the next attempt to synthesize *N*-alkylated indole derivatives consisted on the introduction of the alkyl chain *via* epoxide opening, using the commercial (2,2,2-trifluoro-ethyl)-oxirane, that afforded compounds **15a-c**. Mesylation of the resulting alcohol and elimination by treatment with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) afforded compounds **16a-c** (scheme III.12). However, the isolated products presented a double bond conjugated with the indole ring instead of the desired allylic chain.



Scheme III.12 – Preparation of the compounds 16a-c from the reaction of 3-(*p*-halobenzyl)-5-(methylsulfonyl)-1*H*-indole derivatives 11a-c with (2,2,2-trifluoro-ethyl)-oxirane.

For the preparation of pattern B (compounds **17a-f**), compounds **5** and **7** were dialkylated using the same procedure used to prepare compounds **11a-f**, but with an excess of 3 equivalents of the different *p*-halobenzyl bromides and heating the reaction up to 70ºC. Formation of compounds **17a-f** was also observed in small amount (η ≈ 15%) during mono-alkylation reaction of **5** and **7**.

Scheme III.13 depicts the synthetic routes adopted for the synthesis of the indole based library.



i) $ClSO_3H$, 70ºC; **ii)** NH_3 (aq), THF, rt; **iii)** HCl (aq), MeOH, rfx; **iv)** Co(salen), MeOH, rt; **v)** CuI(I), L-proline, $MeSO_2Na$, DMSO, 80ºC; **vi)** *p*-halobenzyl bromide (1 equiv.), $Zn(OTf)_2$, TBAI, DIPEA, toluene, 50ºC; **vii)** *p*-halobenzyl bromide (3 equiv.), $Zn(OTf)_2$, TBAI, DIPEA, toluene, 70ºC; **viii)** NaH, THF, 0ºC then (2,2,2-trifluoro-ethyl)-oxirane, rfx; **ix)** MsCl, TEA, THF, 0ºC to rt; **x)** DBU, THF, rfx.

Scheme III.13 - Synthetic routes adopted for the synthesis of the indole based library.

Compounds **11a-g**, **15a-c**, **16a-c** and **17a-f** were tested *in vitro* for COX-1 and COX-2.

III.2.1.2 Biological assays

Chemical structures of all tested compounds are depicted in figure III.10.

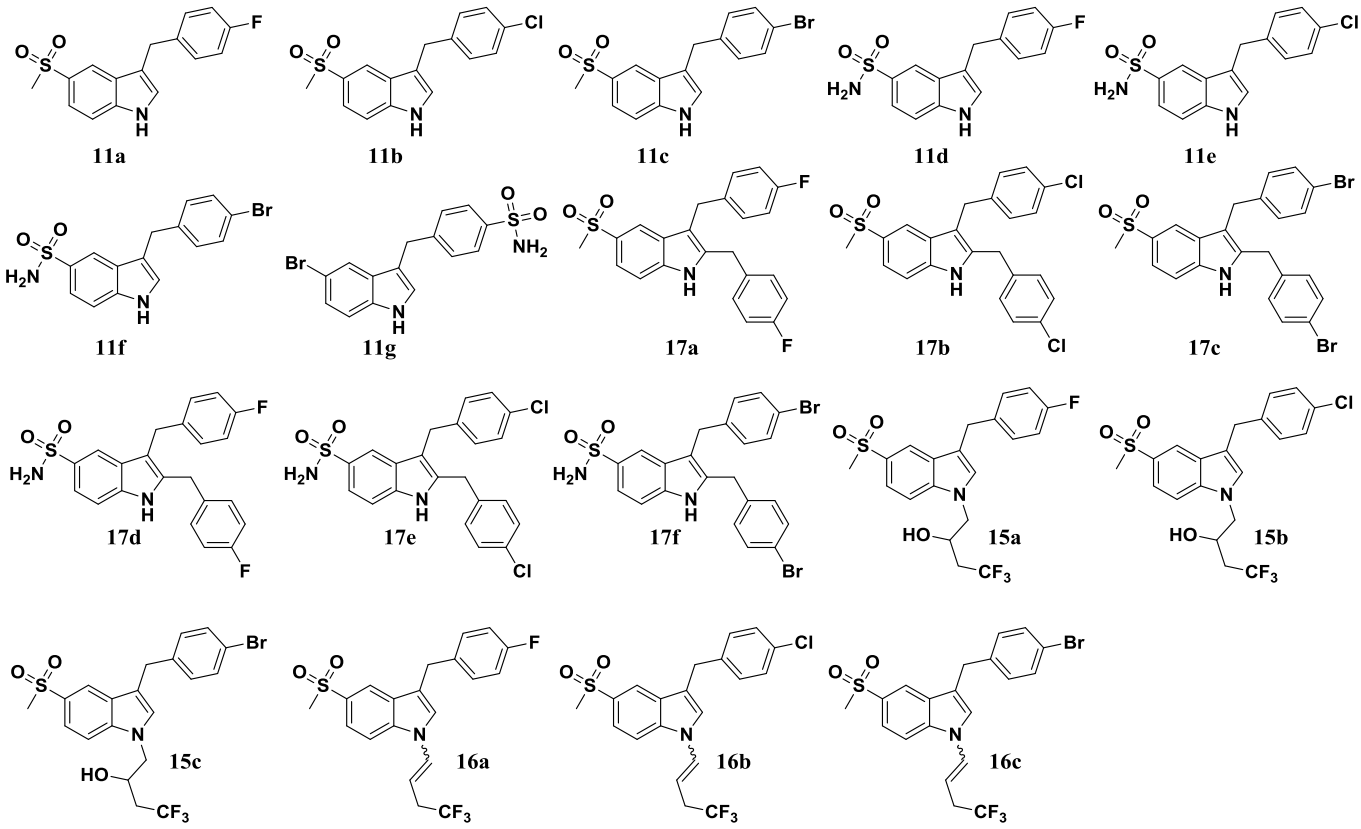
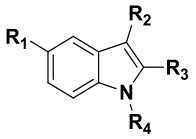


Figure III.10 - Chemical structures of the tested compounds.

Inhibition of COX-1 and COX-2 by the synthesized compounds is expressed as the percentage of inhibition of control COX-1 or COX-2 activity as displayed in table III.1. The known COX inhibitors indomethacin (non-selective) and celecoxib (COX-2 selective) were used as positive controls. The inhibitory activity of the studied compounds was first tested at 50 µM however, since some compounds were not active at this concentration, the inhibitory activity was also tested at 100 µM. Though, compounds were insoluble at this concentration.

Table III.1 – Percent inhibition of control COX-1 or COX-2 activity, determined by EIA. Each value represents mean ± SEM of at least 4 experiments performed in duplicate.



Compound	R_1	R_2	R_3	R_4	COX-1	COX-2	COX-1	COX-2
					50 μM		100 μM	
11a	SO_2Me	*p*-$CH_2(C_6H_5)F$	H	H	INS	INS	---	---
11b	SO_2Me	*p*-$CH_2(C_6H_5)Cl$	H	H	NA	NA	28 ± 7	24 ± 16
11c	SO_2Me	*p*-$CH_2(C_6H_5)Br$	H	H	NA	9 ± 5	13 ± 7	26 ± 16
11d	SO_2NH_2	*p*-$CH_2(C_6H_5)F$	H	H	40 ± 9	32 ± 9	31 ± 8	20 ± 10
11e	SO_2NH_2	*p*-$CH_2(C_6H_5)Cl$	H	H	21 ± 4	32 ± 11	19 ± 5	31 ± 8
11f	SO_2NH_2	*p*-$CH_2(C_6H_5)Br$	H	H	19 ± 7	23 ± 5	INS	INS
11g	Br	*p*-$CH_2(C_6H_5)SO_2NH_2$	H	H	11 ± 6	22 ± 1	33 ± 8	27 ± 2
15a	SO_2Me	*p*-$CH_2(C_6H_5)F$	H	$CH_2CHOHCH_2CF_3$	14 ± 11	16 ± 7	41 ± 10	9 ± 2
15b	SO_2Me	*p*-$CH_2(C_6H_5)Cl$	H	$CH_2CHOHCH_2CF_3$	8 ± 4	20 ± 8	NA	34 ± 8
15c	SO_2Me	*p*-$CH_2(C_6H_5)Br$	H	$CH_2CHOHCH_2CF_3$	12 ± 8	23 ± 5	20 ± 6	15 ± 9
16a	SO_2Me	*p*-$CH_2(C_6H_5)F$	H	$CHCHCH_2CF_3$	8 ± 5	12 ± 7	48 ± 11	18 ± 28
16b	SO_2Me	*p*-$CH_2(C_6H_5)Cl$	H	$CHCHCH_2CF_3$	11 ± 10	NA	26 ± 15	23 ± 22
16c	SO_2Me	*p*-$CH_2(C_6H_5)Br$	H	$CHCHCH_2CF_3$	20 ± 10	21 ± 6	16 ± 10	19 ± 10
17a	SO_2Me	*p*-$CH_2(C_6H_5)F$	*p*-$CH_2(C_6H_5)F$	H	NA	NA	NA	NA
17b	SO_2Me	*p*-$CH_2(C_6H_5)Cl$	*p*-$CH_2(C_6H_5)Cl$	H	25 ± 4	24 ± 4	42 ± 1	NA
17c	SO_2Me	*p*-$CH_2(C_6H_5)Br$	*p*-$CH_2(C_6H_5)Br$	H	NA	NA	NA	NA
17d	SO_2NH_2	*p*-$CH_2(C_6H_5)F$	*p*-$CH_2(C_6H_5)F$	H	18 ± 9	67 ± 5	INS	INS
17e	SO_2NH_2	*p*-$CH_2(C_6H_5)Cl$	*p*-$CH_2(C_6H_5)Cl$	H	41 ± 6	46 ± 1	INS	INS
17f	SO_2NH_2	*p*-$CH_2(C_6H_5)Br$	*p*-$CH_2(C_6H_5)Br$	H	35 ± 8	8 ± 4	84 ± 5	NA
Positive Controls					1 μM		10 μM	
Celecoxib					---	---	---	75 ± 8
Indomethacin					46 ± 5	78 ± 3	---	---

NA – no activity was found; INS – insoluble compound

Some compounds, like **15b** or **16a** (50 μM, COX-1) displayed inhibition values less than 10%, which were considered to be not significant. Nevertheless, most of the obtained results were well above this percentage. Additionally it was observed that some compounds such as **11d**, inhibit both isoforms at the higher concentration to a lesser extent than at the lower. In fact, some compounds with low activities, it was difficult to achieve a concentration-dependent effect, due to the high values of SEM. Indomethacin (1 μM) inhibits COX-1 (46 ± 5%) and COX-2 (78 ± 3%) while celecoxib (10 μM) inhibits only the last one (75 ±8%).

From the C-5, C-3 disubstituted compounds (**11a-g**) only the sulfonamide derivatives (**11d-g**) showed to be active at 50 μM, although not selective (figure III.11).

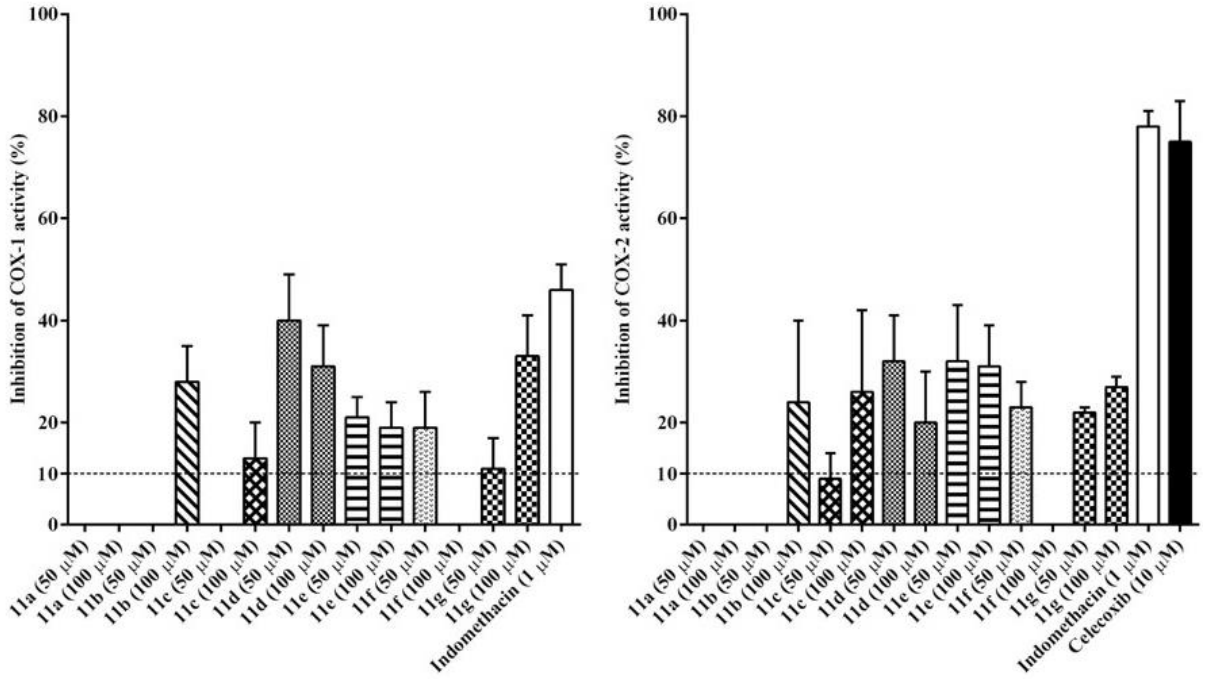


Figure III.11 – Inhibition of COX-1 and COX-2 activity (%), respectively, of the synthesized compounds 11a-g, indomethacin and celecoxib (only for COX-2).

N-alkylation, in compounds **15a-c** and **16a-c** (figure III.9, pattern A), did not result in an improvement of activity/selectivity. At 100 μM, compounds **15a** and **16a** showed inhibitory activity against COX-1 (41 ± 10% and 48 ± 11%, respectively) (figure III.12).

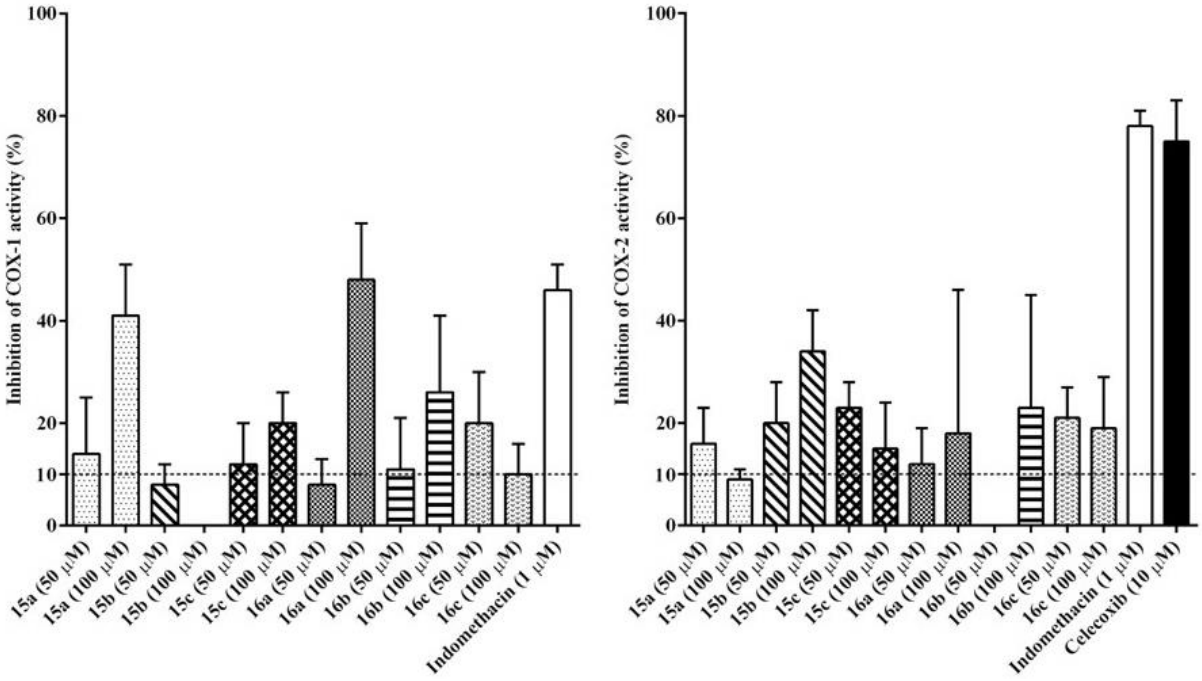


Figure III.12 - Inhibition of COX-1 and COX-2 activity (%), respectively, of the synthetized compounds 15a-c and 16a-c, indomethacin and celecoxib (only for COX-2).

Compounds **17a-f** allowed the investigation of the C-5, C-3, C-2 substitution pattern (figure III.9, pattern B). Compound **17f** showed the strongest inhibition of COX-1 enzyme's activity at the concentration of 100 µM (84 ± 5%). In addition, **17d** resulted in 67 ± 5% (50 µM) of COX-2 and 18 ± 9% of COX-1 inhibition, revealing its relative selectivity to inhibit COX-2. Compound **17e** inhibits both enzyme isoforms at 50 µM (41 ± 6% and 46 ± 1% for COX-1 and COX-2, respectively) (figure III.13).

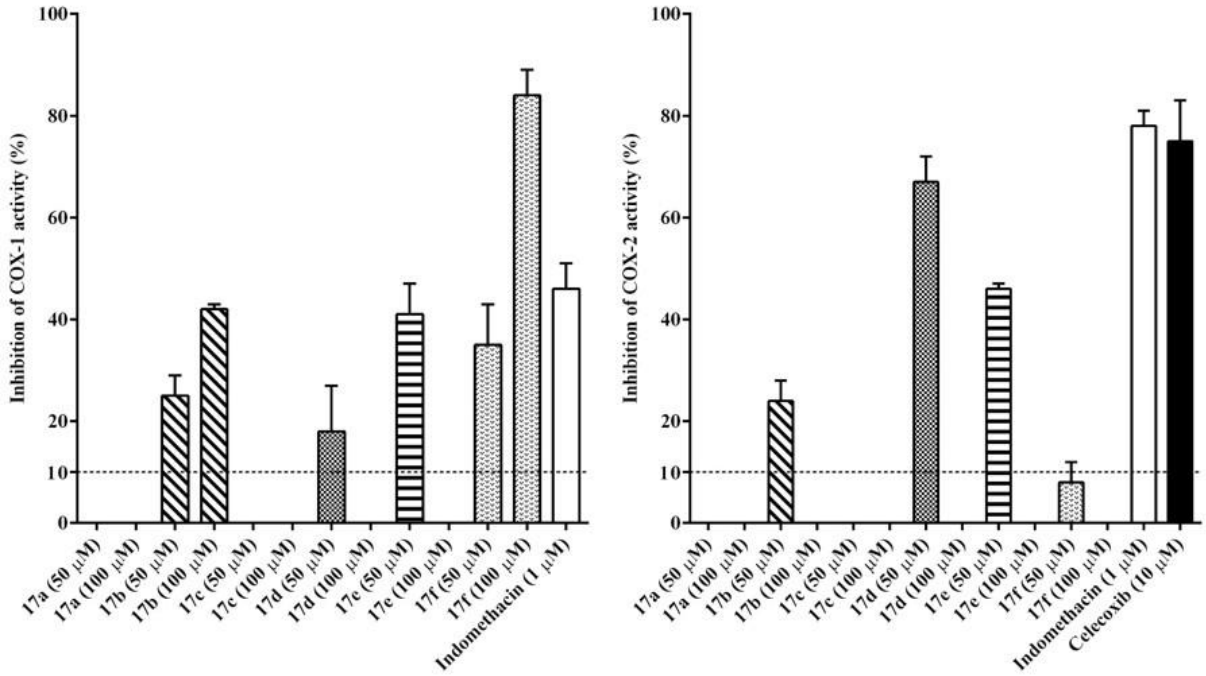


Figure III.13 - Inhibition of COX-1 and COX-2 activity (%), respectively, of the synthetized compounds 15a-c and 16a-c, indomethacin and celecoxib (only for COX-2).

III.2.1.3 Docking studies

All molecules were docked to the active site of both COX-1 and COX-2 using the docking program Autodock4 (this work was performed by Dr. Stefan Erhardt from Edinburgh University). The docking study suggested that most studied compounds are more active for COX-1 which is in agreement with the observed inhibitory activity. Indeed, compounds **11a-g** were all found to be COX-1 selective. They docked inside the binding pocket for both COXs isoforms. Overall, compounds **11a-g** bind less strongly to COX-2 than to COX-1. This can be explained by the fact that they use the existing space in COX-1 binding pocket, with the sulfonamide/methylsulfone close to Arg120 and Tyr355, similar to the binding of the acid group in indomethacin, while the benzyl chain binds in the hydrophobic pocket. The selectivity is reversed for the dialkylated compounds **17a-f** when compared to the mono-alkylated ones **11a-f**. Compounds **17a-f** were the most promising compounds by studies, which is almost consistent with the COX-2 inhibition evaluation assays. Due to the increased flexibility introduced by the benzyl group, the 2,3-dibenzyl substituted indoles show a potential higher affinity to COX-2. According to the docking results, compounds **17d-f**, with a sulfonamide at C-5, are more active than the corresponding methylsulfones **17a-c**, what is in accordance with the experimental data. From the **17** series, the computational study indicated that the most promising compounds are the fluorinated ones **17a** and **17d**, with a methylsulfone and a sulfonamide, respectively. In both cases these compounds strongly bind to COX-2, but not COX-1. Also they do bind in the same orientation as the selective SC-558 inhibitor,

such as **17d** in which the sulfonamide group binds to the side pocket next to Val523 and might establish hydrogen bonding *via* the oxygen atoms with Arg513 (Figure III.14A).

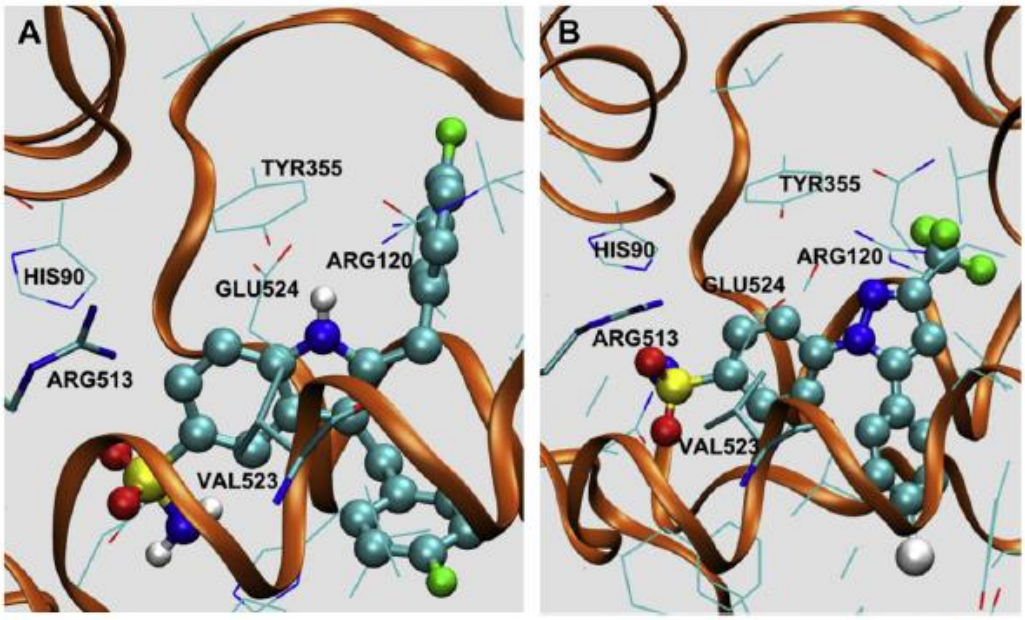


Figure III.14 – Docking of compound 17d (A) and selective inhibitor SC-558 (B) in COX's-2 active site.

Although compounds **17** only binds inside the COX-2 binding pocket, some very strong interactions in the region of the entrance channel can occur with COX-1. Thus, their strong binding close to the entrance channel, suggests that they have the potential to block COX-1 effectively. Moreover, it was found that the binding affinities for COX-2 do not strongly depend on the type of halogen present in the aromatic ring. Nevertheless, the same was not observed in the docking for COX-1, where stronger binding is observed for heavier halogens.

For compounds **15a-c** and **16a-c**, the biological screening results showed only low activity. Additionally the docking predicts that they do not fit inside the binding pocket, due to their bulkiness.

III.2.1.4 NMR studies

In order to have a deeper insight about the mode of interaction of the mono-alkylated (**11a-g**) and the di-alkylated compounds (**17a-f**) with both enzyme isoforms it was performed saturation-transfer difference NMR (STD-NMR) experiments.[94,95] Recently it was demonstrated that STD-NMR can be effectively used to characterize the binding of the anti-inflammatory drugs ibuprofen, diclofenac and ketorolac to COX-1 and COX-2.[96] The STD-NMR experiment is based on the nuclear Overhauser effect and in the observation of the ligand resonance signals.[94,95,97] This technique can be used not only for screening ligands with dissociation constants K_D ranging from ca. 10^{-8} to 10^{-3} M but also to provide

insight about the moieties of the ligand that are most important for binding, since it is expected that the regions of the ligand having the strongest contact to the protein will show the most intense STD NMR signals.[97]

The STD-NMR of known drugs with COX-2 has been reported[96] and demonstrates that COX-2 can be used under those conditions to identify key substituents, although it was not possible to study celecoxib due to solubility reasons.

Considering the percentage of inhibition presented in table III.1 and the results of the docking studies, compounds **11d** and **17d** were chosen for this study. Compound **11d** is a mono-alkylated compound with a higher selectivity for COX-1 and **17d** is, from the di-alkylated series, the one that shows the highest selectivity to COX-2, binding well inside the COX-2 pocket, according to the docking studies.

The STD-NMR results are presented in figure III.15. STD signals were observed for both compounds and for both enzyme isoforms. The fact that STD responses were obtained for all cases is in accordance with **11d** and **17d** binding reversibly to COX-1 and COX-2, as well as with the inhibition and docking results.

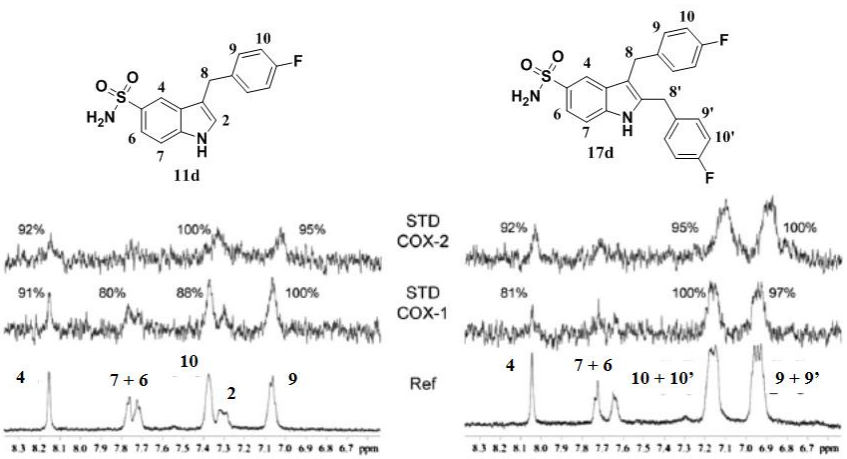


Figure III.15 – Expansions of the aromatic region of the STD-NMR spectra of the compounds 11d and 17d with COX-1 and COX-2. STD intensities relative to the corresponding reference intensities are shown in each signal as percentage.

When considering the interaction of **11d**, from the comparison of the STD spectra with the reference spectra it can be seen that not all protons gave identical STD responses. For this compound, the most intense STD signals originate from the aryl moiety and the response of protons 6 and 7 of the indole ring is higher in COX-1 than in COX-2. These results may be indicative of a more extended and uniform interaction of **11d** with COX-1 than with COX-2. When considering **17d**, the most intense STD signals originate also from the aryl moieties. In COX-1 there is an appreciable decrease in STD response from proton 4 of the indole ring. This may evidence that the sulfonamide moiety in **17d** may be more

important to promote the association with COX-2 than with COX-1 and partly responsible for the selectivity observed in the inhibition and docking studies. Also, contrary to **11d**, it is clear that protons 6 and 7 from the indole ring receive less saturation from the protein, what can be indicative of a different conformation in the binding site. These results are in good agreement with the inhibition and docking studies and they provide an experimental validation of the methodology.

From the library of the 19 new indole based compounds synthesized, the biological tests revealed that the presence of a sulfonamide (**11d-g**) was more favorable for interaction with both COXs, being more active than the corresponding methylsulfones (**11a-c**). These sulfonamides **11d-g** when docked have a similar binding mode as the selective inhibitor SC-558, but the docking studies predicted a slight preference for COX-1 binding. Indeed **11d** demonstrated a percent of inhibition higher for COX-1 (40 ± 9%) than for COX-2 (32 ± 10%) at 50 μM. This observation was further supported by the STD-NMR study that demonstrated that compound **11d**, despite interacting with both COX isoforms, displays a more extended STD response for COX-1, confirming a superior interaction with this enzyme.

These results indicate that the selective inhibitory activity associated with the introduction of a sulfonamide group is dependent on the overall geometry of the molecule. The orientation of the sulfonamide group towards the selective pocket is determined by the shape of the molecule. Thus, the Y shape is necessary for the correct positioning of the groups in the pocket.

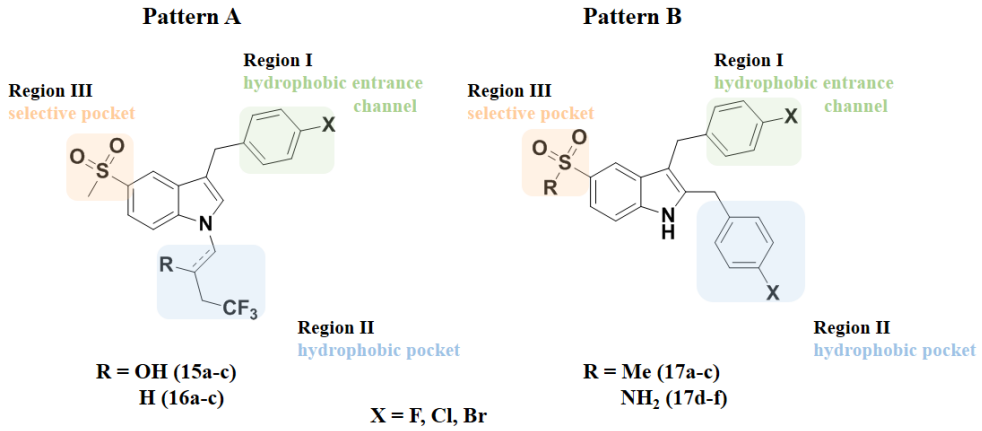


Figure III.16 - Indole-based library tested as selective COX-2 inhibitor.

With compounds **15a-c** and **16a-c** (figure III.16, pattern A) it was tested if a trifluoromethyl allylic chain would introduce flexibility to the Y shape, when compared to the benzoyl group present in indomethacin. The presence of the CF_3 group was thought to establish favorable interactions with the key residue Arg120 in COX-2. The biological evaluation of these compounds only revealed low activity and no selectivity for COX-2. The docking results show that these compounds are too bulky to enter the binding pocket, although interactions can be established and a partial blocking can occur, what would

explain the low activity found. This shows that the bulkiness of the substituents is important for binding. If the substituents are too bulky the molecule will not enter the binding site. However, once in the binding pocket they need to be bulky enough to orient the sulfonamide to the selective pocket.

The second library prepared (figure III.16, pattern B) envisaged exploration of C-2 substitution with a group that has enough volume to fill the hydrophobic pocket interacting with residues in this region. This substitution pattern (C-2, C-3 and C-5 - compounds **17a-f**) also leaves the indolic NH free allowing a possible stabilization of the binding with the enzyme via hydrogen bonding, which was shown by docking to be important to interact with the hydroxyl group of with Tyr355 (figure III.14).

As also shown by the calculations, **17a-f** bind to COX-2 in a similar manner to that of SC-558, and different from indomethacin. Furthermore, these studies indicate that the volume, nature and shape of the benzyl group at C-2 are the most appropriate for stable binding to hydrophobic pocket. The activity results for compounds **17a-f**, confirm that substitution at C-2 with a benzyl group possessing a halogen at *para* position, is important. The results obtained also indicate that the halogen atom at the aromatic side chain is a key substituent. Compound **17d** was found to be the most promising showing 67 ± 5% COX-2 inhibition at 50 μM, demonstrating that fluorine seems to be crucial, this inhibition percentage is close to the one of celecoxib (75 ± 8%) at 10 μM.

The lack of activity of the methylsulfones **17a** and **17c** is most probably related to the lower electronic density of the sulfone oxygen atoms when compared with the corresponding sulfonamides (**17d-f**).

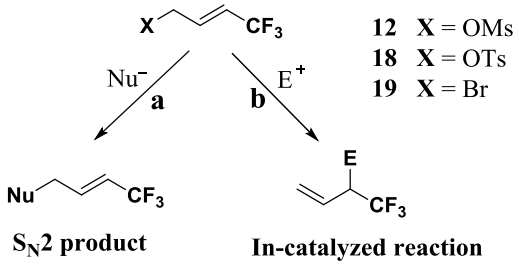
STD-NMR studies performed with **17d** highlighted that the sulfonamide group is indeed important to promote the interaction with COX-2 rather than with COX-1, as evidenced by the close proximity of the indolic proton H4 to COX-2, supporting the observed selectivity in the inhibition evaluation.

In summary, the studies undertaken with the indole-based library served as a platform for the design of new compounds with improved selectivity.

III.2.2 Mechanistic investigation of the reaction of indole with trifluoromethylated olefins

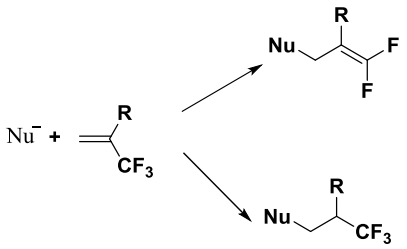
The unexpected reactivity found on the introduction of a trifluoromethylated olefin at the nitrogen atom of the indole (scheme III.11, page 79) was further investigated.

In the literature it is shown that trifluoromethylated allyl methanesulfonate **12** and the toluene-4-sulfonic acid derivative **18** afford exclusively the S_N2 product (scheme III.14, route a) when reacted with nucleophiles.[98-101] Trifluoromethylated allyl bromide **19** has been used in indium-mediated allylations to afford γ-coupling products when reacting with aldehydes (scheme III.14, route b) (S_N2 product was observed only in a small amount).[102,103]



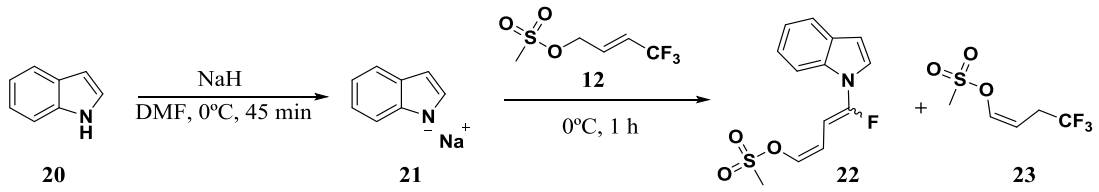
Scheme III.14 – Reactions of β-substituted trifluoropropenes.

It is also reported that trifluoromethylated alkenes, without other reactive functional groups easily react via S_N2' mechanisms, in the presence of nucleophiles (preferentially metallic) (scheme III.15).[104-106]



Scheme III.15 – Reactions of trifluoropropene derivatives with nucleophiles.

Surprisingly, under the experimental conditions of the reaction of compound **11b** and (*E*)-4,4,4-trifluorobut-2-en-1-yl methanesulfonate (**12**) fluorine is a better leaving group then mesyl (scheme III.11, page 79). The same reaction conditions were tested with the indole (**20**). Product **22** was isolated as a mixture of *Z*/*Z* and *Z*/*E* isomers (1:0.6) with 38% yield. It was also isolated a little amount of isomerized compound **23** in 16% yield (scheme III.16).



Scheme III.16 – Reaction of indole (20) with (*E*)-4,4,4-trifluorobut-2-en-1-yl methanesulfonate (12).

In order to understand the role of the solvent and reaction outcome, several reaction conditions were tested (table III.2).

Table III.2 – Conditions of the reaction of indole (20) with (*E*)-4,4,4-trifluorobut-2-en-1-yl methanesulfonate (12).

Entry	Solvent	Base (equiv.)	Products	Yield (%)
1	DMF	NaH (1)	22(*Z*/*Z*)(*Z*/*E*) (1:0.6) 23	38 16
2	DMF	NaH (0.25)	22(*Z*/*Z*)(*Z*/*E*) (1:0.6)	12
3	DMF	NaH (0.5)	22(*Z*/*Z*)(*Z*/*E*) (1:0.6)	22
4[a]	DMF	NaH (2)	22(*Z*/*Z*)(*Z*/*E*) (1:0.5)	83
5[b]	DMF	NaH (1)	22(*Z*/*Z*)(*Z*/*E*) (1:0.6) 23	47 1
6[c]	DMF	NaH (1)	22(*Z*/*Z*)(*Z*/*E*) (1:0.5) 23	32 40
7	MeCN	NaH (1)	22(*Z*/*Z*)(*Z*/*E*) (1:0.5)	31
8[d]	MeCN	NaH (1)	22(*Z*/*Z*)(*Z*/*E*) (1:0.6) 23	40 7
9	THF	NaH (1)	22(*Z*/*Z*)(*Z*/*E*) (1:0.6) 23	3 9
10[d]	THF	NaH (1)	22(*Z*/*Z*)(*Z*/*E*) (1:0.6) 23	25 19
11[d]	THF	^{t}BuOK (1)	22(*Z*/*Z*)(*Z*/*E*) (1:0.6) 23	29 6
12[e]	THF	*n*-BuLi (1)	---	---

[a] 2 equiv of 20 were used; [b] 5 times diluted; [c] inversion of reagents addition; [d] addition of crown ether (1 mol%); [e] no reaction occurred.

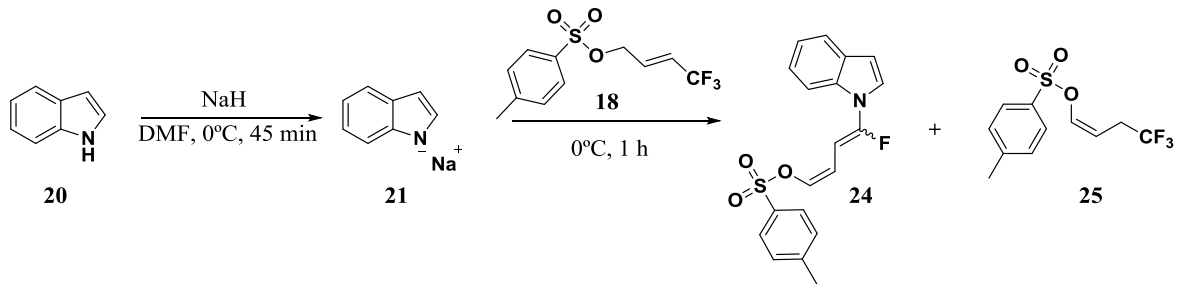
First experiments were carried in DMF and the base loading was investigated (table III.2, entries 1 to 4). The yield of **22** is dependent on the amount of NaH, with smaller amounts leading to lower product yields. In all experiments unreacted **20** was recovered. The dilution of the mixture had no influence on the reaction outcome (table III.2, entry 5). However, the use of 2 equivalents of the sodium salt of indole **21** (table III.2, entry 4), significantly increased the yield of **22**.

The nature of the metal revealed to be crucial for the fluorine elimination, since the formation of **22** was suppressed when *n*-BuLi was used (table III.2, entry 12), but the formation of products **22** and **23** still occurred when ^{t}BuOK was used (table III.2, entry 11).

The change in solvent influenced the products formation (table III.2, entries 7 and 9). When MeCN or THF were used, the yield of **22** decreased, and this might be due the poor solubility of compound **21** in these solvents. When crown ether was used (table III.2, entries 8 and 10) the yield of **22** increased. This yield improvement is due the formation of a complex between crown ether and the cations Na^+ or K^+, increasing the solubility of the formed salt.

The best results were obtained when DMF was used as solvent. Inverting the order of the reagents addition, addition of a suspension of **21** to a solution of **12**, enhanced the yield of the isomerized **23** supporting, that two competitive mechanisms take place.

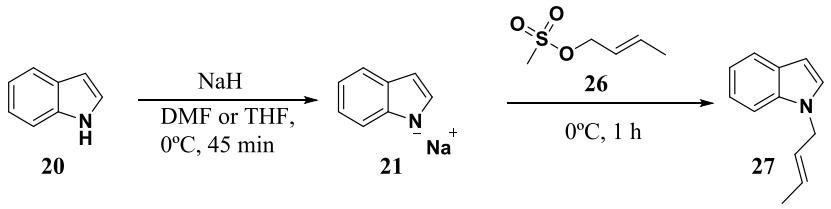
When (*E*)-4,4,4-trifluorobut-1-en-1-yl 4-methylbenzenesulfonate (**18**) was used the corresponding products **24** (17% yield as a mixture of *Z/Z*:*Z/E* 1:0.3) and **25** (51% yield) were isolated (Scheme III.17).



Scheme III.17 - Reaction of indole (20) with (E)-4,4,4-trifluorobut-1-en-1-yl 4-methylbenzenesulfonate (18).

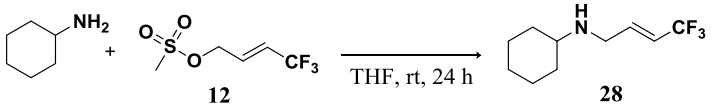
In any case was observed the formation of the S_N2 product. The importance of the electron-withdrawing effect of the CF_3 group on the reaction outcome was confirmed, as when this group was replaced by a methyl group (compound **26**). This reaction was performed using NaH as base, in DMF

and also using *n*-BuLi, in THF. In both cases, the S_N2 product **27** was obtained in 34 and 18% yield, respectively (scheme III.18).



Scheme III.18 - Reaction of indole (20) with but-2-en-1-yl methanesulfonate (26).

The corresponding S_N2 product **28** was also isolated, in 72% yield, when **12** reacted with cylohexylamine (scheme III.19).



Scheme III.19 – Reaction of cyclohexylamine with (*E*)-4,4,4-trifluorobut-2-en-1-yl methanesulfonate (12).

In all experiments the **23** *Z/Z* isomer was the major product. Structure **22** (*Z/Z* and *Z/E*) was assigned by ^1H, ^{13}C, ^{19}F NMR, and 2D experiments.

Figure III.17 shows an expansion of the allylic region of the ^1H-NMR spectrum of compound **22**. Each isomer presents only 3 protons in this region, the resonance signal of proton Hc appears as a doublet, and the resonance signals of protons Ha and Hb appear as double doublets.

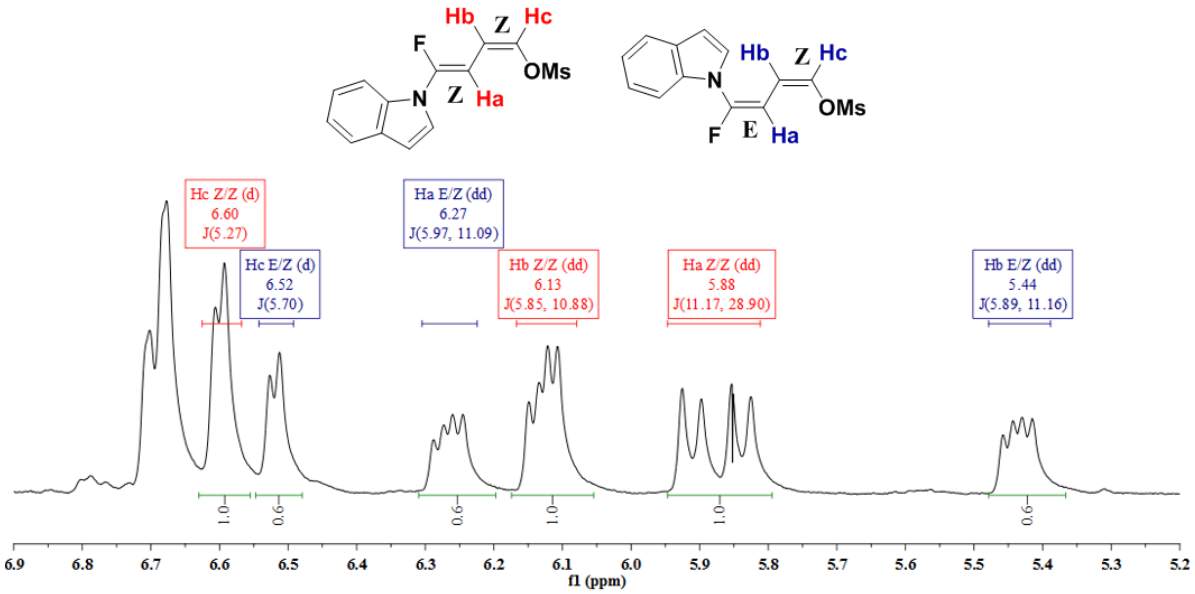


Figure III.17 – ^{1}H-NMR expansion of the allylic region (6.9-5.2 ppm) of the two isomers of compound 22, *Z/Z* and *Z/E*.

Isomers **22** *Z/Z* and *Z/E* were separated by HPLC and each isomer was characterized by ^{1}H, ^{13}C, ^{19}F NMR and 2D techniques and also by mass spectrometry.

The ^{1}H-NMR signal of proton Ha of the *Z/Z* isomer shows a coupling constant of 28.9 Hz with fluorine, while the *E/Z* isomer has a coupling constant of 5.9 Hz with this nucleus. These constants are in accordance with the ^{19}F-NMR of these two species (figure III.18) and confirm the proposed stereochemistry.[107]

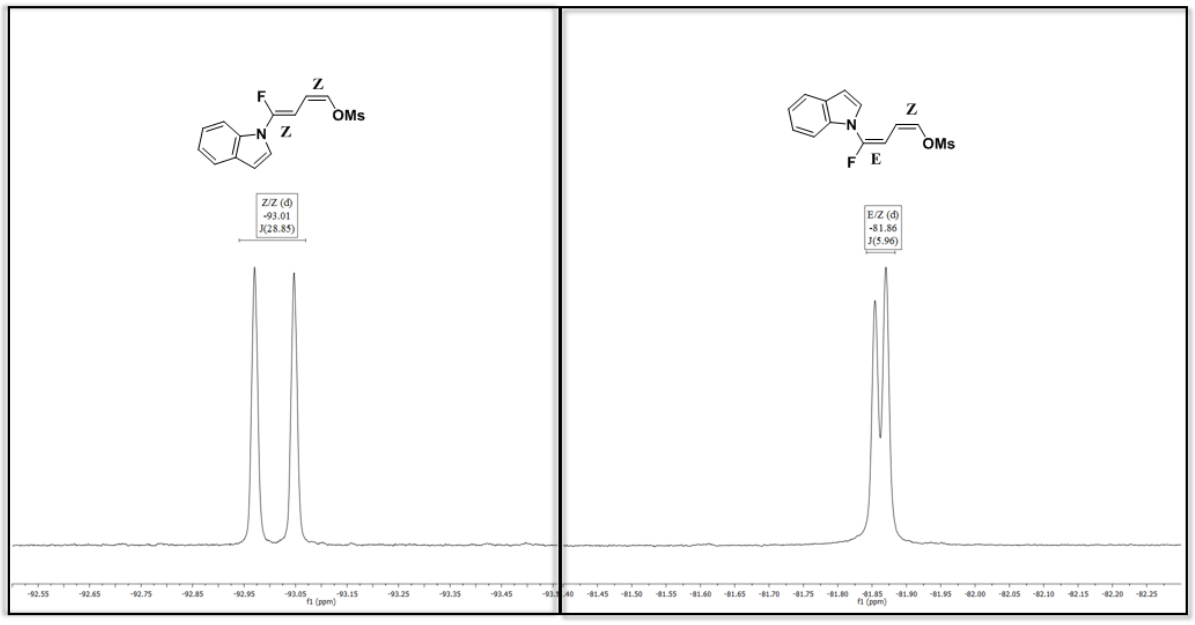


Figure III.18 – ^{19}F-NMR of the isomers *Z/Z* and *E/Z*, respectively, of compound 22.

The doublets found in ^{13}C-NMR confirm that only one fluorine atom is present (figure III.19).

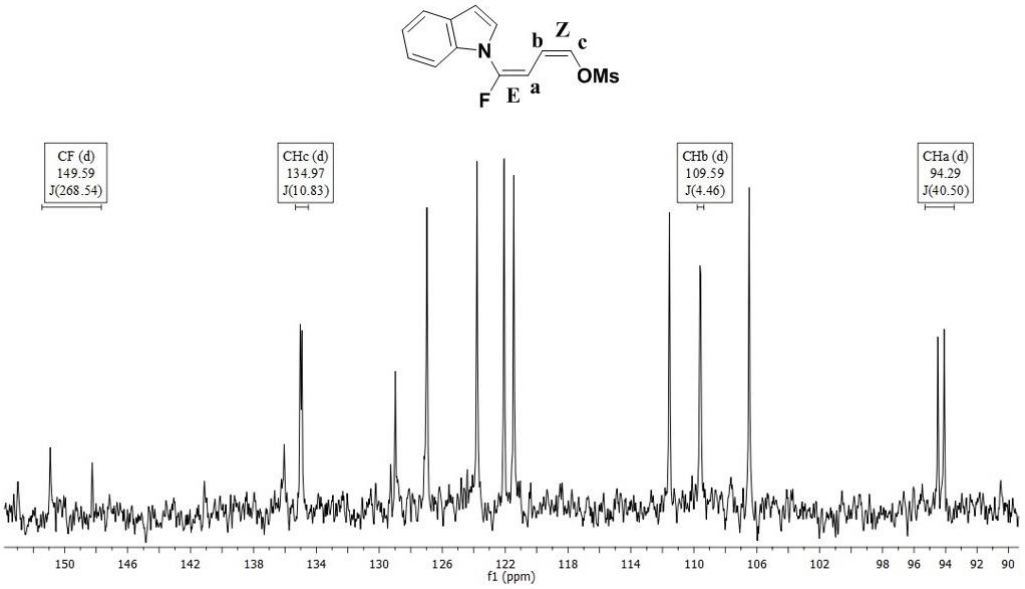


Figure III.19 – ^{13}C-NMR expansion of the isomer 22 *E/Z*.

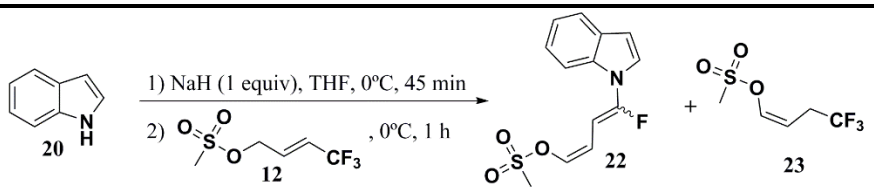
As stated above, fluoride elimination from trifluoro allylic chains is known, as a side product, under metal-mediated reactions. Yamazaki *et al*[108] reported the formation of difluorinated products during the Michael addition of organocopper species to 3-[(*E*)-4,4,4-trifluorobut-2-enoyl]oxazolidin-2-ones. The same group observed the formation of a difluorinated diene as a product in S_N2 reactions of Grignard reagents toward CF_3-containing allylic acetates, in the presence of CuCN and TMSCl.[109-112]

In contrast with the literature known substrates, compounds **12** and **18** have a good leaving group at β-position (mesyl or tosyl group, respectively), and as it is accepted that the C-F bond is strong and chemically stable, the formation of **22** from **12** under these experimental conditions was an unexpected reaction outcome.

In order to understand the mechanism of this reaction, GC-MS studies (GC-FI and GC-EI) were performed, since the identification of minor products and intermediates would be helpful in the disclosure of the mechanism.

Three assays were performed using the GC-FI technique consisting on the analysis of the: *a)* reaction crude without work-up; *b)* reaction crude after work-up but without purification and; *c)* reaction products isolated by PTLC (table III.3). For GC-EI, only the reaction crude without work-up was analyzed [condition *a)*]. These reactions were performed according to the reaction conditions described in table III.2, entry 9. Despite better yields were observed using DMF as solvent (table III.2) THF was used instead due to spectrometer requirements.

Table III.3 – GC-MS assays of the reaction of indole (20) with compound 12.



		Relative intensity (%)			
Retention time (min)	**$[M]^+$ (FI)**	**a)**	**b)**	**c)***	**Fragmentations (EI)****
11.77	203.99	49	64	100	126.02; 78.97
11.83	183.99			---	105.01
12.79	204.00	15	12	24	126.03; 108.99; 78.98
13.31	204.00; 184.00	17	22	---	125.01; 109.01; 78.97; 64.96
15.63	225.04	28	13	---	156.08; 116.05
16.15	225.06	32	27	---	156.08
17.93	117.06	100	100	---	90.02; 63.01; 58.52
20.21	195.05	11	8	---	116.04
20.77	225.08	5	---	---	---

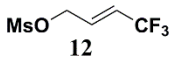
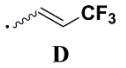
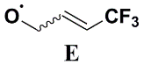
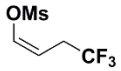
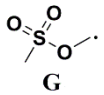
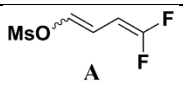
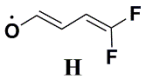
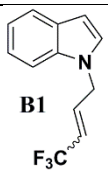
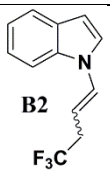
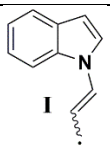
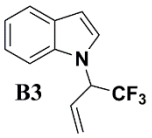
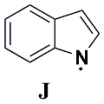
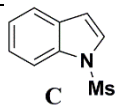
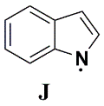
a) reaction crude without work-up; *b)* reaction crude after work-up but without purification and; *c)* reaction products isolated by PTLC.

--- not detected; *between 20-28 min there is a broad peak corresponding to a compound with a MW of 281.05. This occurs due to the retention of the compound in the column; **only for condition *a)*.

GC-MS spectra of the starting materials, indole (**20**) and (*E*)-4,4,4-trifluorobut-2-en-1-yl methanesulfonate (**12**), were also performed. Therefore, the peaks with a retention time of 17.93 min and 13.31 min corresponds to indole (**20**) and (*E*)-4,4,4-trifluorobut-2-en-1-yl methanesulfonate (**12**), respectively. Compound **12** co-elutes with a compound with MW of 184.00.

In table III.4 are represented some proposed structures and its fragmentations of the observed peaks obtained for the reaction crude without work-up [condition *a)*], by the GC-EI technique.

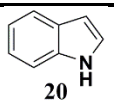
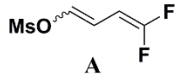
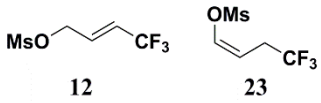
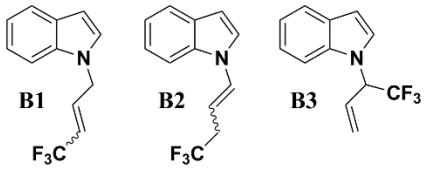
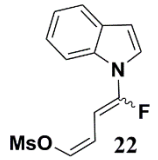
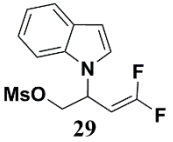
Table III.4 – Proposed structures for the peaks obtained from the GC-MS assays.

$[M]^+$	Proposed structure	Fragmentations	Proposed fragmentation structure
204.00; 203.99	 **12**	109.01	 **D**
		125.01	 **E**
	 **23**	78.97	 **F**
		108.99	 **G**
183.99; 184.00	 **A**	105.01	 **H**
225.04; 225.06	 **B1**  **B2**	156.08	 **I**
	 **B3**	116.05	 **J**
195.05	 **C**	116.05	 **J**

The same peaks were observed before and after work-up (table III.3, *a)* and *b)*, respectively), although with different relative intensities. Meaning that all the products are formed during the reaction. After purification by PTLC (table III.3, *c)*) two peaks corresponding to compounds **23** (100%) and **12** (24%) were observed. Also a broad peak with MW of 281.05, which corresponds to product **22**, was detected.

Once product **22** is retained in the column, and possibly other compounds, the same three assays *a)*, *b)* and *c)* were performed using MS-FI (table III.5, without heating and temperature on probe = 50ºC).

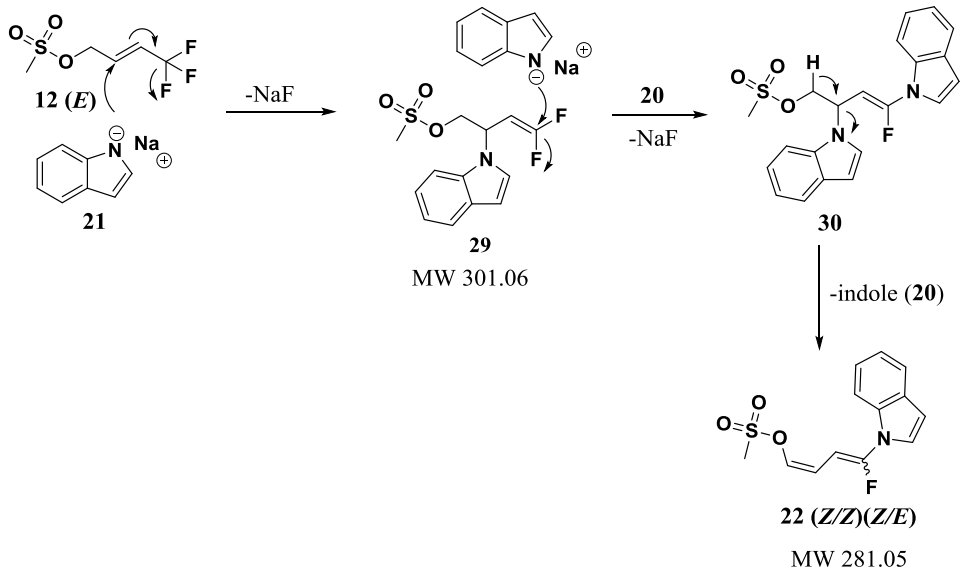
Table III.5 – MS (FI) assays of the reaction of indole (20) with compound 12, without heating and Tprobe = 50°C.

Relative intensity (%)								
[M+]	a)		b)		c)			Proposed structure
	without Δ	T = 50°C	without Δ	T = 50°C	without Δ	T = 50°C 1st	T = 50°C 2nd	
117.06	100	4	100	---	---	---	---	 20
184.00	4	1	15	---	---	11	---	 A
195.04	14	14	1	---	---	---	---	 C
203.99	25	7	34	5	100	100	5	 12 23
225.08	45	7	8	---	---	---	---	 B1 B2 B3
281.05	---	100	---	100	---	---	100	 22
301.06	---	12	---	---	---	---	5	 29

--- not detected *a)* reaction crude without work-up; *b)* reaction crude after work-up but without purification and; *c)* the mixture of isomers after purification by PTLC.

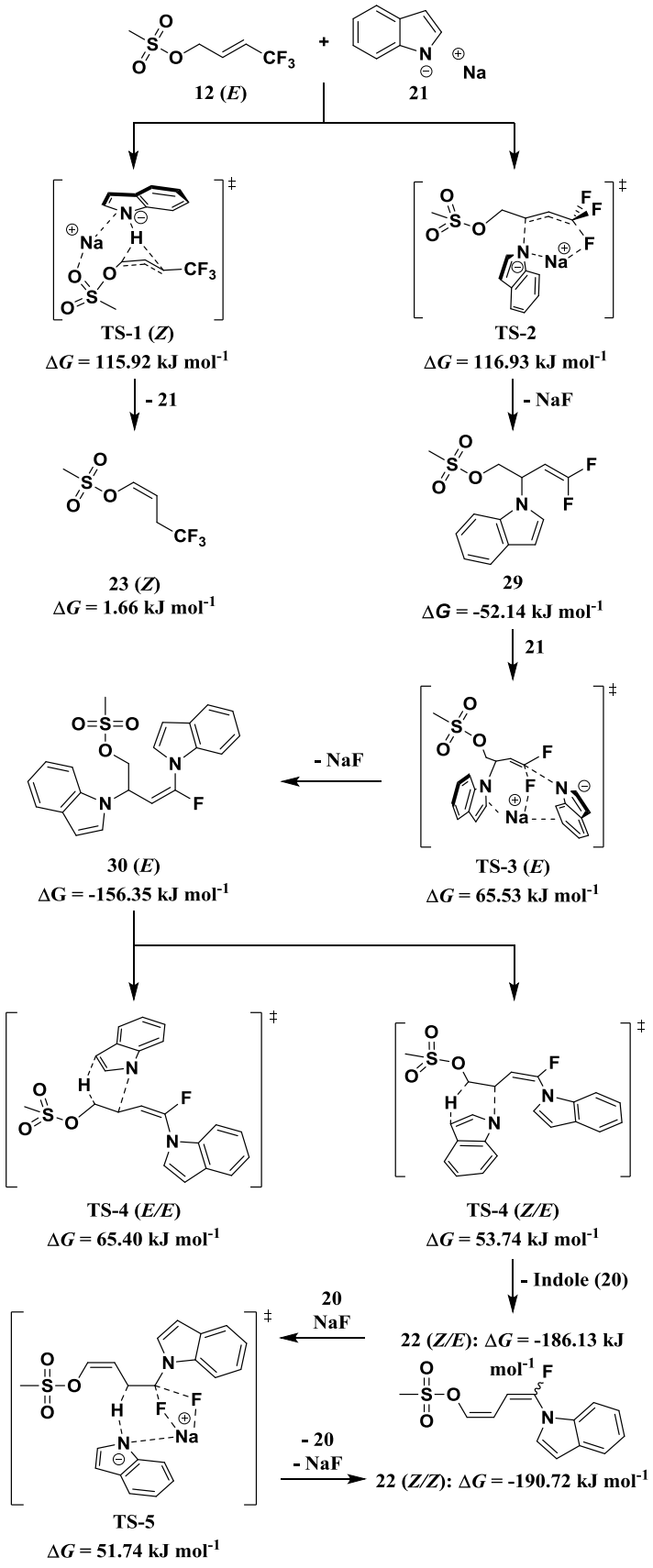
A new peak was observed corresponding to a compound with MW 301.06, but all the other peaks are the same observed using the GC-MS technique. Using the collected experimental data during reaction optimization and also the results from the mass spectrometry analysis, an explanation for the reaction mechanism was attempted.

To achieve a proposal of a possible mechanism, a full density functional theory (DFT) study was performed,[113] by calculating all the intermediates (some of them observed by MS) and transition states (TSs) along the possible reaction pathways (these calculations were performed by Dr. Filipe Duarte under the supervision of Prof. Gil Santos from FCT-UNL). In scheme III.20 is depicted a proposed mechanism on the basis of the calculated data, which indicates that the reaction occurs in three steps.



Scheme III.20 – Proposed mechanism for the formation of 22.

Scheme III.21 details the mechanism by presenting all the important TSs and intermediates as well as their relative energies. In all TSs it is very important to consider the explicit participation of a sodium ion. In the absence of this ion the TS energies become extremely high or the TS structures cannot even be obtained, which is in agreement with the experimental data.



Scheme III.21 - Proposed mechanism for the formation of 22. B3LYP/6-311++G(2df,2p)//B3LYP/6-31G(d,p), DMF, T = 25 °C (similar conclusions at 0 °C), radii = uaks.

The energy difference between the double-bond migration (**TS-1**) and the indole addition with concerted elimination of fluorine (**TS-2**) is minimal. Thus, these two pathways can co-exist and one or the other can be dominant depending on the reaction conditions (solvent, temperature, concentration), as was experimentally observed. The *Z* isomer of **23** is preferentially formed due to strong restrictions on the TS conformation, as the sodium ion has to coordinate between the mesyl and the indole groups.

While compound **23** (*Z*) is a dead-end, intermediate **29** can undergo flourine substitution by indole anion to afford intermediate **30**. According to calculations, only the *E* isomer of **30** can be formed, due to the lack of important electrostatic interactions in the TS with Z configuration. An alternative mechanism for the formation of **30** was tested, via an addition-elimination process, in which the indole anion adds to the intermediate **29** to form an anionic intermediate that undergoes fluorine elimination to originate **30**.

However, this possibility is not discussed, as it originates considerably higher energetic TSs. Indole can be eliminated from intermediate **30** via two diastereomeric concerted TSs (**TS-4**), but high selectivity is expected, as **TS-4** (*Z/E*) is ca. 11.5 kJ mol^{-1} less energetic than **TS-4** (*E/E*). Thus, product **22** (*Z/E*) is predicted to be preferentially formed, which does not fully agree with the experiment. Indeed, while the double-bond at the mesyl group side was experimentally obtained in *Z* configuration, the second double-bond was obtained as a *Z/E* mixture, with preference for the *Z* configuration. Though, this result can be explained, as the indole can catalyze the conversion of **22** (*Z/E*) into **22** (*Z/Z*), via **TS-5**, thus allowing for a mixture of configurations experimentally observed. The conclusion is that while structure **22** (*Z/E*) is formed under kinetic control, isomer **22** (*Z/Z*) is formed under thermodynamic control, which explains the relative variable amounts of both isomers, depending on the reaction conditions.

An unexpected reactivity was observed when compounds **12** and **18** were treated with indole sodium salt (**21**). A mechanism was proposed, based on DFT studies, which fully rationalizes the experimental data. The proposed mechanism identifies the importance of the metal ion and the reaction conditions on the final reaction outcome.

III.2.3 Studies towards a new methodology for the synthesis of sulfonyl-containing compounds.

Recently, new strategies have been introduced to progress the sustainability of organic synthesis (scheme III.1, page 70). The main objective of this study was the development of a novel methodology for carbon-sulfur bond formation *via* a sustainable route. This would constitute a platform for the efficient synthesis of compounds with a sulfonamide group – a crucial and unique functional group present in many relevant therapeutic drugs, such as COX-2 selective inhibitors.

Recently, there has been significant interest in the coupling of sulfinic acid salts with aryl halides to prepare sulfones and vinyl sulfones,[114] and an improvement in the metal-mediated coupling of sulfinic acid salts,[115,116] such as the Ullmann-type reaction.[85] However, these methods are extremely limited, since only a small number of sulfinic acid salts are commercially available (tolyl, phenyl and methyl), or require the preparation of complex template starting materials, suffer from harsh conditions, high costs of metal reagents and/or corresponding ligands. Furthermore, most of these methods are not atom economic.

A proposal to overcome these drawbacks is the development of an atom economy approach to the synthesis of sulfones, by developing a versatile and recyclable sulfonylation agent.

The strategy consisted on the immobilization of a heterocycle scaffold such as a benzotriazole ring, or equivalent, on a solid-support, and prepare the corresponding sulfinic acid salt. Next step would consist on the use transition metal-mediated coupling of this sulfinic acid salt with several carbon electrophiles (halides, boronic acids) allowing *in situ* formation of an intermediate that can be further converted to sulfones, sulfonamides, and other derivatives, in a one-pot procedure by addition of a suitable nucleophile. The ultimate formation of the sulfone, or derivative, would be the detachment of the product from the solid-support leaving the benzotriazole, or equivalent, moiety ready for the next sulfinic acid formation (figure III.20). This approach would enable recycling of the intermediate used without any loss of organic material, constituting a new platform for the preparation of a very versatile sulfinic acid salt.

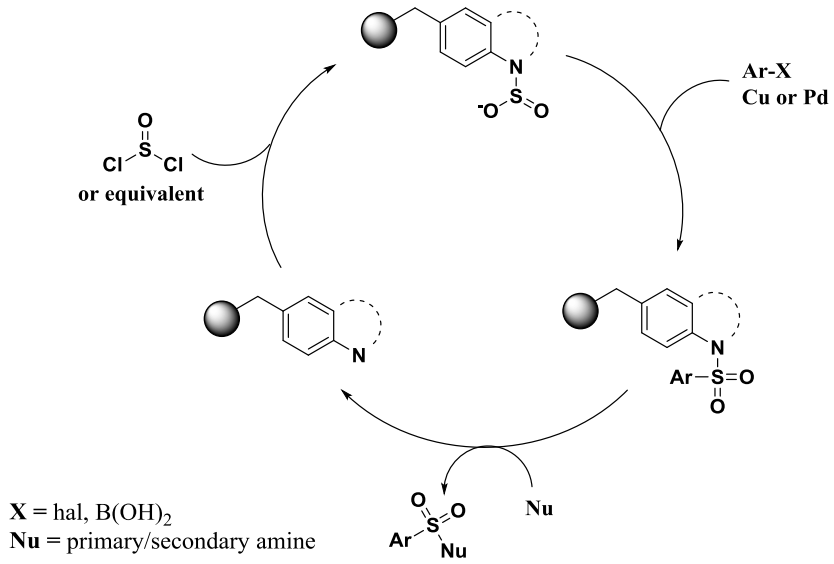
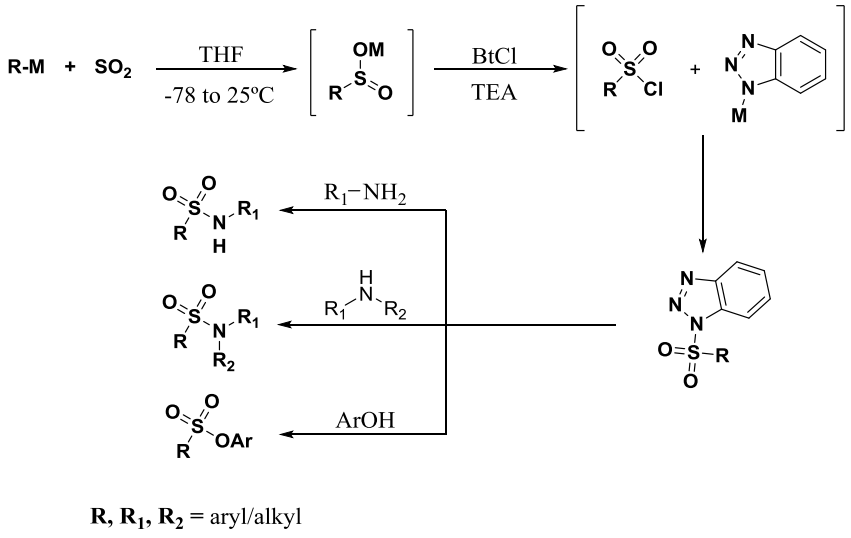


Figure III.20 – Proposed strategy for the sulfonylation.

From the synthetic strategies available for C-S bond formation, the Ulmann reaction, direct coupling of aryl halide with a sulfinic acid salt, shows some advantages.

Thus, the first objective of this study was to synthesize an intermediate that would be both versatile and react under mild conditions. A solution-phase approach was first attempted to establish the sulfonylation route. The use of *N*-substituted benzotriazole unit, an interesting scaffold for sulfinic acid salt formation, was investigated. In order to prepare the key sulfonic acid salt, several routes were explored.

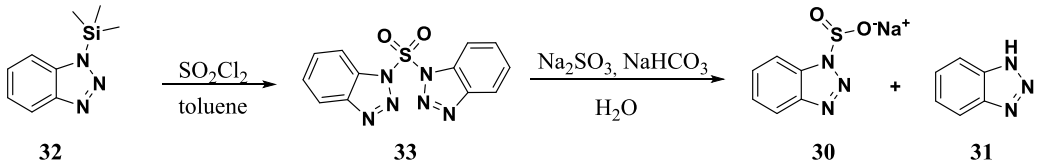
The first choice relied on the use the sulfonylbenzotriazole moiety,[72] due to its interesting properties as leaving group in its reactions with *N*- and *O*-nucleophiles, as well as it replaces the highly reactive and often difficult to access sulfonyl halide unit. Katriztky has demonstrated the use of benzotriazole (Bt) in the preparation of sulfonamides. However, the preparation of the sulfonylated derivative consisted on a multi-step synthesis, involving the use of Grignard reagents and SO_2 (scheme III.22).[72]



R, R_1, R_2 = aryl/alkyl

Scheme III.22 – The use of benzotriazole in the preparation of sulfonamides, reported by Katriztky *et al*.[72]

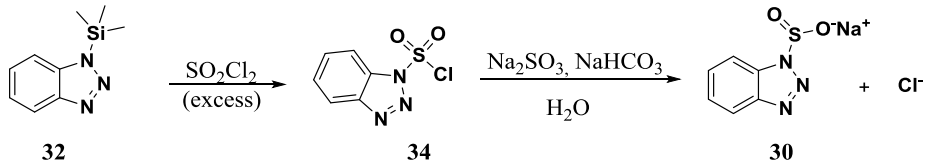
Therefore, based on a different approach developed by the same author[117] the first attempt consisted on the synthesis of sodium benzotriazolesulfinate (**30**) (scheme III.23). Thus, this molecule has a nucleophilic sulfur atom (sulfinate) and a good leaving group (benzotriazole, **31**), allowing the attack from a nucleophile in an advanced stage. The strategy adopted consisted on the treatment of 1-trimethylsilylbenzotriazole (**32**) with sulfuryl chloride, affording 1,1'-sulfonylbis(1*H*-benzotriazole) (Bt_2SO_2, **33**), as described in the literature.[117] Then, compound **33** was treated with sodium sulfite and sodium hydrogenocarbonate, in aqueous media[118] (scheme III.23).



Scheme III.23 – Synthetic pathway adopted for the synthesis of compound 30.

It was expected to obtain compound **30** and benzotriazole (**31**). However after several attempts to separate **31** from the crude, such as: filtration with diethyl ether or acetone; hot filtration with dichloromethane in a soxhlet extractor; recrystallization with methanol/diethyl ether; and purification by HPLC; only benzotriazole (**31**) was isolated and identified.

To avoid having benzotriazole (**31**) as a side product, it was envisaged that benzotriazole-1-sulfonyl chloride (**34**) could be achieved from **32** after treatment with an excess of sulfuryl chloride. This way, after treatment with sodium sulfite and sodium hydrogenocarbonate, the leaving group would be the chlorine anion instead of benzotriazole (**31**) (scheme III.24).

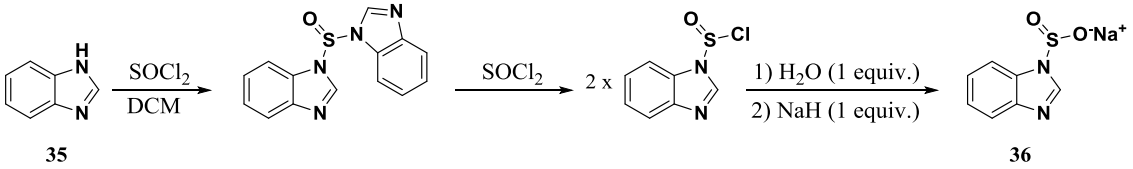


Scheme III.24 – Another synthetic pathway adopted for the synthesis of compound 30.

When a slight excess (1.1 equiv.) of sulfuryl chloride was used, compound **33** was afforded, in 41% yield, instead of the desired product **34**. To overcome that result, an excess (5 equiv.) of sulfuryl chloride was used. However, benzotriazole (**31**) was isolated, independently of the order of addition of the reagents (addition of SO_2Cl_2 to compound **32** or addition of compound **32** to SO_2Cl_2).

An alternative approach was attempted with benzimidazole (**35**) because it was envisaged that the corresponding sulfinic acid salt would be more stable under the reaction conditions.

A method described in the literature for the synthesis of 1-substituted benzimidazoles,[119] was adopted to synthesize sodium benzimidazolesulfinate (**36**) (scheme III.25).



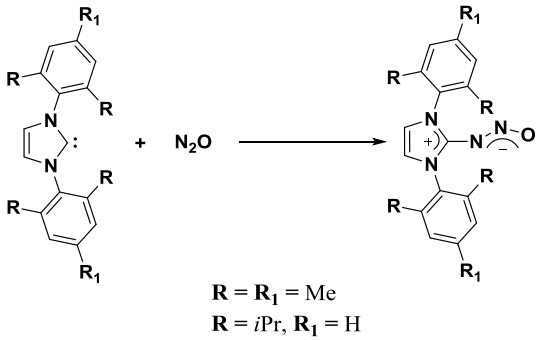
Scheme III.25 - Synthetic pathway adopted for the synthesis of compound 36.

Unfortunately, instead of obtaining the sulfinic sodium salt (**36**), the product isolated was the sodium salt of the benzimidazole.

Despite all efforts to synthesize either sodium benzotriazole or benzimidazole sulfinic salts, all the attempts revealed unsuccessful, thus this strategy was abandoned.

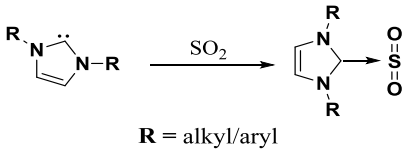
After the unsuccessful results obtained, an alternative method was investigated and the following route was focused on the use of *N*-heterocyclic carbenes (NHCs). Since the first reports of stable nucleophilic carbenes by Arduengo *et al.*[120] in the early 1990s, the broad application of NHCs in organic synthesis has been widely demonstrated and their corresponding metal complexes have attracted enormous attention in the past two decades.[121,122] The broad application of NHCs in organic synthesis has been impressively demonstrated. The usefulness of NHCs is due to the relative ease of synthetic preparations, nearly independent manipulations of the electronic and steric properties, and the strong σ-donating character. Recently, it has been demonstrated that NHC·CO_2 adducts are readily formed by reaction of virtually all types of NHCs with carbon dioxide.[123-125] This zwitterionic adducts have been

used as NHC transfer agents in organometallic chemistry for the synthesis of NHC metal complexes and lately as organic (pre)catalyst. Moreover, the formation of NHCs adducts with sulfur dioxide[126] and more recently with nitrous oxide[127] (scheme III.26) have been also reported.



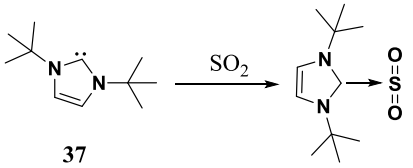
Scheme III.26 – Reaction of NHCs with nitrous oxide.[127]

Therefore, the strategy adopted in this study consisted on reacting a preformed NHC with sulfur dioxide, in order to afford a carbene·SO_2 adduct (scheme III.27). This adduct would result from SO_2 capture by the NHC for a subsequent transfer of this moiety to an aromatic unit.



Scheme III.27 – Proposed formation of the carbene·SO_2 adduct.

The first choice relied on the use of carbene 1,3-di-*tert*-butylimidazol-2-ylidene (**37**) (scheme III.28), that is a stable carbene and was readily available (kindly provided by Professor Teresa Avilés, from FCT-UNL).

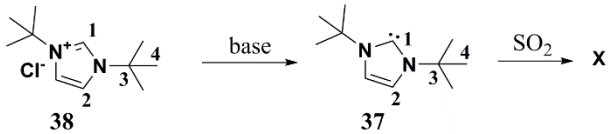


Scheme III.28 – Reaction of carbene 37 with SO_2.

For the SO_2 capture different reaction conditions were tested, such as neat conditions (the solvent was liquid SO_2) or use of dry and degassed organic solvents (toluene or Et_2O and bubbling SO_2 gas). In table III.6 are listed the chemical shifts of the corresponding imidazolium chloride **38**,[128] the corresponding carbene **37**,[129] as well as the spectroscopic data and elemental analysis of the obtained

crudes (**X**) from the three different reaction conditions. The reaction crudes were analyzed after evaporation of the reaction solvent, and without further purification.

Table III.6 - Chemical shifts and elemental analysis of 1,3-di-*tert*-butylimidazolium chloride (38), 1,3-di-*tert*-butylimidazol-2-ylidene (37) and reaction crudes (X) from the different tested conditions.



38 37

		Compound **38**[128]	Compound **37**[129]	**Reaction conditions (X)**		
				neat	toluene	Et_2O
NMR solvent		*DMSO-d_6*	*C_6D_6*	*$CDCl_3$*	*DMSO-d_6*	*DMSO-d_6*
^{1}H-NMR (δ ppm)	H1	9.37 (s, 1H)	---	10.17 (s, < 1H) 9.59 (s, < 1H)	9.88 (s, < 1H) 9.17 (s, < 1H) 9.06 (s, < 1H)	9.88 (s, < 1H) 9.05 (s, < 1H)
	H2	8.11 (s, 2H)	6.79 (s, 2H)	7.56 (s, 2H)	8.07 (s, 2H)	8.07 (s, 2H)
	H4	1.60 (s, 18H)	1.51 (s, 18H)	1.71 (s, 18H)	1.61 (s,18H)	1.61 (s, 18H)
^{13}C-NMR (δ ppm)	C1	132.7	212.9	134.2	132.2	132.3
	C2	120.4	115.0	120.2	120.4	120.5
	C3	59.6	55.8	60.9	59.6	59.6
	C4	29.1	31.5	30.1	29.1	29.1
Elemental analysis		---	---	Calcd.: C, 54.07; N, 11.46; H, 8.25; S, 13.12 Found: C, 47.11; N, 9.62; H, 6.88; S, 10.50	---	Calcd.: C, 54.07; N, 11.46; H, 8.25; S, 13.12 Found: C, 49.20; N, 10.13; H, 7.33; S, 11.27

All the reaction crudes (**X**) presented in the ^{1}H NMR spectra signals that correspond to the resonance of H1 proton. However, the final result from the signals integration showed that this signal does not integrate for one proton (figure III.21). This fact indicated that there are different species of the imidazolium salt presented in the final mixture. Even though, the elemental analysis revealed the presence of sulfur atom. Additionally, IR spectra showed bands at 1040 and 968 cm^{-1}, characteristics of S-O bond stretching. All the data collected might indicate that the sulfur dioxide is interacting with the imidazolium salt (perhaps is coordinating with the nitrogen atoms -NCN- of the imidazolium salt), instead of being coordinated with the -NCN- carbon as it would be expected. The crudes composition, from the different reaction conditions used, revealed themselves to be very similar, since the ^{1}H-NMR spectra were similar. This indicates that the formation of these species was independent of the solvent used.

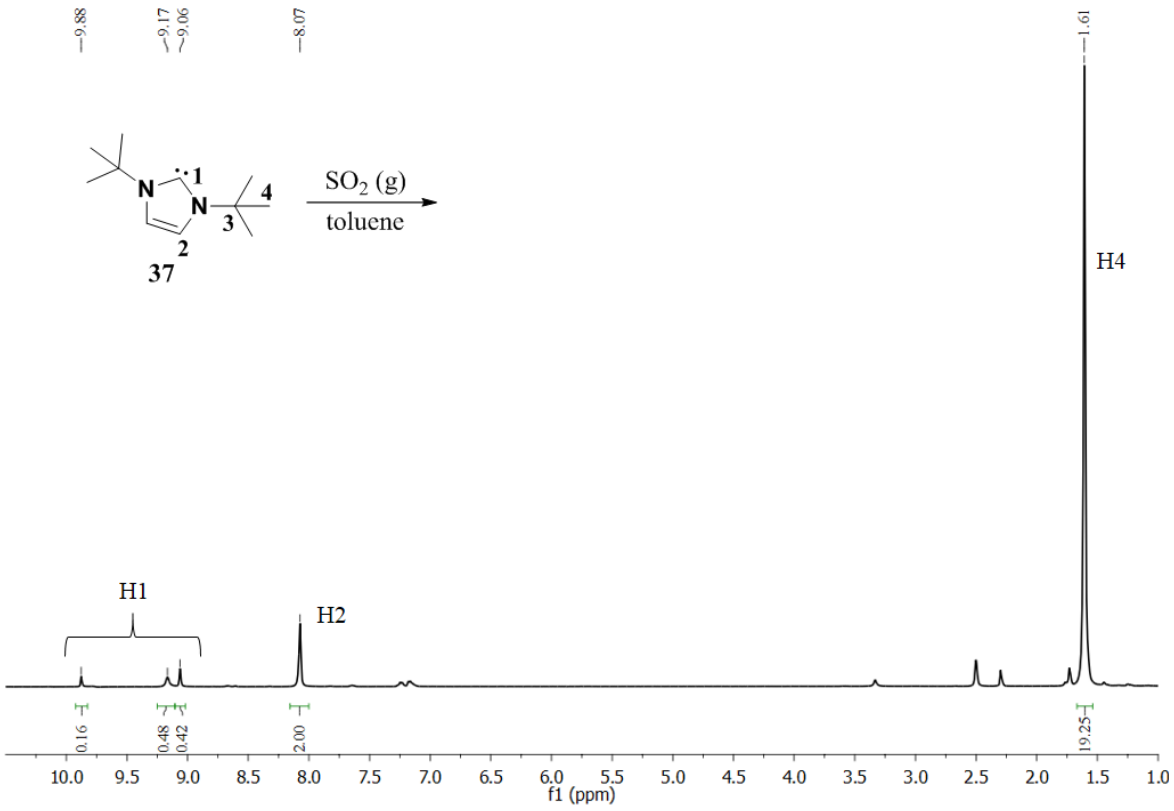
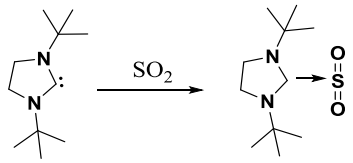


Figure III.21 – ^{1}H-NMR spectrum of the crude from the reaction of carbene 37 with SO_2, in toluene, performed in *DMSO-d_6*.

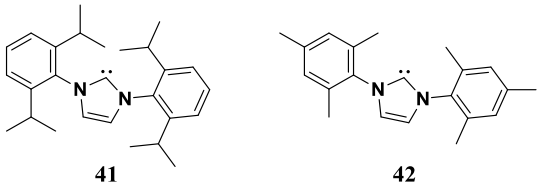
Another NHC, 1,3-di-*tert*-butylimidazolidine-2-ylidene (**39**), was chosen, since according to the literature,[126] this would afford a stable adduct with SO_2 (scheme III.29).



39 **40**

Scheme III.29 – Reaction of carbene 39 with SO_2.

Despite the authors claimed that the product is a stable adduct, it was not possible, in this work, to isolate compound **40**. Due to the difficulties to reproduce the reported procedure it was conceived that this NHC was not nucleophilic enough and/or did not afford a stable product. Thus, two different carbenes were tested, 1,3-bis-(2,6-di-*iso*-propylphenyl)imidazolin-2-ylidene (**41**) and 1,3-bis(2,4,6-trimethylphenyl)imidazol-2-ylidene (**42**) (figure III.22) (kindly provided by Professor Teresa Avilés, from FCT-UNL). Both possess a substituted benzene ring in the nitrogen atoms that would confer more stability to the carbene, as well as to the product.

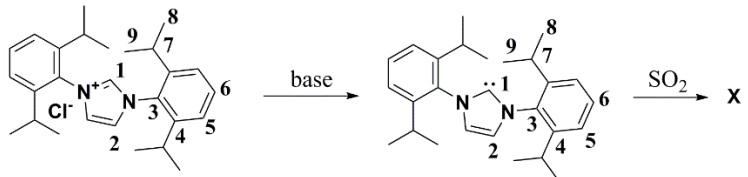


41 **42**

Figure III.22 – Structures of the carbenes 41 and 42, respectively.

Three experiments were performed with carbene **41**: *a)* reaction of **41** in liquid SO_2 (neat conditions); *b)* reaction of a solution of **43**, in dry and degassed THF, bubbled with SO_2 (g) and; *c)* a one-pot reaction which consisted on the treatment of a solution of 1,3-bis(2,6-di-*iso*-propylphenyl)imidazolinium chloride (**43**), in dry THF, with LiHMDS and stirred for 2 h, followed by bubbling of SO_2 (g). The crude from the reaction performed under conditions *a)* was analyzed after removal of the solvent and without further purification, while the obtained crude from the reaction performed under conditions *c)* was washed with dry Et_2O and was analyzed without further purification. Table III.7 summarizes the spectroscopic data collected for compounds **41** and **43** and for the crudes (**X**). ^{1}H-NMR spectrum of the reaction condition *b)* showed a complex mixture of products and therefore this was not further analyzed.

Table III.7 - Chemical shifts of 1,3-bis(2,6-di-*iso*-propylphenyl)imidazolinium chloride (43), 1,3-bis-(2,6-di-*iso*-propylphenyl)imidazolin-2-ylidene (41) and, reaction crudes (X) from the different tested conditions.



43 41

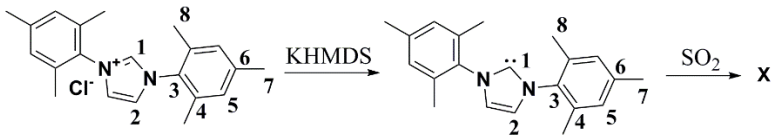
		Compound **43**	Compound **41**	**Reaction conditions (X)**	
				a)	c)
NMR solvent		*$CDCl_3$*	*C_6D_6*	*d_8-THF*	*DMSO-d_6*
^{1}H-NMR (δ ppm)	H1	10.02 (s, 1H)	---	10.60 (s, 1H)	10.60 (s, 1H)
	H2	8.13 (s, 2H)	6.61 (s, 2H)	8.23 (s, 2H)	8.55 (s, 2H)
	H5	7.35 (d, *J* = 7.7 Hz, 4H)	7.16 (4H, *under the solvent peak*)	7.39 (d, *J* = 7.7 Hz, 4H)	7.52 (d, *J* = 7.6 Hz, 4H)
	H6	7.57 (t, *J* = 7.7 Hz, 2H)	7.31-7.28 (m, 2H)	7.54 (t, *J* = 7.7 Hz, 2H)	7.68 (t, *J* = 7.6 Hz, 2H)
	H7	2.50-2.38 (m, 4H)	2.61-2.47 (m, 4H)	2.61-2.47 (m, 4H)	2.36-2.32 (m, 4H)
	H8	1.28 (d, *J* = 6.5 Hz, 12H)	1.28 (d, *J* = 7.0 Hz, 12H)	1.26-1.25 (m, 24H)	1.25 (d, *J* = 6.8 Hz, 12H)
	H9	1.24 (d, *J* = 6.6 Hz, 12H)	1.18 (d, *J* = 7.0 Hz, 12H)		1.15 (d, *J* = 6.8 Hz, 12H)
^{13}C-NMR (δ ppm)	C1	138.7	220.4	141.7	---
	C2	126.9	121.4	127.5	
	C3	130.0	138.8	131.9	
	C4	145.1	146.1	146.4	
	C5	124.9	123.5	125.2	
	C6	132.3	128.8	132.4	
	C7	29.3	28.6	30.0	
	C8	24.9	24.6	24.8	
	C9	23.9	23.5	24.1	

a) reaction of **41** in liquid SO_2 (neat conditions); *c)* a one-pot reaction which consisted on the treatment of a solution of **43**, in dry THF, with LiHMDS and stirred for 2 h, followed by bubbling of SO_2 (g).

Both reaction crudes, from reaction conditions *a)* and *c)*, showed, on the ^{1}H NMR spectra, a signal that corresponds to a proton bonded to C1, and which the chemical shift is similar to the one from the starting imidazolium **43.** Also, the IR spectra did not present any bands at ≈ 1030, ≈ 980 cm^{-1}, characteristics of the S-O bond, contrary to the preliminary reactions carried with carbene **37**, which indicates the absence of SO_2 in these mixtures.

One experiment was still performed with 1,3-bis(2,4,6-trimethylphenyl)imidazol-2-ylidene (**42**), that consisted in reacting carbene **42** (pre-formed by treatment of **44** with KHMDS, in dry toluene, followed by filtration through celite and removal of the solvent), in a solution of dry THF, with a saturate atmosphere of SO_2 (g). The obtained crude was washed with dry pentane and dried under vaccum, affording a beige solid that was analyzed without further purification. Table III.8 summarizes the elemental analysis data and NMR data obtained for the 1,3-bis(2,4,6-trimethylphenyl)imidazolinium chloride (**44**), for the carbene **42** and for the reaction crude (**X**).

Table III.8 - Chemical shifts and elemental analysis of 1,3-bis(2,4,6-trimethylphenyl)imidazolinium chloride (44), 1,3-bis(2,4,6-trimethylphenyl)imidazol-2-ylidene (42) and reaction crude (X).



44 **42**

		Compound **44**	Compound **42**	Reaction crude (**X**)
NMR solvent		*$CDCl_3$*	*C_6D_6*	*$CDCl_3$*
^{1}H-NMR (δ ppm)	H1	10.62 (s, 1H)	---	9.37 (s, 1H)
	H2	7.66 (s, 2H)	6.81 (s, 4H)	7.67 (s, 2H)
	H5	6.94 (s, 4H)	6.49 (s, 2H)	6.99 (s, 4H)
	H7	2.27 (s, 6H)	2.16 (s, 18H)	2.31 (s, 6H)
	H8	2.10 (s, 12H)		2.10 (s, 12H)
^{13}C-NMR (δ ppm)	C1	130.7	219.2	130.7
	C2	124.8	120.6	125.3
	C3	141.2	137.3	141.4
	C4	134.1	135.4	134.3
	C5	129.9	129.1	129.9
	C6	139.4	139.3	138.2
	C7	21.1	21.1	21.3
	C8	17.6	18.1	17.5
Elemental analysis		---	---	Calcd.: C, 68.45; N, 7.60; H, 6.56; S, 8.70 Found: C, 60.63; N, 6.55; H, 6.28; S, 7.01

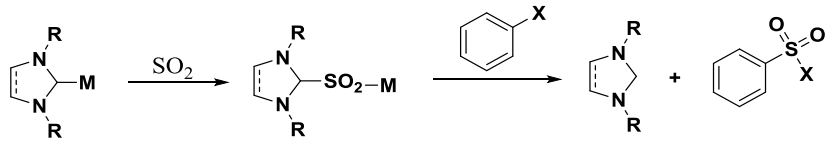
Once more the ^{1}H NMR of the crude showed a proton bonded to C1, nevertheless elemental analysis proved that the crude contains sulfur. Moreover, there is a difference, of more than 1 ppm, of the chemical shift of the H1 proton of the imidazolium **44** and the chemical shift of the same proton of the obtained crude **X** (10.62 and 9.37 ppm, respectively). Despite that, the chemical shifts of the C1 carbon for both species are the same, 130.7 ppm. The obtained results by NMR spectroscopy suggest that the sulfur dioxide is present in the crude **X** by interaction with the imidazolium salt (perhaps by coordination with the nitrogen atoms -NCN- of the imidazolium salt).

Unfortunately, all the attempts to isolate carbene·SO_2 were ineffective. Also, it was not possible to identify the structure of the formed reaction product(s).

According to the literature, sulfur dioxide has the ability to insert between carbon-metal bonds (R-M).[130] Sulfur dioxide insertion reactions may be represented by the equation:

$$M\text{-}R + SO_2 \longrightarrow M\text{-}(SO_2)\text{-}R$$

where M stands for a metal together with its ancillary ligands and R is an alkyl or a related σ-bonded carbon group. Thus, for future work, one way to isolate the SO_2·carbene adduct could consist on the trapping the sulfur dioxide *via* in a carbon-metal bond for further transfer to an aromatic moiety (scheme III.30).



R = alkyl/aryl

Scheme III.30 – Reaction of insertion of sulfur dioxide in a carbene-metal bond and further transfer to an aromatic moiety.

III.3 Conclusions

An indole based library was synthesized and its ability to selectively inhibit COX-2 was evaluated. From the 19 new indole based compounds synthesized, the biological tests revealed that the presence of a sulfonamide was more favorable for interaction with both COXs, being more active than the corresponding methylsulfones. Compound **17d** was found to be the most promising, showing 67 ± 5% COX-2 inhibition at 50 μM, suggesting that fluorine seems to be crucial, as this inhibition percentage is close to the one of celecoxib (75 ± 8%) at 10 μM. STD-NMR studies performed with **17d** highlighted that the sulfonamide group is indeed important to promote the interaction with COX-2 rather than with COX-1, supporting the observed selectivity in the inhibition evaluation and this fact was also confirmed by docking studies of compound **17d** (figure III.23).

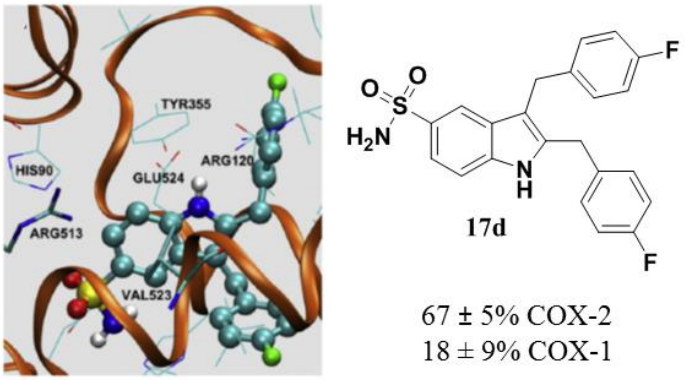


Figure III.23 – Docking and chemical structure of compound 17d.

During the preparation of the fluorinated compounds from the indole-based library, an unexpected reactivity was observed when compounds **12** and **18** were treated with indole sodium salt (**21**). A mechanism was proposed, based on DFT studies, which fully rationalizes the experimental data. The proposed mechanism identifies the importance of the metal ion and the reaction conditions on the final reaction outcome.

The prepared and tested indole-based library required the presence of a sulfonamide or amethyl sulfone groups. Thus, several attempts were made to synthesize a new versatile reagent for the synthesis of sulfonamides. The first attempt consisted on the synthesis of sodium benzotriazole/benzimidazole sulfinates, by reaction of benzotriazole/benzimidazole with sulfuryl or tionyl chloride. The second attempt relied on the use of a carbene·SO_2 adduct. . Both approaches revealed to be ineffectual for the proposed goal, and further work must be performed to explore the metal-catalyzed approaches to generate a versatile sulfonylating reagent.

III.4 Experimental

III.4.1 Synthesis, biological evaluation, docking and STD-NMR studies of an indole based library as COX-2 selective inhibitors

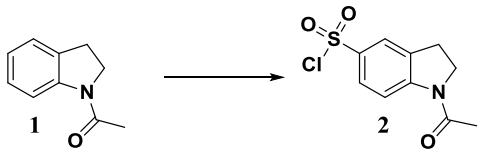
III.4.1.1 General

Melting points were determined on a Reichert Thermovar apparatus and are uncorrected. ^{1}H- and ^{13}C-NMR spectra were recorded in *$(CD_3)_2CO$*, *DMSO* or *$CDCl_3$* on a Bruker ARX 400 spectrometer at 400 and 100.62 MHz respectively. Chemical shifts are expressed in parts per million (ppm) relative to tetramethylsilane (TMS). The coupling constants (*J*) are reported in Hertz (Hz). High resolution mass spectra were recorded on an AutoSpecQ spectrometer. IR spectra were run on an FT PerkinElmer 683 instrument, with absorption frequencies expressed in reciprocal centimeters. The progress of all reaction was monitored by thin-layer chromatography, which was performed on Merck silica gel 60 F254 plates. Flash column chromatography was carried out on Merck silica gel 60 (230-400 mesh). For preparative thin layer chromatography was used Merck silica gel 60 GF_{254} in 20x20 glass plates. Anhydrous solvents were dried as described[131] and freshly distilled. All the tested compounds possess a purity of at least 95% as determined by HPLC. Analytical HPLC was run on a Merck Hitachi system consisting of an L-7100 pump, Rheodyne type injector, a D-7000 interface and an L-7450 diode array spectrometric detector, equipped with LiChrospher®100 RP-18 column. Eluent system was: 20% A (H_2O/TFA pH 2.5), 80% B (MeOH) to 10% A, 90% B; flow rate = 1 mL/min.

A microplate reader (Synergy HT, BIO-TEK), was used to perform the spectrophotometric readings in COX-1 and COX-2 inhibition assays. The COX-1 and COX-2 assay kit was obtained from Cayman Chemical Co.

The STD-NMR spectra were acquired at 37ºC in a Bruker Avance III spectrometer operating at 600.13 MHz, with a 5 mm triple resonance cryogenic probehead.

*III.4.1.2 Synthesis of 1-acetylindoline-5-sulfonyl chloride (**2**)[84]*



In a round bottom flask containing chlorosulfonic acid (1.1 mL, 15.5 mmol), cooled in an ice bath, was added *N*-acetylindoline (**1**) (500 mg, 3.1 mmol) portion wise. The mixture was warmed to room temperature and then to 70ºC for 45 min. After that time it was cooled and poured onto ice and the solid was collected by suction filtration, and washed several times with water. The solid was recrystallized from ^{i}PrOH giving 668.23 mg (83%) of **2** as a white solid.

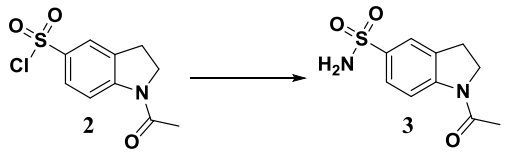
m.p. 163-165ºC (^{i}PrOH) [lit.[84] 167-169ºC (^{i}PrOH)]

^{1}H NMR (400 MHz, *DMSO-d_6*) δ 7.92 (d, *J* = 8.2 Hz, 1H), 7.41 (s, 1H), 7.37 (d, *J* = 8.2 Hz, 1H), 4.08 (t, *J* = 8.3, 2H), 3.11 (t, *J* = 8.3 Hz, 2H), 2.13 (s, 3H);

IR (KBr) 1673, 1377, 1183 cm^{-1};

Spectral data were in accordance with the literature.[84]

*III.4.1.3 Synthesis of 1-acetylindoline-5-sulfonamide (**3**)[132]*



To a solution of **2** (100 mg, 0.38 mmol), in THF (2.5 mL), was added a 25% ammonium hydroxide solution (0.4 mL) at room temperature. The mixture was stirred for 3 h. After that time the solvent was removed under vacuum and the crude recrystallized from MeOH affording 70.3 mg (76%) of **3** as a white solid.

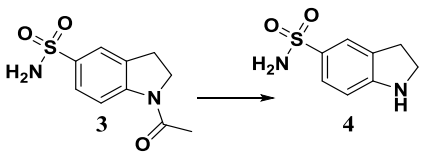
m.p. 225-229ºC (MeOH) [lit.[132] 228-229ºC (EtOH)]

^{1}H NMR (400 MHz, *DMSO-d_6*) δ 8.10 (d, *J* = 8.0 Hz, 1H), 7.63-7.60 (m, 3H), 7.20 (s, 2H, NH_2), 4.14 (t, *J* = 8.2, 2H), 3.20-3.16 (m, 2H), 2.17 (s, 3H);

IR (KBr) 3294, 1668, 1396, 1183 cm^{-1}.

Spectral data were in accordance with the literature.[132]

*III.4.1.4 Synthesis of indoline-5-sulfonamide (**4**)[84]*



To a suspention of **3** (500 mg, 2.1 mmol), in MeOH (4.7 mL), was added a 37% aqueous solution of HCl (0.4 mL). The mixture was refluxed for 1 h 30 min. After that time it was allowed to cool and the solvent was removed under reduced pressure. The crude was dissolved in water and the pH was adjusted to 7 with 1 N sodium hydroxide. The precipitate was collected by suction filtration, washed several times with water, affording 276.3 mg (67%) of **4** as a white solid.

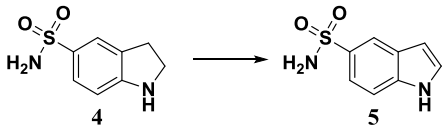
m.p. 155-160ºC (lit.[132] 163-165ºC)

^{1}H NMR (400 MHz, *DMSO-d_6*) δ 7.38 (s, 1H), 7.34 (d, *J* = 8.2 Hz, 1H), 6.86 (s, 2H, NH_2), 6.45 (d, *J* = 8.2 Hz, 1H), 6.19 (s, 1H, NH), 3.49 (t, *J* = 8.5, 2H), 2.94 (t, *J* = 8.5, 2H);

IR (KBr) 3321, 3257, 1309, 1140 cm^{-1}.

Spectral data were in accordance with the literature.[132]

*III.4.1.5 Synthesis of 1H-indole-5-sulfonamide (**5**)[84]*



To a solution of **4** (100 mg, 0.50 mmol), in MeOH (0.5 mL) was added Co(salen) hydrate (12.1 mg, 0.03 mmol). Air was bubbled through the solution for 24 h, at room temperature. The solvent was removed under reduced pressure and the residue was purified by flash chromatography (EtOAc) affording **5** (77.2 mg, 78%) was a white solid.

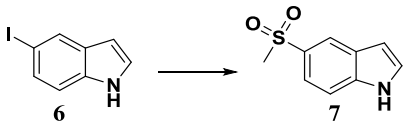
m.p. 200-205ºC (EtOAc) [lit.[132] 208ºC (acetone)]

^{1}H NMR (400 MHz, *DMSO-d_6*) δ 11.49 (s, 1H, NH), 8.05 (s, 1H), 7.56-7.50 (m, 3H), 7.09 (s, 2H, NH_2), 6.59 (s,1H);

IR (KBr) 3322, 3208, 1326, 1148 cm^{-1}.

Spectral data were in accordance with the literature.[132]

III.4.1.6 Synthesis of 5-(methylsulfonyl)-1H-indole (7)[85]



In a sealed tube, containing a solution of 5-iodoindole (**6**) (1 g, 4.11 mmol), in dry DMSO (8.2 mL), were added sodium methanesulfinate (546 mg, 5.35 mmol), copper iodide (157 mg, 0.82 mmol) and L-proline (189 mg, 1.64 mmol), under argon atmosphere. The mixture was stirred at 80ºC for 3 days. The reaction was quenched with saturated aqueous NH_4Cl, diluted with distilled water and extracted with EtOAc. The combined organic layers were washed with water and brine, dried over Na_2SO_4, filtered and concentrated. The crude was purified by flash chromatography (EtOAc/*n*-hexane 1:1) affording **7** (738 mg, 92%) as a white solid.

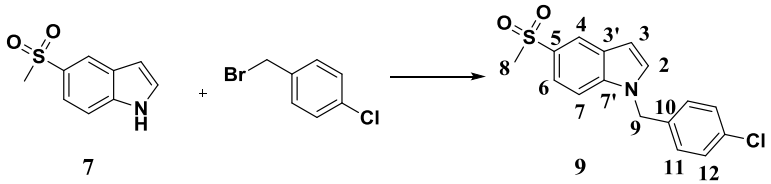
m.p. 166-170ºC (EtOAc/*n*-hexane);

^{1}H NMR [400 MHz, *$(CD_3)_2CO$*] δ 10.79 (s, 1H, NH), 8.22 (s, 1H), 7.68-7.63 (m, 2H), 7.56-7.55 (m, 1H), 6.71-6.70 (m, 1H), 3.06 (s, 3H).

IR (KBr) 1673, 1377, 1183 cm^{-1};

Spectral data were in accordance with the literature.[133]

III.4.1.7 Reaction of 5-(methylsulfonyl)-1H-indole (7) with p-chlorobenzyl bromide and n-BuLi



To a solution of **7** (50 mg, 0.26 mmol), in dry THF (0.5 mL), was added a 1.5 M solution of *n*-BuLi in *n*-hexane (0.19 mL, 0.28 mmol), at -78ºC. The mixture was stirred at this temperature for 30 min. After this time a solution of 4-chlorobenzyl bromide (57.9 mg, 0.28 mmol), in dry THF (0.2 mL) was added. The mixture was allowed to warm up to room temperature. The reaction was quenched with distilled water and extracted with EtOAc. The combined organic layers were dried over Na_2SO_4, filtered and concentrated. The crude was purified by flash chromatography (EtOAc/*n*-hexane 1:1) affording 1-(4-chlorobenzyl)-5-(methylsulfonyl)-1*H*-indole (**9**) (51.4 mg, 63%) was a white solid.

m.p. 136-138ºC (EtOAc/*n*-hexane);

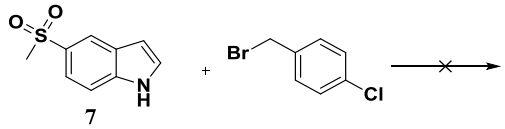
^{1}H NMR (400 MHz, *DMSO-d_6*) δ 8.18 (s, 1H, H4), 7.76 (d, *J* = 3.1 Hz, 1H, H2), 7.71 (d, *J* = 8.0 Hz, 1H, H7), 7.62 (d, *J* = 8.0 Hz, 1H, H6), 7.38 (d, *J* = 8.4 Hz, 2H, H12), 7.23 (d, *J* = 8.3 Hz, 2H, H11), 6.75 (d, *J* = 3.0 Hz, 1H, H3), 5.52 (s, 2H, H9), 3.15 (s, 3H, H8);

^{13}C NMR (101 MHz, *DMSO-d_6*) δ 137.6 (C7'), 136.7 (C10), 132.2 (CCl), 131.9 (C2), 131.8 (C5), 128.9 (2 x C11), 128.6 (2 x C12), 127.7 (C3'), 120.8 (C4), 119.5 (C6), 110.9 (C7), 103.0 (C3), 48.6 (C9), 44.4 (C8);

IR (KBr) 2924, 1491, 1289, 1136, 972, 755 cm^{-1};

HRMS (ESI) m/z 320.0509 $[M+H]^+$ (calcd for $C_{16}H_{15}ClNO_2S$ 320.0512).

*III.4.1.8 Reaction of 5-(methylsulfonyl)-1H-indole (**7**) with p-chlorobenzyl bromide and Grignard reagents*



7

To a solution of **7** (50 mg, 0.26 mmol), in dry THF (0.5 mL), was added a 1 M solution of PhMgBr in THF (0.3 mL, 0.28 mmol) or a 3 M solution of MeMgI in Et_2O (0.1 mL, 0.28 mmol), at -78ºC. The mixture was stirred at this temperature for 30 min. After this time a solution of 4-chlorobenzyl bromide (57.9 mg, 0.28 mmol), in dry THF (0.2 mL) was added. The mixture was allowed to warm up to room temperature for the reaction with PhMgBr or up to 50ºC for the reaction with MeMgI, for 24 h. After TLC control no reaction was observed.

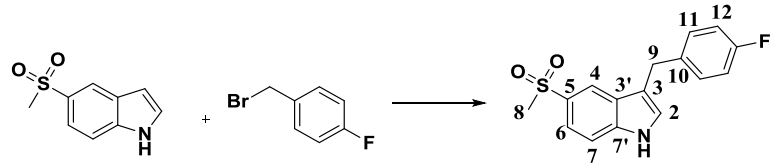
*III.4.1.9 General procedure for the preparation of 3-p-halo-benzylated indoles derivatives (**11a-g**) and 2,3-p-halo-benzylated indoles derivatives (**17a-f**)*[87]

Procedure A: To a solution of 1*H*-indole-5-sulfonamide (**5**)/5-(methylsulfonyl)-1*H*-indole (**7**) (2 equiv.), $Zn(OTf)_2$ (1.2 equiv.) and TBAI (1 equiv.) in dry toluene (ca. 3 mL per mmol of indole) was added DIPEA (2.2 equiv.) under argon. The reaction mixture was heated at 50ºC for 30 min, followed by addition of the *p*-halo-benzyl bromide (1 equiv.). The mixture was stirred overnight at 50ºC under argon. The reaction was quenched with saturated aqueous NH_4Cl, diluted with distilled water and extracted with EtOAc. The combined organic layers were washed with water and brine, dried over Na_2SO_4, filtered and concentrated. The samples were further purified by flash chromatography followed by PTLC.

Procedure B: To a solution of 1*H*-indole-5-sulfonamide (**5**)/5-(methylsulfonyl)-1*H*-indole (**7**) (1 equiv.), $Zn(OTf)_2$ (1.2 equiv.) and TBAI (1 equiv.) in dry toluene (ca. 3 mL per mmol of indole) was added DIPEA (2.2 equiv.) under argon. The reaction mixture was heated at 70ºC for 30 min, followed by addition of the *p*-halo-benzyl bromide (3 equiv.). The mixture was stirred overnight at 70ºC under argon. The reaction was quenched with saturated aqueous NH_4Cl, diluted with distilled water and

extracted with EtOAc. The combined organic layers were washed with water and brine, dried over Na_2SO_4, filtered and concentrated. The samples were further purified by flash chromatography.

III.4.1.9.1 Synthesis of 3-(4-fluorobenzyl)-5-(methylsulfonyl)-1*H*-indole (**11a**)



7 11a

Procedure A: Purified by flash chromatography (gradient CH_2Cl_2; CH_2Cl_2/EtOAc 9:1) followed by PTLC (EtOAc/*n*-hexane 1:1) affording **11a** as a white solid in 47% yield.

m.p. 136-136ºC (EtOAc/*n*-hexane);

^{1}H NMR [400 MHz, *$(CD_3)_2CO$*] δ 10.65 (s, 1H,NH), 8.13 (s, 1H, H4), 7.67 (d, *J* = 8.5 Hz, 1H, H6), 7.62 (d, *J* = 8.5 Hz, 1H, H7), 7.42-7.28 (m, 3H, H2 and H11), 7.03 (t, *J* = 8.7 Hz, 2H, H12), 4.17 (s, 2H, H9), 3.04 (s, 3H, H8);

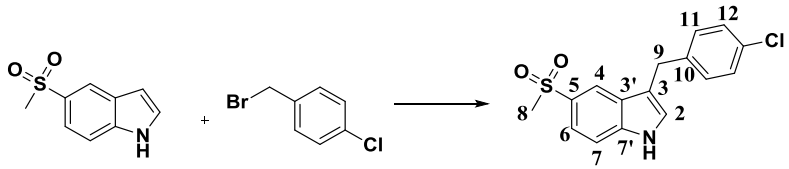
^{13}C NMR [101 MHz, *$(CD_3)_2CO$*] δ 162.1 (d, *J* = 240 Hz, CF), 139.9 (C7'), 138.0 (C10), 132.6 (C5), 131.0 (d, *J* = 7 Hz, 2 x C11), 127.7 (C3´), 126.7 (C2), 120.7 (C6), 120.1 (C4), 117.3 (C3), 115.7 (d, *J* = 21 Hz, 2 x C12), 112.9 (C7), 45.1 (C8), 30.8 (C9);

IR (KBr) 3370, 2928, 1283, 1132, 1090 cm^{-1};

HRMS (ESI) m/z 304.0802 $[M+H]^+$ (calcd for $C_{16}H_{15}FNO_2S$ 304.0808);

Purity 97%

III.4.1.9.2 Synthesis of 3-(4-chlorobenzyl)-5-(methylsulfonyl)-1*H*-indole (**11b**)



7 11b

Procedure A: Purified by flash chromatography (gradient CH_2Cl_2; CH_2Cl_2/EtOAc 9:1) followed by PTLC (EtOAc/*n*-hexane 1:1) affording **11b** as a white solid in 41% yield.

m.p. 155-157ºC (EtOAc/*n*-hexane);

^{1}H NMR [400 MHz, *$(CD_3)_2CO$*] δ 10.67 (s, 1H, NH), 8.12 (s, 1H, H4), 7.66 (d, *J* = 8.6 Hz, 1H, H6), 7.62 (d, *J* = 8.6 Hz, 1H, H7), 7.38 (s, 1H, H2), 7.34 (d, *J* = 8.5 Hz, 2H, H12), 7.30 (d, *J* = 8.5 Hz, 2H, H11), 4.19 (s, 2H, H9), 3.03 (s, 3H, H8);

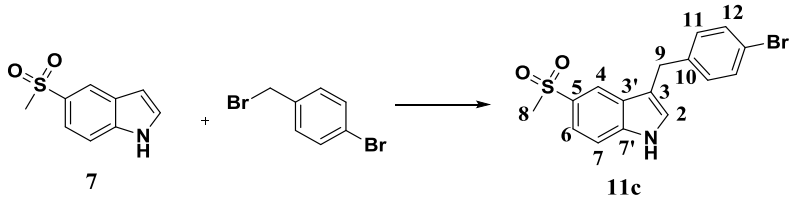
^{13}C NMR [101 MHz, *$(CD_3)_2CO$*] δ 141.0 (C10), 139.9 (C7'), 132.7 (C5), 131.9 (CCl), 131.0 (2 x C11), 129.1 (2 x C12), 126.8 (C2 and C3'), 120.8 (C6), 120.1 (C4), 116.8 (C3), 112.9 (C7), 45.1 (C8), 30.9 (C9);

IR (KBr) 3308, 2926, 1281, 1141, 1130, 1094, 763 cm^{-1};

HRMS (ESI) m/z 320.0507 $[M+H]^+$ (calcd for $C_{16}H_{15}ClNO_2S$ 320.0512);

Purity 95%

III.4.1.9.3 Synthesis of 3-(4-bromobenzyl)-5-(methylsulfonyl)-1*H*-indole (**11c**)



7 11c

Procedure A: Purified by flash chromatography (gradient CH_2Cl_2; CH_2Cl_2/EtOAc 9:1) followed PTLC (EtOAc/*n*-hexane 1:1) affording **11c** as a white solid in 50% yield.

m.p. 169-171ºC (EtOAc/*n*-hexane);

^{1}H NMR [400 MHz, *$(CD_3)_2CO$*] δ 10.69 (s, 1H, NH), 8.11 (s, 1H, H4), 7.66 (d, *J* = 8.3 Hz, 1H, H6), 7.61 (d, *J* = 8.3 Hz, 1H, H7), 7.45 (d, *J* = 7.6 Hz, 2H, H12), 7.38 (s, 1H, H2), 7.29 (d, *J* = 7.6 Hz, 2H, H11), 4.18 (s, 2H, H9), 3.03 (s, 3H, H8);

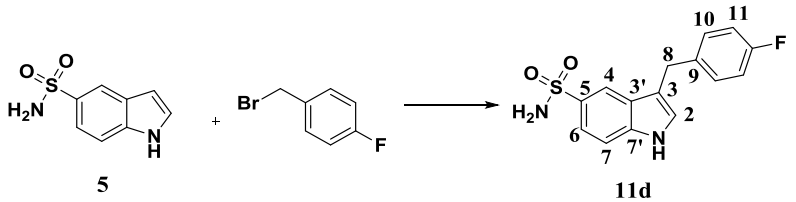
^{3}C NMR [101 MHz, *$(CD_3)_2CO$*] δ 141.4 (C10), 139.8 (C7'), 132.7 (C5), 132.1 (2 x C12), 131.4 (2 x C11), 127.6 (C3'), 126.8 (C2), 120.8 (C6), 120.0 (C4 and CBr), 116.8 (C3), 112.9 (C7), 45.1 (C8), 31.0 (C9);

IR (KBr) 3307, 2926, 1278, 1140, 1095, 764 cm^{-1};

HRMS (ESI) m/z 364.0001 $[M+H]^+$ (calcd for $C_{16}H_{15}BrNO_2S$ 364.0007);

Purity 98%

III.4.1.9.4 Synthesis of 3-(4-fluorobenzyl)-1*H*-indole-5-sulfonamide (**11d**)



5 11d

Procedure A: Purified by flash chromatography ($CHCl_3$; $CHCl_3$/MeOH 2 to 10%) followed by PTLC (EtOAc/*n*-hexane 3:2) affording **11d** as a white solid in 39% yield.

m.p. 153-155ºC (EtOAc/*n*-hexane);

^{1}H NMR [400 MHz, *$(CD_3)_2CO$*] δ 10.53 (s, 1H, NH), 8.09 (s, 1H, H4), 7.66 (d, *J* = 8.3 Hz, 1H, H6), 7.53 (d, *J* = 8.3 Hz, 1H, H7), 7.41-7.25 (m, 3H, H2 and H10), 7.02 (t, *J* = 8.0 Hz, 2H, H11), 6.33 (s, 2H, NH_2), 4.14 (s, 2H, H8);

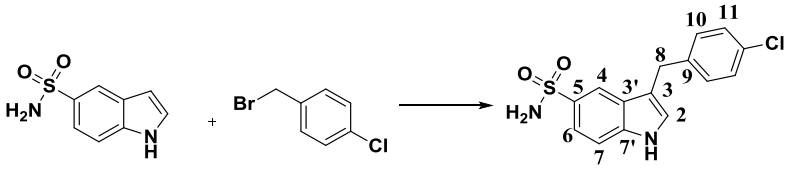
^{13}C NMR [101 MHz, *$(CD_3)_2CO$*] δ 162.1 (d, *J* = 240 Hz, CF), 139.1 (C7'), 138.2 (C9), 135.7 (C5), 131.0 (d, *J* = 7 Hz, 2 x C10), 127.3 (C3'), 126.2 (C2), 120.2 (C6), 118.5 (C4), 116.9 (C3), 115.7 (d, *J* = 21 Hz, 2 x C11), 112.5 (C7), 30.8 (C8);

IR (KBr) 3379, 3291, 1313, 1147, 1096 cm^{-1};

HRMS (ESI) m/z 305.0755 $[M+H]^+$ (calcd for $C_{15}H_{14}FN_2O_2S$ 305.0760);

Purity 99%

III.4.1.9.5 Synthesis of 3-(4-chlorobenzyl)-1*H*-indole-5-sulfonamide (**11e**)



5 **11e**

Procedure A: Purified by flash chromatography ($CHCl_3$; $CHCl_3$/MeOH 2 to 10%) followed by PTLC (EtOAc/*n*-hexane 3:2) affording **11e** as a white solid in 46% yield.

m.p. 205-206ºC (EtOAc/*n*-hexane);

^{1}H NMR [400 MHz, *$(CD_3)_2CO$*] δ 10.55 (s, 1H, NH), 8.08 (s, 1H, H4), 7.66 (d, *J* = 8.4 Hz, 1H, H6), 7.53 (d, *J* = 8.4 Hz, 1H, H7), 7.38-7.23 (m, 5H, H2, H10 and H11), 6.32 (s, 2H, NH_2), 4.15 (s, 2H, H8);

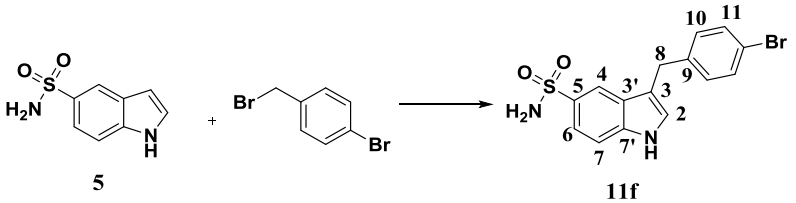
^{13}C NMR [101 MHz, *$(CD_3)_2CO$*] δ 141.1 (C9), 139.1 (C7'), 135.8 (C5), 131.9 (CCl), 131.0 (2 x C10), 129.1 (2 x C11), 127.3 (C3'), 126.3 (C2), 120.2 (C6), 118.5 (C4), 116.4 (C3), 112.5 (C7), 32.0 (C8);

IR (KBr) 3336, 3267, 1324, 1154, 1090, 806 cm^{-1};

HRMS (ESI) m/z 321.0459 $[M+H]^+$ (calcd for $C_{15}H_{14}ClN_2O_2S$ 321.0465);

Purity 99%

III.4.1.9.6 Synthesis of 3-(4-bromobenzyl)-1*H*-indole-5-sulfonamide (**11f**)



5 **11f**

Procedure A: Purified by flash chromatography ($CHCl_3$; $CHCl_3$/MeOH 2 to 10%) followed by PTLC (EtOAc/*n*-hexane 3:2) affording **11f** as a white solid in 42% yield.

m.p. 220-221ºC (EtOAc/*n*-hexane);

^{1}H NMR [400 MHz, $(CD_3)_2CO$] δ 10.57 (s, 1H, NH), 8.08 (s, 1H, H4), 7.66 (d, *J* = 8.5 Hz, 1H, H6), 7.54 (d, *J* = 8.5 Hz, 1H, H7), 7.44 (d, *J* = 8.1 Hz, 2H, H11), 7.34 (s, 1H, H2), 7.26 (d, *J* = 8.1 Hz, 2H, H10), 6.33 (s, 2H, NH_2), 4.14 (s, 2H, H8);

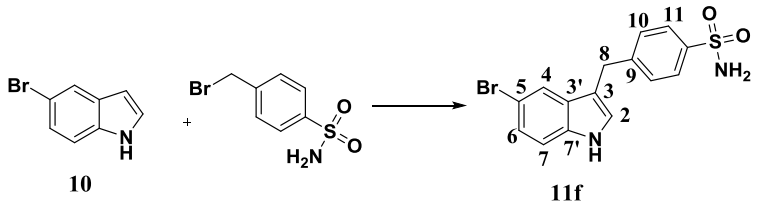
^{13}C NMR [101 MHz, $(CD_3)_2CO$] δ 141.7 (C9), 139.1 (C7'), 135.8 (C5), 132.1 (2 x C11), 131.4 (2 x C10), 127.3 (C3'), 126.4 (C2), 120.2 (C6 and CBr), 118.5 (C4), 116.3 (C3), 112.5 (C7), 32.0 (C8);

IR (KBr) 3331, 3266, 1323, 1153, 1099, 803 cm^{-1};

HRMS (ESI) m/z 364.9954 $[M+H]^+$ (calcd for $C_{15}H_{14}BrN_2O_2S$ 364.9959);

Purity 97%

III.4.1.9.7 Synthesis of 4-[(5-bromo-1*H*-indol-3-yl)methyl]benzenesulfonamide (**11g**)



10 **11f**

Procedure A: The reaction was performed at room temperature. Purified by flash chromatography (EtOAc/*n*-hexane 1:1) affording **11g** as a white solid in 79% yield.

m.p. 157-159ºC (EtOAc/*n*-hexane);

^{1}H NMR [400 MHz, $(CD_3)_2CO$] δ 10.36 (s, 1H, NH), 7.80 (d, *J* = 7.4 Hz, 2H, H11), 7.62 (s, 1H, H4), 7.48 (d, *J* = 7.4 Hz, 2H, H10), 7.38 (d, *J* = 8.4 Hz, 1H, H7), 7.29 (s, 1H, H2), 7.21 (d, *J* = 8.4 Hz, 1H, H6), 6.49 (s, 2H, NH_2), 4.19 (s, 2H, H8);

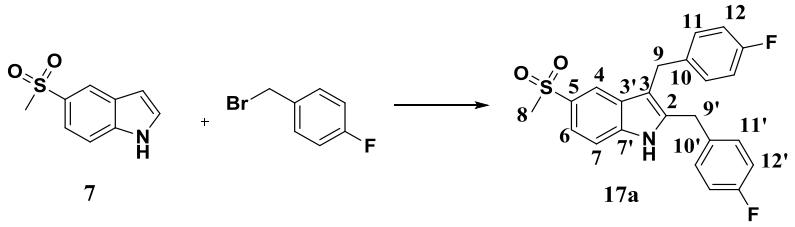
^{13}C NMR [101 MHz, $(CD_3)_2CO$] δ 146.9 (C9), 142.7 (CSO_2NH_2), 136.5 (C7'), 130.0 (C5), 129.7 (2 x C10), 127.0 (2 x C11), 125.8 (C2), 124.9 (C6), 121.8 (C4), 114.3 (C3'), 114.1 (C7), 112.5 (C3), 31.6 (C9);

IR (KBr) 3401, 2923, 1325, 1158, 1095, 882 cm^{-1};

HRMS (ESI) m/z 364.9954 $[M+H]^+$ (calcd for $C_{15}H_{14}BrN_2O_2S$ 364.9959);

Purity 99%

III.4.1.9.8 Synthesis of 2,3-bis(4-fluorobenzyl)-5-(methylsulfonyl)-1*H*-indole (**17a**)



7 **17a**

Procedure A: Purified by flash chromatography (gradient CH_2Cl_2; CH_2Cl_2/EtOAc 9:1) followed by PTLC (EtOAc/*n*-hexane 1:1) affording **17a** as a white solid in 9% yield.

m.p. 165-168ºC (EtOAc/*n*-hexane);

^{1}H NMR [400 MHz, $(CD_3)_2CO$] δ 10.61 (s, 1H, NH), 8.02 (s, 1H, H4), 7.60 (d, *J* = 8.5 Hz, 1H, H6), 7.50 (d, *J* = 8.5 Hz, 1H, H7), 7.29-7.18 (m, 4H, H12 and H12'), 7.09-6.89 (m, 4H, H11 and H11'), 4.24 (s, 4H, H9 and H9'), 3.01 (s, 3H, H8);

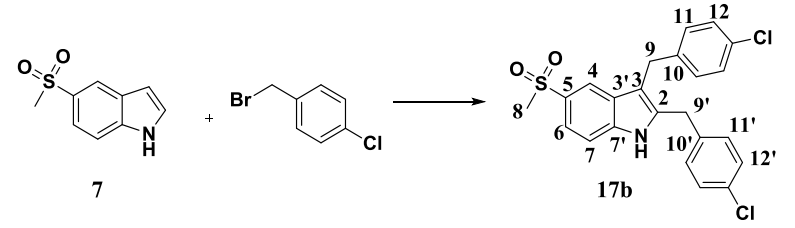
^{13}C NMR [101 MHz, $(CD_3)_2CO$] δ 162.4 (d, *J* = 240 Hz, CF), 162.1 (d, *J* = 239 Hz, CF), 139.3 (C7'), 138.4 (C10 or C10'), 138.2 (C2), 135.9 (C10 or C10'), 132.9 (C5), 131.2 (d, *J* = 7 Hz, 2 x C11 or C11'), 130.8 (d, *J* = 7 Hz, 2 x C11 or C11'), 129.0 (C3'), 120.5 (C6), 119.7 (C4), 115.9 (m, 4 x C12 and C12'), 113.0 (C3), 112.2 (C7), 45.2 (C8), 32.0 (C9 or C9'), under the solvent peak (C9 or C9');

IR (KBr) 3320, 2929, 1291, 1222, 1140 cm^{-1};

HRMS (ESI) m/z 412.1177 $[M+H]^+$ (calcd for $C_{23}H_{20}F_2NO_2S$ 412.1183);

Purity 95%

III.4.1.9.9 Synthesis of 2,3-bis(4-chlorobenzyl)-5-(methylsulfonyl)-1*H*-indole (**17b**)



7 **17b**

Procedure A: Purified by flash chromatography (gradient CH_2Cl_2; CH_2Cl_2/EtOAc 9:1) followed by PTLC (EtOAc/*n*-hexane 1:1) affording **17b** as a white solid in 7% yield.

m.p. 210-211ºC (EtOAc/*n*-hexane);

^{1}H NMR [400 MHz, $(CD_3)_2CO$] δ 10.61 (s, 1H, NH), 8.03 (s, 1H, H4), 7.61 (d, *J* = 8.4 Hz, 1H, H6), 7.51 (d, *J* = 8.4 Hz, 1H, H7), 7.35-7.12 (m, 8H, H11, H11', H12 and H12'), 4.25 (s, 4H, H9 and H9'), 3.01 (s, 3H, H8);

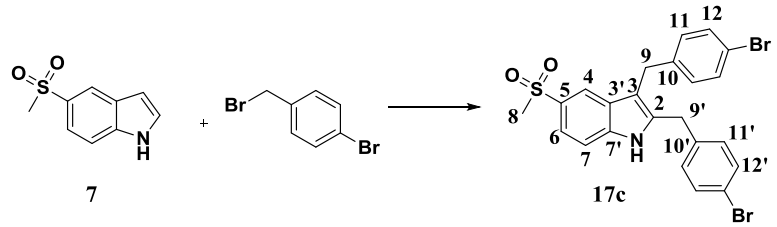
^{13}C NMR [101 MHz, $(CD_3)_2CO$] δ 141.1 (C10 or C10'), 139.2 (C7'), 138.7 (C2), 138.5 (C10 or C10'), 132.9 (C5), 132.6 (CCl or CCl'), 131.9 (CCl or CCl'), 131.2 (2 x C11 or C11'), 130.8 (2 x C11 or C11'), 129.3 (2 x C12 or C12'), 129.1 (2 x C12 or C12'), 128.9 (C3'), 120.6 (C6), 119.6 (C4), 112.8 (C3), 112.2 (C7), 45.2 (C8), 32.1 (C9 or C9'), under the solvent peak (C9 or C9');

IR (KBr) 3310, 2918, 1290, 1142, 765 cm^{-1};

HRMS (ESI) m/z 444.0586 $[M+H]^+$ (calcd for $C_{23}H_{20}Cl_2NO_2S$ 444.0592);

Purity 95%

III.4.1.9.10 Synthesis of 2,3-bis(4-chlorobenzyl)-5-(methylsulfonyl)-1*H*-indole (**17c**)



Procedure A: Purified by flash chromatography (gradient CH_2Cl_2; CH_2Cl_2/EtOAc 9:1) followed by PTLC (EtOAc/*n*-hexane 1:1) affording **17c** as a white solid in 15% yield.

m.p. 189-193ºC (EtOAc/*n*-hexane);

^{1}H NMR [400 MHz, $(CD_3)_2CO$] δ 10.77 (s, 1H, NH), 8.03 (s, 1H, H4), 7.60 (d, *J* = 8.5 Hz, 1H, H6), 7.52 (d, *J* = 8.5 Hz, 1H, H7), 7.43-7.37 (m, 4H, H12 and H12'), 7.20-7.13 (m, 4H, H11 and H11'), 4.23 (s, 4H, H9 and H9'), 3.01 (s, 3H, H8);

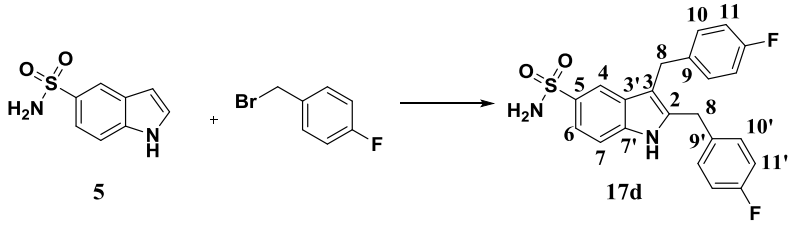
^{13}C NMR [101 MHz, $(CD_3)_2CO$] δ 141.6 (C10 or C10'), 139.2 (C7' and C10 or C10'), 138.4 (C2), 132.9 (C5), 132.3 (2 x C12 or C12'), 132.1 (2 x C12 or C12'), 131.5 (2 x C11 or C11'), 131.2 (2 x C11 or C11'), 128.9 (C3'), 120.5 (C6 and 2 x CBr), 119.5 (C4), 112.6 (C3), 112.2 (C7), 45.1 (C8), 32.1 (C9 or C9'), under the solvent peak (C9 or C9');

IR (KBr) 3316, 2922, 1289, 1142, 1072, 1011, 765 cm^{-1};

HRMS (ESI) m/z 531.9576 $[M+H]^+$ (calcd for $C_{23}H_{20}Br_2NO_2S$ 531.9581);

Purity 96%

III.4.1.9.11 Synthesis of 2,3-bis(4-fluorobenzyl)-1*H*-indole-5-sulfonamide (**17d**)



Procedure A: Purified by flash chromatography ($CHCl_3$; $CHCl_3$/MeOH 2 to 10%) followed by PTLC (EtOAc/*n*-hexane 3:2) affording **17d** as a white solid in 11% yield.

m.p. 183-185ºC (EtOAc/*n*-hexane);

^{1}H NMR [400 MHz, $(CD_3)_2CO$] δ 10.46 (s, 1H, NH), 8.01 (s, 1H, H4), 7.61 (d, *J* = 8.4 Hz, 1H, H6), 7.43 (d, *J* = 8.4 Hz, 1H, H7), 7.22 (m, 4H, H10 and H10'), 7.08-6.91 (m, 4H, H11 and H11'), 6.29 (s, 2H, NH_2), 4.22 (s, 2H, H8 or H8'), 4.20 (s, 2H, H8 or H8');

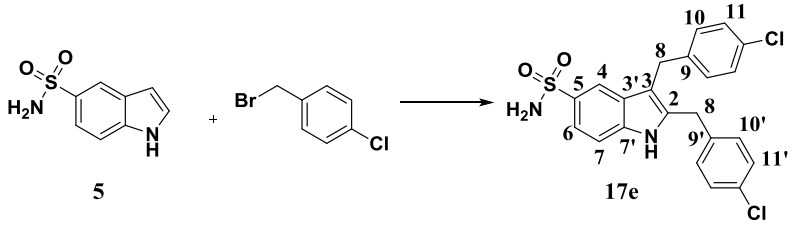
^{13}C NMR [101 MHz, *$(CD_3)_2CO$*] δ 162.4 (d, *J* = 240 Hz, CF), 162.0 (d, *J* = 239 Hz, CF), 138.3 (C7', C9 or C9' and C2), 135.9 (C5 and C9 or C9'), 131.2 (d, *J* = 6 Hz, 2 x C10 or C10'), 130.7 (d, *J* = 7 Hz, 2 x C10 or C10'), 128.6 (C3'), 119.9 (C6), 118.1 (C4), 116.0-115.6 (m, 4 x C11 and C11'), 112.6 (C3), 111.8 (C7), under the solvent peak (2 x C8 and C8');

IR (KBr) 3396, 3267, 2926, 1327, 1217, 1154 cm^{-1};

HRMS (ESI) m/z 413.1130 $[M+H]^+$ (calcd for $C_{22}H_{18}F_2N_2O_2S$ 413.1135);

Purity 95%

III.4.1.9.12 Synthesis of 2,3-bis(4-chlorobenzyl)-1*H*-indole-5-sulfonamide (**17e**)



5 **17e**

Procedure A: Purified by flash chromatography ($CHCl_3$; $CHCl_3$/MeOH 2 to 10%) followed by PTLC (EtOAc/n-hexane 3:2) affording **17e** as a white solid with 15% yield.

m.p. 172-173ºC (EtOAc/n-hexane);

^{1}H NMR [400 MHz, *$(CD_3)_2CO$*] δ 10.48 (s, 1H, NH), 8.01 (s, 1H, H4), 7.62 (d, *J* = 8.4 Hz, 1H, H6), 7.44 (d, *J* = 8.4 Hz, 1H, H7), 7.33-7.12 (m, 8H, H10, H10', H11 and H11'), 6.31 (s, 2H, NH_2), 4.23 (s, 2H, H8 or H8'), 4.20 (s, 2H, H8 or H8');

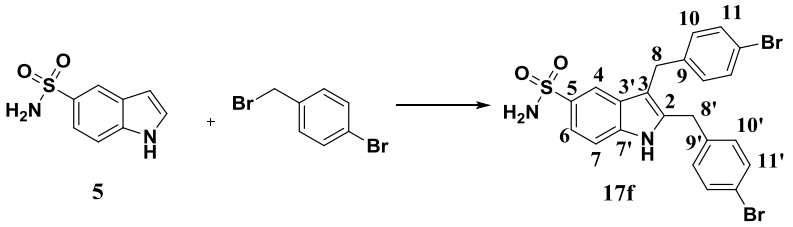
^{13}C NMR [101 MHz, *$(CD_3)_2CO$*] δ 141.2 (C9 or C9'), 138.8 (C9 or C9'), 138.4 (C7'), 137.9 (C2), 135.9 (C5), 132.5 (CCl or CCl'), 131.8 (CCl or CCl'), 131.1 (2 x C10 or C10'), 130.7 (2 x C10 or C10'), 129.3 (2 x C11 or C11'), 129.0 (2 x C11 or C11'), 128.5 (C3'), 119.9 (C6), 118.0 (C4), 112.3 (C3), 111.8 (C7), 32.0 (C8 or C8'), under the solvent peak (C8 or C8');

IR (KBr) 3317, 2909, 1317, 1156, 1014, 800 cm^{-1};

HRMS (ESI) m/z 467.0358 $[M+Na]^+$ (calcd for $C_{22}H_{18}Cl_2N_2NaO_2S$ 467.0364);

Purity 95%

III.4.1.9.13 Synthesis of 2,3-bis(4-bromobenzyl)-1*H*-indole-5-sulfonamide (**17f**)



Procedure B: Purified by flash chromatography ($CHCl_3$; $CHCl_3$/MeOH 2 to 10%) affording **17f** as a white solid in 89% yield.

m.p. 213-216ºC (EtOAc/n-hexane);

^{1}H NMR [400 MHz, *$(CD_3)_2CO$*] δ 10.49 (s, 1H, NH), 8.01 (s, 1H, H4), 7.62 (d, *J* = 8.3 Hz, 1H, H6), 7.48-7.34 (m, 5H, H7, H11 and H11'), 7.15 (d, *J* = 7.3 Hz, 4H, H10 and H10'), 6.30 (s, 2H, NH_2), 4.21 (s, 2H, H8 or H8'), 4.19 (s, 2H, H8 or H8').;

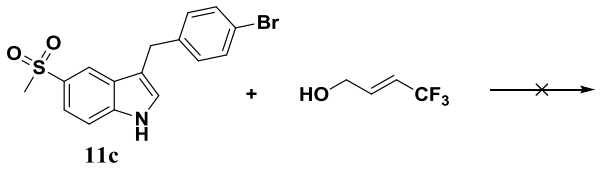
^{13}C NMR [101 MHz, *$(CD_3)_2CO$*] δ 141.6 (C9 or C9'), 139.3 (C7'), 138.4 (C2), 137.8 (C9 or C9'), 135.9 (C5), 132.3 (2 x C11 or C11'), 132.0 (2 x C11 or C11'), 131.5 (2 x C10 or C10'), 131.1 (2 x C10 or C10'), 128.5 (C3'), 120.6 (2 x CBr), 119.9 (C6), 118.0 (C4), 112.3 (C3), 111.8 (C7), 32.1 (C8 or C8'), under the solvent peak (C8 or C8');

IR (KBr) 3336, 3270, 2924, 1320, 1157, 1011, 802 cm^{-1};

HRMS (ESI) m/z 554.9348 $[M+Na]^+$ (calcd for $C_{22}H_{18}Br_2N_2NaO_2S$ 554.9353);

Purity 96%

*III.4.1.10 Reaction of (E)-4,4,4-trifluorobut-2-en-1-ol with 3-(4-bromobenzyl)-5-(methylsulfonyl)-1H-indole (**11c**)*

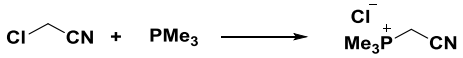


III.4.1.10.1 Classical Mitsunobu reaction[92]

To a solution of **11c** (44 mg, 0.14 mmol), in a dry MeCN (1.3 mL), was added (*E*)-4,4,4-trifluorobut-2-en-1-ol (23.7 mg, 0.19 mmol) and PPh_3 (60.7 mg, 0.23 mmol), under argon atmosphere. The mixture was allowed to stir at room temperature for 10 min and then cooled in an ice bath followed by addition of DEAD (70 µL, 0.16 mmol). The ice bath was removed and the mixture was stirred at room temperature, under argon, for 24 h. After TLC control no reaction was observed.

III.4.1.10.2 Modified Mitsunobu reaction[93]

III.4.1.10.2.1 Synthesis of cyanomethyltrimethylphosphonium chloride[134]



A solution of 1 M PMe_3 in THF (6.6 mL, 6.60 mmol) was slowly added to chloroacetonitrile (0.5 mL, 7.92 mmol). The reaction was maintained at *ca* 40ºC by cooling (water bath). The reaction mixture was stirred at room temperature for 2 days during which a white precipitate was formed. The solid was recrystallized from ^{i}PrOH and collected by filtration affording cyanomethyltrimethylphosphonium chloride in quantitative yield.

m.p. 288-290ºC (decomp.) (^{i}PrOH) [lit.[132] 208-253ºC (decomp.) (^{i}PrOH)];

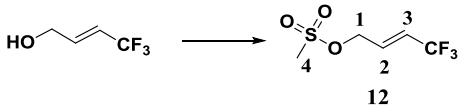
^{1}H NMR (400 MHz, *DMSO-d_6*) δ 4.47 (d, *J* = 16.8 Hz, 2H), 2.12 (d, *J* = 15.4 Hz, 9H);

IR (KBr) 2970, 2887, 2251, 1634, 1292, 975 cm^{-1}.

Spectral data were in accordance with the literature.[134]

In a dried ACE pressure tube under an argon atmosphere, containing a solution of cyanomethyltrimethylphosphonium chloride (62.2 mg, 0.41 mmol), in dry THF (1.8 mL), was added KH (14.5 mg, 0.36 mmol). The mixture was stirred for 2 h at room temperature. Then **11c** (50 mg, 0.16 mmol) and (*E*)-4,4,4-trifluorobut-2-en-1-ol (41.4 mg, 0.33 mmol) were added. The reaction mixture was stirred at 100°C, for 24 h. After TLC control no reaction was observed.

*III.4.1.11 Synthesis of (E)-4,4,4-trifluorobut-2-en-1-yl methanesulfonate (**12**)*



12

To a solution of (*E*)-4,4,4-trifluorobut-2-en-1-ol (1 g, 7.93 mmol), in dry DCM (4.4 mL), cooled with an ice bath, was added TEA (1.2 mL, 1.72 mmol). The mixture was stirred for 10 min at this temperature, then methanesulfonyl chloride (0.67 mL, 1.72 mmol) was added. The reaction was stirred for 30 min at 0ºC and the ice bath was removed and the mixture was stirred for 1 h, at room temperature. The reaction was quenched with distilled water and extracted with EtOAc. The combined organic layers were dried over Na_2SO_4, filtered and concentrated. The crude was purified by flash chromatography (Et_2O/*n*-hexane 3:2) affording **12** (1.39 mg, 86%) as a colorless oil.

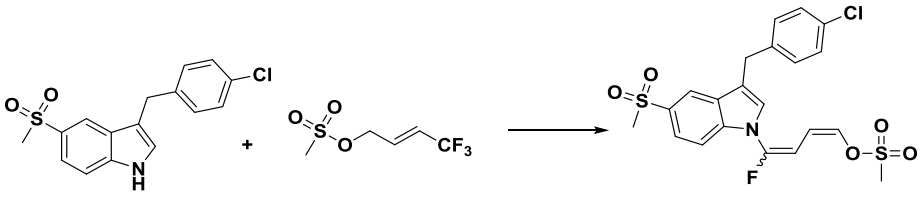
^{1}H NMR (400 MHz, *$CDCl_3$*) δ 6.47-6.42 (m, 1H, H2), 6.05-5.95 (m, 1H, H3), 4.83-4.82 (m, 2H, H1), 3.08 (s, 3H, H4);

^{13}C NMR (101 MHz, *$CDCl_3$*) δ 132.1 (q, *J* = 6.5 Hz, C2), 122.2 (q, *J* = 269.7 Hz, CF_3), 121.6 (q, *J* = 34.7 Hz, C3), 65.9 (C1), 38.0 (C4);

IR (KBr) 2920, 1312, 1118, 936 cm^{-1};

MS (FI) m/z 204.00 $[M]^+$ (calcd for $C_5H_7F_3O_3S$ 204.01).

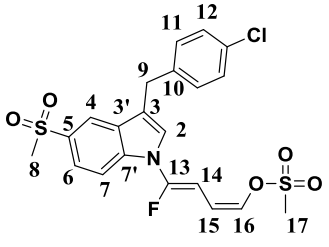
*III.4.1.12 Reaction of 3-(4-chlorobenzyl)-5-(methylsulfonyl)-1H-indole (**11b**) with (E)-4,4,4-trifluorobut-2-en-1-yl methanesulfonate (**12**)*



11b **12** **14** (Z/Z)(Z/E)

To a solution of **11b** (22.6 mg, 0.07 mmol), in dry DMF (1 mL), was added NaH (1.7 mg, 0.07 mmol) at 0ºC, under argon. The reaction mixture was stirred at that temperature for 45 min. Then compound **12** (14.4 mg, 0.07 mmol) was added and mixture was stirred, at 0ºC under argon, for 1 h. The reaction was quenched with distilled water and extracted with EtOAc. The combined organic layers were washed with water and brine, dried over Na_2SO_4, filtered and concentrated. The crude was purified by PTLC (toluene/EtOAc 4:1) affording a mixture of compounds **14**(*Z*/*Z*) and **14**(*Z*/*E*) (1:0.5) as light yellow oils in 27% yield (9.7 mg). These compounds were separated by HPLC.

*(1Z,3Z)-4-[3-(4-chlorobenzyl)-5-(methylsulfonyl)-1H-indol-1-yl]-4-fluorobuta-1,3-dien-1-yl methanesulfonate [**14(Z/Z)**]*

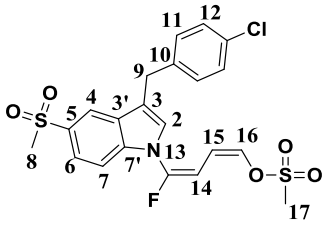


14 (*Z*/*Z*)

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.14 (s, 1H, H4), 7.85 (d, *J* = 8.7 Hz, 1H, H6), 7.72 (d, *J* = 8.7 Hz, 1H, H7), 7.30 (d, *J* = 8.3 Hz, 2H, H12), 7.21 (d, *J* = 8.3 Hz, 2H, H11), 7.05 (s, 1H, H2), 6.63 (d, *J* = 5.9 Hz, 1H, H16), 6.07 (dd, *J* = 11.1, 5.9 Hz, 1H, H15), 5.88 (dd, *J* = 28.5, 11.2 Hz, 1H, H14), 4.09 (s, 2H, H9), 3.13 (s, 3H, H17), 3.06 (s, 3H, H8);

^{13}C NMR (101 MHz, $CDCl_3$) δ 148.4 (d, *J* = 267 Hz, C13), 138.8 (C7'), 137.3 (C10), 134.7 (C16), 134.2 (C5), 132.6 (CCl), 130.1 (2 x C12), 129.0 (2 x C11), 128.4 (C3'), 125.9 (C2), 122.8 (C6), 120.3 (C3 and C4), 113.1 (C7), 108.1 (C15), 90.5 (d, *J* = 18.5 Hz, C14), 45.2 (C8), 38.1 (C17), 30.6 (C9).

*(1Z,3E)-4-[3-(4-chlorobenzyl)-5-(methylsulfonyl)-1H-indol-1-yl]-4-fluorobuta-1,3-dien-1-yl methanesulfonate [**14(Z/E)**]*



14 (*Z/E*)

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.16 (s, 1H, H4), 7.85 (d, *J* = 8.7 Hz, 1H, H6), 7.46 (d, *J* = 8.6 Hz, 1H, H7), 7.30 (d, *J* = 8.4 Hz, 2H, H12), 7.21 (d, *J* = 8.4 Hz, 2H, H11), 7.01 (s, 1H, H2), 6.55 (d, *J* = 6.0 Hz, 1H, H16), 6.33 (dd, *J* = 11.5, 6.0 Hz, 1H, H15), 5.27 (dd, *J* = 11.5, 6.1 Hz, 1H, H14), 4.10 (s, 2H, H9), 3.16 (s, 3H, H17), 3.07 (s, 3H, H8);

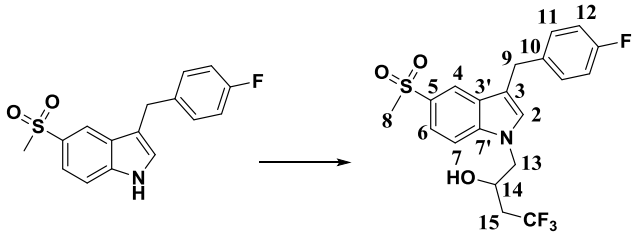
^{13}C NMR (101 MHz, $CDCl_3$) δ 148.0 (d, *J* = 267 Hz, C13), 138.8 (C7'), 137.3 (C10), 135.9 (C16), 134.2 (C5), 132.6 (CCl), 130.1 (2 x C12), 129.0 (2 x C11), 128.4 (C3'), 127.1 (C2), 122.7 (C6), 120.4 (C3), 120.2 (C4) 112.4 (C7), 108.3 (C15), 95.9 (d, *J* = 39 Hz, C14), 45.2 (C8), 38.2 (C17), 30.6 (C9);

MS (EI) m/z 483.03 $[M]^+$ (calcd for $C_{21}H_{19}ClFNO_5S_2$ 483.04).

*III.4.1.13 General procedure for the preparation of 1-[3-(4-halobenzyl)-5-(methylsulfonyl)-1H-indol-1-yl]-4,4,4-trifluorobutan-2-ol (**15a-c**)*

To a solution of 3-*p*-halo-benzylated-5-(methylsulfonyl)indole **11a-c** (1 equiv.), in dry THF (*ca* 8 mL per mmol of indole derivative), was added NaH (0.9 equiv.), at 0ºC under argon. The reaction mixture was stirred at that temperature for 45 min then (2,2,2-trifluoro-ethyl)-oxirane (1.1 equiv.) was added and mixture was refluxed for 2 h. The reaction was quenched with distilled water and extracted with EtOAc. The combined organic layers were washed with water and brine, dried over Na_2SO_4, filtered and concentrated. The crude was purified by PTLC (CH_2Cl_2/MeOH 2%) affording compounds **15a-c**.

III.4.1.13.1 Synthesis of 1-[3-(4-fluorobenzyl)-5-(methylsulfonyl)-indol-1-yl]-4,4,4-trifluorobutan-2-ol (**15a**)



11a **15a**

White solid; 62% yield;

m.p. 75-79 ºC;

^{1}H NMR [400 MHz, *$(CD_3)_2CO$*] δ 8.10 (s, 1H, H4), 7.77-7.63 (m, 2H, H6 and H7), 7.36 (m, 3H, H2 and H11), 7.05-7.01 (m, 2H, H12), 4.53-4.37 (m, 2H, H13), 4.37-4.25 (m, 1H, H14), 4.18 (s, 2H, H9), 3.04 (s, 3H, H8), 2.68-2.36 (m, 2H, H15);

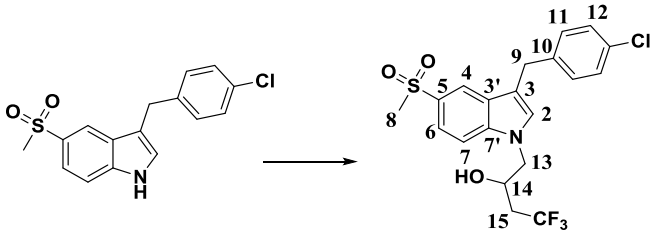
^{13}C NMR [101 MHz, *$(CD_3)_2CO$*] δ 162.2 (d, *J* = 240 Hz, CF), 140.2 (C7'), 137.9 (C10), 132.8 (C5), 131.1 (d, *J* = 7 Hz, 2 x C11), 130.9 (C2), 128.2 (C3'), 120.7 (C6), 120.2 (C4), 116.9 (C3), 115.7 (d, *J* = 21 Hz, 2 x C12), 111.5 (C7), 66.1 (C14), 52.7 (C13), 45.1 (C8), 39.1-38.8 (m, C15), 30.7 (C9);

IR (KBr) 3481, 1510, 1292, 1148, 1132 cm^{-1};

HRMS (ESI) m/z 430.1095 $[M+H]^+$ (calcd for $C_{20}H_{20}F_4NO_3S$ 430.1100);

Purity 99%

III.4.1.13.2 Synthesis of 1-[3-(4-chlorobenzyl)-5-(methylsulfonyl)-indol-1-yl]-4,4,4-trifluorobutan-2-ol (**15b**)



11b **15b**

White solid; 46% yield;

m.p. 115-117 ºC;

^{1}H NMR [400 MHz, *$(CD_3)_2CO$*] δ 8.10 (s, 1H, H4), 7.72 (d, *J* = 8.4 Hz, 1H, H6), 7.69 (d, *J* = 8.4 Hz, 1H, H7), 7.46-7.22 (m, 5H, H2, H11 and H12), 4.53-4.37 (m, 2H, H13), 4.37-4.25 (m, 1H, H14), 4.18 (s, 2H, H9), 3.04 (s, 3H, H8), 2.74-2.30 (m, 2H, H15);

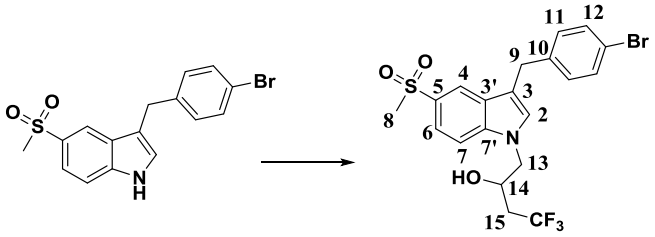
^{13}C NMR [101 MHz, *$(CD_3)_2CO$*] δ 140.8 (C10), 140.1 (C7'), 132.8 (C5), 132.1 (CCl), 131.1 (2 x C11), 131.0 (C2), 129.1 (2 x C12), 128.1 (C3'), 120.8 (C6), 120.2 (C4), 116.5 (C3), 111.5 (C7), 66.1 (C14), 52.7 (C13), 45.1 (C8), 39.1-38.8 (m, C15)), 30.8 (C9);

IR (KBr) 3484, 2926, 1741, 1408, 1290, 1148 cm^{-1};

HRMS (ESI) m/z 446.0799 $[M+H]^+$ (calcd for $C_{20}H_{20}ClF_3NO_3S$ 446.0805);

Purity 97%

III.4.1.13.3 Synthesis of 1-[3-(4-bromobenzyl)-5-(methylsulfonyl)-indol-1-yl]-4,4,4-trifluorobutan-2-ol (**15c**)



11c **15c**

White solid; 49% yield;

m.p. 139-141 ºC;

^{1}H NMR [400 MHz, *$(CD_3)_2CO$*] δ 8.11 (s, 1H, H4), 7.74-7.67 (m, 2H, H6 and H7), 7.44 (d, *J* = 8.0 Hz, 2H, H12), 7.37 (s, 1H, H12), 7.29 (d, *J* = 8.0 Hz, 2H, H11), 4.54-4.37 (m, 2H, H13), 4.37-4.24 (m, 1H, H14), 4.16 (s, 2H, H9), 3.05 (s, 3H, H8), 2.76-2.35 (m, 2H, H15);

^{13}C NMR [101 MHz, *$(CD_3)_2CO$*] δ 141.3 (C10), 140.1 (C7'), 132.7 (C5), 132.1 (2 x C12), 131.5 (2 x C11), 131.0 (C2), 128.1 (C3'), 120.7 (C6), 120.2 (C4 and CBr), 116.3 (C3), 111.5 (C7), 66.5 (C14), 52.7 (C13), 45.0 (C8), 39.0 (q, *J* = 26.7 Hz, C15), 30.9 (C9);

IR (KBr) 3486, 2928, 1290, 1146, 1133 cm^{-1};

HRMS (ESI) m/z 490.0294 $[M+H]^+$ (calcd for $C_{20}H_{20}BrF_3NO_3S$ 490.0299);

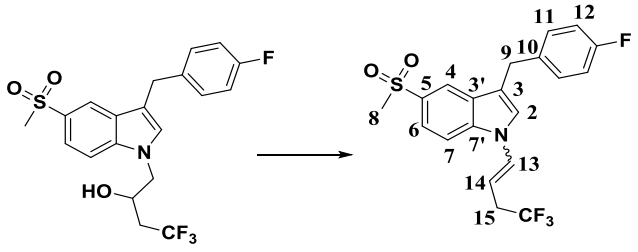
Purity 98%

*III.4.1.14 General Procedure for the preparation of 3-(4-halobenzyl)-5-(methylsulfonyl)-1-(4,4,4-trifluorobut-1-en-1-yl)-1H-indole (**16a-c**)*

To a solution of previous compounds **15a-c**, in dry THF (ca. 7 mL per mmol of indole derivative), TEA (2 equiv.) and mesyl chloride (1.3 equiv.) were added at 0ºC under argon. The mixture was stirred at that temperature for 30 min and then allowed to warm at room temperature and stirred for another 1 h 30 min. The solvent was removed under reduced pressure and the residue was dissolved in EtOAc, washed with distilled water, dried over Na_2SO_4, filtered and concentrated.

The crude was dissolved in dry THF, and DBU (1.3 equiv.) was added at room temperature under argon. The reaction mixture was refluxed for 30 min. After this time work-up was performed as described above. The crude was purified by silica flash chromatography (CH_2Cl_2) affording compounds **16a-c**.

III.4.1.14.1 Synthesis of 3-(4-fluorobenzyl)-5-(methylsulfonyl)-1-(4,4,4-trifluorobut-1-en-1-yl)indole (**16a**)



15a **16a**

White solid; 66% yield (0.6:0.4, *Z*/*E*);

m.p. 83-86 ºC;

^{1}H NMR [400 MHz, $(CD_3)_2CO$] δ 8.15 (s, 2H, H4), 7.84-7.78 (m, 3H, H6 and H13*E*), 7.62 (d, *J* = 8.7 Hz, 2H, H7), 7.51 (s, 2H, H2), 7.42-7.31 (m, 5H, H11 and H13*Z*), 7.08-7.00 (m, 4H, H12), 6.01-5.87 (m, 1H, H14*E*), 5.67-5.59 (m, 1H, H14*Z*), 4.21 (s, 4H, H9), 3.44-3.29 (m, 2H, H15*Z*), 3.30-3.15 (m, 2H, H15*E*), 3.07 (s, 6H, H8);

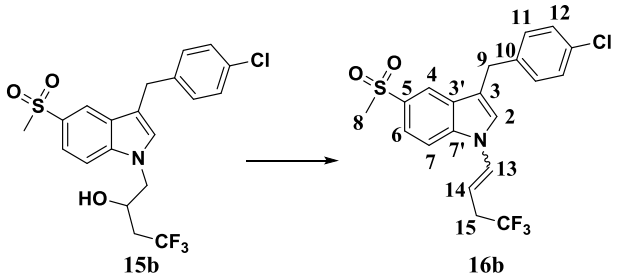
^{13}C NMR [101 MHz, $(CD_3)_2CO$] δ 162.2 (d, *J* = 240 Hz, 2 x CF), 139.7 (2 x C7'), 137.3 (2 x C10), 134.4 (2 x C5), 131.1 (4 x C11), 128.7 (2 x C2 and C13*Z*), 128.3 (2 x C3'), 125.3 (C13*E*), 122.1 (2 x C6), 120.5 (2 x C4), 119.5 (2 x C3), 115.8 (d, *J* = 21 Hz, 4 x C12), 112.1 (C14*Z*), 111.7 (2 x C7), 103.6 (C14*E*), 45.0 (2 x C8), 38.3 (q, *J* = 29 Hz, C15*E*), 32.7 (q, *J* = 30 Hz, C15*Z*), 30.6 (2 x C9);

IR (NaCl) 2928, 1674, 1509, 1303, 1137, 759 cm^{-1};

HRMS (ESI) m/z 412.0989 $[M+H]^+$ (calcd for $C_{20}H_{18}F_4NO_2S$ 412.0994);

Purity 98%

III.4.1.14.2 Synthesis of 3-(4-chlorobenzyl)-5-(methylsulfonyl)-1-(4,4,4-trifluorobut-1-en-1-yl)indole (**16b**)



15b **16b**

White solid; 83% yield (0.6:0.4, *Z*/*E*);

m.p. 97-100ºC;

^{1}H NMR [400 MHz, $(CD_3)_2CO$] δ 8.15 (s, 2H, H4), 7.86-7.77 (m, 3H, H6 and H13*E*), 7.64 (d, *J* = 8.6 Hz, 2H, H7), 7.54 (s, 2H, H2), 7.45-7.27 (m, 9H, H11, H12 and H13*Z*), 5.97-5.90 (m, 1H, H14*E*), 5.68-5.58 (m, 1H, H14*Z*), 4.23 (s, 4H, H9), 3.46-3.28 (m, 2H, H15*Z*), 3.28-3.16 (m, 2H, H15*E*), 3.07 (s, 6H, H8);

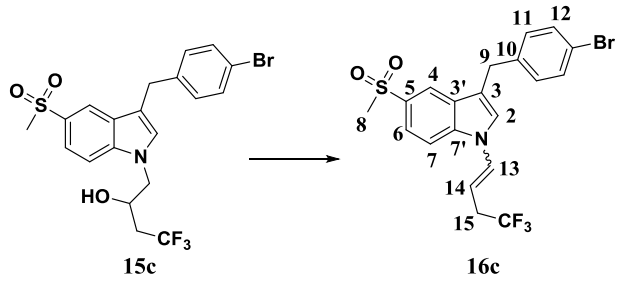
^{13}C NMR [101 MHz, *$(CD_3)_2CO$*] δ 140.3 (2 x C10), 140.0 (2 x C7'), 134.5 (2 x C5), 132.3 (2 x CCl), 131.1 (4 x C11), 129.3 (4 x C12), 128.8 (2 x C2), 128.7 (C13*Z*), 128.4 (2 x C3'), 125.5 (C13*E*), 122.1 (2 x C6), 120.5 (2 x C4), 119.0 (2 x C3), 112.2 (C14*Z*), 111.8 (2 x C7), 103.7 (C14*E*), 44.9 (2 x C8), 35.1 (q, *J* = 29 Hz, C15*E*), 32.7 (q, *J* = 30 Hz, C15*Z*), 30.7 (2 x C9);

IR (NaCl) ν 2928, 1301, 1136 cm^{-1};

HRMS (ESI) m/z 428.0693 $[M+H]^+$ (calcd for $C_{20}H_{18}ClF_3NO_2S$ 428.0699);

Purity 99%

III.4.1.14.3 Synthesis of 3-(4-bromobenzyl)-5-(methylsulfonyl)-1-(4,4,4-trifluorobut-1-en-1-yl)indole (**16c**)



White solid; 69% yield (0.6:0.4, *Z*/*E*);

m.p. 94-97ºC;

^{1}H NMR [400 MHz, *$(CD_3)_2CO$*] δ 8.15 (s, 2H, H4), 7.85-7.77 (m, 3H, H6 and H13*E*), 7.63 (d, *J* = 8.6 Hz, 2H, H7), 7.54 (s, 1H, H2), 7.44 (d, *J* = 7.2 Hz, 4H, H12), 7.34-7.30 (m, 5H, H11 and H13*Z*), 6.04-5.87 (m, 1H, H14*E*), 5.69-5.58 (m, 1H, H14*Z*), 4.20 (s, 4H, H9), 3.45-3.29 (m, 2H, H15*Z*), 3.23 (m, 2H, H15*E*), 3.07 (s, 6H, H8);

^{13}C NMR [101 MHz, *$(CD_3)_2CO$*] δ 140.8 (2 x C10), 139.7 (2 x C7'), 134.5 (2 x C5), 132.2 (4 x C12), 131.5 (4 x C11), 128.8 (2 x C2), 128.7 (C13*Z*), 128.2 (2 x C3'), 125.5 (C13*E*), 122.1 (2 x C6), 120.5 (2 x C4), 120.3 (2 x CBr), 118.9 (2 x C3), 112.3 (C14*Z*), 111.8 (2 x C7), 103.7 (C14*E*), 45.0 (2 x C8), 34.9 (q, *J* = 29 Hz, C15*E*), 32.7 (q, *J* = 29 Hz, C15*Z*), 30.8 (2 x C9);

IR (NaCl) 2925, 1301, 1136 cm^{-1};

HRMS (ESI) m/z 472.0188 $[M+H]^+$ (calcd for $C_{20}H_{18}BrF_3NO_2S$ 472.0194);

Purity 98%

III.4.1.15 COXs inhibition tests

The inhibition of COX-1 (ovine) and COX-2 (human recombinant), by the synthesized compounds was determined in a cell-free system by quantifying the levels of PGF 2α, produced by catalysis of arachidonic acid, using an Enzyme Immunoassay (EIA) kit (COX Inhibitor Screening Assay) supplied by Cayman Chemical Co. The known COX inhibitors indomethacin and celecoxib were used as positive controls. The results are expressed as the percent inhibition of control COX-1 or COX-2 activity. For each experimental condition, were performed at least four independent assays, in duplicate.

III.4.1.16 Docking studies

For all docking calculations Autodock 4.2[135] (Release 4.2.2.1 in combination with Autogrid 4.2.2.) was used for the flexible ligand docking into two X-ray crystal structures 2AYL[136] and 3PGH[20] for COX-1 and COX-2, respectively.

III.4.1.17 NMR studies

The STD-NMR spectra were acquired with a standard pulse sequence from the Bruker library with a spin-lock ($T_{1\rho}$) for protein background suppression and water suppression with excitation sculpting with gradients. 1024 transients were acquired in a matrix of 32 k data points in t2 using a spectral window of 12019 Hz centered at 2812.4 Hz. A 2 kHz spin lock filter with a length of 20 ms was used. Selective saturation of protein resonances was performed by irradiating at -300 Hz (on resonance spectrum) using a series of 51 Eburp2.1000 shaped 90º pulses (50 ms, 1 ms delay between pulses), for a total saturation time of 2.5 s. For the off resonance spectrum irradiation was performed at 20,000 Hz. All data was processed with Bruker Topspin 2.1 and the STD spectra were obtained after subtraction of the on-resonance spectra from the off-resonance spectra. The relative STD effect for a given hydrogen -(I_{STD}/I_0) x 100, where I_0 and I_{STD} are the intensities of the reference (off resonance) and difference (STD-NMR) spectra respectively e was normalized using the highest intensity STD response as a reference for every spectrum.

COX-1 from ram seminal vesicles and COX-2 from sheep placenta were purchased from Cayman Chemical Co. The proteins are supplied in 80 mM TriseHCl, pH 8.0, 0.1% Tween 20 and 300 mM diethyldithiocarbamate and were used as such. Stock solutions of compounds 14d and 15d (2 mM) were prepared in *DMSO-d_6*. The samples for STD-NMR experiments were prepared by adding the appropriate amount of ligand stock solution to a 3 mm NMR tube containing the enzyme. Final concentrations were in the range of 3 mM of COX and 300 mM of ligand(s) in a total volume of 300 mL, when necessary volumes were corrected with 80 mM TriseHCl buffer at pH 8.0.

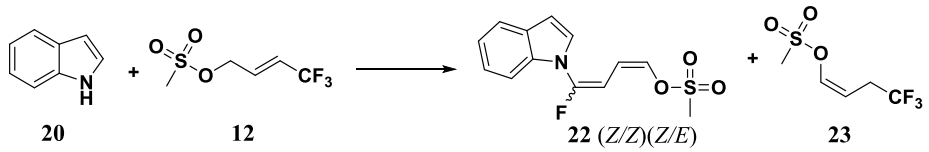
III.4.2 Mechanistic investigation of the reaction of indole with trifluoromethylated olefins

III.4.2.1 General

Melting points were determined on a Reichert Thermovar apparatus and are uncorrected. ^{1}H-, ^{13}C- and ^{19}F-NMR spectra were recorded in *$CDCl_3$* on a Bruker ARX 400 spectrometer at 400, 101.62 and 377 MHz respectively.Chemical shifts are expressed in parts per million (ppm) relative to tetramethylsilane (TMS). The coupling constants (*J*) are reported in Hertz (Hz). High resolution mass spectra were recorded on an AutoSpecQ spectrometer. GC-FID spectra were recorded on a Konik HRGC 4000B spectrometer equipped with a DB-WAX column (30 m; 0.32 I.D.; 0.25 μm film). GC-TOF-MS spectra were recorded on a Micromass GCT spectrometer. IR spectra were run on an FT PerkinElmer 683 instrument, with absorption frequencies expressed in reciprocal centimeters. The progress of all reaction was monitored by thin-layer chromatography, which was performed on Merck silica gel 60 F254 plates. Flash column chromatography was carried out on Merck silica gel 60 (230-400 mesh). For preparative thin layer chromatography was used Merck silica gel 60 GF_{254} in 20x20 glass plates. Anhydrous solvents were dried as described[131] and freshly distilled. Semi-preparative HPLC was run on a Merck Hitachi system consisting of an L-7100 pump, Rheodyne type injector, a D-7000 interface and an L-7450 diode array spectrometric detector, equipped with LiChrospher®100 RP-18 column. Eluent system was: 20% A (H_2O/TFA pH 2.5), 80% B (MeOH) to 10% A, 90% B; flow rate = 1 mL/min.

DFT calculations were performed with Gaussian 09, Revision. B.01.[113]

*III.4.2.2 Reaction of indole (**20**) with (E)-4,4,4-trifluorobut-2-en-1-yl methanesulfonate(**12**)*



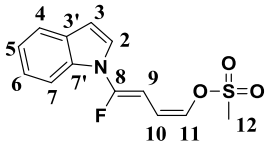
General procedure (table III.9, entry 1):

To a solution of **20** (20 mg, 0.17 mmol) in dry DMF (1.3 mL) was added NaH (6.8 mg, 0.17 mmol) at 0ºC under argon. The reaction mixture was stirred at that temperature for 45 min. **12** (38.3 mg, 0.19 mmol) was added and mixture was stirred, at 0ºC under argon, for 1 h. The reaction was quenched with distilled water and extracted with EtOAc. The combined organic layers were washed with water and brine, dried over Na_2SO_4, filtered and concentrated. The crude was purified by flash chromatography (*n*-hexane/Et_2O 4:1 to 3:2) affording 24.4 mg a mixture of compounds **22 (*Z*/*Z*)(*Z*/*E*)** and **23** (1:0.6:0.6). Isomers **22(*Z*/*Z*)(*Z*/*E*)** were later separated by HPLC. Compound **23** could not be isolated.

Table III.9 – Reaction conditions.

Entry	Solvent	Base (equiv.)	Products	Yield (%)	Observations
1	DMF	NaH (1)	22(*Z*/*Z*)(*Z*/*E*) (1:0.6) 23	38 16	---
2	DMF	NaH (0.25)	22(*Z*/*Z*)(*Z*/*E*) (1:0.6)	12	---
3	DMF	NaH (0.5)	22(*Z*/*Z*)(*Z*/*E*) (1:0.6)	22	---
4	DMF	NaH (2)	22(*Z*/*Z*)(*Z*/*E*) (1:0.5)	83	2 equiv. of 20 were used
5	DMF	NaH (1)	22(*Z*/*Z*)(*Z*/*E*) (1:0.6) 23	47 1	5x diluted
6	DMF	NaH (1)	22(*Z*/*Z*)(*Z*/*E*) (1:0.5) 23	32 40	inversion of reagents addition
7	MeCN	NaH (1)	22(*Z*/*Z*)(*Z*/*E*) (1:0.5)	31	---
8	MeCN	NaH (1)	22(*Z*/*Z*)(*Z*/*E*) (1:0.6) 23	40 7	addition of crown ether (1 mol%)
9	THF	NaH (1)	22(*Z*/*Z*)(*Z*/*E*) (1:0.6) 23	3 9	---
10	THF	NaH (1)	22(*Z*/*Z*)(*Z*/*E*) (1:0.6) 23	25 19	addition of crown ether (1 mol%)
11	THF	^{t}BuOK (1)	22(*Z*/*Z*)(*Z*/*E*) (1:0.6) 23	29 6	addition of crown ether (1 mol%)
12	THF	*n*-BuLi (1)	---	---	no reaction occurred

*(1Z,3Z)-4-fluoro-4-(indol-1-yl)buta-1,3-dien-1-yl methanesulfonate [**22(Z/Z)**]*



22 (*Z/Z*)

Isolated as a pale yellow oil;

^{1}H NMR (400 MHz, *CDCl$_3$*) δ 7.65-7.58 (m, 2H, H2 and H5), 7.35-7.28 (m, 1H, H6), 7.26-7.20 (m, 2H, H4 and H7), 6.66 (d, *J* = 3.4 Hz, 1H, H3), 6.59 (d, *J* = 5.9 Hz, 1H, H11), 6.11 (ddd, *J* = 11.2, 5.9 Hz, 1H, H10), 5.86 (dd, *J* = 28.9, 11.2 Hz, 1H, H9), 3.11 (s, 3H, H12);

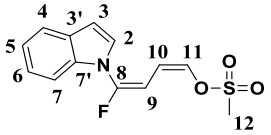
^{13}C NMR (101 MHz, *CDCl$_3$*) δ 149.7 (d, *J* = 267.3 Hz, C8), 135.1 (C7'), 133.7 (d, *J* = 4.8 Hz, C11)), 129.7 (C3'), 125.4 (C2), 124.0 (C6), 122.2 (C5), 121.6 (C4), 112.1 (C7), 109.2 (d, *J* = 1.8 Hz, C10), 106.8 (C3), 88.5 (d, *J* = 19.1 Hz, C9), 37.9 (C12);

^{19}F NMR (377 MHz, $CDCl_3$) δ -93.01 (d, *J* = 28.9 Hz);

IR (NaCl) 2926, 1688, 1633, 1456, 1367, 1182, 967 cm^{-1};

HRMS (HPLC-ESI-TOF) m/z 282,0595 $[M+H]^+$ (calcd for $C_{13}H_{13}FNO_3S$ 282,06002).

*(1Z,3E)-4-fluoro-4-(indol-1-yl)buta-1,3-dien-1-yl methanesulfonate [**22(Z/E)**]*



22 (*Z/E*)

Isolated as a pale yellow oil;

^{1}H NMR (400 MHz, *CDCl$_3$*) δ 7.64 (d, *J* = 7.9 Hz, 1H, H4), 7.37 (d, *J* = 7.9 Hz, 1H, H7), 7.30 (t, *J* = 7.5 Hz, 1H, H6), 7.23 (t, *J* = 7.5 Hz, 1H, H5), 7.19 (d, *J* = 3.4 Hz, 1H, H2), 6.69 (d, *J* = 3.4 Hz, 1H, H3), 6.51 (d, *J* = 6.0 Hz, 1H, H11), 6.25 (dd, *J* = 11.4, 6.0 Hz, 1H, H9), 5.42 (dd, *J* = 11.4, 6.0 Hz, 1H, H10), 3.14 (s, 3H, H12);

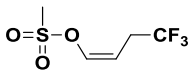
^{13}C NMR (101 MHz, *CDCl$_3$*) δ 149.6 (d, *J* = 268.5 Hz, C8), 136.0 (C7'), 135.0 (d, *J* = 10.8 Hz, C11), 129.0 (C3'), 127.0 (C2), 123.8 (C6), 122.1 (C5), 121.4 (C4), 111.5 (C7), 109.6 (d, *J* = 4.5 Hz, C10), 106.5 (C3), 94.3 (d, *J* = 40.5 Hz, C9), 38.1 (C12);

^{19}F NMR (377 MHz, *CDCl$_3$*) δ -81.86 (d, *J* = 6.0 Hz);

IR (NaCl) 2925, 1687, 1629, 1456, 1367, 1182, 964 cm^{-1};

HRMS (HPLC-ESI-TOF) m/z 282,0595 $[M+H]^+$ (calcd for $C_{13}H_{13}FNO_3S$ 282,06002).

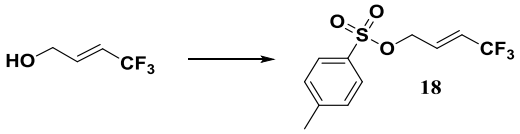
*(Z)-5,5,5-trifluoropent-1-en-1-yl methanesulfonate (**23**)*



23

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.78 (d, *J* = 5.7 Hz, 1H), 5.14 (dd, *J* = 13.6, 7.3, 1H), 3.10 (s, 3H), 3.06-2.95 (m, 2H).

*III.4.2.3 Synthesis of (E)-4,4,4-trifluorobut-1-en-1-yl 4-methylbenzenesulfonate (**18**)*

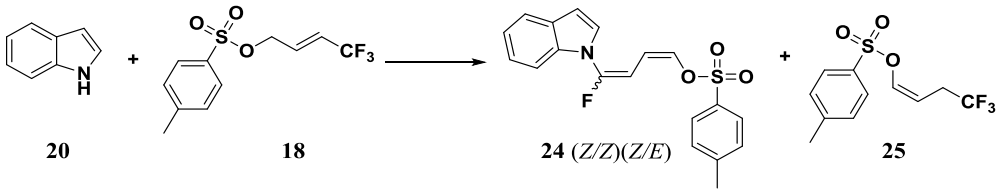


18

To a solution of (*E*)-4,4,4-trifluorobut-2-en-1-ol (500 mg, 3.96 mmol), in dry DCM (5 mL), was added TEA (0.6 mL, 4.36 mmol), in an ice bath. The mixture was stirred for 10 min at this temperature, and then tosyl chloride (831.7 mg, 4.36 mmol) was added. The reaction was stirred for 30 min at 0ºC and the ice bath was removed and the mixture was stirred for 1 h, at room temperature. The reaction was quenched with distilled water and extracted with EtOAc. The combined organic layers were dried over Na_2SO_4, filtered and concentrated. The crude was purified by flash chromatography (*n*-hexane/Et_2O 4:1) affording **18** (712.1 mg, 64% yield) as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80 (d, *J* = 8.3 Hz, 2H), 7.37 (d, *J* = 8.1 Hz, 2H), 6.35-6.25 (m, 1H), 5.95-5.82 (m, 1H), 4.65-4.63 (m, 2H), 2.46 (s, 3H).

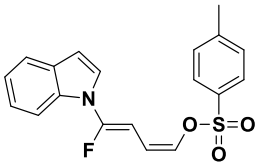
*III.4.2.4 Reaction of indole (**20**) with (E)-4,4,4-trifluorobut-1-en-1-yl 4-methylbenzenesulfonate (**18**)*



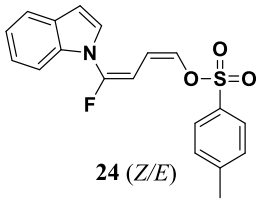
20 **18** **24** (*Z*/*Z*)(*Z*/*E*) **25**

To a solution of **20** (20 mg, 0.17 mmol) in dry DMF (1.3 mL) was added NaH (6.8 mg, 0.17 mmol) at 0ºC under argon. The reaction mixture was stirred at that temperature for 45 min. Then **18** (38.3 mg, 0.19 mmol) was added and mixture was stirred, at 0ºC under argon, for 1 h. The reaction was quenched with distilled water and extracted with EtOAc. The combined organic layers were washed with water and brine, dried over Na_2SO_4, filtered and concentrated. The crude was purified by flash column chromatography (hexane/Et_2O 4:1 to 3:2) affording a mixture of compounds **24(*Z*/*Z*)(*Z*/*E*)** (1:0.3) (10.3 mg,17% yield) and compound **25** (18.8 mg, 51% yield).

*(4-fluoro-4-(5-(methylsulfonyl)-1H-indol-1-yl)buta-1,3-dien-1-yl 4-methylbenzenesulfonate [**24**(Z/Z)(Z/E)]*



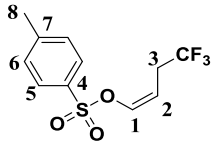
24 (*Z*/*Z*)



24 (*Z*/*E*)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.89-7.76 (m, 4H), 7.61 (d, *J* = 7.4 Hz, 2H), 7.50-7.32 (m, 6H), 7.32-7.17 (m, 4H), 7.14 (d, *J* = 3.5 Hz, 1H), 7.09 (d, *J* = 3.4 Hz, 1H), 6.66-6.61 (m, 2H), 6.47 (d, *J* = 5.4 Hz, 1H for *Z*/*Z*), 6.38 (d, *J* = 6.0 Hz, 1H for *E*/*Z*), 6.07 (dd, *J* = 11.4, 6.1 Hz, 1H for *E*/*Z*), 5.98 (dd, *J* = 11.0, 5.6 Hz, 1H for *Z*/*Z*), 5.64 (dd, *J* = 29.2, 11.2 Hz, 1H for *Z*/*Z*), 5.32-5.24 (m, 1H for *E*/*Z*), 2.50-2.40 (m, 6H).

*(Z)-4,4,4-trifluorobut-1-en-1-yl 4-methylbenzenesulfonate (**25**)*



25

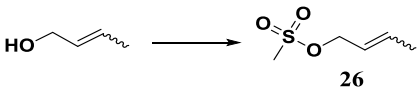
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79 (d, *J* = 8.2 Hz, 2H, H5), 7.36 (d, *J* = 8.1 Hz, 2H, H6), 6.64 (d, *J* = 5.9 Hz, 1H, H1), 5.00 (dd, *J* = 13.6, 7.1 Hz, 1H, H2), 2.91-2.72 (m, 2H, H3), 2.45 (s, 3H, H8).

^{13}C NMR (101 MHz, $CDCl_3$) δ 146.0 (C7), 139.0 (C1), 132.3 (C4), 130.2 (2 x C5), 128.0 (C6), 125.3 (q, *J* = 276.7 Hz, CF_3), 106.0 (C2), 29.6 (q, *J* = 31.2 Hz, C3), 21.77 (C8);

IR (NaCl) 1377, 1193, 1181, 1056, 984 cm^{-1}

MS (EI) m/z 280.02 $[M]^+$ (calcd for $C_{11}H_{11}F_3O_3S$ 280.04).

*III.4.2.5 <u>Synthesis of but-2-en-1-yl methanesulfonate (**26**)</u>*



26

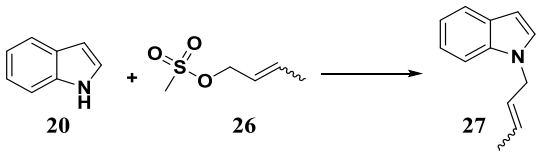
To a solution of but-2-en-1-ol (200 mg, 2.77 mmol), in dry DCM (2 mL), was added TEA (0.4 mL, 3.05 mmol), in an ice bath. The mixture was stirred for 10 min, at this temperature, and then mesyl chloride (0.2 mL, 3.05 mmol) was added. The reaction was stirred for 30 min at 0ºC, then the ice bath was removed and the mixture was stirred for 1 h, at room temperature. The reaction was quenched with distilled water and extracted with EtOAc. The combined organic layers were dried over Na_2SO_4, filtered

and concentrated. The crude was purified by flash chromatography (*n*-hexane/Et_2O 4:1) affording **26** (332.8 mg, 80% yield) was a colorless oil.

^{1}H NMR (400 MHz, *$CDCl_3$*) δ 5.94-5.85 (m, 1H), 5.64-5.56 (m, 1H), 4.61 (d, *J* = 6.6 Hz, 2H), 2.95 (s, 3H), 1.72 (d, *J* = 6.1 Hz, 2H).

Spectral data were in accordance with the literature.[137]

*III.4.2.6 Reaction of indole (**20**) with but-2-en-1-yl methanesulfonate (**26**)*



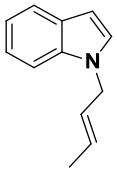
III.4.2.6.1 In DMF using NaH as base

To a solution of **20** (20 mg, 0.17 mmol) in dry DMF (1.3 mL) was added NaH (6.8 mg, 0.17 mmol) at 0ºC under argon. The reaction mixture was stirred at that temperature for 45 min. Then **26** (28.2 mg, 0.19 mmol) was added and mixture was stirred, at 0ºC under argon, for 1 h. The reaction was quenched with distilled water and extracted with EtOAc. The combined organic layers were washed with water and brine, dried over Na_2SO_4, filtered and concentrated. The crude was purified by flash chromatography (*n*-hexane/Et_2O 3:2) affording **27**, as a colorless oil, in 34% yield (10mg).

III.4.2.6.2 In THF using *n*-BuLi as base

The same procedure, as described above, was performed in THF (1.3 mL), using a 1.5 M solution of *n*-BuLi in *n*-hexane (0.1 mL, 0.17 mmol) as base. Product **27** was obtained in 18% yield (5.3 mg).

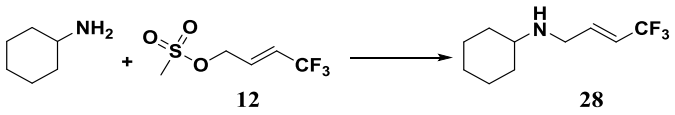
*1-(but-2-en-1-yl)-1H-indole (**27**)*



^{1}H NMR (400 MHz, *$CDCl_3$*) δ 7.72 (d, *J* = 7.9 Hz, 1H), 7.41 (d, *J* = 7.9 Hz, 1H), 7.28 (t, *J* = 7.5 Hz, 1H), 7.20-7.16 (m, 2H), 6.58 (d, *J* = 2.6 Hz, 1H), 5.77-5.64 (m, 2H), 4.71 (s, 2H), 1.77 (s, 3H).

Spectral data were in accordance with the literature.[138]

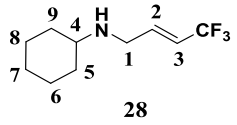
*III.4.2.7 Reaction of cyclohexylmanine with (E)-4,4,4-trifluorobut-2-en-1-yl methanesulfonate (**12**)*



12 **28**

To a solution cyclohexylamine (150 mg, 1.51 mmol) in dry THF (6 mL), was added **12** (102.9 mg, 0.50 mmol) and the mixture was stirred, at room temperature under argon, for 24 h. The reaction was quenched with distilled water and extracted with DCM. The combined organic layers were washed with water and brine, dried over Na_2SO_4, filtered and concentrated. The crude was purified by flash chromatography (*n*-hexane/Et_2O 1:1) affording **28**, as a colorless oil, in 72% yield (74.6 mg).

*(E)-N-(4,4,4-trifluorobut-2-en-1-yl)cyclohexanamine (**28**)*



28

^{1}H NMR (400 MHz, *$CDCl_3$*) δ 6.45-6.39 (m, 1H, H2), 5.84-5.75 (m, 1H, H3), 3.37-3.35 (m, 2H, H1), 2.44-2.37 (m, 1H, H4), 1.86-1.83 (m, 2H, H5 and H9), 1.72-1.69 (m, 2H, H6 and H8), 1.60-1.57 (m, 1H, H7), 1.25-0.99 (m, 5H, H5, H6, H7, H8 and H9);

^{13}C NMR (101 MHz, *$CDCl_3$*) δ 139.7 (q, *J* = 6.2 Hz, C2), 123.3 (q, *J* = 269.1 Hz, CF_3), 118.6 (q, *J* = 33.6 Hz, C3), 56.4 (C4), 46.8 (C1), 33.7 (C5 and C9), 26.2 (C7), 25.0 (C6 and C8).

III.4.2.8 Mass spectrometry studies

III.4.2.8.1 GC-MS studies

The temperature program used was: 60ºC (2 min), 10ºC/min till 250ºC (10 min); $T_{injector}$: 250ºC; Splitless time of 1 min; $T_{interface}$: 250ºC; Solvent delay of 5 min; $V_{injected}$: 1µL.

Three assays were performed in GC-MS: *a)* reaction crude without work-up; *b)* reaction crude after work-up but without purification and; *c)* reaction products isolated by PTLC.

The procedure used is described above in section III.4.3.1, using the conditions mentioned in table III.9, entry 9.

Table III.10 – GC-MS studies.

		Relative intensity (%)			
Retention time (min)	**$[M]^+$ (FI)**	**a)**	**b)**	**c)***	**Fragmentations (EI)**
11.77	203.99	49	64	100	126.02; 78.97
11.83	183.99			---	105.01
12.79	204.00	15	12	24	126.03; 108.99; 78.98
13.31**	204.00; 184.00	17	22	---	125.01; 109.01; 78.97; 64.96
15.63	225.04	28	13	---	156.08; 116.05
16.15	225.06	32	27	---	156.08
17.93***	117.06	100	100	---	90.02; 63.01; 58.52
20.21	195.05	11	8	---	116.04
20.77	225.08	5	---	---	---

*Between 20-28 min there is a broad peak that corresponds to a compound with a MW of 281.05. This observation is due to the retention of the compound in the column.

Corresponds to compound **12. Co-elution with a compound with MW of 184.00.

***Corresponds to compound **20**.

III.4.2.8.2 MS studies

The crudes *a)* and *b)* as well as the reaction products isolated by PTLC (*c)*) previously analyzed by GC-MS (section III.4.3.7.1) were submitted to MS analysis. The assays were carried out without heating on probe and also with temperature on probe = 50ºC.

Table III.11 – MS studies.

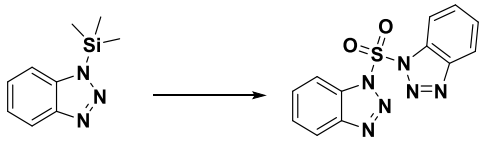
	Relative intensity (%)						
	Without Δ			**Tprobe = 50ºC**			
$[M]^+$	**a)**	**b)**	**c)**	**a)**	**b)**	**c)**	
						1st spectrum	2nd spectrum
117.06	100	100	---	4	1	---	---
184.00	4	15	---	1	---		
195.04	14	1	---	14	---		
203.99	25	34	100	7	5	100	5
225.08	45	8	---	7	---	---	---
281.05	---	---	---	100	100	---	100
301.06	---	---	---	12	3	---	5

III.4.3 Studies towards a new methodology for the synthesis of sulfonyl-containing compounds

III.4.3.1 General

Melting points were determined on a Reichert Thermovar apparatus and are uncorrected. ^{1}H- and ^{13}C-NMR spectra were recorded in *DMSO*, $CDCl_3$, d_8-*THF or* C_6D_6 on a Bruker ARX 400 spectrometer at 400 and 100.62 MHz respectively. Chemical shifts are expressed in parts per million (ppm) relative to tetramethylsilane (TMS). The coupling constants (*J*) are reported in Hertz (Hz). High resolution mass spectra were recorded on an AutoSpecQ spectrometer. Elemental analysis spectra were recorded on a Thermo Finnigan-CE Instruments, model Flash EA 1112 CHNS series. IR spectra were run on an FT PerkinElmer 683 instrument, with absorption frequencies expressed in reciprocal centimeters. Anhydrous solvents were dried as described[131] and freshly distilled and degassed. All the air-sensitive reactions were performed or prepared in a glovebox.

*III.4.3.2 Synthesis of 1,1'-sulfonylbis(benzotriazole) (**33**)*[117]



32 **33**

To a solution of 1-(trimethylsilyl)-1*H*-benzotriazole (**32**) (1.4 mL, 7.84 mmol), in dry toluene (2 mL), cooled with an ice bath, was slowly added SO_2Cl_2 (0.32 mL, 3.92 mmol), under argon atmosphere. The mixture was allowed to warm to room temperature and was stirred for 24 h. After that time the solid was filtered and washed with Et_2O, affording **33** with 48% yield (1.13 g) as a white solid.

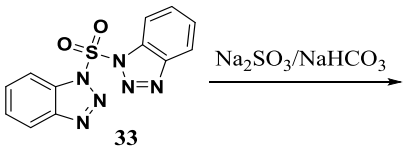
m.p. 110-113ºC (Et_2O) [lit.[115] 165-166ºC (dec.) (Et_2O)];

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.24 (d, *J* = 8 Hz, 2H), 8.09 (d, *J* = 8.0 Hz, 2H), 7.80-7.76 (m, 2H), 7.57-7.53 (m, 2H);

IR (KBr) 1442, 1204, 905, 751 cm^{-1};

Spectral data were in accordance with the literature.[117]

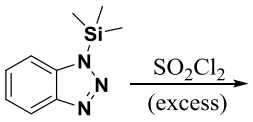
*III.4.3.3 Reaction of 1,1'-sulfonylbis(benzotriazole) (**33**) with sodium sulfite and sodium hydrogenoarbonate*[118]



33

A solution of Na_2SO_3 (29.4 mg, 0.23 mmol), in H_2O (0.3 mL), was stirred for 10 min at room temperature. Then $NaHCO_3$ (39.2 mg, 0.46 mmol) was added and the mixture was stirred at 50ºC for 1 h. Compound **33** (70 mg, 0.23 mmol) was added and the reaction mixture was stirred for 3 h at 50ºC. The solvent was removed under reduced pressure, redissolved in MeOH and the solid was filtered. This reaction afforded a complex mixture of products that were not possible to identify.

*III.4.3.4 Reactions of 1-(trimethylsilyl)-1H-benzotriazole (**32**) with an excess of sulfuryl chloride*



32

III.4.3.4.1 In toluene, using 1.1 equivalents of sulfuryl chloride

To a solution of 1-(trimethylsilyl)-1*H*-benzotriazole (**32**) (0.19 mL, 1.04 mmol), in dry toluene (0.4 mL), cooled with an ice bath, was slowly added SO_2Cl_2 (93.2 µL, 1.15 mmol), under argon atmosphere. The mixture was allowed to warm to room temperature and was stirred for 24 h. After that time the solid was filtered and washed with Et_2O, affording **33** with 41% yield (129.3 mg) as a white solid.

m.p. 105-110ºC (Et_2O) [lit.[117] 165-166ºC (dec.) (Et_2O)];

^{1}H NMR (400 MHz, *CDCl*$_3$) δ 8.24 (d, *J* = 8.1 Hz, 2H), 8.09 (d, *J* = 8.1 Hz, 2H), 7.78 (t, *J* = 7.5 Hz, 2H), 7.55 (t, *J* = 7.6 Hz, 2H);

IR (KBr) 1442, 1205, 905, 752 cm^{-1}.

III.4.3.4.2 Neat conditions, using 5 equivalents of sulfuryl chloride

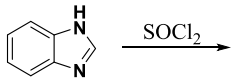
In a round bottom flask containing SO_2Cl_2 (0.42 mL, 5.23 mmol), cooled with an ice bath, was slowly added 1-(trimethylsilyl)-1*H*-benzotriazole (**32**) (0.19 mL, 1.04 mmol), under argon atmosphere. Then the ice bath was removed and the mixture was stirred for 24 h. After this time Et_2O was added and the solid was filtered and washed with Et_2O, affording benzotriazole (**31**) in 75% yield (93.7 mg) as a white solid.

m.p. 95-97ºC (lit.[139] 96-99ºC);

^{1}H NMR (400 MHz, *$CDCl_3$*) δ 7.95-7.92 (m, 2H), 7.43-7.40 (m, 2H);

IR (KBr) 3085, 2800, 1212, 743 cm^{-1}.

*III.4.3.5 Reaction of benzimidazole (**35**) with tionyl chloride[119]*



35

To a solution of **35** (250 mg, 2.12 mmol), in dry DCM (2 mL), was slowly added $SOCl_2$ (38.5 µL, 0.53 mmol), under argon atmosphere. The mixture was stirred at room temperature for 30 min. After that time the solution was filtered, under argon, and the solid was washed with dry DCM (5 mL). $SOCl_2$ (16.2 µL, 0.22 mmol) was slowly added to the solution. The mixture was stirred for 10 min then NaH (17.8 mg, 0.44 mmol) and H_2O (8 µL, 0.44 mmol) were added. The solid was filtered and washed with dry DCM, affording benzimidazole sodium salt, as a white solid.

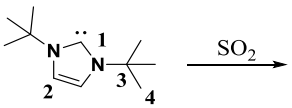
^{1}H NMR (400 MHz, *DMSO-d_6*) δ 8.10 (s, 1H), 7.54-7.51 (m, 2H), 7.10-7.08 (m, 2H);

IR (KBr) 2930, 1458, 1410, 1246, 744 cm^{-1}.

Data are in accordance with authentic sample.

III.4.3.6 Studies towards SO_2 capture using NHCs

III.4.3.6.1 Reaction of 1,3-di-tert-butylimidazol-2-ylidene (**37**) with SO_2



37

III.4.3.6.1.1 Neat conditions

In a dried schlenk containing **37** (20 mg, 0.11 mmol), cooled at -20ºC, fitted with a condenser, cooled at -78ºC, was introduced SO_2 gas till the solid was complete covered with SO_2 liquid. The sulfur dioxide flow was stopped and the flask allowed to warm up to -10ºC and left stirring for 1h. The cooling baths were removed and a flow of argon was introduced in the system, till complete evaporation of SO_2, affording 15 mg of a white solid that was analyzed without further purification.

m.p. 145-150ºC;

^{1}H NMR (400 MHz, *$CDCl_3$*) δ 10.17 (s, < 1H, H1), 9.59 (s, < 1H, H1'), 7.56 (s, 2H, H2), 1.71 (s, 18H, H4);

^{13}C-NMR (101 MHz, *$CDCl_3$*) δ 134.2 (C1), 120.2 (C2), 60.9 (C3), 30.1 (C4);

IR (KBr) 3055, 2974, 1547, 1377, 1205, 1136, 1041, 968, 782 cm^{-1};

Elemental analysis Calcd.: C, 54.07; N, 11.46; H, 8.25; S, 13.12; Found: C, 47.11; N, 9.62; H, 6.88; S, 10.50.

III.4.3.6.1.2 Reaction in solution

General: In a dried schlenk containing a 0.1 M solution of **37**, cooled at -20ºC, was bubbled SO_2 gas, for 5 min. The sulfur dioxide flow was stopped and the mixture was left stirring for 1 h. The cooling baths were removed and a flow of argon was introduced in the system, till complete evaporation of SO_2. Then the solvent was removed under vacuum and the solid collected was analyzed without further purification.

In toluene

m.p. hygroscopic;

^{1}H NMR (400 MHz, *DMSO-d_6*) δ 9.88 (s, < 1H, H1), 9.17 (s, <1 H, H1'), 9.06 (s, < 1H, H1''), 8.07 (s, 2H, H2), 1.61 (s, 18H, H4);

^{1}H NMR (400 MHz, *DMSO-d_6+D_2O*) δ 8.95 (s, 1H, H1), 7.96 (s, 2H, H2), 1.58 (s, 18H, H4)

^{13}C-NMR (101 MHz, *DMSO-d_6*) δ 132.2 (C1), 120.4 (C2), 59.6 (C3), 29.1 (C4);

IR (KBr) 3432 (br), 2978, 1640, 1545, 1379, 1203, 1124, 1096, 970 cm^{-1}.

In diethyl ether

m.p. 199-204ºC;

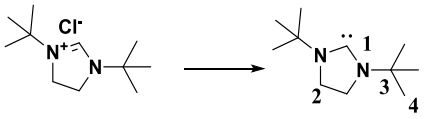
^{1}H NMR (400 MHz, *DMSO-d_6*) δ 9.88 (s, < 1H, H1), 9.05 (s, <1 H, H1'), 8.07 (s, 2H, H2), 1.61 (s, 18H, H4);

^{13}C-NMR (101 MHz, *DMSO-d_6*) δ 132.3 (C1), 120.5 (C2), 59.6 (C3), 29.1 (C4);

IR (KBr) 3055, 1205, 1124, 968, 810 cm^{-1};

Elemental analysis Calcd.: C, 54.07; N, 11.46; H, 8.25; S, 13.12; Found: C, 49.20; N, 10.13; H, 7.33; S, 11.27.

III.4.3.6.2 Synthesis of 1,3-di-tert-butylimidazolidine-2-ylidene (**39**)



39

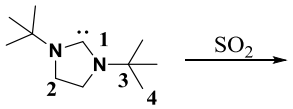
To a solution of 1,3-bis(*tert*-butyl)ylimidazolinium chloride (200 mg, 0.91 mmol), in dry toluene (2.7 mL), was added KHMDS (182.4 mg, 0.91 mmol). The mixture was stirred for 2 h at room temperature. After this time the crude was filtered under celite. The solvent was removed under vacuum, affording **39**, in 80% yield (132.4 mg) as a beige solid.

^{1}H NMR (400 MHz, C_6D_6) δ 3.03 (s, 4H, H2), 1.36 (s, 18H, H4);

^{13}C NMR (101 MHz, C_6D_6) δ 218.3 (C1), 54.0 (C2), 44.5 (C3), 30.0 (C4);

Spectral data were in accordance with the literature.[140]

III.4.3.6.3 Reaction of 1,3-di-tert-butylimidazolidine-2-ylidene (**39**) with SO_2



39

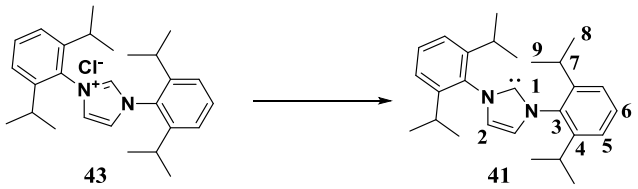
In a dried schlenk containing **39** (100 mg, 0.55 mmol), in dry THF (2 mL), was bubbled SO_2 gas, for 5 min. The sulfur dioxide flow was stopped and the mixture was left stirring for 20 min. A flow of argon was introduced in the system, till complete evaporation of SO_2. Then the solvent was removed under vacuum, affording 103.1 mg of a white solid that was analyzed without further purification.

m.p. hygroscopic

^{1}H NMR (400 MHz, *DMSO-d_6*) δ 8.15 (s, 1H, H1), 3.92 (s, 4H, H2), 1.35 (s, 18H, H4);

IR (KBr) 2972, 1634, 1210, 1173, 972 cm^{-1}.

III.4.3.6.4 Synthesis of 1,3-bis-(2,6-di-iso-propylphenyl)imidazolin-2-ylidene (**41**)



43 **41**

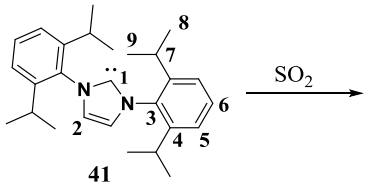
To a solution of 1,3-bis(2,6-di-*iso*-propylphenyl)imidazolinium chloride (**43**) (200 mg, 0.47 mmol), in dry toluene (2.5 mL), was added KHMDS (133.5 mg, 0.47 mmol). The mixture was stirred for 2 h at room temperature. After this time the crude was filtered under celite and the solvent was removed under vacuum, affording **41**, in 52% yield (96 mg) as a beige solid.

^{1}H NMR (400 MHz, C_6D_6) δ 7.31-7.28 (m, 2H, H6), 7.16 (4H, H5, *under the solvente peak*), 6.61 (s, 2H, H2), 2.61-2.47 (m, 6H, H7), 1.28 (d, *J* = 7 Hz, 12H, H8), 1.18 (d, *J* = 7 Hz, 12H, H9);

^{13}C NMR (101 MHz, C_6D_6) δ 220.4 (C1), 146.1 (C4), 138.8 (C3), 128.8 (C6), 123.5 (C5), 121.4 (C2), 28.6 (C7), 24.6 (C8), 23.5 (C9).

Spectral data were in accordance with the literature.[141]

III.4.3.6.5 Reaction of 1,3-bis-(2,6-di-iso-propylphenyl)imidazolin-2-ylidene (**41**) with SO_2



41

III.4.3.6.5.1 Neat conditions

In a dried schlenk containing **41** (80 mg, 0.20 mmol) cooled at -20ºC, fitted with a condenser cooled at -78ºC, was introduced SO_2 gas till the solid was complete covered with SO_2 liquid. The sulfur dioxide flow was stopped and the flask allowed to warm up to -10ºC and left stirring for 1 h. The cooling baths were removed and a flow of argon was introduced in the system, till complete evaporation of SO_2, affording 49.2 mg of a white solid that was analyzed without further purification.

m.p. 289-300ºC (dec.);

^{1}H NMR (400 MHz, *d_8-THF*) δ 10.60 (s, 1H, H1), 8.23 (s, 2H, H2), 7.54 (t, *J* = 7.7 Hz, 2H, H6), 7.39 (d, *J* = 7.7 Hz, 4H, H5), 2.61-2.47 (m, 6H, H7), 1.26-1.25 (m, 24H, H8 and H9);

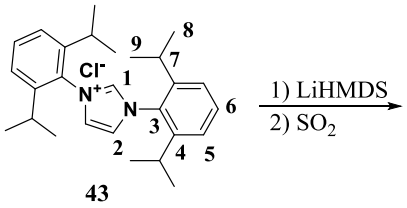
^{13}C-NMR (101 MHz, *d_8-THF*) δ 146.4 (C4), 141.7 (C1), 132.4 (C6), 131.9 (C3), 127.5 (C2), 125.2 (C5), 30.0 (C7), 24.8 (C8), 24.1 (C9);

IR (KBr) 2965, 1545, 1199, 1177, 1059 cm^{-1};

III.4.3.6.5.2 Reaction in solution

In a dried schlenk containing **41** (45 mg, 0.12 mmol), in dry THF (2 mL), was bubbled SO_2 gas, for *ca* 5 min. The sulfur dioxide flow was stopped and the mixture was left stirring for 1 h. A flow of argon was introduced in the system, till complete evaporation of SO_2. Then the solvent was removed under vacuum, affording 52.6 mg of a beige solid. ^{1}H-NMR spectrum of this solid showed a complex mixture of products that was no further investigated.

III.4.3.6.6 Reaction of 1,3-bis(2,6-di-iso-propylphenyl)imidazolinium chloride (**43**) with SO_2



43

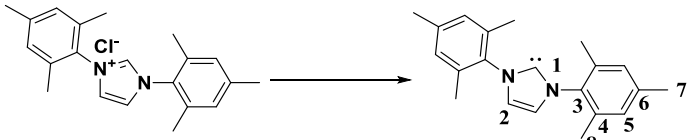
In a dried schlenk containing **43** (100 mg, 0.23 mmol), in dry THF (2 mL), was added a 1 M solution of LiHMDS (0.26 mL, 0.26 mmol). The solution was stirred for 2 h at room temperature. After that time, the mixture was cooled at -50ºC and SO_2 was bubbled for *ca* 5 min, then stirred for 10 min, then SO_2 was bubbled for another 5 min. The reaction was stirred for 30 min. The cooling bath was removed, and a flow of argon was introduced till complete evaporation of SO_2. The solvent was removed under vacuum and the solid washed with dry Et_2O, affording 89.3 mg a beige solid that was analyzed without further purification.

m.p. 278-285ºC

^{1}H NMR (400 MHz, *DMSO-d_6*) δ 10.16 (s, 1H, H1), 8.55 (s, 2H, H2), 7.68 (t, *J* = 7.6 Hz, 2H, H6), 7.52 (d, *J* = 7.6 Hz, 4H, H5), 2.36-2.32 (m, 4H, H7), 1.25 (d, *J* = 6.8 Hz, 12H, H8), 1.15 (d, *J* = 6.8 Hz, 12H, H9);

IR (KBr) 2969, 1537, 948, 810 cm^{-1}.

III.4.3.6.7 Synthesis of 1,3-bis(2,4,6-trimethylphenyl)imidazol-2-ylidene (**42**)



44 **42**

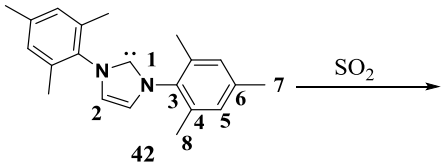
To a solution of 1,3-bis(2,4,6-trimethylphenyl)imidazolinium chloride (**44**) (200 mg, 0.59 mmol), in dry toluene (2.5 mL), was added KHMDS (133.5 mg, 0.59 mmol). The mixture was stirred for 2 h at room temperature. After this time the crude was filtered under celite and the solvent was removed under vacuum, affording **42** as a beige solid, in 57% yield (103.2 mg).

^{1}H NMR (400 MHz, C_6D_6) δ 6.81 (s, 4H, H5), 6.49 (s, 2H, H2), 2.16 (s, 18H, H7 and H8);

^{13}C NMR (101 MHz, C_6D_6) δ 219.2 (C1), 139.3 (C6), 137.3 (C3), 135.4 (C4), 129.1 (C5), 120.6 (C2), 21.1 (C7), 18.1 (C8).

Spectral data were in accordance with the literature.[142]

III.4.3.6.8 Reaction of 1,3-bis(2,4,6-trimethylphenyl)imidazol-2-ylidene (**42**) with SO_2



42

In a dried schlenk containing **42** (80 mg, 0.26 mmol), was added dry THF (8 mL). The schlenk was sealed and connected to a vacuum line. Then it was connected to the SO2 system and the schlenk was filled with this gas. The solution was stirred for 24 h at room temperature. After that time, a flow of argon was introduced till complete evaporation of SO_2. The solvent was removed under vacuum, till 1/3 of the initial volume, then pentane was added. The solid were washed with pentane and dried under vacuum, affording a beige solid (52.3 mg) that was analyzed without further purification.

m.p. > 300ºC (dec.)

^{1}H NMR (400 MHz, *$CDCl_3$*) δ 9.37 (s, 1H, H1), 7.67 (s, 2H, H2), 6.99 (s, 4H, H5), 2.31 (s, 6H, H7), 2.10 (s, 12H, H8);

^{13}C NMR (101 MHz, *$CDCl_3$*) δ 141.4 (C3), 138.2 (C6), 134.3 (C4), 130.7 (C1), 129.9 (C5), 125.3 (C2), 21.3 (C7), 17.5 (C8);

IR (KBr) 3432, 2923, 1541, 1230, 1035 cm^{-1};

Elemental analysis Calcd.: C, 68.45; N, 7.60; H, 6.56; S, 8.70; Found: C, 60.63; N, 6.55; H, 6.28; S, 7.01.

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Chapter IV New indole derivatives as antitubercular agents: synthetic studies

My contribution for this work was the preparation of all synthetic compounds.

IV.1 Introduction

Tuberculosis (TB) is a contagious and deadly disease that spreads through air, and has reached pandemic proportions.[1]

TB is caused predominantly by the bacillus *Mycobacterium tuberculosis (M.tb)*, which is an aerobic pathogenic bacterium that establishes its infection preferentially to the pulmonary system. It is a weak Gram-positive rod-shaped bacterium that has no flagellum, does not form spores nor produces toxins and it has no capsule. Currently, there are 60 known species among the *Mycobacterium* genera and a minority of these species are pathogenic to humans, causing TB (*M. tuberculosis, M. bovis* and *M. africanum*) and leprosy (*M. leprae*).[2] Mycobacteria produce an extremely uncommon cell wall structure. Its peptidoglycan contains *N*-glycolylmuramic acid instead of the usual *N*-acetylmuramic acid. Another distinctive feature is that up to 60% of the mycobacterial cell wall is composed of lipids that consists almost of unusually long-chain fatty acids with 60 to 90 carbons, the mycolic acids. Mycolic acids are branched fatty acids that have a short and a long branch, with 22 to 24 and 40 to 64 carbons, respectively. They are covalently bonded to the polysaccharide that composes the cell wall, the arabinogalactan, which is bonded to peptidoglycan by a phosphodiester link. Approximately 10% of the arabinose residues in the arabinogalactan are substituted by mycolic acids. The cell wall also contains several other lipids that are not covalently bonded to this basal skeleton (the mycolycarabinogalactan-peptodoglycan complex). Although mycobacteria have several cell wall lipid types, some are limited to specific species, such as sulfolipids, only present in *M.tb* and being involved in its pathogenicity (figure IV.1).[3-5]

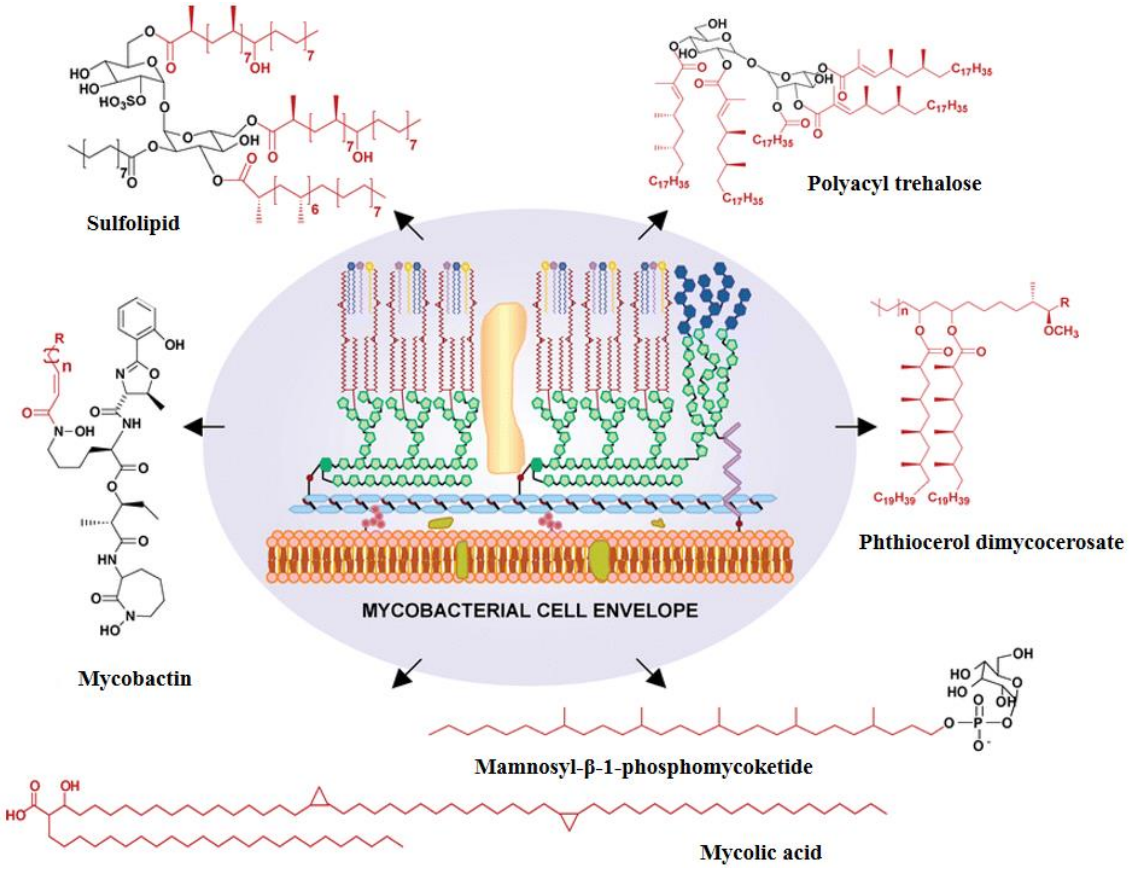


Figure IV.1 – Schematic representation of the mycobacterial cell envelope (adapted from Gokhale, 2007).[6]

The unique structure of the cell wall of *M.tb* allows it to lie dormant for many years as a latent infection, particularly as it can grow readily inside macrophages, hiding it from the host immune system. It usually affects lungs (pulmonary TB), but in about 25% of cases (immunosuppressed people and young children) the bacteria enters the blood and infects other parts of the body, such as pleura, meninges, lymphatic system, genitourinary system and bones and joints.[2]

TB occurs in every part of the world. According to the World Health Organization (WHO), in 2012, 8.6 million people fell ill with TB and 1.3 million died from this disease. Over 95% of TB deaths occur in low- and middle-income countries, and it is among the top three causes of death for women aged 15 to 44. The largest number of new TB cases occurred in Asia, accounting for 60% of new cases globally. However, sub-Saharan Africa carried the greatest proportion of new cases *per* population with over 255 cases *per* 100 000 population in 2012. (figure IV.2).[1]

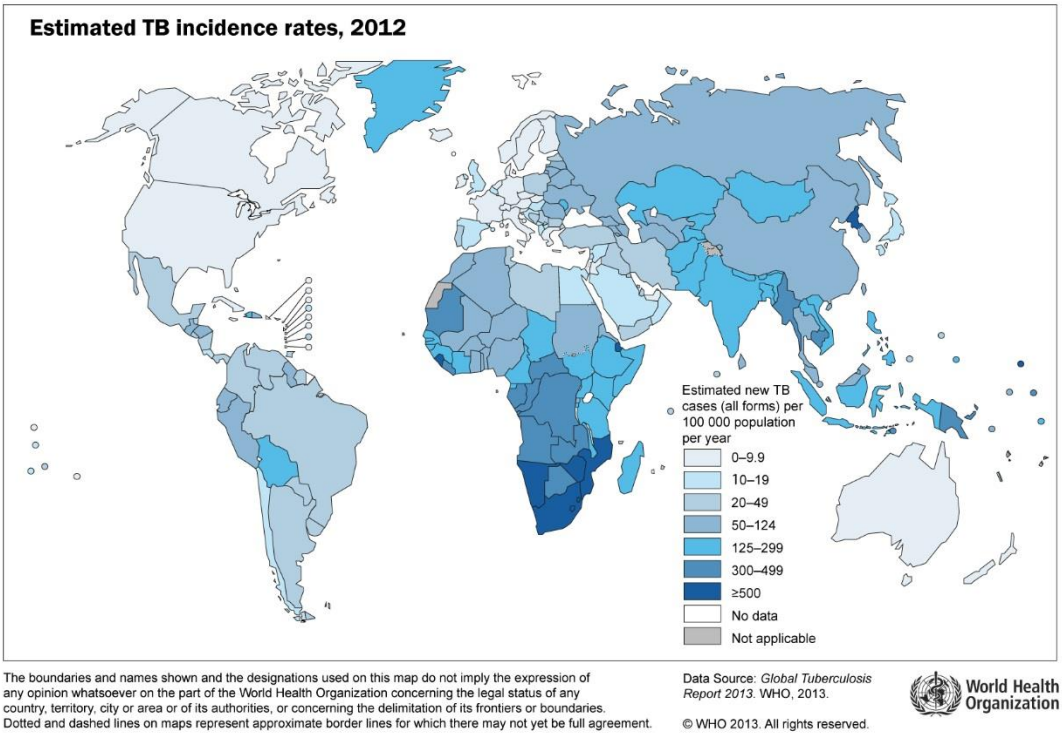


Figure IV.2 – Estimated TB incidence rates in 2012.[1]

Prior to the explosive spread of human immunodeficiency virus (HIV), TB held the distinction of being the single most deadly pathogen for the most part of the 20th century. By the time HIV surpassed TB in the late 1990s, the synergy among these two pathogens was apparent: HIV and TB form a lethal combination, each speeding the other's progress. Someone who is infected with HIV and TB is much more likely to become sick with active TB. In 2012 about 320 000 people died of HIV-associated TB. Almost 25% of deaths among people with HIV are due to TB. There were an estimated 1.1 million new cases of HIV-positive/TB, 75% of whom living in Africa.[1]

The current first-line TB drug regimen is more than 40 years old and consists of rifampicin, isoniazid, pyrazinamide and ethambutol (figure IV.3). These antibiotics are effective in active, drug-susceptible TB, provided that patients complete the course. There is, however, poor patient compliance due to the cost of drugs, adverse effects, the long time required for full treatment and the required number of drug doses. Non-compliance has contributed to the appearance of multi-drug resistant (MDR) and extensively drug resistant (XDR) TB strains. Infection by MDR-TB strains is resistant to second-line drugs such as kanamycin, amikacin, capreomycin, *p*-aminosalicylate, fluoroquinolones (*e.g.*, levofloxacin), ethionamide, and cycloserine (figure IV.3). Thus, XDR-TB exhibits resistance to both first and second-lines drugs and is virtually incurable.[7,8]

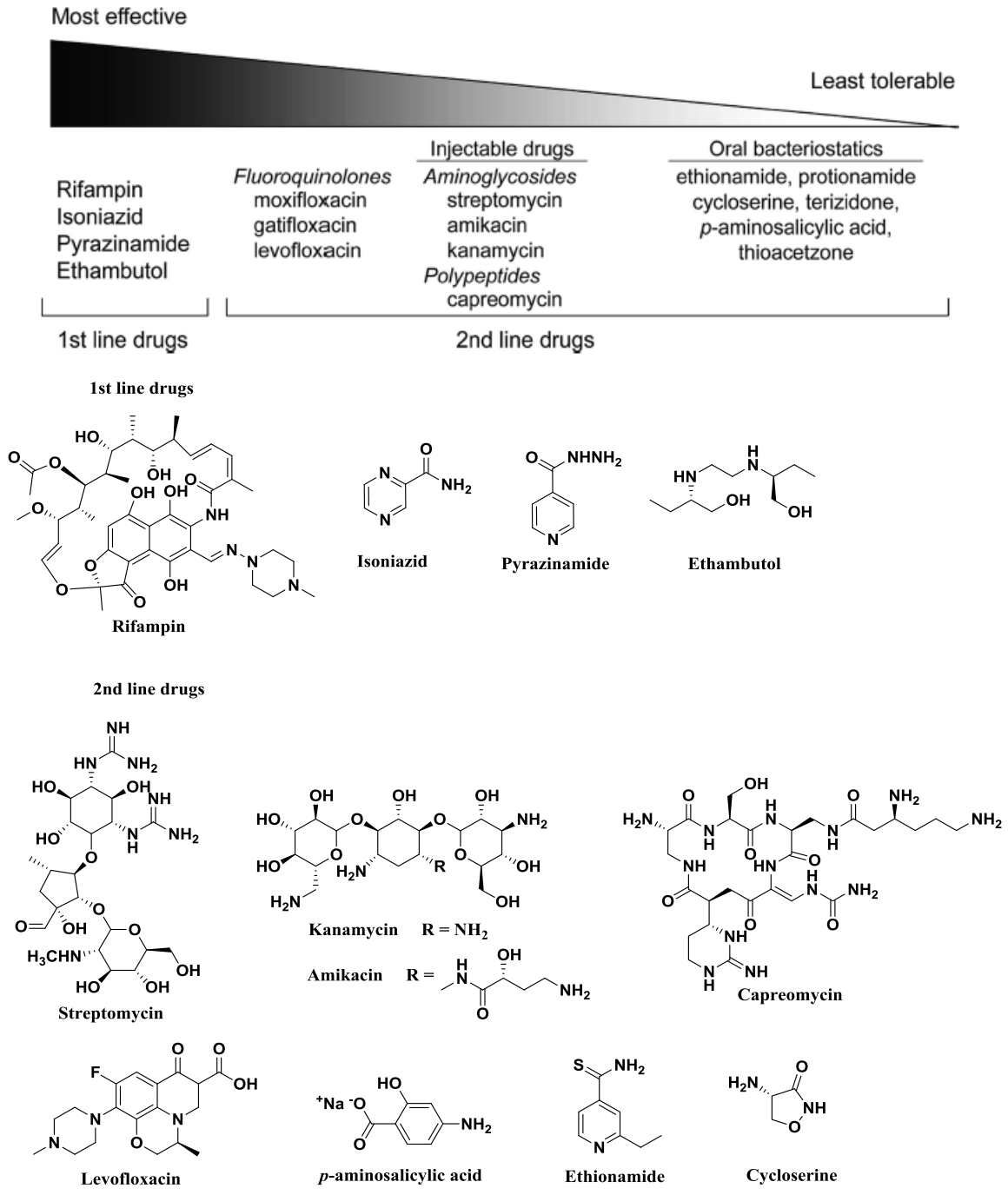


Figure IV.3 – Currently prescribed antitubercular drugs (adapted from Bewley, 2008).[8]

Globally, in 2012, an estimated 450 000 people developed MDR-TB and there was an estimated 170 000 death toll. The number of people diagnosed with MDR-TB nearly doubled between 2011 and 2012, and reached 94 000 worldwide. At least one case of XDR-TB has been reported by 92 countries by the end of 2012. On average, an estimated 9.6% of MDR-TB cases have XDR-TB.[1] The incidence of MDR- and XDR-TB demands renewed efforts in the development of new classes of anti-TB drugs.

Table IV.1 summarizes some of the most important attributes that a new drug must have to contribute to a future regimen.

Table IV.1 – Important attributes that new TB drug candidates should have and their therapeutic objectives (adapted from Wang, 2010).[9]

	Desired attributes	Therapeutic objectives
Mechanism of action	Novel mode of action	Active against MDR and XDR TB
Potency and efficacy	Active against both replicating and non-replicating *M.tb*	Shorten treatment; effective against latent infection, and prevent development of resistance to co-administered drugs
Drug-drug interaction	Reduced interaction with P450 enzymes	Co-administration with antiretroviral drugs
Pharmacokinetics	Orally bioavailable, acceptable pharmacokinetic and pharmacodynamic profiles	Suitable for oral, once daily or less frequent administration
Safety and tolerability	Improved safety and tolerability profiles	Acceptable for treatment of drug-susceptible and drug-resistant TB, including acceptability for treatment of children and pregnant women
Cost	Low cost	Ensure affordability

At present, there are several drugs being evaluated in clinical trials.[9,10] Some of these represent novel chemical entities, such as TMC207 and PA824, whereas others are approved drugs being redeveloped or repurposed for TB (rifapentine, gatifloxin) (figure IV.4).

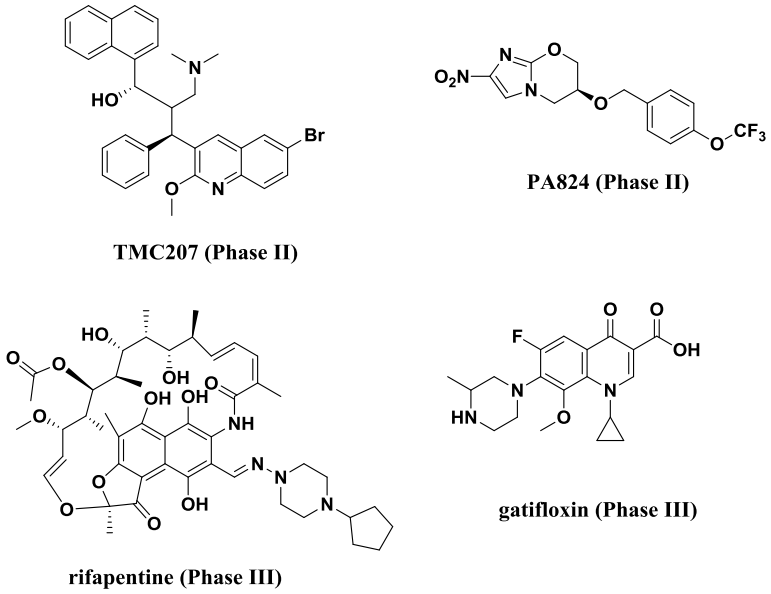


Figure IV.4 – Compounds in clinical development for the treatment of active TB.

The United States Food and Drug Administration (FDA) agency recently approved TMC207[11] for the treatment of MDR-TB (including XDR-TB) in adults when no other treatment options are effective. This is the first antitubercular drug to be approved by FDA in the last four decades. Unfortunately, like other known second-line antitubercular drugs, it has been reported to possess unwanted side effects. A singular FDA approval in 40 years coupled with the staggering volume of mortality from TB stimulate the impetus for the discovery and development of new TB chemotherapeutics.

For the development of a new class of anti-TB drugs the choice of a scaffold and its substitution pattern are of almost importance. When considering the vast amount of work published concerning new antitubercular agents, several heterocyclic scaffolds have been investigated, such as benzopyranones, thiadiazoles, oxadiazoles, triazoles, hydroxyquinolines, pyrimidines, pyrazoles, and nitrofurans (figure IV.5).[12,13]

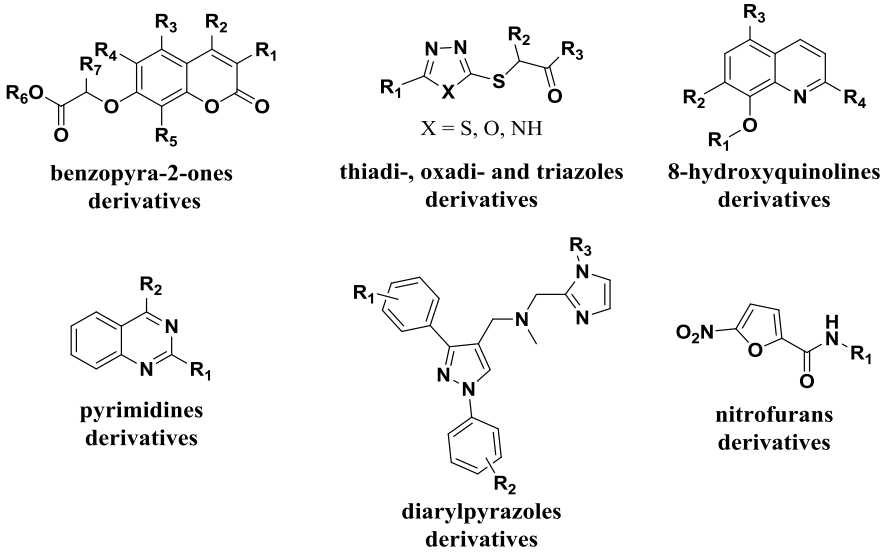


Figure IV.5 – Several scaffolds proposed as antitubercular agents.

Although the indole ring being considered a privileged structure and an attractive scaffold for drug discovery, it has been scarcely explored as antitubercular agents (figure IV.6). Moreover, these works have been published mostly on the last five years.[14-21] Thus, further investigation should be carried on that direction. The aim of this work was the development of novel compounds with antitubercular activity and indole was selected as scaffold for that purpose.

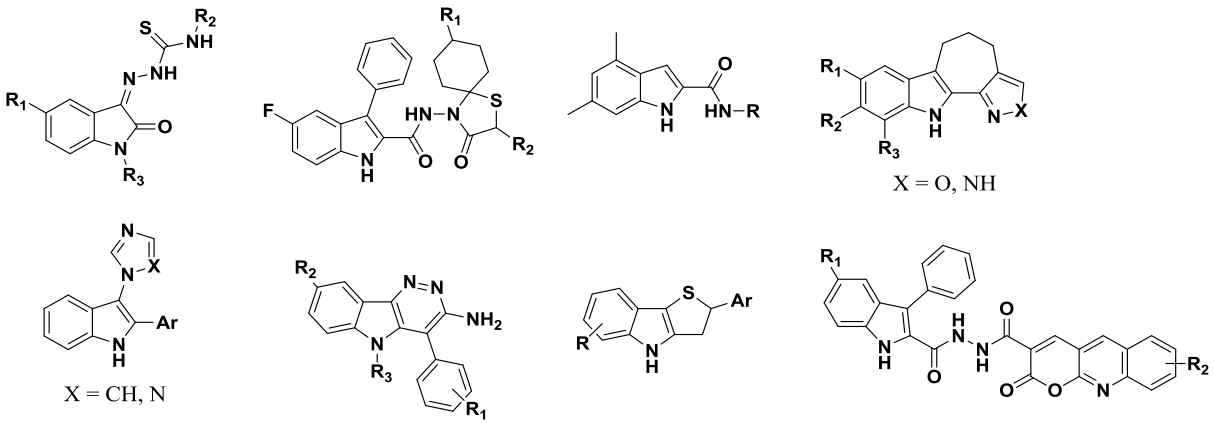


Figure IV.6 – Chemical structures of some indole derivatives reported in literature possessing antitubercular activity.

A QSAR approach was used to develop classification and regression models to assist in the design and selection of new potentially antitubercular agents. Several QSAR models were built using Random Forests (RFs) and Associative Neural Networks (AsNNs) as machine learning techniques, and

different types of descriptors.[22] Several different training and test sets were built from publicly available data sets of compounds, including recent data deposited in the PubChem database.

Therefore, an indole-based library was synthesized involving the substitution patterns proposed by the previously QSARs studies.

IV.2 Results and discussion

The most relevant structural features responsible for the compounds' biological activities were identified after a careful analysis of the literature.[12,13] Therefore, a set of virtual molecules was computationally generated as a combinatorial library, which comprised analogues of 1355 with distinct substitution patterns (the set of generated structures was comprised in the general formula of figure IV.7 but not all possible structures were generated). The molecular structures were produced by in-house developed software that combines SMILES strings of molecular fragments. The data set of the compounds was screened by the developed classification and regression models (studies performed by the group of Prof. J. Aires de Sousa, from FCT-UNL).

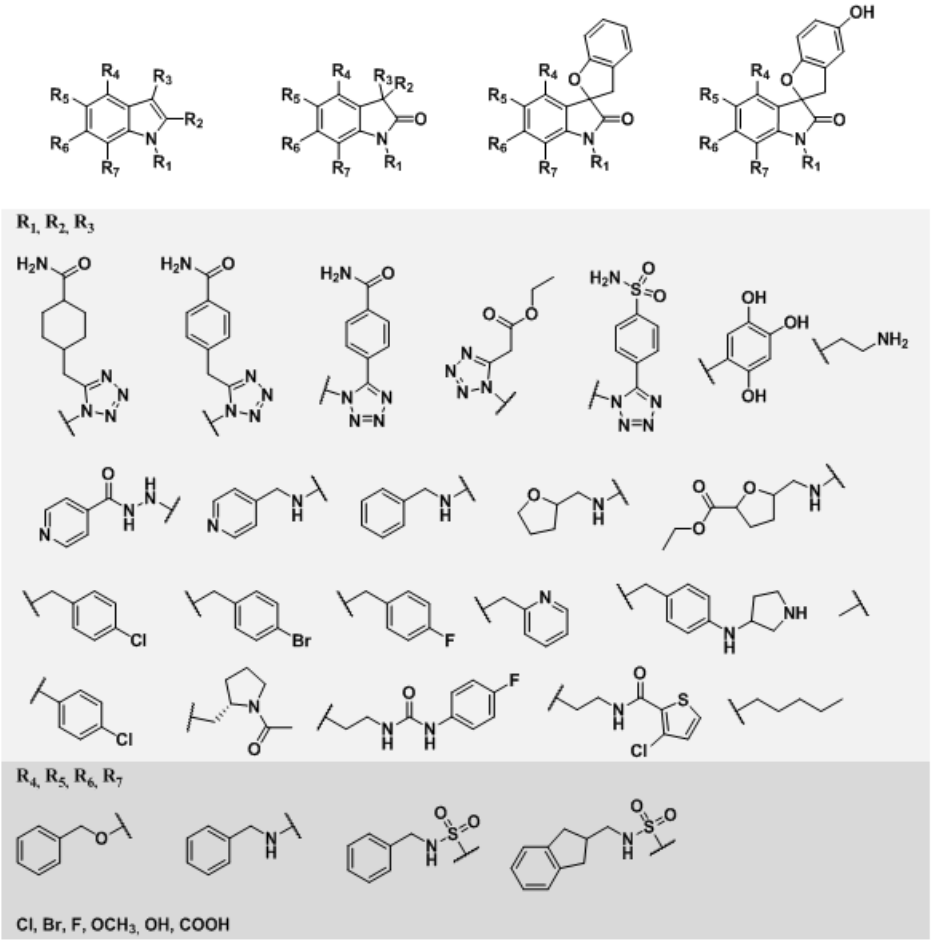


Figure IV.7 – Proposed structures submitted for computational screening.

The proposed structures were encoded and their minimum inhibitory concentration (MIC) values were predicted. Further, the compounds were divided into two classes, active (MIC ≤ 5 μM) and inactive (MIC > 5 μM) compounds. From those, only compounds with low predicted MIC values were suggested for further evaluation. Subsequently, the synthetic strategies required for each compound

were analysed and five different compounds were considered, based on their predicted MIC and synthetic feasibility and promptitude (figure IV.8).

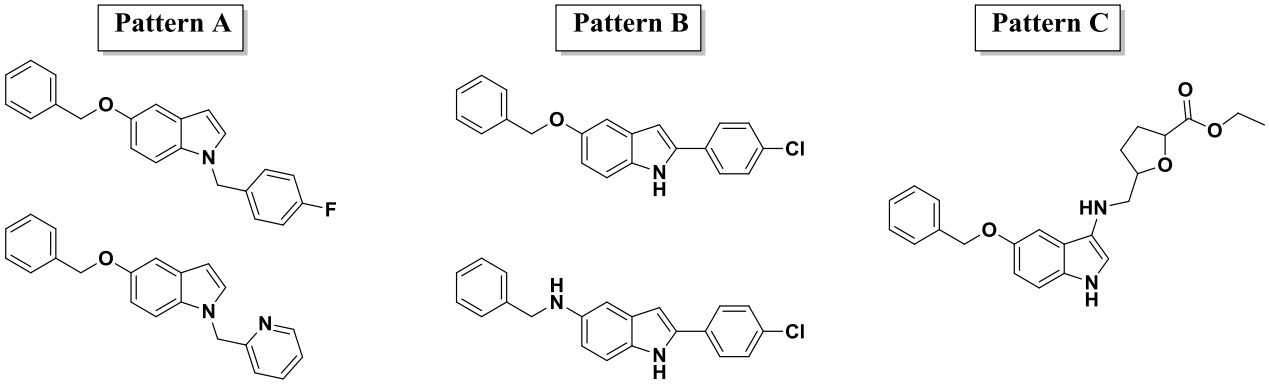
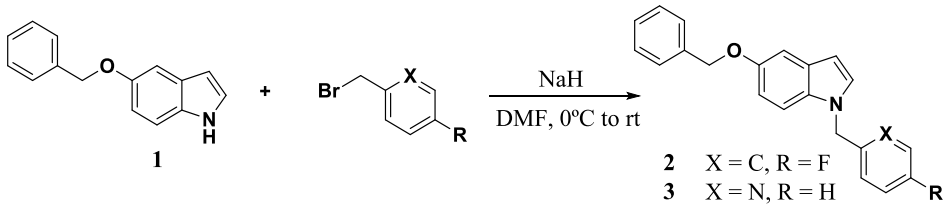


Figure IV.8 – Chemical structures of the proposed compounds, chosen for further synthesis and antitubercular activity analysis.

The compounds substituted at N-1 and C-5 (figure IV.8, pattern A) were readily synthesized from the reaction of the 5-(benzyloxy)-1*H*-indole (**1**) sodium salt with 4-fluorobenzyl bromide or 2-(bromomethyl)pyridine hydrobromide, all commercially available (scheme IV.1). 5-(Benzyloxy)-1-(4-fluorobenzyl)-1*H*-indole (**2**) and 5-(benzyloxy)-1-(pyridin-2-ylmethyl)-1*H*-indole (**3**) were obtained in 97% and 84% yield, respectively.



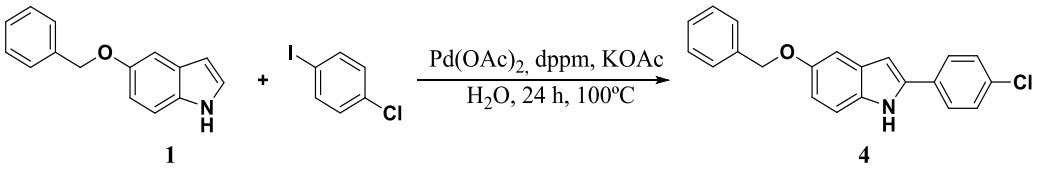
Scheme IV.1 – Synthesis of compounds 2 and 3.

The compounds possessing a 4-chlorobenzene at position C-2 (figure IV.8, pattern B) can be achieved by two main pathways: functionalization of the indole ring by direct C-2 arylation or; by indole ring assembly using a precursor possessing the aryl group in its structure (*e.g.* Fischer indolization).

A procedure described in the literature by Larrosa *et al.*,[23] for the direct C-2 arylation of indole with aryl iodides, was applied. For the synthesis of 5-(benzyloxy)-2-(4-chlorophenyl)-1*H*-indole (**4**), 5-(benzyloxy)-1*H*-indole (**1**) was treated with 1-chloro-4-iodobenzene, in an organic media catalyzed by palladium(II) acetate, silver (I) oxide and *o*-nitrobenzoic acid. Unfortunately no products formation was observed by TLC control, after 48 h. This result can be explained by the lower reactivity of free N-H

indoles, under these reaction conditions, observed by the authors. In fact, when the reaction of indole with iodobenzene was performed, higher temperature and reaction time was required, 50ºC for 38 h instead of 25ºC for 15 h, and the product was obtained only in moderate yield.[23]

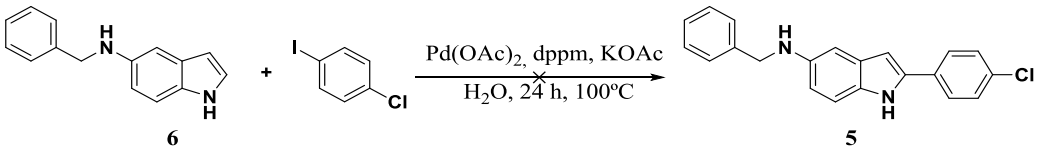
Next, a procedure reported by Djakovitch *et al.*,[24] that consists on the direct and site-selective C-H arylation of (NH)-indoles catalyzed by palladium, was applied. Thus, indole derivative **1** was treated with 1-chloro-4-iodobenzene, in the presence of palladium(II) acetate, 1,1-bis(diphenylphosphino)methane (dppm) and potassium acetate, and compound **4** was obtained in 56% yield (scheme IV.2).



1 **4**

Scheme IV.2 – Synthesis of 5-(benzyloxy)-2-(4-chlorophenyl)-1*H*-indole (4).

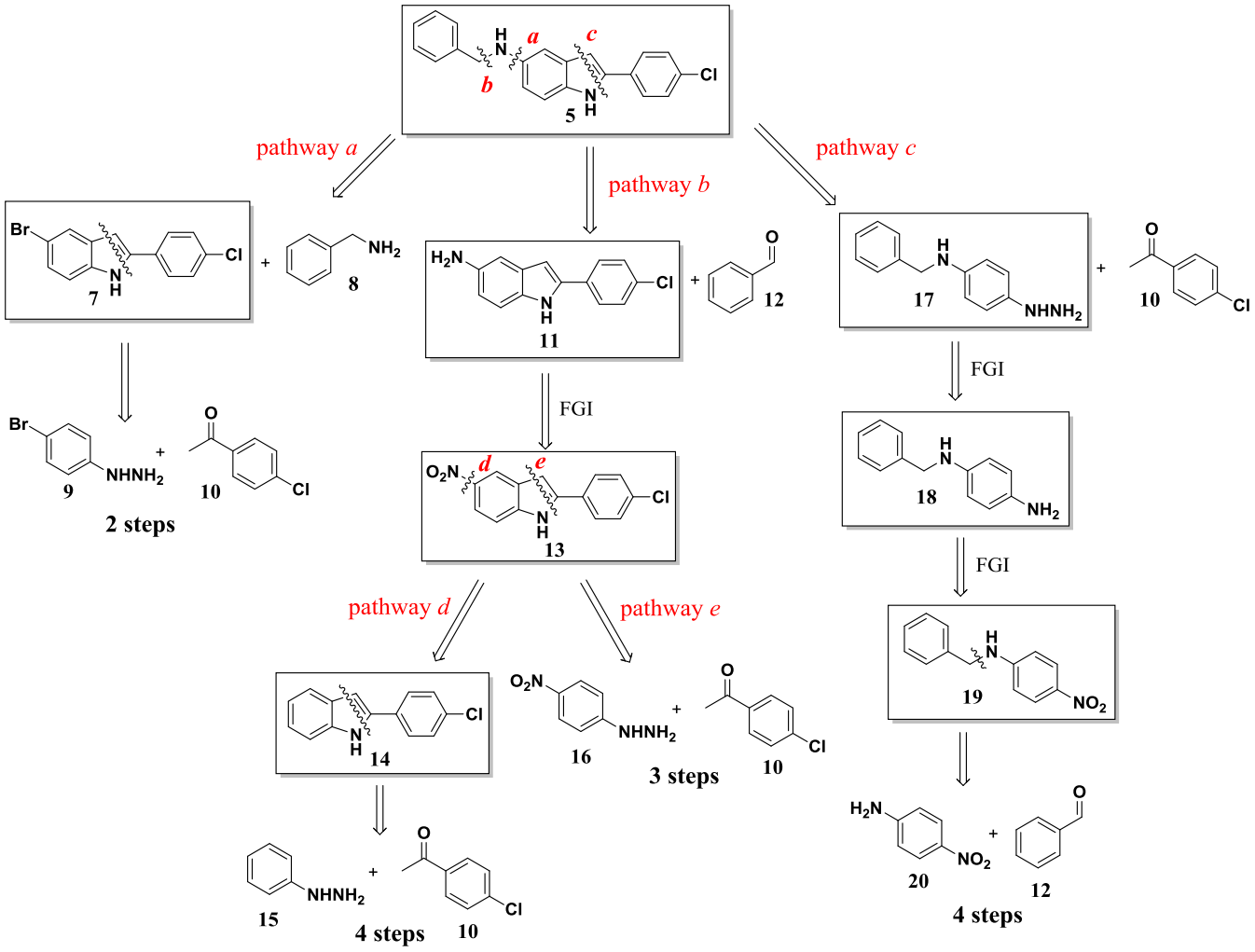
For the synthesis of the other C-2 substituted indole derivative, *N*-benzyl-2-(4-chlorophenyl)-1*H*-indol-5-amine (**5**), the same conditions were applied. However, no reaction occurred during the treatment of *N*-benzyl-1*H*-indol-5-amine (**6**) with 1-chloro-4-iodobenzene, in the presence of palladium(II) acetate, dppm and potassium acetate (scheme IV.3).



6 **5**

Scheme IV.3 – Reaction of *N*-benzyl-1*H*-indol-5-amine (6) with 1-chloro-4-iodobenzene.

Since direct C-2 arylation of indole compound **6** was not well succeeded, the next approach to synthesize compound **5** consisted on a Fischer indole synthesis. Indole derivative **5** has two substituted position, C-2 and C-5, thus the retrosynthetic analysis of this molecule was evaluated in order to select the easiest, faster and less expensive pathway. Three different and conceivable approaches are depicted on scheme IV.4.

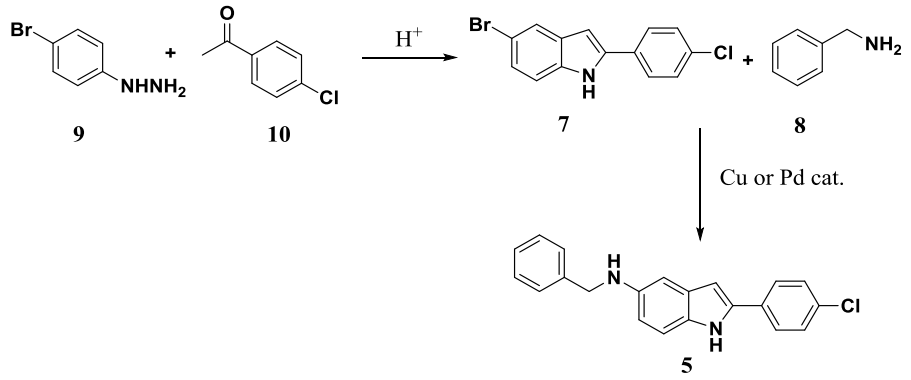


Scheme IV.4 – Retrosynthetic analysis of compound 5.

Pathway *a* consists on a C-N disconnection at position C-5, leading to 5-bromo-2-(4-chlorophenyl)-1*H*-indole (**7**), followed by a C-N/C-C disconnection. This disconnection leads to the reagents required for the Fischer indolization, a phenyl hydrazine and a ketone. Both (4-bromophenyl)hydrazine (**9**) and 4-chloroacetophenone (**10**) are commercially available. The first disconnection of pathway *b* results on the indole derivative **11** which, through a functional group interconversion (FGI), leads to 2-(4-chlorophenyl)-5-nitro-1*H*-indole (**13**). Next, two different disconnection are possible: the C-N disconnection at C-5, leading to indole derivative **14** which can be prepared by a Fischer indolization by reaction of phenylhydrazine (**15**) and 4-chloroacetophenone (**10**) (pathway *d*); and the disconnection at C-N/C-C bonds, resulting in two commercially available reagents: (4-nitrophenyl)hydrazine (**16**) and 4-chloroacetophenone (**10**) (pathway *e*). Pathway *b/d* consists in four synthetic steps whereas pathway *b/e* can afford the desired indole **5** in three synthetic steps. A C-N/C-C disconnection of indole **5** (pathway *c*) results in the *N*-benzyl-4-hydrazinylaniline (**17**) and 4-chloroacetophenone (**10**). A FGI leads **17** to the corresponding aniline, the *N*-benzylbenzene-1,4-

diamine (**18**). This compound can be acquired from a few chemical suppliers, however it is offered at high costs. Thus, a FGI results in the, also commercially available, *N*-benzyl-4-nitroaniline (**19**), however, this reagent is also very expensive. To prepare **19**, a C-N disconnection leads to the affordable 4-nitroaniline (**20**).

Pathway *a* was the first choice to synthesize indole derivative **5** due to its simplicity and readiness. Scheme IV.5 depicts the synthetic route proposed for the preparation of compound **5** through pathway *a*.

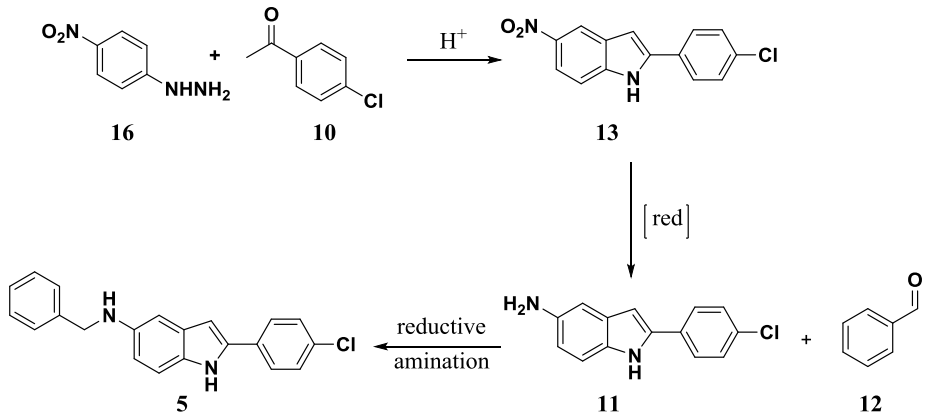


9 10 7 8 5

Scheme IV.5 – Synthetic route proposed for the preparation of compound 5 through pathway *a*.

Treatment of (4-bromophenyl)hydrazine hydrochloride (**9**) with 4-chloroacetophenone (**10**), in polyphosphoric acid (PPA) afforded 5-bromo-2-(4-chlorophenyl)-1*H*-indole (**7**) in 85% yield. Next, the C-N coupling reaction was attempted. Indole **7** was treated with benzylamine (**8**), in the presence of copper (I) iodide, L-proline and potassium carbonate, under the conditions described by Ma *et al.*[25] However no product formation resulted from this reaction. Therefore, the reaction conditions reported for the Pd-catalyzed amination reactions of heterocycles, described by Buchwald *et al.*[26] were tested. These involve the reaction of indole derivative **7** with benzylamine (**8**) using a BrettPhos based catalyst system and lithium hexamethyldisilazide (LiHMDS) in THF as the base. Despite the fact that several examples for the C-N coupling of different halo-indoles with varied amines are stated under these conditions, no reaction occurred between indole **7** and amine **8**.

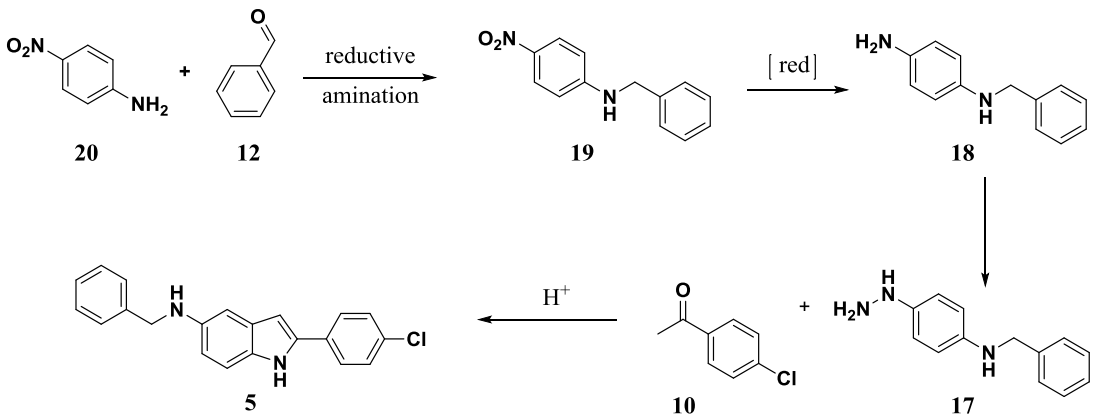
Since the compound **7** could not be accomplished through this synthetic route, pathway *b/e* (scheme IV.4) was then considered. This involves three synthetic steps: a Fisher indolization by reaction of (4-nitrophenyl)hydrazine (**16**) with 4-chloroacetophenone (**10**), next a reduction of the nitro group and lastly a reductive amination reaction of compound **11** with benzaldehyde (**12**) (scheme IV.6).



Scheme IV.6 – Proposed synthetic route for the preparation of compound 5 through pathway *b/e*.

The (4-nitrophenyl)hydrazine hydrochloride (**16**) was treated with 4-chloroacetophenone (**10**), in PPA. Unfortunately, no reaction took place under these conditions. In fact, only one report was found in the literature describing a Fischer indolization reaction of hydrazine **16** with acetophenone.[27] The conditions described herein were then applied. However, treatment of hydrazine **16** with ketone **10**, in the presence of phosphomolybdic acid, did not afford any product.

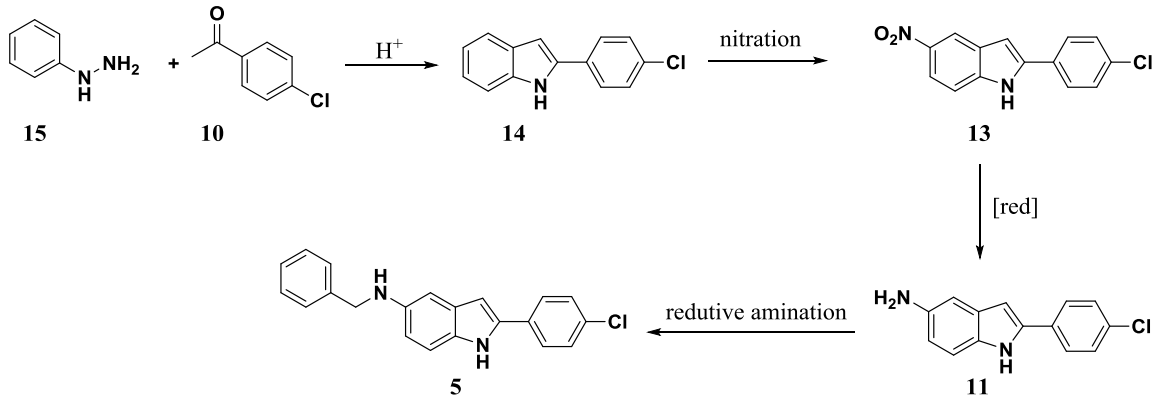
Probably, the observed absence of reactivity is due to the presence of the nitro group, which is a strongly deactivator group, at *para*-position. For that reason, it was envisaged that the presence of the benzylamine group, an activator group, at *para*-position, would improve the reactivity of the hydrazine and the desired product would be successfulafforded through the Fischer indole synthesis (scheme IV.4, pathway *c*). The first requirement of this route is to synthesize *N*-benzyl-4-hydrazinylaniline (**17**). The synthesis of compound **17** can be accomplished from the reductive amination reaction of the 4-nitroaniline (**20**) with benzaldehyde (**12**), followed by reduction of the nitro group, affording *N*-benzylbenzene-1,4-diamine (**18**), and finally conversion of the amine to hydrazine (scheme IV.7). Lastly, the desired compound **5** can be obtained from the reaction of the synthesized hydrazine **17** with 4-chloroacetophenone (**10**) *via* a Fischer indolization reaction (scheme IV.7).



Scheme IV.7 - Proposed synthetic route for the preparation of compound 5 through pathway *c*.

Synthesis of *N*-benzyl-4-nitroaniline (**19**) was performed in 89% yield using solvent-free conditions, by grounding 4-nitroaniline (**20**), benzaldehyde (**12**), sodium borohydride and silica-gel-supported sulfuric acid (SSA). Then, compound **19** was reduced, using a Fe/NH_4Cl system, affording *N*-benzylbenzene-1,4-diamine (**18**) in 95% yield. Treatment of aniline **18** with sodium nitrite in aqueous HCl, gave the corresponding aryl diazonium salt that was readily converted in the aryl hydrazine hydrochloride **17**, in the presence of tin (II) chloride and aqueous HCl, in 40% yield. The next step consisted on the reaction of **17** with 4-chloroacetophenone (**10**), in PPA. However, this reaction did not afford the desired indole derivative **5**, instead decomposition of starting material **17** was observed.

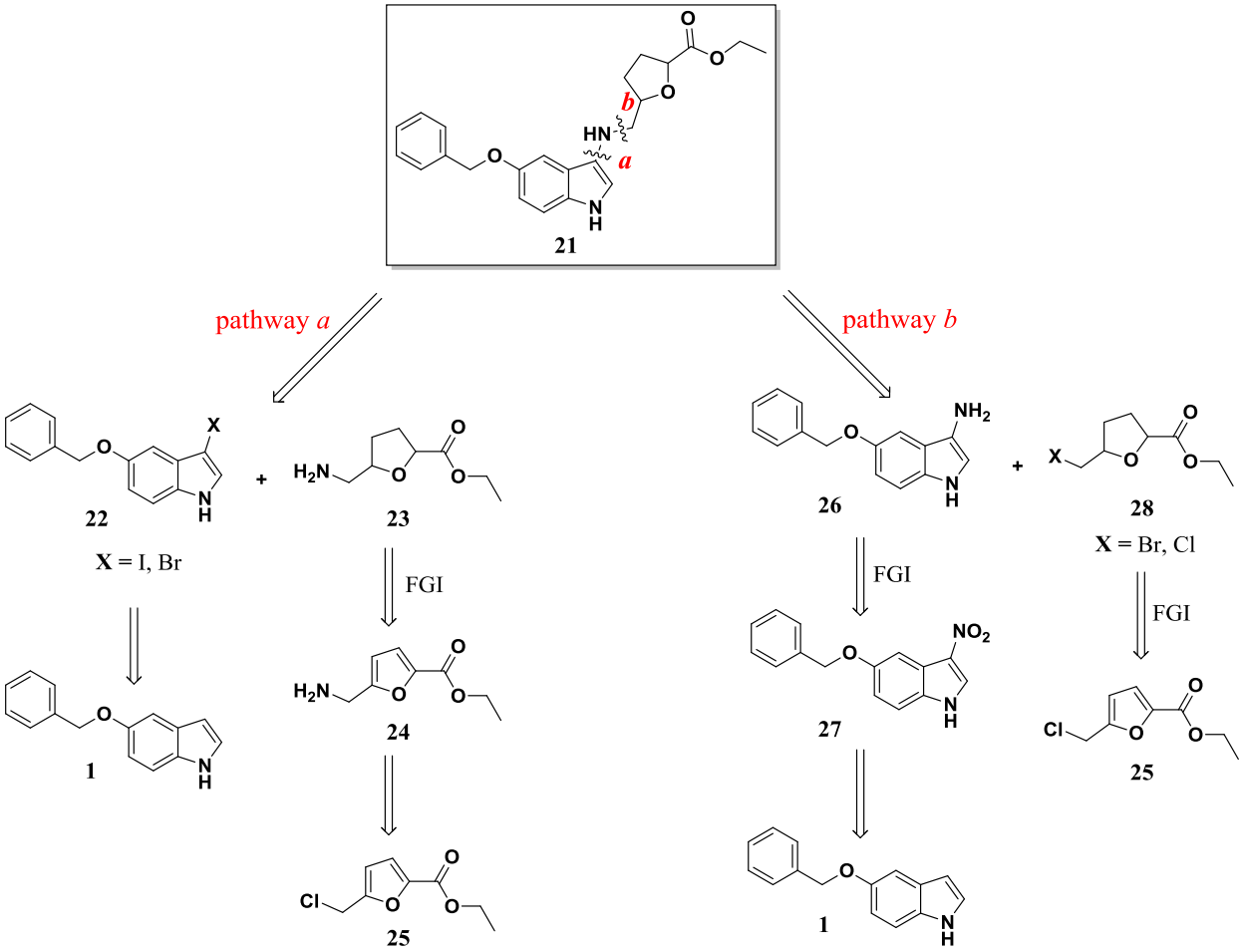
Since it was not possible to perform a Fischer indolization reaction neither with the nitro group nor with the benzilamine group at *para*-position of the hydrazine, due to its low reactivity or instability, respectively, under these reaction conditions, pathway *b/d* (scheme IV.4) was then considered. This route consists in four steps: a Fisher indolization by reaction of phenylhydrazine (**15**) with 4-chloroacetophenone (**10**), followed by a regioselective nitration of position C-5 of **14**, affording 2-(4-chlorophenyl)-5-nitro-1*H*-indole (**13**), subsequent reduction of the nitro group, and lastly a reductive amination reaction of compound **11** with benzaldehyde (scheme IV.8).



Scheme IV.8 - Proposed synthetic route for the preparation of compound 5 through pathway *b/d*.

The synthesis of 2-phenylindole as well as its regioselective nitration at C-5 position is described by Smith *et al.*[28] Therefore, reaction of the phenylhydrazine (**15**) with 4-chloroacetophenone (**10**), in PPA afforded 2-(4-chlorophenyl)-1*H*-indole (**14**) in 66% yield. 2-(4-chlorophenyl)-5-nitro-1*H*-indole (**13**) was obtained in 82% yield, by treatment of indole **15** with a solution of sodium nitrate in sulfuric acid. The next step consisted on the reduction of the nitro group present in compound **13**, using a Pd/C, H_2 system. A mixture of the desired indole derivative **11** along with the dechlorinated product was obtained. Therefore, a Fe/NH_4Cl system was used, affording exclusively 2-(4-chlorophenyl)-1*H*-indol-5-amine (**11**), in 59% yield. For the reductive amination reaction, the indole derivative **11** was treated with benzaldehyde, in the presence of $NaBH_3CN$, affording *N*-benzyl-2-(4-chlorophenyl)-1*H*-indol-5-amine (**5**) in 78% yield.

Concerning the preparation of the ethyl 5-({[5-(benzyloxy)-1*H*-indol-3-yl]amino}methyl)tetrahydrofuran-2-carboxylate (**21**), two different substituents were required: the benzyloxy group at C-5, and an alkyl amine derivative at C-3 (figure IV.8, pattern C). The substitution at C-5 did not require any synthetic studies since 5-(benzyloxy)-1*H*-indole (**1**) is commercially available. Thus, only the introduction of the alkyl amine derivative at C-3 position was considered for the retrosynthetic analysis of compound **21** (scheme IV.9).



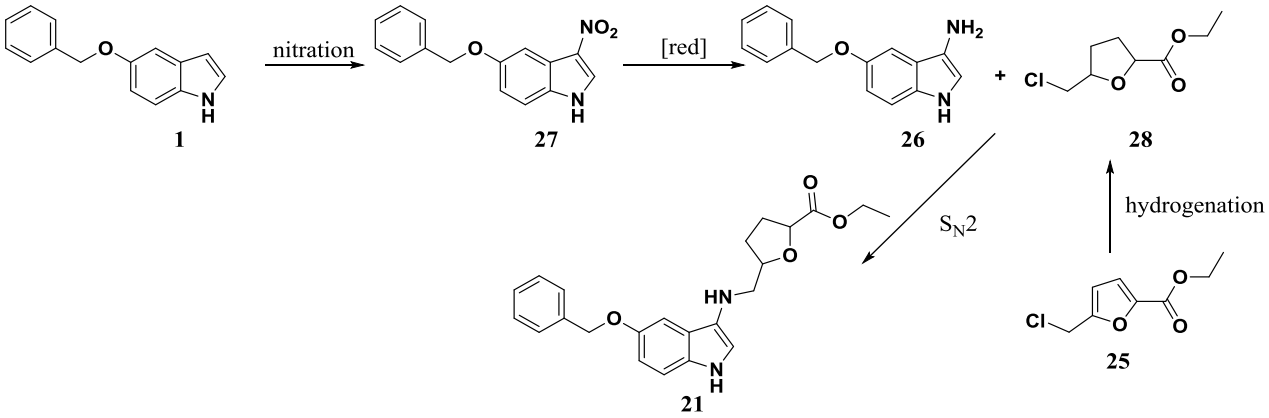
Scheme IV.9 - Retrosynthetic analysis of compound 21 for the introduction of the alkyl amine derivative at C-3.

Two different approaches were considered. Pathway *a* consists on the C-N coupling of the 3-bromo or iodo indole derivative **22** with the alkyl amine derivative **23**. Compound **22** can be obtained from the C-3 halogenation of 5-(benzyloxy)-1*H*-indole (**1**). A FGI of compound **23** results on the corresponding furan derivative **24**, which can be attained from the 5-(chloromethyl)furan-2-carboxylate (**25**), that is commercially available. Pathway *b* comprises a S_N2 reaction of the 3-aminoindole derivative **26** with 5-(halomethyl)furan-2-carboxylate derivative **28**. A FGI of indole **26** leads to the corresponding

3-nitroindole derivative **27**, which can be afforded from the C-3 nitration of 5-(benzyloxy)-1*H*-indole (**1**). A FGI to compound **28** leads to compound **25**.

Despite the existence of several procedures in the literature describing C-N coupling reactions of aryl halides with amines,[26,29,30] amides,[31-33] or carbamates,[34,35] by catalysis of either copper or palladium complexes, no report of the C-N coupling at C-3 position of the indole ring was described to date. Therefore, the first attempt to synthesize indole derivative **21** relied on pathway *b* (scheme IV.9) since it was envisaged that would be a more straightforward route.

The synthetic approach required to synthesize indole derivative *via* pathway *b*, consists in a convergent synthetic route, as depicted on scheme IV.10. Thus, the 3-aminoindole derivative **26** can be afforded from the regioselective nitration reaction at position C-3 of the 5-(benzyloxy)-1*H*-indole (**1**), followed by reduction of the nitro group present in the indole derivative **27**. The desired product **21** can be obtained from a S_N2 reaction of indole derivative **26** with compound **28**, that can be prepared from hydrogenation of **25** (scheme IV.10).

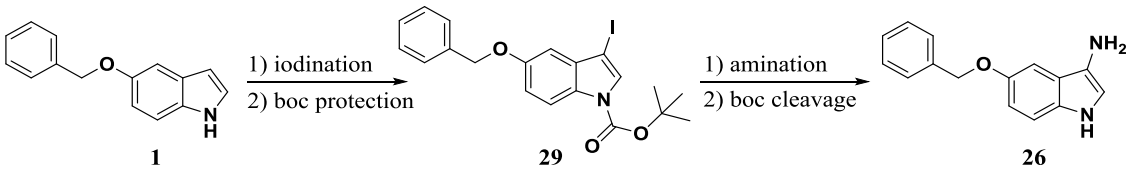


Scheme IV.10 - Proposed synthetic route for the preparation of compound 21 *via* pathway *b*.

Concerning to the nitration reaction, indole was tested as a model molecule, since it is cheaper than 5-(benzyloxy)-1*H*-indole (**1**) and this reaction is described in the literature.[36,37] The nitration of C-3 position of the indole ring was attempted from two different conditions: reaction of indole with a reactive intermediate specie, generated *in situ*, from chlorodiphenylphosphine (CDP), molecular iodine and silver nitrate[36], or from silver nitrate and benzoyl chloride[37]. Both approaches afforded a slurry mixture of products which was not possible to separate.

In 2008, Chang *et al.*[38] firstly reported a procedure for the Cu-catalyzed amination of aryl halides using ammonium salts (ammonium chloride or aqueous ammonia) as the nitrogen source. Further, other methodologies were published employing aqueous ammonia as the nitrogen source for the amination of aryl halides.[39-42] Thus, it was envisaged that 5-(benzyloxy)-1*H*-indol-3-amine (**26**) could be afforded

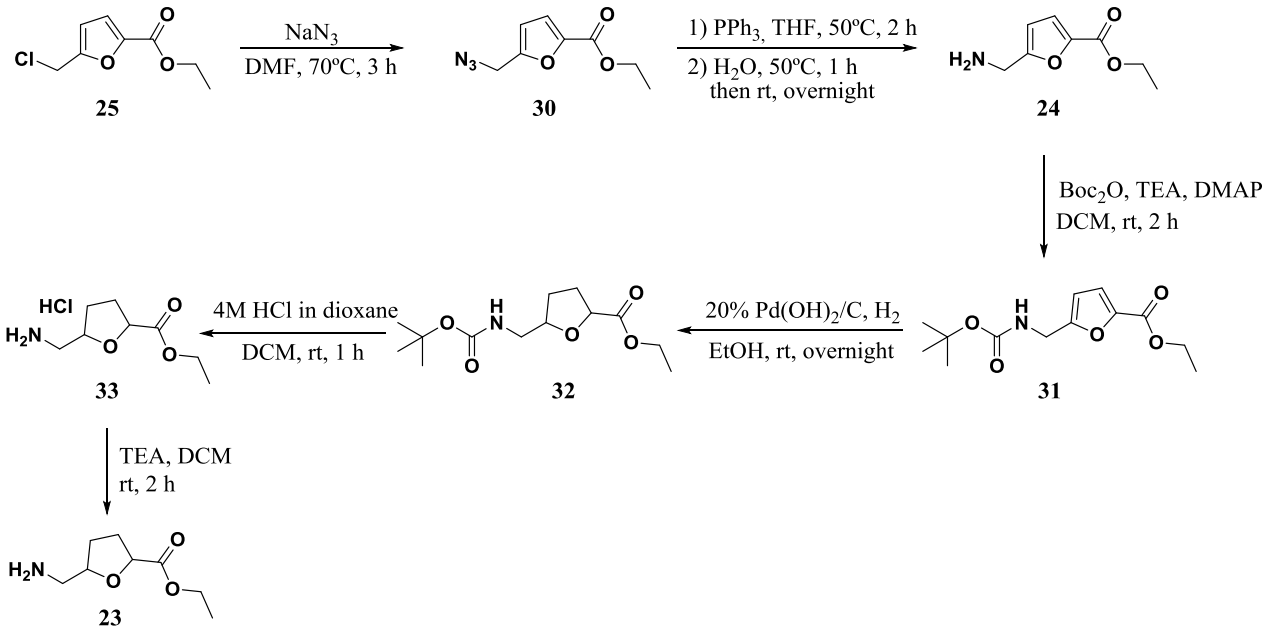
from the amination reaction of the *tert*-butyl 5-(benzyloxy)-3-iodo-1*H*-indole-1-carboxylate (**29**) (scheme IV.11).



Scheme IV.11 – Alternative approach for the synthesis of compound 26.

Treatment of indole **1** with potassium hydroxide and molecular iodine afforded the corresponding 3-iodoindole derivative that was then treated with Boc anhydride, in the presence of triethylamine and DMAP, affording *tert*-butyl 5-(benzyloxy)-3-iodo-1*H*-indole-1-carboxylate (**29**) in 80% yield. Two different experiments were carried attempting the synthesis of compound **26**. The reaction of indole **29** with aqueous ammonia catalyzed by: a copper (I) oxide/NMP system[41], and an iron (III) oxide/copper (I) iodide system[42]. The first catalytic system did not afford any product and from the second only the product resulting from the removal of the Boc group was observed. Unfortunately, despite the good yields reported for the amination reaction of aryl halides, these reactions conditions could not be applied for the tested substrate.

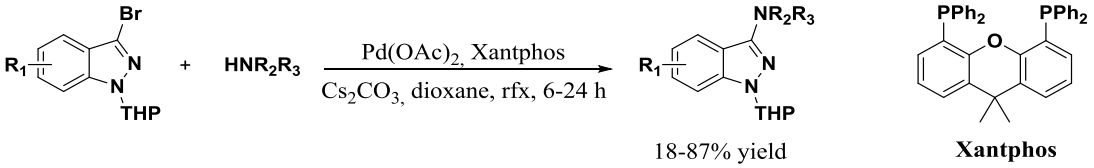
Due to the difficulties in synthesizing compound **26**, pathway *b* was abandoned and pathway *a* was then considered. Pathway *a* also consists in a convergent synthetic route which involves the preparations of the alkyl amine **23** and the indole derivative **22** (scheme IV.9, pathway *a*).



Scheme IV.12 – Synthetic approach for synthesis of the amine 23.

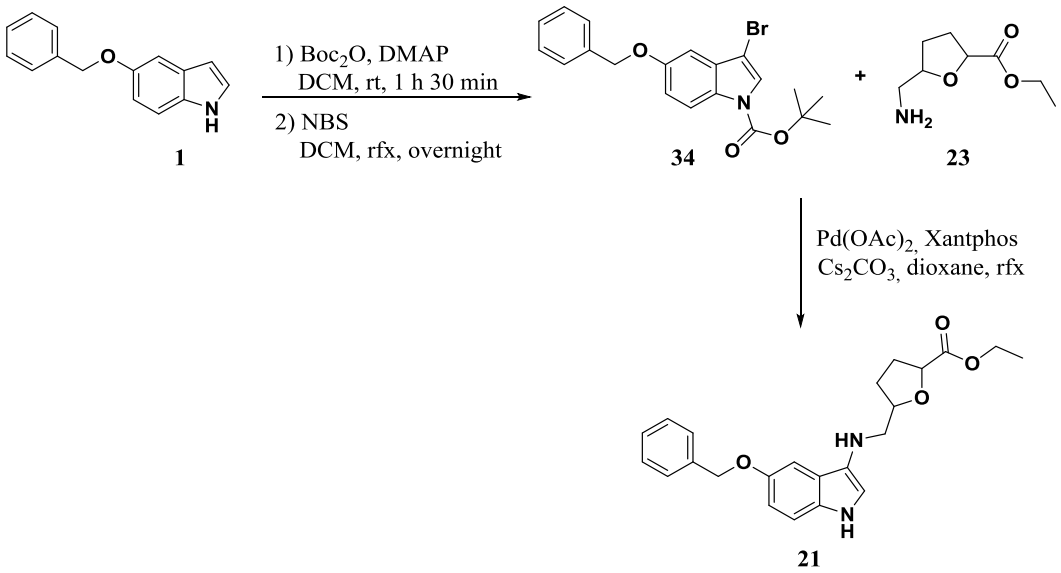
The synthesis of compound **23** consisted on the treatment of ethyl 5-(chloromethyl)furan-2-carboxylate (**25**) with sodium azide, affording the azide derivative **30**, in 90% yield. Subsequent, reduction of the azide to amine **24** with PPh_3/H_2O, followed by protection of the amino group with Boc anhydride, afforded compound **31**, in 56% yield (two steps). Next step involved the hydrogenation of the furan ring, with $Pd(OH)_2/C$ under H_2 atmosphere ,affording compound **32** in 91% yield. Subsequent, treatment of **32** with a 4 M solution of HCl in dioxane afforded ethyl 5-(aminomethyl)tetrahydrofuran-2-carboxylate hydrochloride (**33**), in 92% yield. Since the free alkyl amine derivative **23** is not very stable, this was prepared and immediately used in the next step (scheme IV.12).

Collot *et al.*[43] described a procedure to synthetize 3-aminoindazoles derivatives, in moderate to good yields, *via* Buchwald-Hartwig C-N coupling reaction, using a palladium(II) acetate/Xantphos catalytic system (scheme IV.13).



Scheme IV.13 – Synthesis of 3-aminoindazoles derivatives *via* Buchwald-Hartwig C-N coupling reaction, described by Collot *et al.*[43]

Thus, the first attempt to synthesize indole derivative **21** consisted on the C-N coupling reaction of *tert*-butyl 5-(benzyloxy)-3-bromo-1*H*-indole-1-carboxylate (**34**) with ethyl 5-(aminomethyl)tetrahydrofuran-2-carboxylate (**23**), catalyzed by palladium(II) acetate/Xantphos in the presence of cesium carbonate. Indole derivative **34** was obtained after protecting the indolic nitrogen atom with Boc anhydride, followed by the bromination at C-3 with *N*-bromosuccinimide (NBS), in 67% yield (scheme IV.14).



1 34 23 21

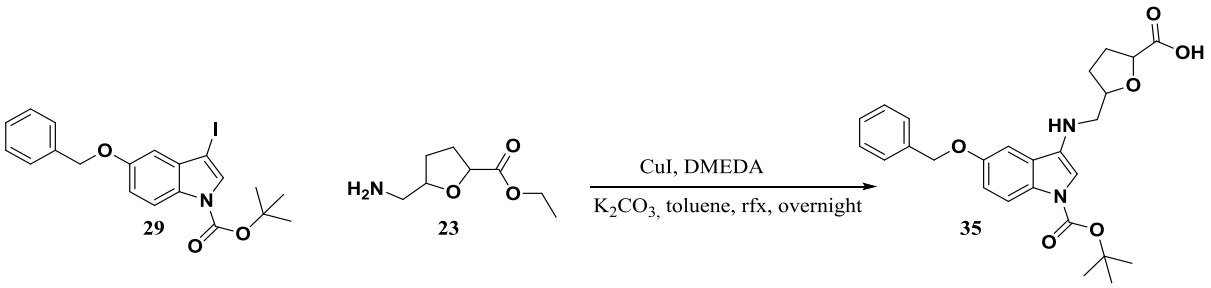
Scheme IV.14 – Proposed synthetic approach of compound 21.

Unfortunately, only the product of debromination of the starting material **34** was observed by TLC control, after 24 h of reaction. Same conditions were applied on the reaction of *tert*-butyl 5-(benzyloxy)-3-bromo-1*H*-indole-1-carboxylate (**34**) with *N*-Boc alkyl amine derivative **32**. In this experiment a product was formed, however by analysis of the ^{1}H-NMR spectra it was possible to conclude that the amine moiety was not present. This product could not be identified due to its instability.

Alternative reaction conditions for the amination reaction towards heterocyclic compounds functionalization, reported by Buchwald *et al.*,[26] were then adopted. The authors performed a C-N coupling at C-4 or C-5 positions of the indole ring, using a BrettPhos based catalystic system. Thus, the 3-bromoindole derivative **34** was treated with ethyl 5-(aminomethyl)tetrahydrofuran-2-carboxylate (**23**), in the presence of the BrettPhos, chloro[2-(dicyclohexylphosphino)-3,6-dimethoxy-2′,4′, 6′-triisopropyl-1,1′-biphenyl][2-(2-aminoethyl)phenyl]palladium(II) (BrettPhos precatalyst) and LiHMDS. However, only removal of the Boc group from the starting **34** was observed by TLC control.

Due to the difficulties in performing the C-N coupling reaction of the indole derivative **34** with the alkyl amine derivative **23** *via* palladium catalysis, a copper catalysis was then considered to attempt the synthesis of compound **21**.

Several Ulmman-type C-N coupling reactions are mediated by a copper (I)/α-amino acid[44] or copper (I)/diamine[45] catalytic systems. Therefore, two different experiments were performed envisaging the synthesis of ethyl 5-({[5-(benzyloxy)-1*H*-indol-3-yl]amino}methyl)tetrahydrofuran-2-carboxylate (**21**). It consisted on the treatment of *tert*-butyl 5-(benzyloxy)-3-iodo-1*H*-indole-1-carboxylate (**29**) with ethyl 5-(aminomethyl)tetrahydrofuran-2-carboxylate (**23**) catalyzed by: a CuI/L-proline; or a CuI/DMEDA catalytic systems. Under the first conditions only the product of de-iodination and removal of the Boc group of the starting material **34** was observed by TLC, while when the second conditions were tested the C-N coupling product was afforded. However, hydrolysis of the ethyl ester occurred and 5-({[5-(benzyloxy)-1-(*tert*-butoxycarbonyl)-1*H*-indol-3-yl]amino}methyl) tetrahydrofuran-2-carboxylic acid (**35**) was isolated in 56% yield (scheme. IV.15).



Scheme IV.15 – Reaction of indole derivative 29 with the alkyl amine derivative 23.

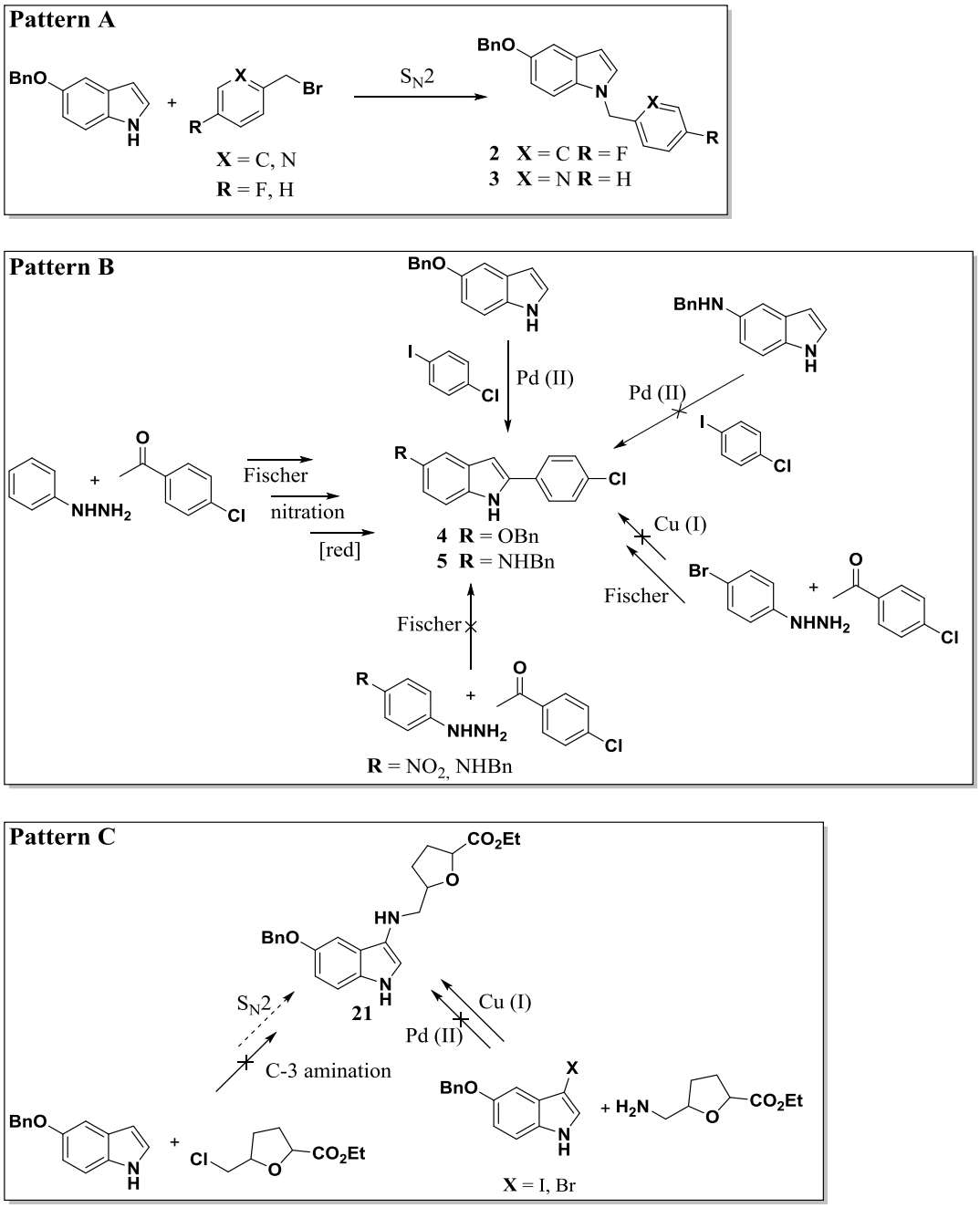
Despite all efforts, such as the use of dry dioxane as solvent, or the use of potassium phosphate as base, it was not possible to avoid the ester hydrolysis. Nevertheless, due to the structural similarity of compounds **21** and **35**, indole derivative **35** was also submitted for biological evaluation together with compounds **2**, **3**, **4** and **5**.

The biological tests are being performed at Instituto de Higiene e Medicina Tropical in collaboration with Faculdade de Ciências da Universidade de Lisboa under the supervision of Prof. Filomena Martins.

IV.3 Conclusions

Five indole-based compounds, predicted by computational methods, were synthesized to be evaluated as potential antitubercular agents. These compounds were divided in three different substitution patterns (figure IV.8): pattern A, C-5 and N-1 substitution (compounds **2** and **3**); pattern B, C-5 and C-2 substitution (compounds **4** and **5**); and pattern C, C-5 and C-3 substitution (compound **21**).

Several approaches and methodologies were applied for the synthesis of these compounds (scheme IV.16).



Scheme IV.16 – Summary of the synthetic approaches performed towards compounds 2, 3, 4, 5 and 21.

The synthesis of compounds **2** and **3** (pattern A) consisted on the N-alkylation reactions of the indole sodium salt of compound **1** with the corresponding alkyl bromide, *via* a S_N2 reaction.

Concerning the synthesis of compound **4** (pattern B), a direct C-2 arylation of **1** with an arylhalide was performed, *via* a palladium catalysis. Due to the impossibility to synthesize compound **5** using the same procedure used to prepare compound **4**, a Fischer indolization reaction was attempted, followed by a regioselective nitration at position C-5 and subsequent reduction of the nitro group and lastly a reductive amination.

In relation to compound **21**, a C-N coupling reaction, at C-3 position of indole, catalyzed by copper, was performed. Despite all efforts to avoid the ester hydrolysis, the indole derivative **35** was also submitted for biological evaluation due to its structural similarity with compound **21**.

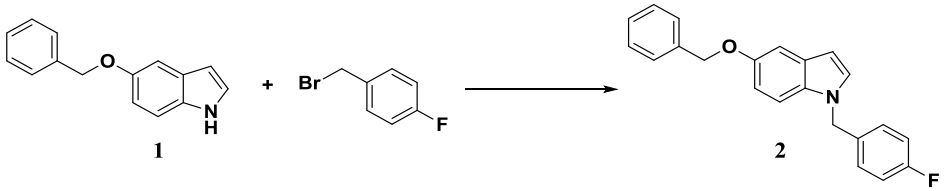
Overall new compounds were synthetized trough useful synthetic routes in order to achieve functionalized indole derivatives. The choice of the substitution patterns was based on preliminary computational studies upon analysis of several known antitubercular agents. Thus, it is expected that the biological evaluation of the synthesized indole derivatives along with the QSARs studies will allow the validation of this strategy, opening a new path to the design of new antitubercular agents with improved activity.

IV.4 Experimental

IV.4.1 General

Melting points were determined on a Reichert Thermovar apparatus and are uncorrected. ^{1}H- and ^{13}C-NMR spectra were recorded in *$(CD_3)_2CO$*, *DMSO-d_6* or *$CDCl_3$* on a Bruker ARX 400 spectrometer at 400 and 100.62 MHz respectively. Chemical shifts are expressed in parts per million (ppm) relative to tetramethylsilane (TMS). The coupling constants (*J*) are reported in Hertz (Hz). High resolution mass spectra were recorded on an AutoSpecQ spectrometer. IR spectra were run on an FT PerkinElmer 683 instrument, with absorption frequencies expressed in reciprocal centimeters. The progress of all reaction was monitored by thin-layer chromatography, which was performed on Merck silica gel 60 F254 aluminum plates. Flash column chromatography was carried out on Merck silica gel 60 (230-400 mesh). For preparative thin layer chromatography was used Merck silica gel 60 GF_{254} in 20x20 glass plates. Anhydrous solvents were dried as described and freshly distilled.[46] All the tested compounds possess a purity of at least 94% as determined by HPLC. Analytical HPLC was run on a Merck Hitachi system consisting of an L-7100 pump, Rheodyne type injector, a D-7000 interface and an L-7450 diode array spectrometric detector, equipped with LiChrospher®100 RP-18 column. Eluent system was: 50% A (H_2O/TFA pH 2.5), 70% B (MeOH) to 30% A, 100% B; flow rate = 1 mL/min.

*IV.4.2 Synthesis of 5-(benzyloxy)-1-(4-fluorobenzyl)-1H-indole (**2**)*



1 **2**

To a solution 5-(benzyloxy)-1*H*-indole (**1**) (100 mg, 0.45 mmol) in dry DMF (1.2 mL) was added NaH (21.5 mg, 0.54 mmol) at 0ºC under argon. The reaction mixture was stirred at that temperature for 45 min. 4-Fluorobenzyl bromide (67 µL, 0.54 mmol) was added and the mixture was stirred, from 0ºC to room temperature, under argon, for 3 h. The reaction was quenched with distilled water and extracted with EtOAc. The combined organic layers were washed with water and brine, dried over Na_2SO_4 anhydrous, filtered and concentrated. The crude was purified by flash chromatography (*n*-hexane/Et_2O 7:3) affording compound **2** in 97% yield (144.6 mg) as a white solid.

m.p. 75-76ºC (*n*-hexane/Et_2O) (lit.[47] 77-78ºC);

^{1}H NMR (400 MHz, *$CDCl_3$*) δ 7.48 (d, *J* = 7.2 Hz, 2H), 7.39 (t, *J* = 7.2 Hz, 2H), 7.32 (t, *J* = 7.2 Hz, 1H), 7.20 (d, *J* = 2.3 Hz, 1H), 7.14 (d, *J* = 8.9 Hz, 1H), 7.09 (d, *J* = 3.1 Hz, 1H), 7.08-7.05 (m, 2H), 6.98 (t, *J* = 8.6 Hz, 2H), 6.93 (dd, *J* = 8.9, 2.3 Hz, 1H), 6.47 (d, *J* = 3.1 Hz, 1H), 5.25 (s, 2H), 5.11 (s, 2H);

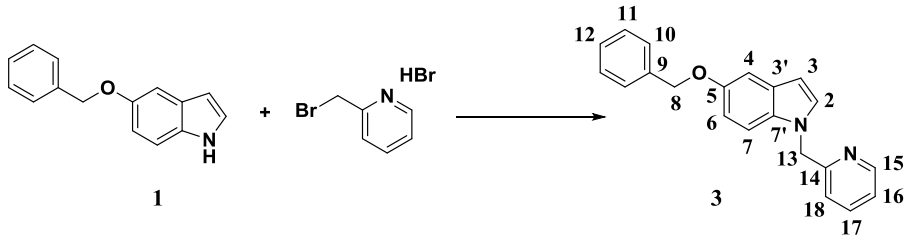
^{13}C NMR (101 MHz, *CDCl$_3$*) δ 162.3 (d, *J* = 246.0 Hz, CF), 153.5, 137.8, 133.4 (d, *J* = 3.2 Hz), 131.8, 129.3, 128.8, 128.6 (2 x CH), 128.5 (d, *J* = 8.1 Hz), 127.9, 127.7 (2 x CH), 115.8 (d, *J* = 21.6 Hz), 112.9, 110.5, 104.4, 101.6, 71.0, 49.8;

IR (KBr) 1487, 1240, 1017, 810 cm^{-1};

Purity 94%

Spectral data were in accordance with the literature.[47]

*IV.4.3 Synthesis of 5-(benzyloxy)-1-(pyridin-2-ylmethyl)-1H-indole (**3**)*



To a solution 5-(benzyloxy)-1*H*-indole (**1**) (100 mg, 0.45 mmol) in dry DMF (1.2 mL), was added NaH (21.5 mg, 0.54 mmol) at 0ºC under argon. The reaction mixture was stirred at that temperature for 45 min. 2-(Bromomethyl)pyridine hydrobromide (135.9 mg, 0.54 mmol) was added and the mixture was stirred, from 0ºC to room temperature, under argon, for 24 h. The reaction was quenched with distilled water and extracted with EtOAc. The combined organic layers were washed with water and brine, dried over Na_2SO_4 anhydrous, filtered and concentrated. The crude was purified by flash chromatography (*n*-hexane/Et_2O 2:3) affording compound **3** in 84% yield (117.8 mg), as a white solid.

m.p. 115-117ºC (*n*-hexane/Et_2O);

^{1}H NMR (400 MHz, *CDCl$_3$*) δ 8.59 (d, *J* = 4.0 Hz, 1H, H15), 7.52 (t, *J* = 7.7, 1H, H17), 7.47 (d, *J* = 7.3 Hz, 2H, H10), 7.39 (t, *J* = 7.3 Hz, 2H, H11), 7.32 (t, *J* = 7.3 Hz, 1H, H12), 7.20 (d, *J* = 2.3 Hz, 1H, H4), 7.18 (d, *J* = 3.2 Hz, 1H, H2), 7.17-7.15 (m, 2H, H7 and H16), 6.92 (dd, *J* = 8.9, 2.3 Hz, 1H, H6), 6.67 (d, *J* = 7.8 Hz, 1H, H18), 6.51 (d, *J* = 3.2 Hz, 1H, H3), 5.43 (s, 2H, H13), 5.10 (s, 2H, H8);

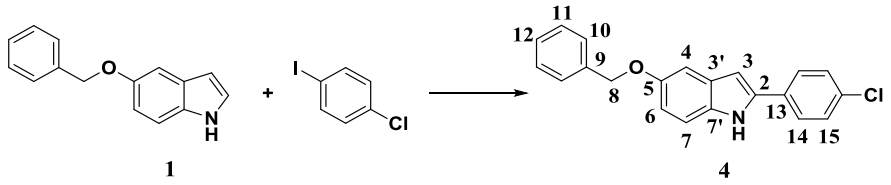
^{13}C NMR (101 MHz, *CDCl$_3$*) δ 157.8 (C14), 153.6 (C5), 149.5 (C15), 137.8 (C9), 137.2 (C17), 131.8 (C7'), 129.3 (C3'), 129.2 (C2), 128.6 (2 x C11), 127.9 (C12), 127.7 (2 x C10), 122.6 (C16), 120.8 (C18), 113.0 (C6), 110.6 (C7), 104.4 (C4), 101.9 (C3), 71.0 (C8), 52.4 (C13);

IR (KBr) 3444, 1484, 1237, 1019 cm^{-1};

HRMS (ESI) m/z 315.1495 $[M+H]^+$ (calcd for $C_{21}H_{19}N_2O$ 315.1497);

Purity 100%

*IV.4.4 Synthesis of 5-(benzyloxy)-2-(4-chlorophenyl)-1H-indole (**4**)*



1 **4**

IV.4.4.1 Reaction catalyzed by palladium(II) acetate, silver(I) oxide and 2-nitrobenzoic acid[23]

A solution of **1** (50.0 mg, 0.22 mmol), $Pd(OAc)_2$ (2.5 mg, 0.01 mmol), Ag_2O (38.9 mg, 0.17 mmol), 2-nitrobenzoic acid (56.1 mg, 0.33 mmol) and 1-chloro-4-iodobenzene (106.8 mg, 0.45 mmol), in dry DMF (0.45 mL), was stirred under argon, at 50ºC, for 48 h. No product formation was observed after TLC control.

IV.4.4.2 Reaction catalyzed by palladium(II) acetate, dppm and potassium acetate[24]

In a dried ACE pressure tube, containing **1** (100.0 mg, 0.45 mmol), 1-chloro-4-iodobenzene (128.2 mg, 0.54 mmol), $Pd(OAc)_2$ (5.0 mg, 0.02 mmol), dppm (8.6 mg, 0.02 mmol) and KOAc (131.9 mg, 1.34 mmol), was added degassed H_2O (1 mL), under an argon atmosphere. The mixture was stirred for 24 h at 100ºC. After this time, the reaction was quenched with 1 M HCl and extracted with EtOAc. The combined organic layers were washed with water and brine, dried over Na_2SO_4 anhydrous, filtered and concentrated. The crude was purified by flash chromatography (*n*-hexane/EtOAc 5 to 20%) affording compound **4** in 56% yield (83.7 mg), as a white solid.

m.p. 180-182ºC (*n*-hexane/EtOAc);

^{1}H NMR [400 MHz, *$(CD_3)_2CO$*] δ 10.60 (s, 1H, NH), 7.83 (d, *J* = 8.7 Hz, 2H, H15), 7.50 (d, *J* = 7.5 Hz, 2H, H10), 7.46 (d, *J* = 8.7 Hz, 2H, H14), 7.39 (t, *J* = 7.5 Hz, 2H, H11), 7.36-7.27 (m, 2H, H7 and H12), 7.17 (d, *J* = 2.3 Hz, 1H, H4), 6.88 (dd, *J* = 8.8, 2.3 Hz, 1H, H6), 6.84 (s, 1H, H3), 5.13 (s, 2H, H8);

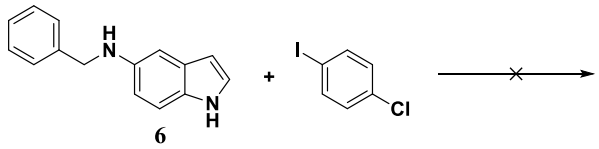
^{13}C NMR [101 MHz, *$(CD_3)_2CO$*] δ 154.4 (C5), 139.1 (C9), 138.1 (C13), 133.8 (C7'), 133.7 (CCl), 133.2 (C2), 130.5 (C3'), 129.8 (2 x C14), 129.2 (2 x C11), 128.4 (C12), 128.3 (2 x C10), 127.3 (2 x C15), 114.1 (C6), 112.8 (C7), 104.3 (C4), 100.5 (C5), 71.0 (C8);

IR (KBr) 3436, 1215, 1096, 731 cm^{-1};

HRMS (ESI) m/z 334.0982 $[M+H]^+$ (calcd for $C_{21}H_{17}ClNO$ 334.0999);

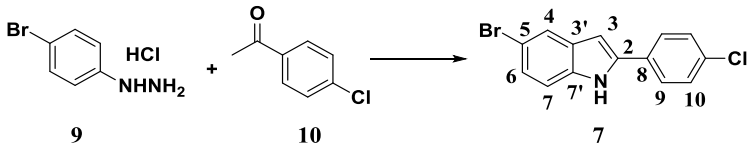
Purity 98%

*IV.4.5 Reaction of N-benzyl-1H-indol-5-amine (**6**) with 1-chloro-4-iodobenzene, catalyzed by palladium(II) acetate, dppm and potassium acetate*[24]



In a dried ACE pressure tube, containing **6** (50.0 mg, 0.22 mmol), 1-chloro-4-iodobenzene (64.1 mg, 0.27 mmol), $Pd(OAc)_2$ (2.8 mg, 0.01 mmol), dppm (4.8 mg, 0.01 mmol) and KOAc (66.4 mg, 0.67 mmol), was added degassed H_2O (0.5 mL), under an argon atmosphere. The mixture was stirred for 24 h at 100ºC. No product formation was observed after TLC control.

*IV.4.6 Synthesis of 5-bromo-2-(4-chlorophenyl)-1H-indole (**7**)*[28]



In a two-neck round bottom flask, equipped with a thermometer, was added (4-bromophenyl)hydrazine hydrochloride (**9**) (200 mg, 0.84 mmol) and 4-chloroacetophenone (**10**) (0.12 mL, 0.84 mmol). The mixture was stirred for 5 min. Then PPA (*ca*. 1 g) was added and the reaction mixture was gently warmed to 110ºC. After 2 h stirring at that temperature, the reaction was poured onto ice. A solution of 2 M NaOH was added till pH ≈ 7. The solid was filtered by suction and purified by flash chromatography (*n*-hexane/Et_2O 7:3) affording compound **7** in 85% yield (233.7 mg), as a white solid.

m.p. 190-192ºC (*n*-hexane/Et_2O 7:3);

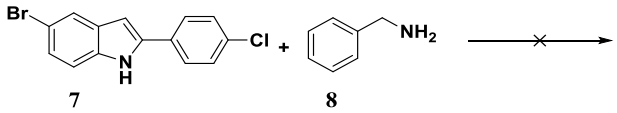
^{1}H NMR [400 MHz, $CD_3)_2CO$] δ 10.96 (s, 1H, NH), 7.91-7.83 (m, 2H, H10), 7.74 (d, *J* = 1.8 Hz, 1H, H4), 7.53-7.46 (m, 2H, H9), 7.38 (d, *J* = 8.6 Hz, 1H, H7), 7.23 (dd, *J* = 8.6, 1.8 Hz, 1H, H6), 6.92 (s, 1H, H3);

^{13}C NMR [101 MHz, $CD_3)_2CO$] δ 139.1 (C8), 137.1 (C7'), 133.9 (CCl), 131.9 (C2 or C5), 131.8 (C2 or C5), 129.9 (2 x C9), 127.7 (2 x C10), 125.5 (C6), 123.5 (C4), 113.9 (C7), 113.4 (C3'), 100.1 (C3);

IR (KBr) 3437, 1440, 1097, 831, 792 cm^{-1};

HRMS (ESI) m/z 305.9662 $[M+H]^+$ (calcd for $C_{14}H_{10}BrClN$ 305.9685).

*IV.4.7 Reaction of 5-bromo-2-(4-chlorophenyl)-1H-indole (**7**) with benzylamine (**8**)*



7 8

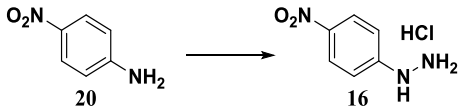
IV.4.7.1 Via copper catalysis[25]

In a dried ACE pressure tube, containing **7** (50 mg, 0.16 mmol), CuI (3.1 mg, 0.016 mmol), L-proline (3.8 mg, 0.032 mmol), K_2CO_3 (45.1 mg, 0.32 mmol) and benzylamine (**8**) (26.7 µL, 0.24 mmol), was added dry DMSO (0.1 mL), under an argon atmosphere. The mixture was stirred for 96 h at 80ºC. No product formation was observed after TLC control.

IV.4.7.2 Via palladium catalysis[26]

Compound **7** (100 mg, 0.33 mmol), BrettPhos (1.7 mg, 0.003 mmol) and chloro[2-(dicyclohexylphosphino)-3,6-dimethoxy-2′,4′, 6′-triisopropyl-1,1′-biphenyl][2-(2-aminoethyl)phenyl] palladium(II) (BrettPhos precatalyst) (2.6 mg, 0.003 mmol) were added in a dried ACE pressure tube. The vial was evacuated and backfilled with argon, then a 1 M solution of LiHMDS (0.8 mL, 0.79 mmol) in THF, and benzylamine (**8**) (42.7 µL, 0.39 mmol) were added. The mixture was stirred at 65ºC for 48 h. No product formation was observed after TLC control.

*IV.4.8 Synthesis of (4-nitrophenyl)hydrazine hydrochloride (**16**)*[48]



20 16

To a solution of 4-nitroaniline (**20**) (500 mg, 3.62 mmol), in HCl (6.3 mL), cooled with an ice bath, was added dropwise a solution of $NaNO_2$ (265.6 mg, 3.85 mmol), in H_2O (1.5 mL), also cooled in an ice bath. Then, a solution of $SnCl_2 \cdot 2H_2O$ (1.81 g, 8.02 mmol), in HCl (1.8 mL) was added, dropwise. The solution was stirred for 2 h. The resulting solid was filtered by suction and washed with Et_2O, affording compound **16** in 81% yield (555.6 mg) as an orange solid.

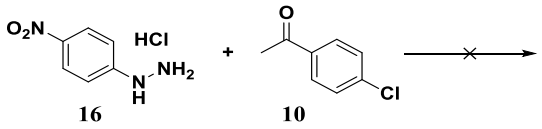
m.p. 199-201ºC (Et_2O) [lit.[49] 201-202ºC (EtOAc)];

^{1}H NMR (400 MHz, *DMSO-d_6*) δ 10.64 (s, 3H, NH_3^+), 9.57 (s, 1H, NH), 8.16 (d, *J* = 9.1 Hz, 2H), 7.06 (d, *J* = 9.1 Hz, 2H);

IR (KBr) 3326, 2880, 2677, 1601, 1336, 1112, 851 cm^{-1};

Spectral data were in accordance with the literature.[49]

*IV.4.9 Reaction of (4-nitrophenyl)hydrazine hydrochloride (**16**) with 4-chloroacetophenone (**10**)*



16 10

IV.4.9.1 With polyphosphoric acid[28]

In a two-neck round bottom flask, equipped with a thermometer, was added compounds **16** (200 mg, 1.05 mmol) and **10** (0.14 mL, 1.05 mmol). The mixture was stirred for 5 min. Then PPA (*ca*. 1 g) was added and the reaction mixture was gently warmed to 110ºC, for 5 h. No product formation was observed after TLC control.

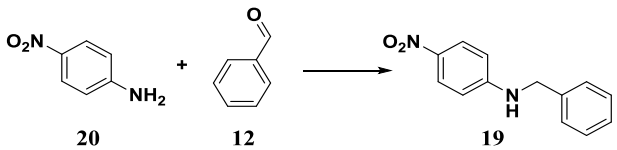
IV.4.9.2 With phosphomolybdic acid[27]

To a solution of 4-chloroacetophenone (**10**) (0.14 mL, 1.05 mmol), in dry $CHCl_3$ (0.5 mL), was added phosphomolybdic acid (385.1 mg, 0.21 mmol). Compound **16** (200 mg, 1.05 mmol) was then slowly added, at room temperature. The reaction mixture was heated to 60ºC, for 24 h. No product formation was observed after TLC control.

IV.4.10 Preparation of silica-gel-supported sulfuric acid (SSA)[50]

To a flask containing silica gel (1 g) was slowly added chlorosulfonic acid (0.22 mL). The mixture was shacked for 30 min, affording SSA, which was used without purification directly on the next step.

*IV.4.11 Synthesis of N-benzyl-4-nitroaniline (**19**)[51]*



20 12 19

In a mortar was added 4-nitroaniline (**20**) (50 mg, 0.36 mmol), $NaBH_4$ (13.7 mg, 0.36 mmol), SSA (72.4 mg) and benzaldehyde (**12**) (36.9 µL, 0.36 mmol). The mixture was grounded for 10 min, then was filtered through celite and washed with EtOAc. The crude was purified by flash

chromatography (*n*-hexane/EtOAc 3:2) affording compound **19** in 89% yield (73.6 mg) as a yellow solid.

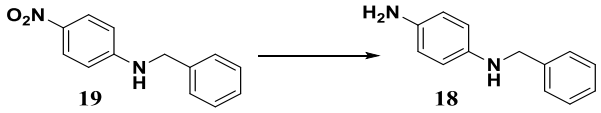
m.p. 147-148ºC (*n*-hexane/EtOAc) [lit.[52] 146-147ºC (EtOAc/petroleum ether)];

^{1}H NMR (400 MHz, *CDCl$_3$*) δ 8.08 (d, *J* = 9.2 Hz, 2H), 7.42-7.28 (m, 5H), 6.58 (d, *J* = 9.2 Hz, 2H), 4.70 (s, 1H, NH), 4.43 (s, 2H);

IR (KBr) 3366, 1604, 1282, 1106 cm^{-1}.

Spectral data were in accordance with the literature.[52]

*IV.4.12 Synthesis of N-benzylbenzene-1,4-diamine (**18**)*[53]



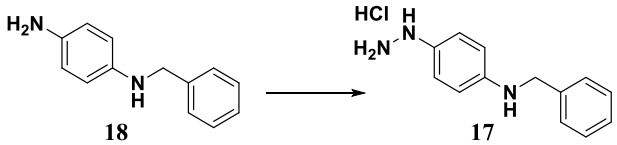
To a solution of NH_4Cl (70.3 mg, 1.31 mmol), in H_2O (0.9 mL), was added Fe (73.4 mg, 1.31 mmol), under argon atmosphere. A solution of **19** (100 mg, 0.44 mmol), in acetone (2.2 mL), was slowly added. The reaction was refluxed overnight. The mixture was diluted in EtOAc, washed with a saturated solution of $NaHCO_3$ and then brine, dried over Na_2SO_4 anhydrous, filtered and concentrated. The crude was purified by flash chromatography (DCM/MeOH 1 to 2%) affording compound **18** in 95% yield (82.8 mg) as beige solid.

^{1}H NMR (400 MHz, *CDCl$_3$*) δ 7.40-7.27 (m, 5H), 6.62 (d, *J* = 8.4 Hz, 2H), 6.55 (d, *J* = 8.4 Hz, 2H), 4.27 (s, 2H), 3.27 (s, 3H, NH and NH_2);

IR (KBr) 3409, 3340, 1516 cm^{-1}.

Spectral data were in accordance with the literature.[54]

*IV.4.13 Synthesis of N-benzyl-4-hydrazinylaniline hydrochloride (**17**)*



To a solution of **18** (122.7 mg, 0.62 mmol), in HCl (1.1 mL), cooled with an ice bath, was added dropwise a solution of $NaNO_2$ (45.3 mg, 0.66 mmol), in H_2O (0.3 mL), also cooled in an ice bath. Then, a solution of $SnCl_2 \cdot 2H_2O$ (310.0 mg, 1.37 mmol), in HCl (0.3 mL) was added, dropwise. The solution was stirred for 2 h. The resulting solid was filtered by suction and washed with Et_2O, affording **17** in 40% yield (62.4 mg) as beige solid.

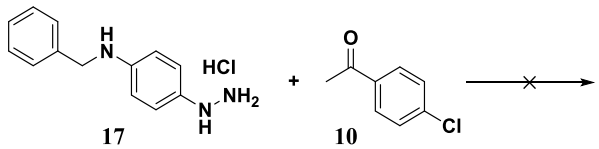
m.p. 173-175ºC (Et_2O);

^{1}H NMR (400 MHz, *DMSO-d_6*) δ 10.02 (s, 3H, NH_3^+), 7.36 (d, *J* = 7.2 Hz, 2H), 7.31 (t, *J* = 7.2 Hz, 2H), 7.22 (t, *J* = 7.2 Hz, 1H), 7.09 (d, *J* = 8.4 Hz, 2H), 6.71 (d, *J* = 8.4 Hz, 2H), 4.29 (s, 2H), 3.40 (s, NH, *under the solvent water peak*);

^{13}C NMR (101 MHz, *DMSO-d_6*) δ132.2, 131.3, 129.5, 129.2, 128.3, 127.6, 126.9, 123.5, 47.1;

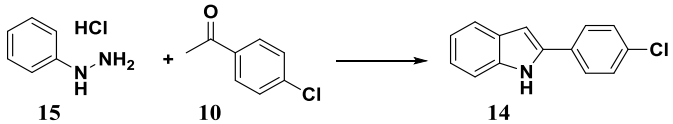
IR (KBr) 3435, 2836, 1508 cm^{-1}.

*IV.4.14 Reaction of N-benzyl-4-hydrazinylaniline hydrochloride (**17**) with 4-chloroacetophenone (**10**)*



In a two-neck round bottom flask, equipped with a thermometer, was added **17** (55 mg, 0.22 mmol) and **10** (28.6 µL, 0.22 mmol). The mixture was stirred for 5 min. Then PPA (*ca*. 150 mg) was added and the reaction mixture was gently warmed to 110ºC. Decomposition of the starting material was observed and no reaction product was formed by TLC control.

*IV.4.15 Synthesis of 2-(4-chlorophenyl)-1H-indole (**14**)[28]*



In a two-neck round bottom flask, equipped with a thermometer, was added phenylhydrazine hydrochloride (**15**) (500 mg, 3.46 mmol) and 4-chloroacetophenone (**10**) (0.44 mL, 3.39 mmol). The mixture was stirred for 5 min. Then PPA (*ca*. 2 g) was added and the reaction mixture was gently warmed to 110ºC. After 1 h stirring at that temperature, the reaction was poured onto ice. A solution of 2 M NaOH was added till pH ≈ 7. The solid was filtered by suction and recrystallized from Et_2O/pentane affording compound **13** in 66% yield (512.1 mg) as white solid.

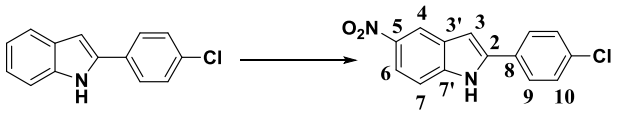
m.p. 208-210ºC (Et_2O/pentane) [lit.[55] 208-210ºC (EtOAc/*n*-hexane)];

^{1}H NMR (400 MHz, *DMSO-d_6*) δ 11.58 (s, 1H, NH), 7.88 (d, *J* = 8.5 Hz, 2H), 7.56-7.49 (m, 3H), 7.40 (d, *J* = 7.7 Hz, 1H), 7.11 (t, *J* = 7.7 Hz, 1H), 7.00 (t, *J* = 7.7 Hz, 1H), 6.93 (s, 1H);

IR (KBr) 3433, 1426, 1094, 798 cm^{-1}.

Spectral data were in accordance with the literature.[55]

*IV.4.16 Synthesis of 2-(4-chlorophenyl)-5-nitro-1H-indole (**13**)*[28]



14 **13**

To a solution of **14** (190 mg, 0.83 mmol), in H_2SO_4 (5.4 mL), cooled with an ice bath, was added, dropwise, a solution of $NaNO_3$ (75.5 mg, 0.89 mmol), in H_2SO_4 (2.7 mL), also cooled in an ice bath. The mixture was stirred, at 0ºC, for 5 min. Then, was poured onto ice and a solution of 2 M NaOH was added till pH ≈ 7. The solid was filtered by suction, redissolved in EtOAc, washed with distilled water and brine, dried over Na_2SO_4 anhydrous, filtered and concentrated. The resulting solid was recrystallized from EtOAc/pentane affording **13** in 82% yield (185.6 mg) as a yellow solid.

m.p. 292-294ºC (EtOAc/pentane);

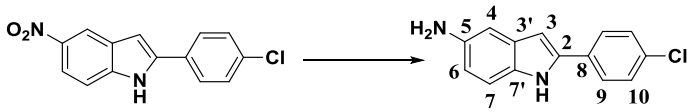
^{1}H NMR (400 MHz, *DMSO-d_6*) δ 12.34 (s, 1H, NH), 8.53 (s, 1H, H4), 8.01 (dd, *J* = 9.0, 2.1 Hz, 1H, H6), 7.92 (d, *J* = 8.4 Hz, 2H, H10), 7.62-7.52 (m, 3H, H7 and H9), 7.21 (s, 1H, H3);

^{13}C NMR (101 MHz, *DMSO-d_6*) δ 141.1 (C5), 140.4 (C7' or C8), 140.2 (C7' or C8), 133.0 (C2), 129.9 (CCl), 129.1 (2 x C9), 127.8 (C3'), 127.1 (2 x C10), 117.2 (C6), 117.1 (C4), 111.7 (C7), 101.5 (C3);

IR (KBr) 3333, 1471, 1334 cm^{-1};

HRMS (ESI) m/z 273.0408 $[M+H]^+$ (calcd for $C_{14}H_{10}ClN_2O_2$ 273.0431).

*IV.4.17 Synthesis 2-(4-chlorophenyl)-1H-indol-5-amine (**11**)*



13 **11**

IV.4.17.1 <u>Reduction with Pd/C, H_2</u>[56]

To a solution of **13** (50 mg, 0.18 mmol), in EtOH (7 mL), was added 10% Pd/C (12.9 mg, 0.01 mmol). The mixture was stirred at room temperature under H_2 atmosphere. After 2 h, the reaction mixture was filtered through celite and concentrated under reduce pressure. The crude was purified by flash chromatography ($CHCl_3$/MeOH 2 to 4%) affording a mixture of 1:0.6 of compounds **11** and the dechlorinated product.

IV.4.17.2 Reduction with Fe/NH_4Cl[53]

To a solution of NH_4Cl (98.1 mg, 1.83 mmol), in H_2O (1.2 mL), was added Fe (102.4 mg, 1.83 mmol), under argon atmosphere. A solution of **13** (100 mg, 0.37 mmol), in THF (1.8 mL), was slowly added. The reaction was refluxed overnight. The mixture was diluted in EtOAc, washed with a saturated solution of $NaHCO_3$ and then brine, dried over Na_2SO_4 anhydrous, filtered and concentrated. The crude was purified by flash chromatography ($CHCl_3$; $CHCl_3$/MeOH 2%) affording compound **15** in 59% yield (52.7 mg) as a beige solid.

m.p. 249-251ºC (EtOAc/pentane);

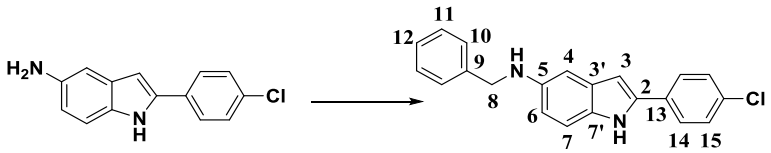
^{1}H NMR [400 MHz, *$(CD_3)_2CO$*] δ 10.30 (s, 1H, NH), 7.79 (d, *J* = 8.6 Hz, 2H, H10), 7.43 (d, *J* = 8.6 Hz, 2H, H9), 7.14 (d, *J* = 8.5 Hz, 1H, H7), 6.81 (s, 1H, H4), 6.67 (s, 1H, H3), 6.62 (dd, *J* = 8.5, 2.2 Hz, 1H, H6), 4.15 (s, 2H, NH_2);

^{13}C NMR [101 MHz, *$(CD_3)_2CO$*] δ 142.6 (C5), 137.3 (C8), 132.9 (CCl), 132.8 (C7'), 131.1 (C2), 129.7 (2 x C9), 127.1 (2 x C10), 114.1 (C6), 112.3 (C7), 112.2 (C3'), 104.7 (C4), 99.5 (C3);

IR (KBr) 3420, 1458, 830, 762 cm^{-1};

HRMS (ESI) m/z 243.0686 $[M+H]^+$ (calcd for $C_{14}H_{12}ClN_2$ 243.0684).

*IV.4.18 Synthesis of N-benzyl-2-(4-chlorophenyl)-1H-indol-5-amine (**5**)*



11 5

To a solution of **11** (70 mg, 0.29 mmol), in dry MeOH (2.2 mL), was added benzaldehyde (29.4 µL, 0.29 mmol), one drop of glacial acetic acid and $NaBH_3CN$ (18.1 mg, 0.29 mmol). The mixture was stirred, at room temperature, under argon atmosphere, overnight. The reaction was quenched with distilled water, extracted with EtOAc, dried over Na_2SO_4 anhydrous, filtered and concentrated. The crude was purified by flash chromatography (*n*-hexane/EtOAc 4:1) affording compound **5** in 78% yield (75.2 mg) as white solid.

m.p. 189-191ºC (*n*-hexane/EtOAc);

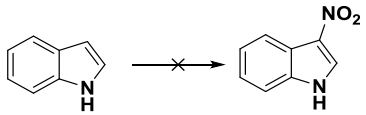
^{1}H NMR [400 MHz, *$(CD_3)_2CO$*] δ 10.32 (s, 1H, NH), 7.79 (d, *J* = 8.7 Hz, 2H, H15), 7.45-7.41 (m, 4H, H10 and H14), 7.31 (t, *J* = 7.4 Hz, 2H, H11), 7.22 (t, *J* = 7.4 Hz, 1H, H12), 7.17 (d, *J* = 8.4 Hz, 1H, H7), 6.72-6.68 (m, 3H, H3, H4 and H6), 5.01 (s, 1H, NH), 4.37 (s, 2H, H8);

^{13}C NMR [101 MHz, *$(CD_3)_2CO$*] δ 144.0 (C5), 141.9 (C9), 137.1 (C13), 132.9 (CCl), 132.7 (C7'), 132.5 (C2), 131.0 (C3'), 129.7 (2 x C14), 129.1 (2 x C11), 128.2 (2 x C10), 127.4 (C12), 127.1 (2 x C15), 113.6 (C6), 112.5 (C7), 101.9 (C4), 99.9 (C3), 49.4 (C8);

IR (KBr) 3432, 1458, 1239, 833 cm^{-1};

HRMS (ESI) m/z 333.1148 $[M+H]^+$ (calcd for $C_{21}H_{18}ClN_2$ 333.1159);
Purity 100%

IV.4.19 Synthetic studies toward C-3 nitration of indole



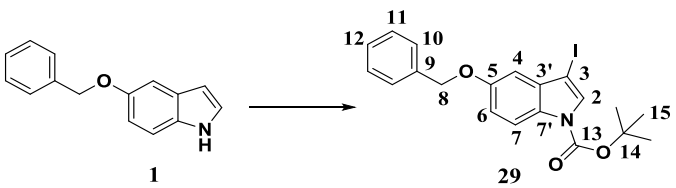
IV.4.19.1 Reaction with chlorodiphenylphosphine and silver nitrate[36]

To a solution of Ph_2PCl (42.6 µL, 0.24 mmol) and I_2 (60.2 mg, 0.24 mmol), in dry DCM (1.1 mL), was added $AgNO_3$ (73.9 mg, 0.47 mmol), at room temperature. Indole (50 mg, 0.43 mmol) was then added to the reaction mixture. The reaction afforded a complex mixture of products, observed by TLC control and no isolation was attempted due to the complexity of the mixture.

IV.4.19.2 Reaction with benzoyl chloride and silver nitrate[37]

To a solution $AgNO_3$ (87 mg, 0.51 mmol), in dry MeCN (0.3 mL), cooled with ice bath, was slowly added a solution of benzoyl chloride (54.5 µL, 0.47 mmol), in dry MeCN (0.2 mL). The resulting mixture was added to a solution of indole (50 mg, 0.43 mmol), in dry MeCN (0.5 mL), cooled ate -10ºC. The reaction mixture was stirred ate this temperature for 1 h and then was allowed to warm to room temperature. The reaction afforded a complex mixture of products, observed by TLC control and no isolation was attempted due to the complexity of the mixture.

*IV.4.20 Synthesis of tert-butyl 5-(benzyloxy)-3-iodo-1H-indole-1-carboxylate (**29**)[57]*



To a solution of 5-(benzyloxy)-1*H*-indole (**1**) (100 mg, 0.45 mmol), in dry DMF (0.8 mL), was added KOH (62.8 mg, 1.12 mmol), under argon atmosphere. The solution was stirred at room temperature for 20 min. After that time, a solution of I_2 (114.8 mg, 0.45 mmol), in dry DMF (0.8 mL), was slowly added. The reaction was stirred for 45 min. The solution was poured onto ice and water

containing ammonia (0.5%) and sodium metabisulphite (0.1%), the precipitate was filtered and washed with cold water, affording 5-(benzyloxy)-3-iodo-1*H*-indole in 98% yield (152.9 mg) as a white solid.

To a solution of 5-(benzyloxy)-3-iodo-1*H*-indole (152.9 mg, 0.44 mmol), in dry DCM (10.2 mL), was added Boc_2O (105.13 mg, 0.48 mmol), TEA (0.18 mL, 1.31 mmol) and DMAP (5.3 mg, 0.04 mmol). The mixture was stirred for 30 min, under argon atmosphere, at room temperature. The reaction mixture was then washed with a 5% aqueous solution of sodium metabisulphite, dried over $MgSO_4$ and concentrated. The crude was purified by flash chromatography (*n*-hexane/Et_2O 4:1) affording compound **29** in 82% yield (161 mg), as a white solid.

m.p. 109-112ºC (*n*-hexane/Et_2O);

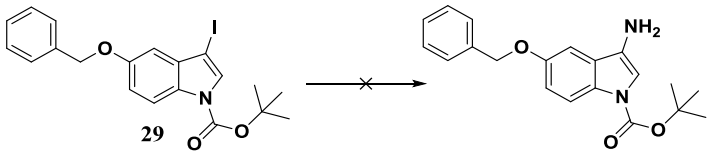
^{1}H NMR (400 MHz, *CDCl*$_3$) δ 8.03-8.01 (m, 1H, H7), 7.70 (s, 1H, H2), 7.49 (d, *J* = 7.2 Hz, 2H, H10), 7.40 (t, *J* = 7.2 Hz, 2H, H11), 7.34 (t, *J* = 7.2 Hz, 1H, H12), 7.05 (dd, *J* = 9.0, 2.5 Hz, 1H, H6), 6.95 (d, *J* = 2.5 Hz, 1H, H4), 5.14 (s, 2H, H8), 1.66 (s, 9H, 3 x H15);

^{13}C NMR (101 MHz, *CDCl*$_3$) δ 155.8 (C5), 148.8 (C13), 137.1 (C9), 133.1 (C7'), 130.8 (C2), 129.7 (C3'), 128.7 (2 x C11), 128.1 (C12), 127.8 (2 x C10), 116.1 (C7), 115.2 (C6), 105.3 (C4), 84.3 (14), 70.7 (C8), 65.3 (C3), 28.3 (3 x C15);

IR (KBr) 1720, 1358, 1271, 1154 cm^{-1};

HRMS (ESI) m/z 450.0557 $[M+H]^+$ (calcd for $C_{20}H_{21}INO_3$ 450.0566).

*IV.4.21 Synthetic studies towards C-3 amination of tert-butyl 5-(benzyloxy)-3-iodo-1H-indole-1-carboxylate (**29**)*



IV.4.21.1 Reaction with NH_4OH and Cu_2O[41]

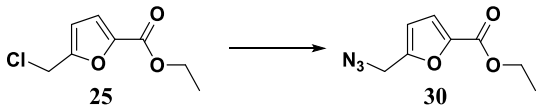
In an ACE pressure tube containing a solution of **29** (100 mg, 0.22 mmol) and Cu_2O (1.6 mg, 0.01 mmol), in NMP (0.2 mL), was added a 25% aqueous solution of NH_3 (0.17 mL, 2.23 mmol). The reaction mixture was heated to 80ºC, for 16 h. No product formation was observed after TLC control.

IV.4.21.2 Reaction with NH_4OH, CuI and Fe_2O_3[42]

In an ACE pressure tube containing a solution of **29** (100 mg, 0.22 mmol), in EtOH (0.4 mL), were added CuI (4.2 mg, 0.02 mmol) and Fe_2O_3 (3.5 mg, 0.02 mmol). A 25% aqueous solution of NH_3 (83.3 µL, 1.11 mmol) and NaOH (17.8 mg, 0.44 mmol) were then added. The reaction mixture was

heated to 90ºC for 24 h. only the product resulting from the hydrolysis of the *tert*-butylcarboxylate group compound **29** was observed by TLC.

*IV.4.22 Synthesis of ethyl 5-(azidomethyl)furan-2-carboxylate (**30**)*[58]



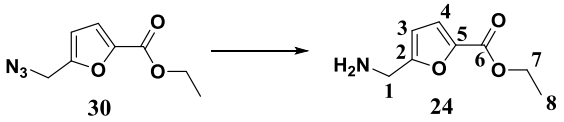
To a solution of ethyl 5-(chloromethyl)furan-2-carboxylate (**25**) (300 mg, 1.59 mmol), in dry DMF (1.1 mL), was added NaN_3 (124.1 mg, 1.91 mmol), under argon atmosphere. The mixture was heated to 70ºC and was stirred for 3 h. After that time the reaction was quenched with distilled water, extracted with EtOAc, dried over Na_2SO_4, filtered and the solvent removed. The crude was purified by flash chromatography (*n*-hexane/Et_2O 4:1 to 3:2) affording compound **30** in 90% yield (299.6 mg) as colorless oil.

^{1}H NMR (400 MHz, *CDCl*$_3$) δ 7.14 (d, *J* = 3.4 Hz, 1H), 6.46 (d, *J* = 3.4 Hz, 1H), 4.37-4.46 (m, 4H, 2 x CH_2), 1.38 (t, *J* = 7.1 Hz, 3H, CH_3);

IR (NaCl) 2100, 1723, 1300 cm^{-1}.

Spectral data were in accordance with the literature.[59]

*IV.4.23 Synthesis of ethyl 5-(aminomethyl)furan-2-carboxylate (**24**)*[58]



To a solution of **30** (300 mg, 1.54 mmol), in dry THF (2.1 mL), was added a solution of PPh_3 (427.3 mg, 1.63 mmol), in dry THF (1.2 mL), under argon atmosphere. The mixture was heated to 50ºC, for 2 h. After that time, H_2O (61 µL) was added. The reaction was stirred at 50ºC for 1 h. Then it was allowed to cool to room temperature and was stirred overnight. The THF was removed under reduced pressure and the crude was purified by flash chromatography ($CHCl_3$; $CHCl_3$/MeOH 1 to 10%) affording compound **24** in 88% yield (228.1 mg) as yellow oil.

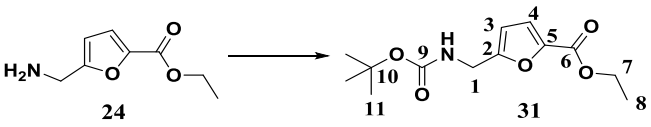
^{1}H NMR (400 MHz, *CDCl*$_3$) δ 7.09 (d, *J* = 3.4 Hz, 1H, H4), 6.26 (d, *J* = 3.4 Hz, 1H, H3), 4.33 (q, *J* = 7.1 Hz, 2H, H7), 3.88 (s, 2H, H1), 1.70 (s, 2H, NH_2), 1.35 (t, *J* = 7.1 Hz, 3H, H8);

^{13}C NMR (101 MHz, *CDCl*$_3$) δ 161.1 (C2), 159.0 (C6), 143.9 (C5), 118.9 (C4), 107.6 (C3), 61.0 (C7), 39.6 (C1), 14.5 (C8);

IR (NaCl) 3371, 1715, 1302 cm^{-1};

HRMS (ESI) m/z 170.0815 $[M+H]^+$ (calcd for $C_8H_{12}NO_3$ 170.0812).

*IV.4.24 Synthesis of ethyl 5-{[(tert-butoxycarbonyl)amino]methyl}furan-2-carboxylate (**31**)*



To a solution of **24** (656 mg, 3.88 mmol), in dry DCM (5.2 mL), was added Boc_2O (931.6 mg, 4.27 mmol), TEA (1.1 mL, 7.76 mmol) and DMAP (47.4 mg, 0.39 mmol), under argon atmosphere. The mixture was stirred for 2 h at room temperature. After that time the mixture was washed with distilled water and brine, the organic layers were dried over $MgSO_4$, filtered and the solvent removed. The crude was purified by flash chromatography (*n*-hexane/Et_2O 7:3) affording compound **31** in 64% yield (670 mg) as yellow oil.

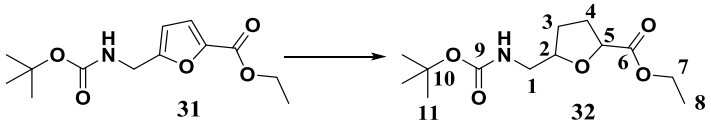
^{1}H NMR (400 MHz, *$CDCl_3$*) δ 7.08 (d, *J* = 3.4 Hz, 1H, H4), 6.33-6.32 (m, 1H, H3), 5.02 (s, 1H, NH), 4.34-4.33 (m, 4H, H1 and H7), 1.43 (s, 9H, H11), 1.35 (t, *J* = 7.1 Hz, 3H, H8);

^{13}C NMR (101 MHz, *$CDCl_3$*) δ 158.9 (C6), 156.7 (C2), 155.6 (C9), 144.2 (C5), 119.0 (C4), 109.2 (C3), 80.1 (C10), 61.1 (C7), 37.9 (C1), 28.4 (C11), 14.5 (C8);

IR (NaCl) 3351, 1712, 1519, 1300, 1138 cm^{-1};

HRMS (ESI) m/z 292.1155 $[M+Na]^+$ (calcd for $C_{13}H_{19}NNaO_5$ 292.1161).

*IV.4.25 Synthesis of ethyl 5-{[(tert-butoxycarbonyl)amino]methyl} tetrahydrofuran-2-carboxylate (**32**)*



To a solution of **31** (633 mg, 2.35 mmol), in dry EtOH (3 mL), was added 20% $Pd(OH)_2/C$ (165.1 mg, 0.23 mmol). The mixture was stirred at room temperature under H_2 atmosphere, overnight. The reaction mixture was filtered through celite and concentrated under reduce pressure. The crude was purified by flash chromatography (*n*-hexane/Et_2O 3:2) affording compound **32** in 91% yield (582.2 mg) as pale yellow oil.

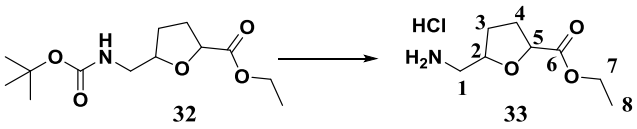
^{1}H NMR (400 MHz, *$CDCl_3$*) δ 5.55 (s, 1H, NH), 4.46 (dd, *J* = 8.8, 4.2 Hz, 1H, H5), 4.25-4.15 (m, 3H, H2 and H7), 3.45-3.37 (m, 1H, H1), 3.30-3.22 (m, 1H, H1), 2.34-2.22 (m, 1H, H4), 2.11-2.03 (m, 1H, H4), 1.97-1.87 (m, 1H, H3), 1.79-1.69 (m, 1H, H3), 1.43 (s, 9H, H11), 1.29 (t, J = 7.1 Hz, 3H, H8);

^{13}C NMR (101 MHz, *$CDCl_3$*) δ 173.9 (C6), 156.5 (C9), 80.3 (C2), 79.2 (C10), 77.4 (C5), 61.3 (C7), 43.7 (C1), 30.9 (C4), 28.5 (C11), 27.2 (C3), 14.3 (C8);

IR (KBr) 3352, 2977, 1714, 1514, 1172 cm^{-1};

HRMS (ESI) m/z 296.1470 $[M+Na]^+$ (calcd for $C_{13}H_{23}NNaO_5$ 296.1474).

*IV.4.26 Synthesis of ethyl 5-(aminomethyl)tetrahydrofuran-2-carboxylate hydrochloride (**33**)[60]*



To a solution of **32** (425 mg, 1.56 mmol), in dry DCM (1.3 mL), was added a 4 M solution of HCl in dioxane (1.6 mL, 6.35 mmol). The reaction mixture was stirred at room temperature, under argon atmosphere, for 4 h. The solvent was removed under reduced pressure. The solid was washed with Et_2O affording compound **33** in 92% yield (326.1 mg) as white solid.

m.p. 111-113ºC (Et_2O);

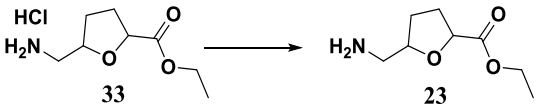
^{1}H NMR (400 MHz, *$CDCl_3$*) δ 8.54 (s, 3H, NH_3^+), 4.59-4.41 (m, 2H, H2 and H5), 4.27-4.10 (m, 2H, H7), 3.46-3.14 (m, 2H, H1), 2.44-2.26 (m, 1H, H4), 2.19-2.02 (m, 2H, H4 and H3), 2.04-1.84 (m, 1H, H3), 1.27 (t, *J* = 7.1 Hz, 3H, H8);

^{13}C NMR (101 MHz, *$CDCl_3$*) δ 175.6 (C6), 77.0 (C2 or C5), 76.6 (C2 or C5), 62.1 (C7), 41.8 (C1), 30.9 (C4), 27.0 (C3), 14.2 (C8);

IR (KBr) 2860, 1717, 1234, 1054 cm^{-1};

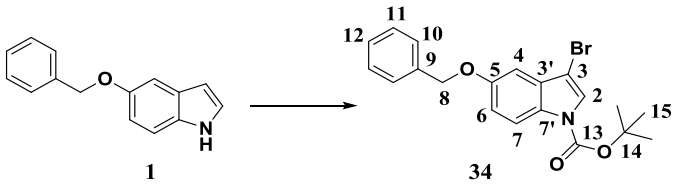
HRMS (ESI) m/z 174.1131 $[M]^+$ (calcd for $C_8H_{16}NO_3$ 174.1130).

*IV.4.27 Synthesis of ethyl 5-(aminomethyl)tetrahydrofuran-2-carboxylate (**23**)*



To a solution of **33** (90 mg, 0.43 mmol), in dry DCM (2.9 mL), was added TEA (60.2 µL, 0.43 mmol). The mixture was stirred for 3 h, at room temperature, under argon atmosphere. After that time, the reaction was cooled at 0ºC, filtered through celite and washed with cold DCM. The solvent was removed under reduced pressure, affording **23** in quantitative yield as a colorless oil. Compound **23** was used in the next step without further purification.

*IV.4.28 Synthesis of tert-butyl 5-(benzyloxy)-3-bromo-1H-indole-1-carboxylate (**34**)*[61]



To a solution of 5-(benzyloxy)-1*H*-indole (**1**) (100 mg, 0.45 mmol), in dry DCM (0.6 mL), was added Boc_2O (106.6 mg, 0.49 mmol) and DMAP (1.1 mg, 0.009 mmol). The mixture was stirred for 1 h 30 min, under argon atmosphere, at room temperature. After this time the solvent was removed under reduced pressure. The crude was redissolved in *n*-hexane and washed with distilled water, dried over Na_2SO_4, filtered and concentrated, affording *tert*-butyl 5-(benzyloxy)-1*H*-indole-1-carboxylate with 96% yield (139.4 mg) as a white solid.

To a solution of *tert*-butyl 5-(benzyloxy)-1*H*-indole-1-carboxylate (130 mg, 0.40 mmol), in dry DCM (6.1 mL), was added NBS (75.8 mg, 0.43 mmol), under argon atmosphere. The reaction mixture was refluxed overnight. Then it was washed with distilled water, dried over Na_2SO_4, filtered and concentrated. The crude was purified by flash chromatography (hexanes; hexanes/Et_2O 2 to 4%) affording 70% yield (122.6 mg) of compound **27** as a white foam.

^{1}H NMR (400 MHz, *$CDCl_3$*) δ 8.03-8.02 (m, 1H), 7.62 (s, 1H), 7.48 (d, *J* = 7.2 Hz, 2H), 7.40 (t, *J* = 7.2 Hz, 2H), 7.34 (t, *J* = 7.2 Hz, 1H), 7.10-7.01 (m, 2H), 5.14 (s, 2H), 1.66 (s, 9H);

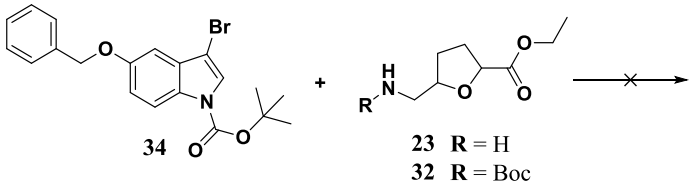
^{13}C NMR (101 MHz, *$CDCl_3$*) δ 155.7, 149.0, 137.1, 130.3, 129.5, 128.7 (2 x CH), 128.13, 127.8 (2 x CH), 125.5, 116.3, 115.4, 103.2, 97.8, 84.3, 70.7, 28.3 (3 x CH_3);

IR (KBr) 3158, 1728, 1477, 1271, 1155, 748 cm^{-1}.

Spectral data were in accordance with the literature.[62]

*IV.4.29 Synthesis of ethyl 5-({[5-(benzyloxy)-1H-indol-3-yl]amino}methyl) tetrahydrofuran-2-carboxylate (**21**)*

IV.4.29.1 Via Pd catalysis



23 **R** = H
32 **R** = Boc

IV.4.29.1.1 $Pd(OAc)_2$/Xantphos system[43]

General procedure: A dried ACE pressure tube, containing a solution of $Pd(OAC)_2$ (2.8 mg, 0.012 mmol), Xantphos (8.6 mg, 0.015 mmol) and compound **23** or **32** (0.15 mmol), in dry dioxane (0.2 mL), under an argon atmosphere, was heated to 100ºC for 5 min. After this time, the mixture was allowed to cool and **34** (50 mg, 0.12 mmol) and Cs_2CO_3 (113.4 mg, 0.35 mmol) were added, under argon atmosphere. The reaction was stirred at 100ºC for 24 h.

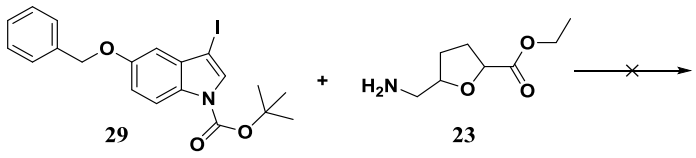
Only debromation of **34** was observed by the TLC control during the reaction of **34** with **23**.

Due to its instability, it was not possible to identify the product formed in the reaction of compound **34** with **32**. However by analysis of the ^{1}H-NMR spectra it was possible to conclude that the amine moiety was not present.

IV.4.29.1.2 BrettPhos based catalyst system[26]

Compounds **34** (50 mg, 0.12 mmol) and **23** (25.8 mg, 0.15 mmol), BrettPhos (0.7 mg, 1.2 µmol) and chloro[2-(dicyclohexylphosphino)-3,6-dimethoxy-2′,4′, 6′-triisopropyl-1,1′-biphenyl][2-(2-aminoethyl)phenyl]palladium(II) (BrettPhos precatalyst) (1.0 mg, 1.2 µmol) were added in a dried ACE pressure tube. The vial was evacuated and backfilled with argon, then a 1 M solution of LiHMDS (0.3 mL, 0.3 mmol) in THF was added. The mixture was stirred at 65ºC, for 4 h. Only removal of the Boc group of the starting material **34** was observed by TLC.

IV.4.29.2 Via copper catalysis



29 23

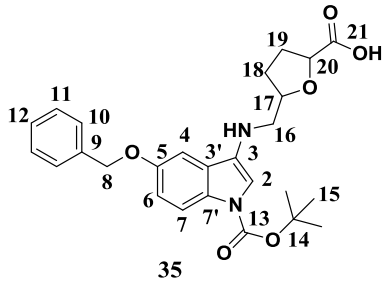
IV.4.29.2.1 CuI/L-proline system[30]

In a dried Ace pressure tube, charged with **29** (50 mg, 0.11 mmol), **23** (57.8 mg, 0.33 mmol), CuI (2.1 mg, 0.011 mmol), L-proline (2.6 mg, 0.022 mmol) and K_2CO_3 (30.8 mg, 0.22 mmol), was added dry DMSO (0.1 mL). The mixture was stirred at 90ºC for 48 h. Only de-iodination and removal of the Boc group of the starting material **29** was observed by TLC.

IV.4.29.2.2 CuI/DMEDA system

In a dried schlenk, containing **29** (50 mg, 0.11 mmol), **23** (28.9 mg, 0.17 mmol), CuI (2.1 mg, 0.011 mmol) and K_2CO_3 (30.8 mg, 0.22 mmol), in dry toluene (0.5 mL), was added DMEDA (2.4 µL, 0.022 mmol), under argon atmosphere. The reaction was stirred at 105ºC, overnight. After that time the mixture was quenched with a saturated aqueous solution of NH_4Cl, diluted with distilled water, extracted with EtOAc, dried over Na_2SO_4 anhydrous, filtered and concentrated. The crude was purified by flash chromatography (*n*-hexanes/EtOAc 1:1) affording of 5-({[5-(benzyloxy)-1-(*tert*-butoxycarbonyl)-1*H*-indol-3-yl]amino}methyl)tetrahydrofuran-2-carboxylic acid (**35**) in 56% yield (28.9 mg), as white solid.

*5-({[5-(benzyloxy)-1-(tert-butoxycarbonyl)-1H-indol-3-yl]amino}methyl)tetrahydrofuran-2-carboxylic acid (**35**)*



35

m.p. 158-160ºC (EtOAc/*n*-hexane)

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.05-8.03 (s, 1H, H7), 7.56 (s, 1H, H2), 7.46 (d, *J* = 7.2 Hz, 2H, H10), 7.39 (t, *J* = 7.2 Hz, 2H, H11), 7.32 (t, *J* = 7.2 Hz, 1H, H12), 7.03 (dd, *J* = 9.1, 2.4 Hz, 1H, H6), 6.85 (d, *J* = 2.4 Hz, 1H, H4), 5.10 (s, 2H, H8), 4.75-4.68 (m, 2H, H17 and H20), 4.02 (dd, *J* = 11.4, 4.1 Hz, 1H, 1 x H16), 3.26 (d, *J* = 11.4 Hz, 1H, 1 x H16), 2.39-2.18 (m, 3H, 1 x H18 and 2 x H19), 2.07-1.98 (m, 1H, 1 x H18), 1.64 (s, 9H, 9 x H15);

^{13}C NMR (101 MHz, *$CDCl_3$*) δ 170.7 (C21), 155.3 (C5), 149.4 (C13), 137.7 (C9), 129.5 (C7'), 128.7 (2 x C11), 128.0 (C12), 127.7 (2 x C10), 127.0 (C3'), 122.5 (C2), 122.1 (C3), 116.6 (C7), 114.6 (C6), 102.9 (C4), 84.2 (C14), 77.7 (C20), 73.0 (C17), 70.8 (C8), 57.2 (C16), 32.0 (C19), 28.3 (3 x C15), 28.2 (C18);

IR (KBr) 3436, 1741, 1727, 1678, 1374, 1156 cm^{-1};

HRMS (ESI) m/z 449.2970 $[M\text{-}OH]^+$ (calcd for $C_{26}H_{26}N_2O_5$ 449.2076).

IV.4.30 QSAR studies

Several QSAR models were built using Random Forests (RFs) and Associative Neural Networks (AsNNs) as machine learning techniques, and different types of descriptors from ADRIANA.Code (Molecular Networks GmbH) and Dragon (Talete s.r.l.) software. Several different training and test sets were built from publicly available data sets of compounds, including recent data deposited in the PubChem database. A set of virtual molecules was computationally generated as a combinatorial library, which comprised analogues of 1355 with distinct substitution patterns (not all possible structures were generated). The molecular structures were produced by in-house developed software that combines SMILES strings of molecular fragments. The data set of 1355 compounds was screened by the developed classification and regression models. The developed classification models were used to classify as active/inactive the virtual compounds and the regression models were used to predict the anti-tubercular activity of the virtual compounds. With predictions from all models a first ranking of these compounds was achieved taking into account their average anti-tubercular activity, determined by the average predictions from the regression models. When the average activity was the same, their average probability to be active, determined by the probability measure of the RFs was taken into account. After this initial ranking, two additional pruning operations were performed: i) virtual compounds classified as inactives by a classification model were excluded; and ii) virtual compounds with average probability of being active lower than 0.8 were also discarded. A further refinement of the virtual screening using the web interface OSIRIS, allowed an evaluation of these compounds in terms of predicted drug relevant properties such as toxicity (mutagenicity, tumorigenicity, irritation and reproductive effectiveness), drug-likeness, drug score, ClogP, solubility and synthetic accessibility.

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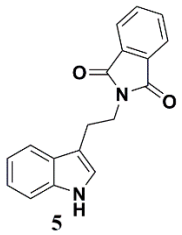
Chapter V General conclusions

The indole scaffold represents one of the most important structural subunits in drug discovery. The demonstration that several alkaloids contain the indole nucleus, and the recognition of the importance of the essential amino acid tryptophan in human nutrition, has led to an extensive research on indole chemistry. Indeed, a vast number of biologically active natural and synthetic indole derivatives have been reported, with a wide range of therapeutic activities, such as antioxidant, anti-inflammatory, antimicrobial, analgesic, anticonvulsant, and antimalarial.

Taking into consideration the unique properties of this scaffold, three indole-based libraries were synthesized and its biological activity investigated, searching for novel drugs, including antioxidants, selective COX-2 inhibitors – anti-inflammatory, and tuberculostatic agents.

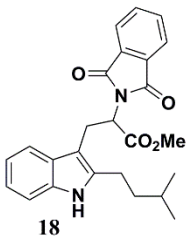
Thus, a library of tryptamine and tryptophan derivatives was prepared and evaluated for the scavenging activity against reactive oxygen species (ROS) and reactive nitrogen species (RNS). The tested indole derivatives share a heteroaromatic ring system, differing among them by the presence of diverse side chains with different functionalization. The results obtained in the present study reveal a strong scavenging effect of tryptophan and tryptamine for the tested ROS and RNS, which activity varies in different extensions, depending on the substituents. Figure V.1 depicts the chemical structure and the respective ORAC or IC_{50} values of the best synthesized compounds evaluated.

For the scavenging of $ROO^{\cdot}$, 7 of the 18 compounds tested were more potent than trolox (which was used as control). The most and the less potent compounds tested for this ROS were tryptophan (ORAC = 2.74 ±0.07) and the tryptophan derivative **3** (ORAC = 0.21 ±0.03), respectively. In addition, the best synthesized compound evaluated against $ROO^{\cdot}$ was the tryptamine derivative **5** with ORAC = 1.46 ±0.08 (figure V.1). Concerning to HOCl, all the tested compounds were found to be active against this ROS, and all hydrogenated compounds (except **15**) were more active than the corresponding unsaturated compounds. The best results were obtained for the tryptophan (**8**) and tryptophan derivative **18** (figure V.1) with IC_{50} of 3.50 ± 0.4 and 3.75 ± 0.52 µM, respectively. The library was also evaluated against RNS. Concerning the $ONOO^{-}$, scavenging activity was observed for all the studied compounds when the study was carried in the presence of $NaHCO_3$, and the best result was obtained for the tryptophan derivative **18**, with an IC_{50} of 14 ± 6.8 µM.



5

ROO$^{\cdot}$ ORAC = 1.46 ±0.08



18

HOCl IC_{50} = 3.75 ± 0.52 µM

$ONOO^{-}$ with $NaHCO_3$ IC_{50} = 14.0 ± 6.8 µM

Figure V.1 - Chemical structures and respective ORAC or IC_{50} values of the most potent synthesized and tested compounds.

The cyclic voltammetry studies showed that all the compounds have an oxidation potential peak lower than that observed for indole (Ep_{ox} = 1.035 V), except for compound **10** (Ep_{ox} = 1.054 V), but higher that those described for melatonin (Ep_{ox} = 0.715 V). In addition, no reversibility was observed in the obtained voltammograms. The lack of reversibility is an advantage, meaning that once oxidized these species do not tend to receive electrons.

Indole scaffold was further explored to find novel anti-inflammatory agents. For this purpose, an indole based library was synthesized and its ability for inhibiting COX-2 selectively was evaluated. From the 19 new indole based compounds synthesized, the biological tests revealed that the presence of a sulfonamide was more favorable for interaction with both COXs, being more active than the corresponding methylsulfones. Compound **17d** was found to be the most promising showing 67 ± 5% COX-2 inhibition at 50 µM, demonstrating that fluorine seems to be crucial for inhibition. Also, the inhibition percentage of **17d** is close to the one of celecoxib (75 ± 8%) at 10 µM. According to the docking results, compounds **17d-f**, with a sulfonamide at C-5, are more active than the corresponding methylsulfones **17a-c**, which is in accordance with the experimental data. From the **17** series, the computational study indicated that the most promising compounds are the fluorinated ones **17a** and **17d**, with a methylsulfone and a sulfonamide, respectively. In both cases these compounds strongly bind to COX-2, but not to COX-1. Saturation transfer difference (STD)-NMR studies performed with **17d** highlighted that the sulfonamide group is indeed important to promote the interaction with COX-2 rather than with COX-1, as evidenced by the close proximity of the indolic proton H4 to COX-2, supporting the observed selectivity in the inhibition evaluation (figure V.2).

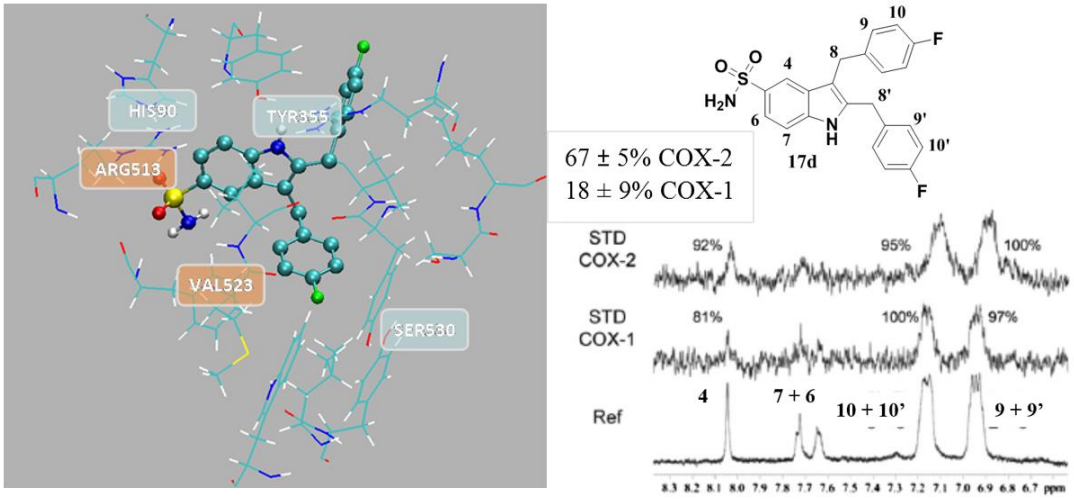


Figure V.2 – Docking of compound 17d at the active site of COX-2, STD-NMR spectra of compound 17d with COX-1 and COX-2 and its inhibitory percentage at 50 µM.

During the preparation of the indole-based library, an unexpected reactivity was observed in the reaction of alkylation at N-1 position of indole with a trifluoromethylated allyl methanesulfonate. Several experiments were carried in order to elucidate the mechanism involved in the formation of a mixture of isomers, (1*Z*,3*E*)-4-fluoro-4-(indol-1-yl)buta-1,3-dien-1-yl methanesulfonate [**22**(***Z*/*E***)] and (1*Z*,3*Z*)-4-fluoro-4-(indol-1-yl)buta-1,3-dien-1-yl methanesulfonate [**22(*Z*/*Z***)].

A mechanism was proposed, based on DFT studies, which fully rationalizes the experimental data. The proposed mechanism identifies the importance of the metal ion (sodium) and the reaction conditions on the final reaction outcome.

During this study the presence of sulfonamide group revealed to be important for the COX-2 inhibition. However, the methods available for sulfonylation of heterocyclic compounds were limited. Due to the lack of versatile methods for the introduction of a sulfonamide group in aromatic systems, several attempts were made to develop a new and versatile reagent for this purpose. The first approach consisted on the synthesis of sodium benzotriazole/benzimidazole sulfinates, by reaction of benzotriazole/benzimidazole with sulfuryl or tionyl chloride. The second approach relied on the use of a carbene·SO_2 adduct. Both approaches reveal unsuccessful for the proposed goal, and further work must be performed to explore the metal-catalyzed approaches to generate a versatile sulfonylating reagent.

Indole scaffold was also investigated in the search for novel and potent antitubercular agents. Thus, five indole-based compounds were synthesized and submitted to biological evaluation as antitubercular agents. Several structures were proposed for computational evaluation according to the structure of known antitubercular agents. The data obtained in the preliminary computational studies allowed establishment of the best candidates to be synthesized, and of the substitution patterns on the indole scaffold. The synthesis efforts involved several approaches for the functionalization of commercial indole derivatives as well as several synthetic approaches for the synthesis of the indole ring system properly functionalized, such as the Fischer indole synthesis.

Thus, it is expected that the biological evaluation of the synthesized indole derivatives along with the quantitative structure-activity relationship (QSAR) studies will allow the validation of this strategy, opening a new path to the design of new antitubercular agents with improved activity.

Overall, new compounds were synthetized through useful synthetic routes in order to achieve functionalized indole derivatives. The choice of the substitution patterns was based on an extensive study of the literature, in case of the antioxidants or anti-inflammatories, or preliminary computational studies for the antitubercular agents. The obtained data was fully rationalized by SAR studies (antioxidant) and validated by docking and STD-NMR studies (anti-inflammatory).

The generated data obtained from this work constitutes a platform for the rational design of new and more potent antioxidant, anti-inflammatory and antitubercular libraries based on the indole scaffold.