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**INDOLE-3-CARBINOL (I3C) DERIVATIVES: DESIGN, SYNTHESIS
AND POTENTIAL BIOLOGICAL APPLICATIONS**

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Preface

The following thesis is divided into 6 chapters.

The first 5 chapters report the medicinal chemistry investigations conducted on indole-3-carbinol, 3,3'-diindolylmethane, and 2,3'-diindolylmethane, aimed at the design, synthesis, and chemical optimization of new compounds, with the objective to identify new lead molecules for the selected targets and define structure–activity relationships (SARs). Chapter 1 provides a general introduction to the broad world of cruciferous vegetables, focusing on the main bioactive compounds of interest and analyzing their pharmacokinetics, pharmacodynamics, and potential biological targets. Chapters 2 and 3 have a strong methodological emphasis due to the development of reactions involving transition metals in the presence of a primary amine core to generate structurally diverse bioactive scaffolds. Chapters 4 and 5 aim to expand the libraries of pharmaceutically relevant compounds through the synthesis of various indole derivatives, according to synthetic methodologies reported in the literature, to obtain a wide range of structures potentially relevant for future biological studies.

Chapter 6 of this thesis describes the results obtained during my visiting period at the Noël Research Group, University of Amsterdam, reporting the development of a new methodology for C–C (sp^3 – sp^2) bond formation under photochemical conditions and subsequently in flow, implementing machine learning to achieve optimized conditions.

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Chapter 1. General Introduction

Among the countless molecules explored in organic chemistry, indole stands out as a timeless source of fascination – simple in appearance, yet endlessly rich in chemistry and reactivity [1]. Commonly referred to as a “privileged structure”, the indole core is present in a wide variety of natural products and pharmacologically active molecules [2]. Its distinctive bicyclic framework, where a benzene ring seamlessly merges with a five-membered pyrrole, creates an unusually stable aromatic system through elegant π -electron delocalization. This balance between simplicity and complexity makes indole a remarkably adaptable platform for chemical innovation [3].

The story of indole chemistry began in the late 19th century, when Adolf von Baeyer first synthesized it by reducing oxindole [4]. What followed was a wave of creativity that shaped modern heterocyclic chemistry: the Fischer, Bischler, Julia, Sundberg, and Bartoli methods [5–9], each a milestone that opened new paths toward the functionalization of this fascinating scaffold. Over the years, these collective efforts have built an extraordinary chemical legacy, granting access to an impressive variety of indole derivatives with diverse structural and electronic features.

This rich diversity has naturally translated into biology. Indole-based molecules display an astonishing range of pharmacological properties [10].

Within this context, the opening chapters of this thesis are dedicated to exploring and expanding the chemistry of indole derivatives, with particular attention to indole-3-carbinol (I3C) and its condensation products, 2,3'-diindolylmethane (2,3'-DIM) and 3,3'-diindolylmethane (3,3'-DIM). By combining the logic of organic synthesis with the purpose of medicinal chemistry, these studies aim to unlock new structural variations and reveal fresh insights into the chemical and biological potential of one of nature's most captivating molecular frameworks.

1.1 Indole-3-carbinol (I3C)

I3C is a natural compound that can be found in cruciferous vegetables such as broccoli, cauliflower, and cabbage, which belong to the *Brassicaceae* family [11]. Over

the years, it has drawn considerable attention because of the wide range of biological effects attributed to it. Several studies have reported cardioprotective, antioxidant, anti-inflammatory, antiangiogenic, and antimicrobial activities, as well as a role in promoting tumor cell apoptosis [12]. Moreover, more recent findings also suggest that I3C can interfere with the viral egress of SARS-CoV-2, including the Omicron variant [13]. From a biochemical point of view, I3C is not directly present in the plant in its intact form. Instead, it is generated when the plant tissue is damaged – for instance, during cutting or chewing – because of the enzymatic activity of myrosinase, a β -thioglucosidase enzyme [14]. This enzyme hydrolyzes the thioglucosidic bond of glucosinolates, mainly glucobrassicin, a secondary metabolite largely found in cruciferous vegetables. As a result, the hydrolysis gives rise to unstable intermediates such as thiohydroximate-*O*-sulfonate and 3-indolylmethyl isothiocyanate. The former eliminates bisulfate and rearranges to give the latter, which, in turn, undergoes an S_N2 displacement of thiocyanate by water to form I3C as the final product (**Figure 1**) [11].

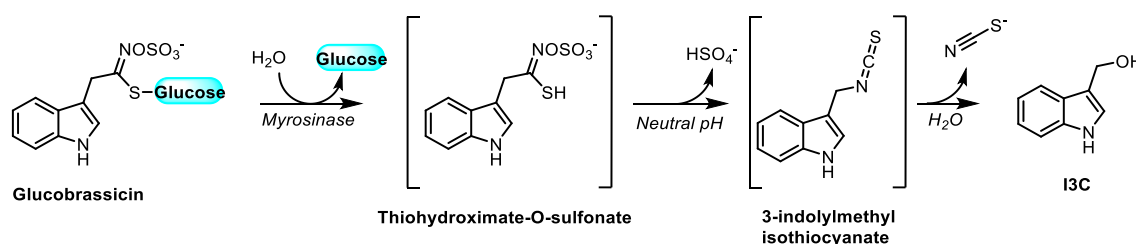


Figure 1. Biosynthesis of I3C.

I3C is commonly included in dietary supplements, as its intake has been associated with a reduced risk of various diseases, including those previously discussed. It remains the focus of extensive biochemical and pharmacological research due to its potential health-promoting effects [13b, 15]. However, when administered orally, I3C undergoes rapid acid-catalyzed transformation in the gastric environment, leading to a diverse array of condensation and oligomerization products (**Figure 2**). Among these, 3,3'-DIM (or DIM) is the predominant dimer formed under acidic conditions [16, 17]. In addition, the trimeric species include the cyclic derivative 5,6,11,12,17,18-hexahydrocyclonona[1,2-*b*:4,5-*b'*:7,8-*b''*]triindole (CTr) [18] and the linear trimer 3,3'-[(1*H*-indole-2,3-diyl)bis(methylene)]bis(1*H*-indole) (LTr) [18, 19], whereas the major tetrameric product is 5,6,11,12,17,18,23,24-octahydrocyclododeca[1,2-*b*:4,5-*b'*:7,8-

b'':10,11-*b'''*]tetraindole (CTet) [20, 21]. Furthermore, other I3C-derived metabolites include indole-3-acetonitrile, indole[3,2-*b*]carbazole (ICZ) [18a, 22], and indole-3-carbaldehyde (I3A) [23]. These represent some of the most significant metabolites resulting from the acid-mediated transformation of I3C. Collectively, they are believed to play a crucial role in modulating its overall bioactivity and pharmacological profile.

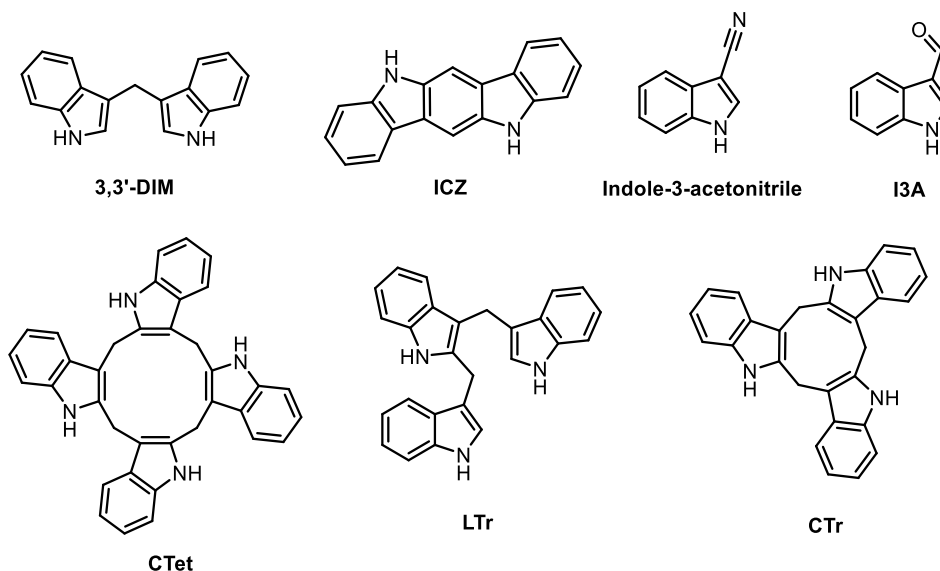


Figure 2. Representative chemical structures of major oligomeric metabolites derived from I3C.

Glucosinolates derived from cruciferous vegetables may undergo hydrolysis during food processing and later in the gastrointestinal tract, including through the action of enzymes produced by the gut microbiota. A portion of glucosinolates escapes hydrolysis, and those that remain intact may reach the colon, where enzymes produced by the gut microbiota can promote further hydrolysis. At this stage, the resulting metabolites can be absorbed or excreted [24]. Once absorbed (**Figure 3**), isothiocyanates undergo phase II metabolism, primarily through conjugation with glutathione (GSH). The main limitation of this entire process lies in the low bioavailability of glucosinolates and isothiocyanates, which is largely due to their chemical structure, as they are highly sensitive to heat, extreme pH conditions, and aqueous instability, as previously discussed [25, 26].

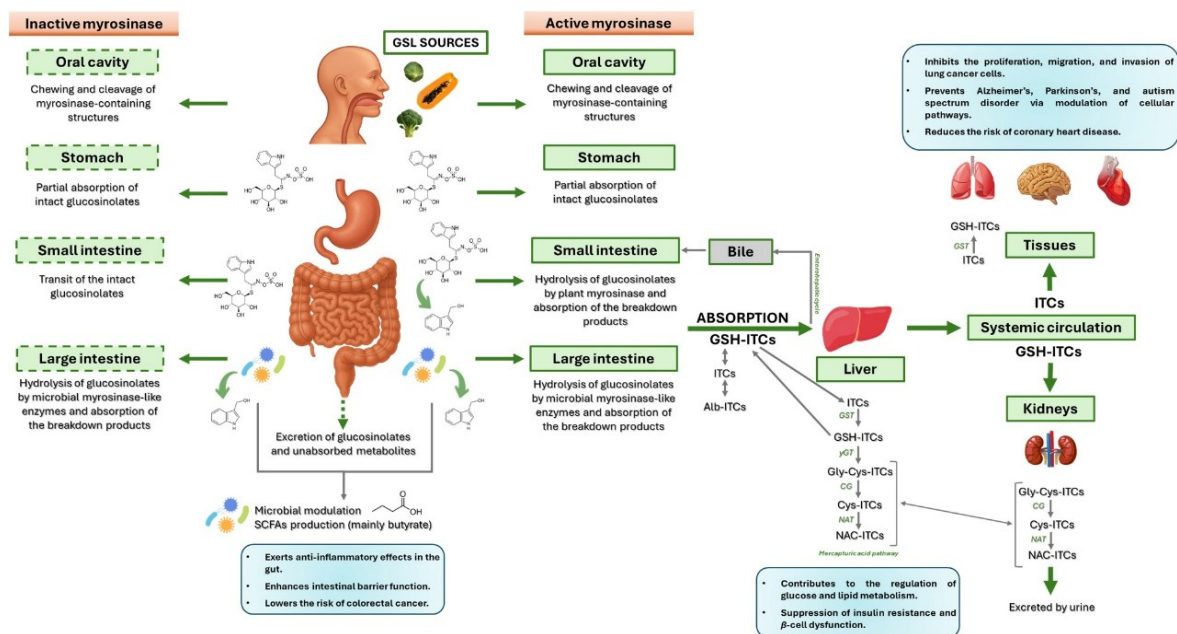


Figure 3. Overview of the metabolism of glucosinolates and their degradation products from dietary sources and their subsequent beneficial effects on target organs [25].

1.2 Biological Activity of I3C and its Derivatives

1.2.1 Antitumor Activity

In breast cancer, conventional treatments are effective but often cause severe side effects due to high toxicity in healthy cells and to the development of resistance [27]. Therefore, the discovery of new therapies – particularly the use of natural compounds – represents a primary objective in breast cancer research, owing to their therapeutic potential with generally fewer adverse effects compared with traditional approaches, such as chemotherapy and radiotherapy [28].

Over the past years, numerous studies have indicated that I3C and its derivatives may constitute antitumor options with negligible toxicity [29-32]. Indeed, many antiproliferative responses appear to be selectively mediated by pathways activated by I3C. I3C can modulate DNA repair, induce cell-cycle arrest and apoptosis, impede cell migration, and influence hormone receptor signaling [33-35]. The first protein target identified for I3C was the serine protease elastase [36]. In human breast cancer, inhibition

of elastase activity by I3C prevents CD40 cleavage, resulting in NF- κ B-dependent blockade of cell-cycle progression and proliferation [37, 38].

Moreover, I3C is involved in DNA damage-repair processes by affecting the expression of tumor-suppressor genes such as BRCA1 and BRCA2 and other DNA repair proteins [39, 40]. In estrogen-dependent tumors (including breast cancer), I3C decreases cyclin-dependent kinase (CDK) expression, leading to G1-phase cell-cycle arrest. I3C has also been reported to induce apoptosis in the human prostate cancer cell line PC-3 through inhibition of Akt (a serine/threonine kinase) activation [41, 42]. Additionally, I3C can counteract metastatic processes, tumor angiogenesis, and cancer cell migration by inhibiting CDK6 and thereby blocking cell proliferation [43].

More recently, I3C has been shown to exert important oncogenic-modulatory effects via ubiquitin-dependent regulation of several protein substrates, including the PTEN homologous phosphatase and tensin [44]. Indeed, members of the HECT family of E3 ubiquitin ligases are frequently overexpressed in human tumors [44], with NEDD4 and WWP1 identified as particularly involved members. Notably, *in vitro* studies have demonstrated that I3C inhibits the ubiquitin ligase activity of NEDD4 and WWP1 [45, 46].

I3C and its metabolites also modulate gene expression by affecting DNA methyltransferases, histone deacetylases, miRNAs, lncRNAs, and transcription factors such as AhR, ER, and NF- κ B, resulting in decreased oncogene expression and increased tumor-suppressor gene expression. This mechanism, observed in murine models and in various human prostate cancer cell lines, suggests a potential chemopreventive role through epigenetic regulation of tumors [47-51].

Recent investigations have identified molecular mechanisms and preclinical evidence supporting the antitumor activity of I3C and its derivatives. Notably, Lee *et al.* demonstrated that I3C inhibits the E3 ligase WWP1, thereby reactivating PTEN and promoting its membrane localization, which in turn suppresses MYC-driven, PI3K-Akt-mediated tumorigenesis in Myc-driven and PTEN-heterozygous mouse models [52]. PTEN deletion by CRISPR-Cas9 in Hi-Myc tumor organoids conferred partial resistance to I3C, supporting a PTEN-dependent component of I3C action. Pharmacokinetic analyses in C57BL/6 male mice after intraperitoneal administration (20 mg/kg) further supported WWP1 targeting by I3C *in vivo* [52].

Numerous murine and xenograft studies have demonstrated antitumor efficacy of I3C and its principal metabolite DIM. In immunodeficient xenograft models, dietary I3C or DIM supplementation reduced tumor cell proliferation over time [53]. For example, in SCID mice bearing human T-ALL CCRF-CEM xenografts, 100 ppm dietary DIM significantly affected tumor size and doubling time, whereas higher dietary I3C doses (500–2000 ppm) were required to observe comparable effects [53, 54].

Safety and outcomes depend on model and dose. Chronic I3C administration induced hepatocarcinogenesis in some rodent studies [55,56], while in neonatal models exposed to diethylnitrosamine I3C markedly reduced hepatic tumor formation [57]. Colorectal cancer studies indicate an AhR-dependent chemopreventive activity for I3C/DIM, potentially influenced by gut microbial production of indole AhR ligands from dietary tryptophan [58, 59]. In DMBA- or MNU-induced mammary carcinoma models, I3C (but not always DIM) showed preventive efficacy [60]. Maternal dietary I3C conferred protection against tumor development in female – but not male – offspring in a manner dependent on ER β expression [61], consistent with an ER-mediated component of I3C/DIM chemoprevention in breast cancer [62].

Overall, preclinical evidence is encouraging and extends beyond liver, breast, and colon cancer to include effects on tobacco-related pulmonary carcinogenesis [63-66]. These findings motivated clinical investigations: a randomized, double-blind placebo-controlled trial in 60 women (50 or 400 mg I3C) reported no significant adverse effects except in subjects with elevated aminotransferases and indicated preventive activity against breast cancer and estrogen-dependent neoplasms [67]. Mechanistically, I3C/DIM modulate estrogen metabolism by increasing the 2-hydroxyestrone/16 α -hydroxyestrone ratio, a biomarker linked to reduced risk of estrogen-dependent tumors and cervical intraepithelial neoplasia (CIN); clinical studies have reported improvements in CIN with I3C/DIM administration.

Metabolic interactions have been described: dietary I3C/DIM affect flavin-containing monooxygenases (FMO) and cytochrome P450 enzymes, with species-specific effects (inhibition of FMO1 in rats and FMO3 in humans) and dose-dependent alterations in FMO/CYP-mediated metabolism of certain substrates [68-74]. Such interactions can modify the disposition of xenobiotics (e.g., nicotine, tamoxifen), potentially enhancing toxicity in some contexts [75].

Despite some promising preclinical and clinical results, important challenges remain. Effective concentrations in preclinical/clinical studies typically exceed levels achievable by diet alone, so supplementation is required to attain pharmacologically relevant exposures. In vivo toxicity assessments reveal sex- and route-dependent tolerability thresholds, with no mortality or histopathological lesions observed below 550 mg/kg (oral) and 250 mg/kg (i.p.) [13b]. Moreover, the biological effects attributed to I3C are often mediated by its decomposition products – particularly DIM – since I3C is rarely detected in plasma after oral intake and DIM itself circulates as further metabolites of uncertain activity [76, 77]. Consequently, many clinical studies have focused on DIM rather than I3C, and epidemiological evidence generally supports an inverse association between cruciferous vegetable intake and various cancers [78-81].

1.2.2 *Anti-inflammatory Activity*

I3C exhibits significant anti-inflammatory activity following ischemia–reperfusion injury by reducing leukocyte–endothelial interactions. I3C suppresses E-selectin expression and decreases NF- κ B transcriptional activity in B cells, leading to fewer adherent leukocytes at the lesion site and marked anti-inflammatory effects in experimental models [82].

This anti-inflammatory property has suggested the use of I3C in chronic inflammatory diseases, such as systemic lupus erythematosus (SLE). I3C is an agonist of the aryl hydrocarbon receptor (AhR), which regulates macrophage function, and it exerts potent anti-inflammatory effects while also influencing estrogen metabolism by shifting the balance toward less toxic metabolites, a mechanism that may help modulate SLE manifestations. Furthermore, I3C regulates T- and B-cell differentiation, producing immunomodulatory effects relevant to SLE [83].

In lupus-prone F1 (NZB $\times$ NZW) mouse models, I3C inhibited disease progression and prolonged survival; treatment induced a transient block in B-cell maturation and delayed T-cell maturation [84].

Supporting clinical translation, a study in 80 female SLE patients receiving oral I3C (125 mg, three times daily for 3 months) demonstrated favorable changes in estrogen metabolism and good tolerability, suggesting potential benefits, though further studies are needed to confirm clinical efficacy [85]. Ex vivo studies on macrophages isolated from

SLE patients also revealed that I3C (100 μ M) promotes AhR nuclear translocation, more pronounced in patients than in controls, supporting the role of I3C as an AhR modulator in macrophages [83].

In conclusion, preclinical evidence and preliminary clinical data indicate that I3C possesses anti-inflammatory and immunomodulatory properties of relevance to SLE.

1.2.3 Antiviral activity

The antiviral properties of I3C (and DIM) have been partly attributed to estrogenic metabolites formed in the acidic environment following oral administration, and these compounds exhibit activity against HPV, with potential therapeutic relevance in recurrent respiratory papillomatosis (RRP) [10a, 76, 86, 87, 88].

Respiratory viral infections (influenza, metapneumovirus, SARS-CoV-2) pose a significant global health burden, driving research efforts toward novel antivirals able to block viral entry, replication, or release. Despite the effectiveness of vaccination, protection tends to wane within months, and emerging variants underscore the need for broad-spectrum antiviral therapies. Multiple strategies are currently under investigation – including spike-targeting peptides, vaccines, and drugs disrupting virus–host interactions – yet a comprehensive understanding of the cellular targets essential for viral replication remains limited [89-99].

Recent findings highlight a critical role of HECT E3 ubiquitin ligases in SARS-CoV-2 pathogenesis: these ligases physically interact with and ubiquitinate the spike protein, are overexpressed in infected cells and COVID-19 mouse models, and rare variants in NEDD4 and WWP1 have been linked to severe disease [13a, 100]. Novelli *et al.* demonstrated that I3C inhibits HECT ligases implicated in disease pathology, blocking viral infection in Vero E6 cells and suggesting an antiviral mechanism based on interference with viral ubiquitination processes [13a, 100, 101].

Further *ex vivo* evidence indicates that I3C treatment reduces viral entry and the expression of innate immunity and inflammation-related genes in human lung organoids (hLORG) [13b]. Collectively, these findings support the potential application of I3C as an antiviral agent and justify targeted clinical studies to assess its efficacy and safety in COVID-19 patients, particularly in therapeutic strategies aimed at ubiquitination pathways critical for viral replication.

1.2.4 Antimicrobial Activity

I3C has also demonstrated antibacterial and antifungal activity against human pathogenic microorganisms. Several drug-resistant Gram-positive bacteria (*Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Enterococcus faecium*), Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*), and fungal species (*Candida albicans*, *Trichosporon beigelii* and *Saccharomyces cerevisiae*) are susceptible to I3C [102]. The minimum inhibitory concentration (MIC) of I3C ranges from 5–20 µg/mL for Gram-positive bacteria, 20–80 µg/mL for Gram-negative bacteria, and 10–20 µg/mL for fungi, exerting its effects through disruption of the plasma membrane. Furthermore, I3C shows significant synergistic activity with the antibiotic ampicillin against both Gram-positive and Gram-negative drug-resistant bacteria [103]. Its antimicrobial action is associated with its ability to bind to the phospholipid membrane (particularly lipopolysaccharides) and to affect the cytoplasmic membrane and DNA [103], although the precise mechanism remains unclear.

I3C's ability to bind DNA also makes it a potent fungicide against *C. albicans* [104]. Dietary supplementation with I3C has been shown to prevent and enhance treatment efficacy against *Clostridium difficile* infection in mice through both AhR-dependent and -independent mechanisms [105].

In conclusion, the antimicrobial activity of I3C is primarily related to plasma membrane disruption, although the exact molecular mechanism remains poorly understood [104].

1.2.5 Antioxidant Activity

Several plants, particularly cruciferous vegetables, have demonstrated antioxidant activity [106]. Lipid peroxidation is the oxidative degradation of lipids, primarily targeting unsaturated chains, and leads to cellular damage that can compromise cell survival. This process is mainly triggered by the presence of free radicals, and in vitro studies have shown that I3C mitigates this damage, thereby enhancing cell survival. These properties were also confirmed in vivo in mice, where I3C administration inhibited carbon tetrachloride (CCl₄)-induced lipid peroxidation, with an IC₅₀ value in the range of 35–40 µM [107].

Molecular analysis revealed that the combination of I3C with crambene (a nitrile product of the glucosinolate progoitrin) synergistically increased hepatic quinone reductase mRNA levels in HepG2 cells through co-activation of antioxidant response elements [108]. It has been hypothesized that I3C and crambene act synergistically to enhance the expression of metabolizing enzymes.

Conversely, other indole derivatives, such as indole-3-aldehyde and indole-3-carboxylic acid, exhibit no antioxidant activity [109].

1.2.6 *Antiobesity Activity and Chronic Diseases*

I3C has potential benefits in the prevention of obesity and metabolic disorders because its action involves multiple mechanisms, including the reduction of adipogenesis and inflammation, as well as the activation of thermogenesis. Adipose tissue is an endocrine organ associated with chronic inflammation and insulin resistance, resulting in inflammation of the adipose tissue itself during normal development and obesity. It also plays a key role in metabolism and is linked to chronic inflammatory states and insulin resistance [110] through the production of cytokines and lipids, with consequences for glucose metabolism, thermogenesis, and adipogenesis [111].

By in vitro experiments I3C reduces lipid droplet accumulation in adipocytes and suppresses adipocyte-induced angiogenesis in endothelial cells [112]. These findings highlight I3C as a potential therapeutic agent for obesity-related diseases such as type 2 diabetes and atherosclerosis, as reported by Chang *et al.* [113].

1.2.7 *Other Activities*

I3C also exerts a neuroprotective effect against neurotoxicity through its anti-inflammatory and antiapoptotic properties. Mohamad *et al.* reported the promising neuroprotective effects of I3C against rotenone-induced Parkinson's disease in a male albino rat model, showing maximal protection at a dose of 100 mg/kg/day. These effects were further characterized behaviorally, neurochemically, and histologically, suggesting that the neuroprotective actions of I3C may be attributed to the activation of the SIRT1–AMPK signaling pathway, which counteracts inflammatory and apoptotic cascades [114].

1.3 Bisindoles

In this section, 3,3'-DIM, 2,3'-DIM and their derivatives will be examined.

As discussed above, 3,3'-DIM is the most relevant active metabolite of I3C, whereas 2,3'-DIM is a synthetic analogue that has gained considerable pharmaceutical interest over the years.

1.3.1 3,3'-Diindolylmethane (3,3'-DIM)

3,3'-DIM (DIM) is the primary metabolite of I3C, as the latter spontaneously dimerizes under acidic conditions. This reaction explains why DIM is found in the body following the consumption of cruciferous vegetables; in a key pharmacokinetic study of I3C [115], women received varying doses of I3C (ranging from 400 to 1200 mg), and plasma samples were analyzed over time. The oral dose of I3C was already undetectable after 1 hour (as it is rapidly absorbed, distributed, and eliminated from tissues), while DIM was only detectable within the first 12 hours after administration. **Figure 4** illustrates how the mean plasma concentrations of DIM change over time at each I3C dose level, whereas **Table 1** reports the mean values of key pharmacokinetic parameters.

- The maximum plasma concentration ($C_{\max}$) and the area under the curve (AUC) increase with I3C doses up to 1000 mg.
- No further increases in $C_{\max}$ and AUC are observed at 1200 mg.
- The changes in these variables (600-1000 mg) are markedly non-linear, with dose-normalized values 2- to 4-fold higher than those calculated for lower I3C doses.
- The half-life of DIM increases with higher I3C doses, though not as dramatically as $C_{\max}$ and AUC.

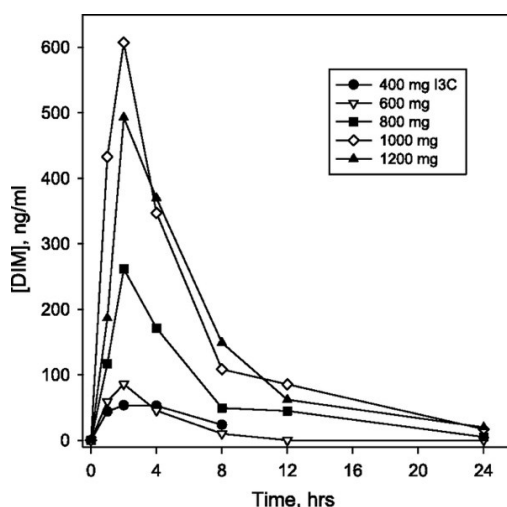


Figure 4. Plasma concentration of DIM following single doses of I3C. Mean plasma [DIM] at each time point are shown for each of the single-dose levels administered. Sampling, preparation and analysis were done as described in Materials and Methods. Error bars are not shown [115].

Table 1. DIM pharmacokinetic data from acute and chronic single doses of I3C [115].

I3C dose (mg)	<i>n</i>	<i>C</i> _{max} (ng/mL)	<i>t</i> _{max} (h)	AUC (0-24 h) (h ng/mL)	<i>t</i> _{1/2} (h)
400	4	61±12 (50-77)	3.0±1.2	329±59 (271-400)*	3.8±0.3
600	4	93±30 (54-127)	2.2±1.2	364±136 (124-502)	2.0±0.7
800	4	262±109 (144-408)	2.0±0.0	1,608±662 (660-2,411)	4.2±1.9
1,000	3	607±561 (274-1,255)	2.0±0.0	3,376±3,542 (698-7,451)	5.7±0.1
1,200	8	510±248 (262-1,025)	2.5±0.9	3,212±1,380 (1,638-5,153)	5.0±3.1
400 mg (chronic)	14	69±42 (11-139)	1.3±1.4	372±180 (107-688)	6.0±4.5

NOTE: Data are presented as mean ± SD. Values in parentheses for *C*_{max} and AUC denote the range (minimum-maximum) of individual values.

* Sampling period for the acute 400-mg dose of I3C was from 0-8 h, as opposed to 0-24 h for subsequent dosing groups.

Based on the analysis of the above pharmacokinetic parameters, it can be concluded that the biological activity of I3C is attributable to the presence of DIM in plasma, rather than to I3C itself [115, 116]. From a biological perspective, DIM exhibits significant antiproliferative and anti-inflammatory properties, as indicated below.

- Antiproliferative activity: through the induction of G1/S cell cycle arrest and apoptosis, DIM exerts antiproliferative effects on numerous cancer cell lines (including breast, prostate, ovarian, lung, pancreatic, colorectal, and head and neck cancers) [117, 118].
- Hormonal modulation: DIM can reduce levels of active estrogens and testosterone by promoting the oxidative pathway of 2-hydroxyestrogen formation; it also acts as an AhR agonist, attenuating estrogenic activity. It is therefore considered an important molecule for the prevention of hormone-dependent cancers [117, 118].
- Other activities: DIM is believed to possess antioxidant properties and antiproliferative effects against pathogens (e.g., antibacterial and anti-Leishmania activity reported in some studies), as well as antiviral and immunomodulatory activity (inhibiting HPV and wart growth in vitro) [118].

- Synthetic derivatives: Numerous synthetic DIM analogues (e.g., DIM molecules bearing aromatic or alkyl substituents) exhibit anticancer potency often superior to that of natural DIM. These aim to enhance DIM's antitumor activity through chemical modifications of the indole scaffold. Other substituted DIM derivatives (e.g., brominated bisindoles) have demonstrated increased antiproliferative potency (up to $\sim 2\times$ compared to DIM) and selectivity toward cancer cells [117, 118].

Taken together, DIM is undoubtedly the most extensively studied metabolite, and over the years, significant efforts have been devoted to developing novel synthetic methodologies for its production, as well as analogs designed to improve its pharmacokinetic and pharmacodynamic profile.

In recent years, our research group has devoted considerable effort to the synthesis and biological evaluation of various derivatives of DIM, with a particular focus on bisindole alkaloids of marine origin.

Marine organisms such as corals, sponges, ascidians, and tunicates represent an exceptionally rich source of biologically active compounds [119]. Their unique chemical architectures and remarkable activity toward multiple pharmacological targets make them highly attractive synthetic challenges as well as promising lead candidates for the development of new therapeutic agents [120].

Among the different structural classes of marine metabolites, indole-based alkaloids have drawn special attention due to their pronounced cytotoxic and antitumor properties, as well as their still largely unexplored potential in neurological and behavioral disorders [120]. In particular, the presence of bromoindole units is frequently associated with enhanced biological activity, further highlighting the pharmacological relevance of these compounds [121].

A growing body of evidence has also revealed that some of these molecules are able to interfere with bacterial biofilm formation key survival and resistance strategy for many microorganisms. Biofilms represent the predominant lifestyle of most bacterial species, characterized by cells adhering to a surface and embedded within an extracellular polymeric substance (EPS) composed of polysaccharides and other macromolecules [122]. The EPS matrix not only promotes adhesion to the substrate but also protects

microbial colonies from harsh environmental conditions, including antimicrobial treatments [123].

Biofilm formation on food-processing surfaces and, more critically, on medical devices poses a serious health concern, as it facilitates microbial colonization and the spread of pathogens through cross-contamination events [124]. Moreover, bacteria organized in biofilms exhibit a markedly reduced susceptibility to antimicrobials compared to their planktonic counterparts, making infections extremely difficult to eradicate [125].

Within this framework, marine natural products have emerged as an invaluable source of bioactive molecules displaying antibacterial, antifungal, antibiofilm, and antiparasitic properties, owing to their extraordinary structural diversity and biological selectivity. Among these, marine bisindole alkaloids – indole dimers mainly produced by sponges and tunicates – have demonstrated a remarkably broad spectrum of biological activities, including cytotoxic, antitumor, antileishmanial, antibacterial, and antibiofilm effects [125, 126].

In this context, one of the most interesting compounds identified and investigated by our research group is the marine alkaloid 2,2-bis(6-bromo-3-indolyl)ethylamine (**1a**, **Figure 5**). This molecule stands out for its distinctive alkylamino side chain, which may play a key role not only in enhancing its pharmacological properties and water solubility but also in improving its chemical stability under acidic conditions. This functional group also provides a versatile site for further structural modifications through amino derivatization or conjugation with other functional moieties, enabling fine-tuning of its biological profile.

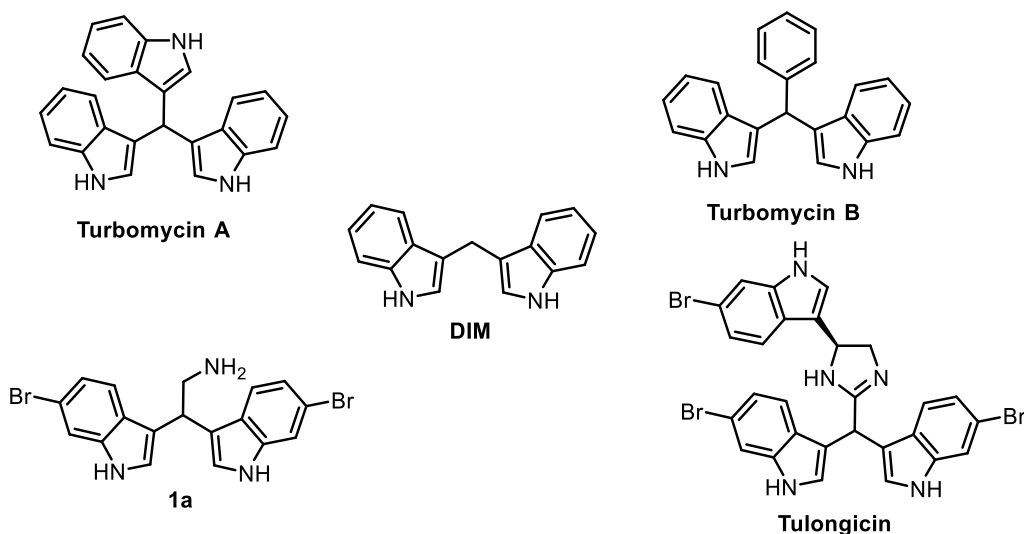


Figure 5. Selection of bisindole alkaloids derivatives, including compounds identified by our research group and structurally related analogues reported in the literature.

Compound **1a**, originally isolated from the Californian tunicate *Didemnum candidum* and the New Caledonian sponge *Orina* spp. [127], has been reported to exhibit significant antileishmanial activity against *Leishmania human* [128], as well as cytotoxic effects toward several human cancer cell lines, including U937 (leukemia), MCF-7 (breast carcinoma), and Caco-2 (colorectal carcinoma) [129].

Interestingly, several natural and synthetic compounds with antibacterial, antibiofilm and antiparasitic properties – such as tulongicin, turbomycin A and turbomycin B (**Figure 5**) – share with compound **1a** the common DIM molecular scaffold [130]. This recurring structural motif strongly suggests that the bisindole core represents a key pharmacophoric unit able to confer multiple biological activities and modulating diverse molecular targets.

Building on these insights, our research group has focused its efforts on the derivatization of **1a**, aiming to design new analogues that are non-toxic to human cells yet display enhanced antileishmanial activity.

Chapters 2 and 3 of this thesis describe in detail the late-stage functionalization strategies developed for the selective modification of the natural compound **1a**, with the goal of optimizing the biological and pharmacokinetic profile of marine bisindole derivatives.

1.3.2 3,3'-Diindolylmethane (2,3'-DIM)

2,3'-DIM is a positional isomer of 3,3'-DIM that can be obtained through chemical synthesis. The need to develop semisynthetic DIM analogs has sparked significant scientific interest in 2,3'-DIM, with the goal of improving the antitumor activity and bioavailability of DIM.

Over the years, bisindole derivatives of 2,3'-DIM have attracted attention, such as the indole[3,2-b]carbazole (ICZ) derivative of I3C, and malassezin, a natural compound isolated from the yeast *Malassezia furfur*, which shares structural similarity with both ICZ and DIM (**Figure 6**). Malassezin has been shown to act as an AhR agonist [131], further expanding research into 2,3'-DIM derivatives, molecules that remain relatively underexplored but have already demonstrated multiple therapeutic potentials.

The AhR is a basic helix–loop–helix transcription factor that is highly conserved across species. It is best known for its ability to bind a wide range of endogenous and xenobiotic polycyclic aromatic hydrocarbons and for mediating the toxic effects of dioxin – TCDD. Once activated by a ligand, the AhR–ligand complex translocates into the nucleus, where it dimerizes with the aryl hydrocarbon receptor translocator – ARNT – and regulates the transcription of multiple target genes, most notably CYP1A1 [132].

Although initially studied for its toxicological implications, AhR has since emerged as a key regulator in various physiological and developmental processes, including immune cell differentiation, intestinal barrier maintenance, and prostate development. For these reasons, AhR is now considered a promising therapeutic target for conditions such as benign prostatic hyperplasia, transplant-related immune suppression, and inflammatory and neoplastic diseases [132].

In this context, AhR also plays a significant chemopreventive effect. It upregulates phase I enzyme CYP1A1 and phase II enzymes such as glutathione S-transferase and NAD(P)H quinone oxidoreductase, which are crucial for metabolizing and detoxifying genotoxic compounds. Moreover, CYP1A1 catalyzes the conversion of estrone into 2-hydroxyestrone, an antiestrogenic metabolite known to inhibit the proliferation of breast cancer cells [133]. These biochemical effects help explain the antitumor potential of AhR agonists, including 2,3'-diindolylmethane (2,3'-DIM)

derivatives, which are now being recognized as highly promising compounds for a range of therapeutic applications.

Among these derivatives, several 2,3'-DIM analogs have also been shown to reduce plasma levels of proprotein convertase subtilisin/kexin type 9 (PCSK9) in HepG2 cell lines, making them attractive candidates for further investigation in the treatment of hypercholesterolemia and cardiovascular disease prevention [134].

Generally, 2,3'-DIM derivatives represent a fascinating biologically active class: selective AhR agonists able of fine-tuning multiple cellular pathways while offering new therapeutic opportunities in oncology, immunology and metabolic regulation.

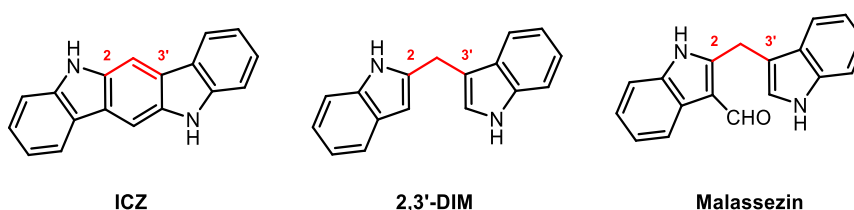


Figure 6. 2,3'-DIM derivatives as agonists of the AhR receptor.

Overall, while 3,3'-DIM has been extensively investigated, 2,3'-DIM derivatives have received comparatively less attention, despite emerging evidence of their diverse biological potential. Recent studies underscore the need for systematic exploration of this scaffold, prompting the development of efficient synthetic strategies and structural analogues aimed at enhancing stability and broadening pharmacological applications.

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Chapter 2. Suzuki-Miyaura Diversification of 2,2-Bis(6-bromo-1*H*-indol-3-yl)ethanamine for the Development of Antileishmanial Lead Compounds.

This chapter is based on: **Buono, A.**; Diotallevi, A.; Maestrini, S.; Verboni, M.; Kiuru, P.; Galluzzi, L.; Duranti, A.; Olivieri, D.; Lucarini, S. Efficient and Rapid Arylation of NH₂-Unprotected Bromobisindole Ethanamines via Suzuki-Miyaura Coupling: Generating New Leads Against *Leishmania*. *Chem. Eur. J.* **2025**, 31: e202500637. <https://doi.org/10.1002/chem.202500637>

2.1 Introduction

Across history, *Leishmania* has followed humankind like a silent shadow, thriving in the cracks of poverty, heat, and neglect. Today, leishmaniasis stands as the second most dangerous parasitic disease after malaria in terms of both mortality and morbidity, silently afflicting millions [1–3]. Around twelve million people are believed to be infected worldwide, and each year more than a million new cases are reported. The World Health Organization warns that nearly one billion people – stretching from the arid plains of Africa and the Middle East to the tropical forests of South America and the vast landscapes of Asia – remain at risk [4].

Even regions that once seemed safe, like the Mediterranean basin, have not been spared. Here, the disease has found new opportunities to spread, carried by the complex tides of climate change, global travel, and human migration [5]. Each factor subtly reshapes the geography of infection, allowing sandflies to expand their range into previously untouched areas and transforming leishmaniasis into a global concern rather than a distant tropical affliction.

This neglected tropical disease is caused by more than twenty species of *Leishmania*, protozoan parasites transmitted to humans through the bite of infected female sandflies. What begins as an almost invisible puncture may evolve into one of three devastating forms. The visceral form (VL), also known as kala-azar, invades the

body's internal organs and is invariably fatal if left untreated. The mucocutaneous form (ML) attacks the mucous membranes of the nose, mouth, and throat, leaving behind disfigurement and stigma. The cutaneous form (CL), the most common, manifests as skin ulcers that can persist for months or even years, often in the most visible and socially damaging places. In Mediterranean Europe, the species *Leishmania infantum* is the primary cause of both VL and CL [6].

Despite the scale of this burden, no effective vaccines are currently available [7]. The treatments we rely on – many developed decades ago – come with high toxicity, limited selectivity, growing resistance, long treatment durations, and complex or costly administration routes [8]. For patients in low-income regions, these challenges often make therapy inaccessible, perpetuating a cycle of suffering. Thus, the search for safer, more effective and affordable drugs has become not just a scientific pursuit, but an urgent humanitarian goal.

Among the diverse molecular architectures explored in recent years, several heterocyclic scaffolds have drawn increasing attention for their potential to combat the parasite [9–11]. Within this landscape, certain bisindole derivatives have emerged as particularly intriguing candidates. Their elegant molecular frameworks seem to interact with key biological targets in *Leishmania*, showing encouraging antileishmanial activity in preliminary studies (**Figure 7**). Such findings illuminate a promising path forward – one that unites chemistry, biology, and human need in the ongoing effort to confront one of humanity's oldest parasitic adversaries.

The first bisindole with antileishmanial activity identified was DIM (**Figure 7**), which showed potent inhibition of *L. donovani* DNA topoisomerase I ($IC_{50} = 1.2 \mu M$) [12]. An ideal point for diversification of DIM is its methylene linker. Bharate and coworkers introduced a phenyl ring, and compound II (**Figure 7**) displayed an IC_{50} below $10 \mu M$ [13]. URB1483 (**Figure 7**) demonstrated strong activity against *L. infantum* promastigotes ($IC_{50} = 3.7 \mu M$) and intracellular amastigotes, with no quantifiable cytotoxicity in mammalian cells (selectivity index [SI] > 55) [14]. However, URB1483 failed in vivo evaluation in a murine infection model, likely due to its poor water solubility [15]. Several bisindole analogues were designed to increase solubility, including compound I and the marine bisindole alkaloid 2,2-bis(6-bromo-1*H*-indol-3-

yl)ethanamine (**1a**, **Figure 7**) [15], both showing strong potency but also toxicity, likely attributed to the presence of bromine substituents.

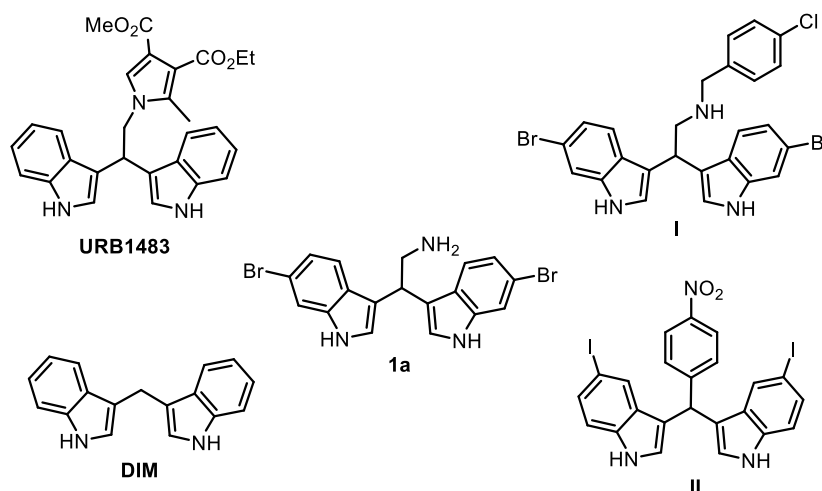


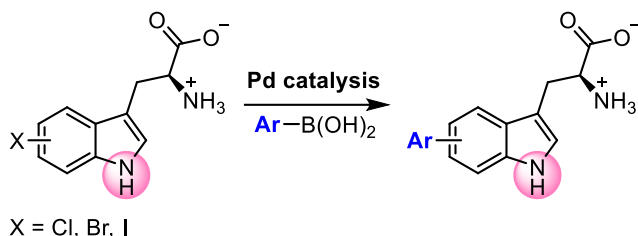
Figure 7. Bisindoles with antileishmanial activity.

Therefore, with the aim to reduce the toxicity of these compounds, cross-coupling reactions [16] with suitable nucleophiles can be performed to replace the bromine atoms. Suzuki–Miyaura coupling [17] allows the straightforward introduction of aryl functionalities starting from inexpensive and readily available boronic acids [18].

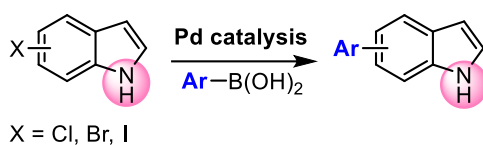
Typically, couplings on substrates containing unprotected N–H groups are highly challenging because the nitrogen atom can coordinate to the metal catalyst [19], making the use of protecting groups unavoidable [20]. Goss and co-workers made significant contributions in this field by reporting Suzuki–Miyaura couplings on unprotected halotryptophans [21, 22] (**Scheme 1a**) and more recently, the same group succeeded in the coupling of unprotected haloindoles with aryl boronic acids (**Scheme 1b**) [23]. Despite the importance of these and others' contributions in the field [24], to the best of our knowledge, no examples of the Suzuki–Miyaura coupling have been reported to date with halotryptamines [25] bearing both an unprotected indole nitrogen and a free amino group, as in the case of the marine bisindole alkaloid **1a**. Literature reports also indicate that substrates containing amino groups can coordinate the catalyst, reducing the catalyst activity [26, 27]. Clearly, the nucleophilicity of the amino group in tryptophane (or amino acid) derivatives is attenuated since the molecule is in its zwitterionic form [28]. Herein, for the first time, we report a Pd-catalyzed Suzuki–Miyaura cross-coupling reaction on brominated bisindoles containing a free primary amine, including compound **1a** (**Scheme**

1c). The same catalytic system was successfully applied to tryptamine, and finally, the biological activity of the newly synthesized bisindole derivatives was evaluated.

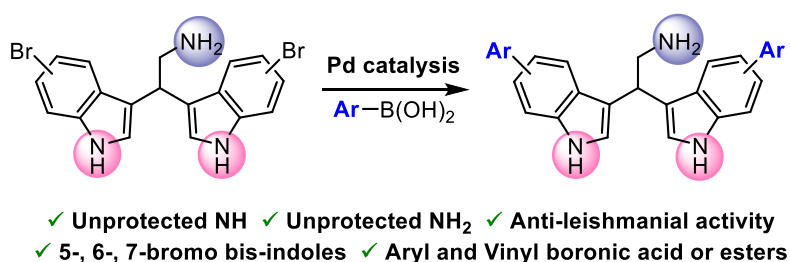
(a) Suzuki-Miyura coupling of unprotected halotryptophans



(b) Suzuki-Miyaura coupling of unprotected haloindoles



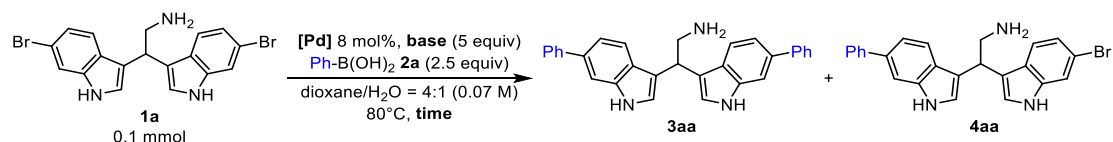
(c) *This work*: Suzuki-Miyura coupling of unprotected bromo bisindoles



Scheme 1. Suzuki-Miyura coupling of unprotected (a) halotryptophans, (b) haloindoles, and (c) bromo bisindoles.

2.2 Results and Discussion

Table 2. Screening of the reaction conditions.^[a]



Entry	[Pd]	Base	Time [h]	Conversion 1a [%] ^[b]	Yield 3aa [%] ^[b]	Yield 4aa [%] ^[b]
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1	Pd(dppf)Cl ₂	K ₃ PO ₄ ·H ₂ O	16	100	61 ^[c]	< 2
2 ^[d]	-	-	16	< 2	0	0
3 ^[d]	Pd(dppf)Cl ₂	K ₃ PO ₄ ·H ₂ O	16	37	0	0
4 ^[d]	-	K ₃ PO ₄ ·H ₂ O	16	37	0	0
5	Pd(dppf)Cl ₂	Cs ₂ CO ₃	16	98	60 ^[c]	< 2
6	Pd(dppf)Cl ₂	KOH	16	n.d.	31 ^[c]	n.d.
7	Pd(dppf)Cl ₂	K ₂ CO ₃	16	98	16 ^[c]	n.d.
8	Pd(dppf)Cl ₂	-	16	15	0	0
9	Pd(dppf)Cl ₂	K ₃ PO ₄ ·H ₂ O	1	100	70 (69 ^[c])	< 2
10	Pd(dppe)Cl ₂	K ₃ PO ₄ ·H ₂ O	1	95	43	25
11	Pd(dppp)Cl ₂	K ₃ PO ₄ ·H ₂ O	1	93	38	16
12	Pd(Xphos) ₂ Cl ₂	K ₃ PO ₄ ·H ₂ O	1	71	11	59
13	<i>t</i> BuXPhos Pd G1	K ₃ PO ₄ ·H ₂ O	1	90	7	0
14	Pd(PPh ₃) ₂ Cl ₂	K ₃ PO ₄ ·H ₂ O	1	100	59	20
15	(PPh ₃) ₄ Pd	K ₃ PO ₄ ·H ₂ O	1	85	18	38
16	Pd(dppf)Cl ₂	Cs ₂ CO ₃	1	100	79 (79 ^[c])	< 2
17	Pd(Binap)Cl ₂	Cs ₂ CO ₃	1	79	11	25
18	Pd(DPEPhos)Cl ₂	Cs ₂ CO ₃	1	95	52	16
19	Pd(dppf)(OAc) ₂ ^[e]	Cs ₂ CO ₃	1	90	29	32
20	Pd(JhonPhos) ₂ (OAc) ₂ ^[e]	Cs ₂ CO ₃	1	55	5	37

[a] Reaction performed under N₂ inert atmosphere using bisindole **1a** (0.1 mmol), boronic acid **2a** (0.25 mmol), the indicated Pd source (8 mol% of catalyst loading), the indicated base (0.5 mmol), in dioxane/H₂O = 4:1 (0.07 M) as the reaction medium at 80 °C for the indicated time. [b] Determined by ¹H NMR analysis of the reaction crude using 1,3,5-trimethoxybenzene as standard. [c] Isolated yield by means of chromatographic column. [d] No boronic acid was utilized. [e] Catalyst was formed in situ by mixing Pd(OAc)₂ and dppf or JhonPhos ligand (ratio Pd:ligand = 1:2). n.d. = not determinable.

The investigation started using the natural compound **1a**, applying conditions similar to those reported by Jin and co-workers [25]. In particular, in the presence of 2.5 equivalents of phenylboronic acid **2a**, 8 mol% of Pd(dppf)Cl₂ as the catalyst, and K₃PO₄·H₂O as the base, in dioxane/H₂O = 4:1 at 80 °C, after 16 hours the diarylated compound 2,2-bis(6-phenyl-1*H*-indol-3-yl)ethan-1-amine (**3aa**) was obtained with an isolated yield of 61% (Table 2, entry 1). Stability tests were performed on compound **1a**

indicate that it is stable in the absence of boronic acid **2a**, catalyst, and base, as it was fully recovered by column chromatography (entry 2). On the other hand, when adding either the catalyst and the base (entry 3) or only the base (entry 4), a partial decomposition of **1a** was observed, suggesting this effect is more likely attributable to the basic environment rather than the presence of the catalyst.

Similar results to entry 1 were reached by replacing the base with Cs₂CO₃ (entry 5), while other bases led to lower yields (entries 6 and 7) and the amino group alone was not sufficient to promote the reaction (entry 8) confirming the necessity of an external base. Surprisingly, reducing the reaction time to 1 hour resulted in complete conversion of **1a** and a slightly higher yield of **3aa** (entry 9). The rapid product formation minimizes decomposition and explains the better yield. Subsequently the reactions were all stopped after 1 hour; typically, the reaction mixture turned dark brown upon complete consumption of **1a** (**Figure 8**). The use of either bidentate ligands (entries 10–11) or monodentate ligands (entries 12–15) did not lead to further improvements, confirming Pd(dppf)Cl₂ as the best catalyst. Finally, replacing the base with Cs₂CO₃ afforded a **3aa** isolated yield of 79% after 1 hour (entry 16) and even with this base Pd(dppf)Cl₂ is confirmed as the optimal catalyst (entries 17–20). Doubling the amount of boronic acid or the catalyst loading did not lead to improvements, whereas a decrease in yield was observed when reducing the base or the catalyst (see Experimental Section).

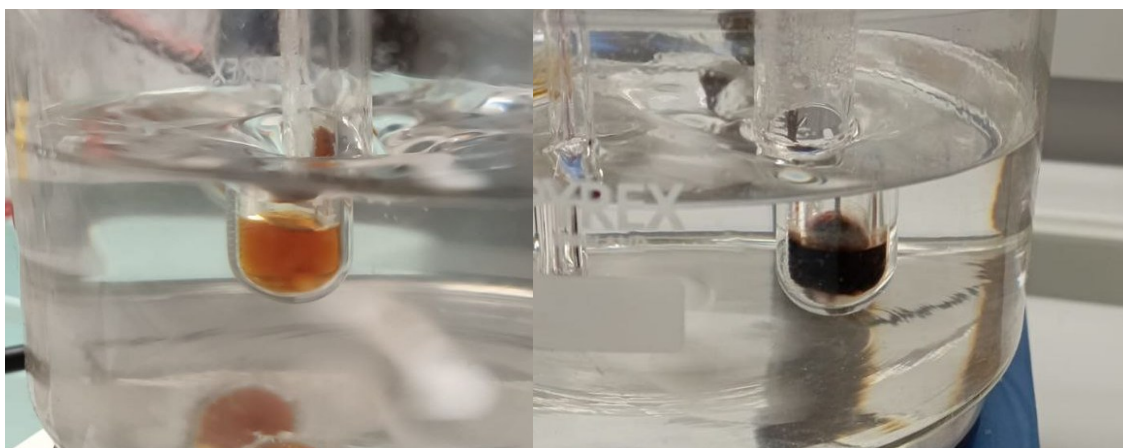
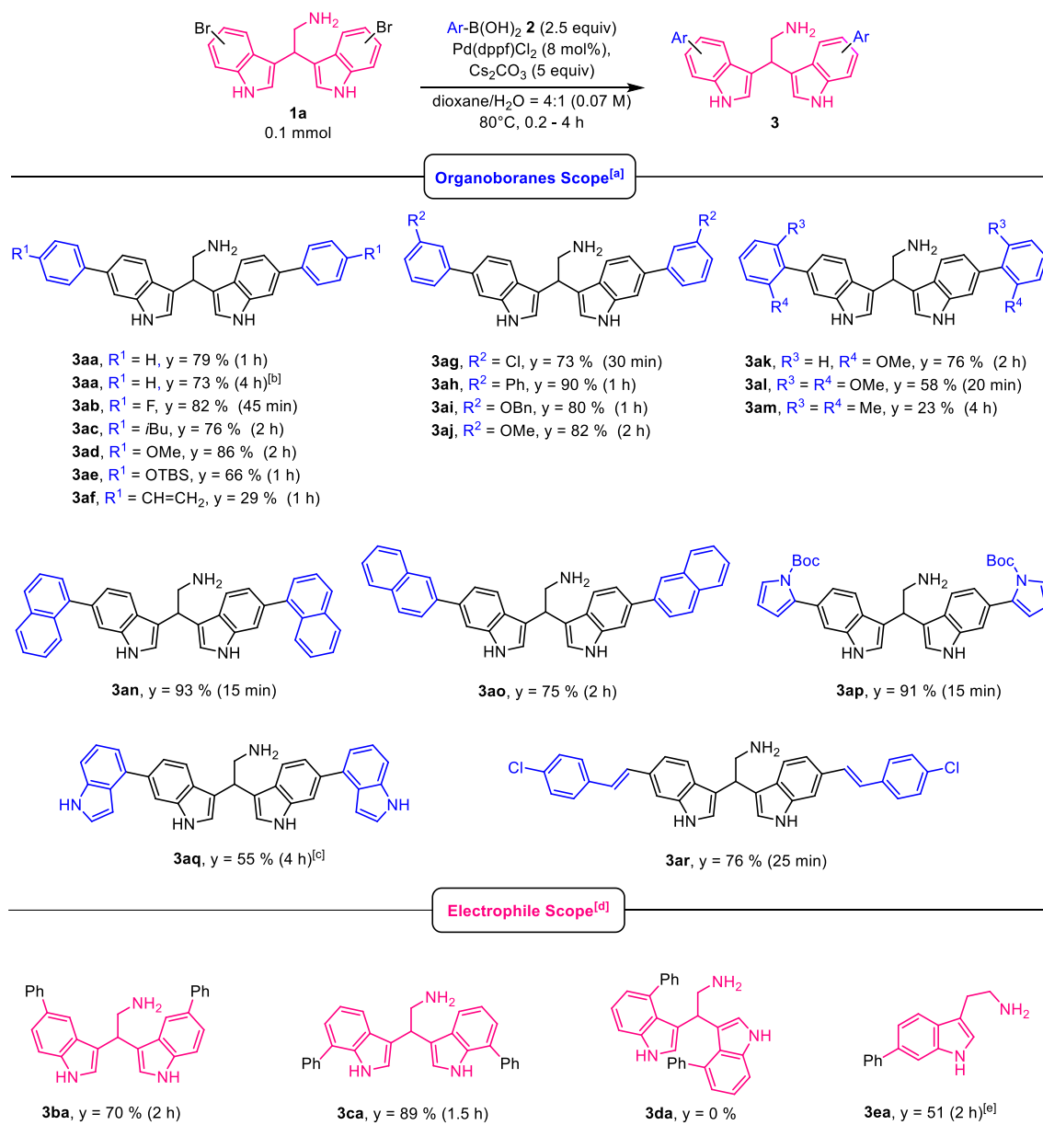


Figure 8. Color change of the reaction after 1 h.

With the optimized conditions (entry 16), we investigated the scope of this late-stage functionalization (**Table 3**).

Table 3. Scope of the Pd-Catalyzed Suzuki-Miyaura coupling of unprotected bisindoles.



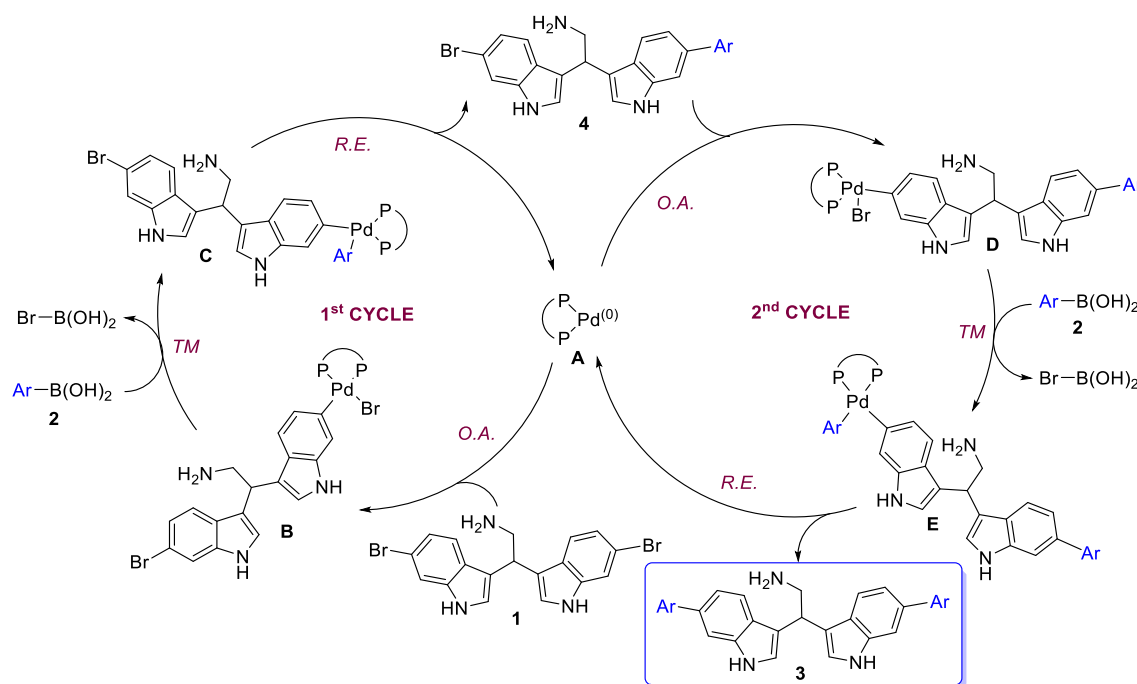
[a] Reaction performed under N₂ inert atmosphere using bisindole **1a** (0.1 mmol), boronic acid **2** (0.25 mmol), 8 mol% of Pd(dppf)Cl₂, 0.5 mmol of Cs₂CO₃, dioxane/H₂O = 4:1 (0.07 M) at 80 °C for the indicated time. Isolated yields are reported. [b] In place of the boronic acid, the phenylboronic acid pinacol ester was utilized. [c] In place of the boronic acid, 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indole was utilized. [d] Reaction performed under N₂ inert atmosphere using 4-, 5- and 7-bromobisindoles **1** or tryptamine (0.1 mmol), phenylboronic acid **2a** (0.25 mmol), 8 mol% of Pd(dppf)Cl₂, 0.5 mmol of Cs₂CO₃, dioxane/H₂O = 4:1 (0.07 M) at 80 °C for the indicated time. Isolated yields are reported. [e] 1.25 equivalents of boronic acid **2a** (0.125 mmol), 4 mol% of catalyst loading, and 2.5 equivalents of base (0.25 mmol) were utilized.

To start with, we tested our reaction conditions using the pinacol ester instead of phenylboronic acid, obtaining a 73% isolated yield in 4 hours, suggesting that the reaction is also accessible using boronic esters, albeit requiring longer reaction times. A series of boronic acids was then tested, with the reaction being stopped upon complete conversion, monitored by TLC, and, in any case, after a maximum of 4 hours to minimize reagent/product degradation. In all cases, formation of the monoarylated product was observed (less than 5%). With *para*-substituted boronic acids, high yields were obtained in the presence of substituents such as fluorine (**3ab**, 82%), isobutyl (**3ac**, 76%), and methoxy (**3ad**, 86%). No product formation was observed using (4-hydroxyphenyl)boronic acid, which made it necessary to protect the hydroxyl group with a *tert*-butyldimethylsilyl (TBS) group, affording **3ae** in 66% isolated yield. Compound **3af** was obtained in only 29%, and no Heck-type product was detected under our developed reaction conditions. The low yield is therefore likely attributable to side reactions involving (4-vinylphenyl)boronic acid, such as polymerization or protodeboronation. With *meta*-substituted boronic acids, yields ranged from 73% to 90% (**3ag**, **3ah**, **3ai**, **3aj**) both with electron-donating and withdrawing groups. In the presence of an *ortho*-methoxy group, the yield was good (**3ak**, 76%) and remained acceptable with two *o*-OMe substituents (**3al**, 58%). Compound **3am** was obtained in a less satisfactory 23% yield, probably due to the more pronounced steric effect of the methyl group compared to methoxy [29]. However, with this hindered substrate, it was also possible to isolate the monoarylated compound **4am** in 27% yield (see Experimental Section). The coupling between naphthalen-1-ylboronic acid and **1a** provided an excellent yield of product **3an** in a very short time (93% in 15 minutes), and good results were also obtained with naphthalen-2-ylboronic acid (**3ao**, 75%). Subsequently, heteroarylboranes containing either a pyrrole or indole scaffold were tested, affording compounds **3ap** and **3aq** in excellent and modest yields, respectively. Notably, our C(sp²)–C(sp²) Suzuki–Miyaura coupling can also be applied to vinyl boronic acids, as bisindole **3ar** was obtained in 76% isolated yield. Overall, these results suggest that longer reaction times are generally required in the presence of electron-donating groups (EDGs), although no clear reactivity trend emerges. Alkylboronic acids were completely unreactive.

Other bisindoles were tested in the presence of boronic acid **2a**: the 5-bromo- and 7-bromobisindoles gave good and high yields, respectively (**3ba** and **3ca**), whereas 4-

bromobisindole was unreactive, likely due to steric hindrance caused by the ethylamino group. This finding further rules out the possibility that the NH_2 group acts as a directing group, as an easier arylation at the 4-position would otherwise have been expected. Finally, for the first time, 6-bromotryptamine was successfully arylated directly (**3ea**, 51%) without the use of any protecting group.

A plausible catalytic cycle based on our results and literature data [30] is shown in **Scheme 2**.



Scheme 2. Proposed catalytic cycle. O.A. = oxidative addition; T.M. = transmetalation; R.E. = reductive elimination.

The catalytic cycle likely begins with oxidative addition of Pd(0) into one of the C–Br bonds of bisindole **1a**, generating intermediate **B**. This is followed by transmetalation with boronic acid **2**. As reported in the literature, the base facilitates this step through two possible pathways: either via formation of the organoboronate species ArB(OH)_3^- or through a Pd–hydroxide complex [30]. In both scenarios, intermediate **C** is formed, which undergoes reductive elimination to regenerate the Pd(0) species **A** and furnish the monoarylated bisindole **4**. The latter can re-enter the catalytic cycle, undergoing a second oxidative addition–transmetalation–reductive elimination sequence to yield the desired diarylated product **3**. Based on the data presented in **Table 3**, both

catalytic cycles appear to operate concurrently. As a matter of fact, in non-optimal condition compound **4aa** can be isolated supporting the proposed catalytic cycle (see experimental section).

Eighteen newly synthesized compounds were screened to evaluate their efficacy against *L. infantum* MHOM/TN/80/IPT1 promastigotes at a single concentration of 20 μ M for 72 hours. All compounds exhibited satisfactory activity (inhibition $\geq$ 92.6%) (Table 4), except for **3ai**, **3ah**, **3ac**, and **3al** (inhibition $\leq$ 51%). The cytotoxicity of all compounds was assessed in THP-1 cells at concentrations of 100, 20, and 4 μ M for 72 hours to estimate the CC₅₀ range, which was determined to be between 4 and 20 μ M for all compounds, except for **3ah**, **3ac**, and **3al**.

Table 4. Summary of bisindole derivatives activity on *L. infantum* promastigotes and THP-1 cells and corresponding SIs.^[a] IC₅₀ and CC₅₀ values are reported as mean and 95% CI, from at least two independent experiments. Each experimental condition was conducted at least in duplicate, and miltefosine (Milt) was used as positive control.

	Inhibition of <i>L.</i> <i>infantum</i> at 20 μ M (%)	Cytotoxicity on THP-1 (100, 20, 4 μ M) (%)	<i>L.</i> <i>infantum</i> IC ₅₀ (μ M) (95% CI)	THP-1 CC ₅₀ (μ M) (95% CI)	SI ^[a]
3af	98.7	78, 97, -14	1.1 (0.9-1.3)	24.0 ^[b]	21.8
3ca	98.0	89, 98, -19	1.7 (1.5-1.9)	18.1 ^[b]	10.7
3an	97.8	79, 95, -15			
3ab	97.1	85, 97, -33			
3aq	96.9	89, 73, -16	2.8 (2.5-3.1)	14.6 (12.2-17.2)	5.2
3ba	96.8	91, 98, -22			
3aj	96.5	85, 97, 2			
3ak	96.5	86, 97, -4			
3aa	96.2	87, 97, -18			
3ad	95.1	86, 96, -12			

3ao	95.1	76, 94, -13			
3ar	95.1	66, 95, -32			
3ag	93.6	84, 97, -7			
3ap	92.6	85, 97, 22			
3al	51.4	84, 47, -5			
3ac	36.6	88, 1, -17			
3ah	25.3	77, 32, -20			
3ai	19.7	72, 90, 1			
Milt	98.5	99, 6, -35	3.7 (3.3-4.1)	36.6 (32.8-40.6)	9.8

[a] Selectivity index = CC_{50}/IC_{50} . [b] CI was not calculable.

Compounds **3af**, **3ca**, and **3aq** (**Figure 9**) were selected for further testing. The first two were chosen for their high activity against the parasite, while the latter was selected due to its lower toxicity. They were further evaluated on the promastigote form with serial dilutions of 2:3 and 1:2. The IC_{50} values of these molecules were found to be lower than that of miltefosine, whereas their cytotoxicity was comparable to that of **1a** [15]. It is noteworthy that, in the calculation of the CC_{50} on THP-1 cells, the 95% confidence interval (CI) was not determined for **3af** and **3ca** due to the steeper slope of the dose–response curves (**Figure 19**). Although **3af**, **3ca**, and **3aq** exhibited relative toxicity toward human cells, their SI values were 21.8, 10.7, and 5.2, respectively. It is worth noting that the SI of miltefosine (the reference antileishmanial drug) is 9.8. These three compounds were also tested against the amastigote form of the parasite, showing a reduction in infection at 2 μ M (**Figure 10**). Despite a slightly higher cytotoxicity compared to miltefosine (as also shown in **Figure 20**, Experimental section), **3af**, **3ca**, and **3aq** emerged as the most promising compounds in our new library, displaying strong inhibitory activity against *L. infantum* promastigotes and intramacrophage amastigotes.

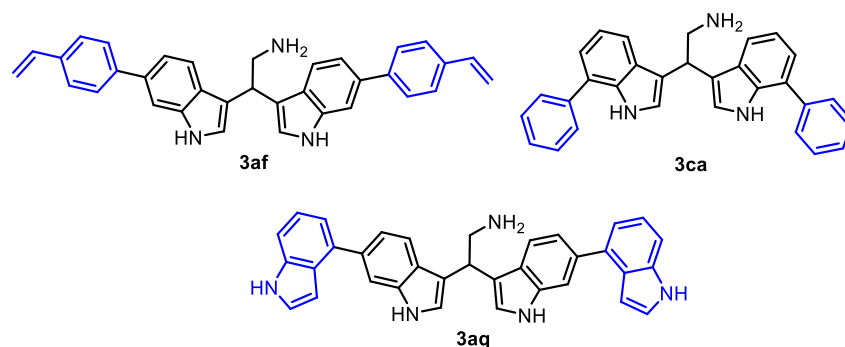


Figure 9. Molecular structures of our best new candidates as antileishmanial agents.

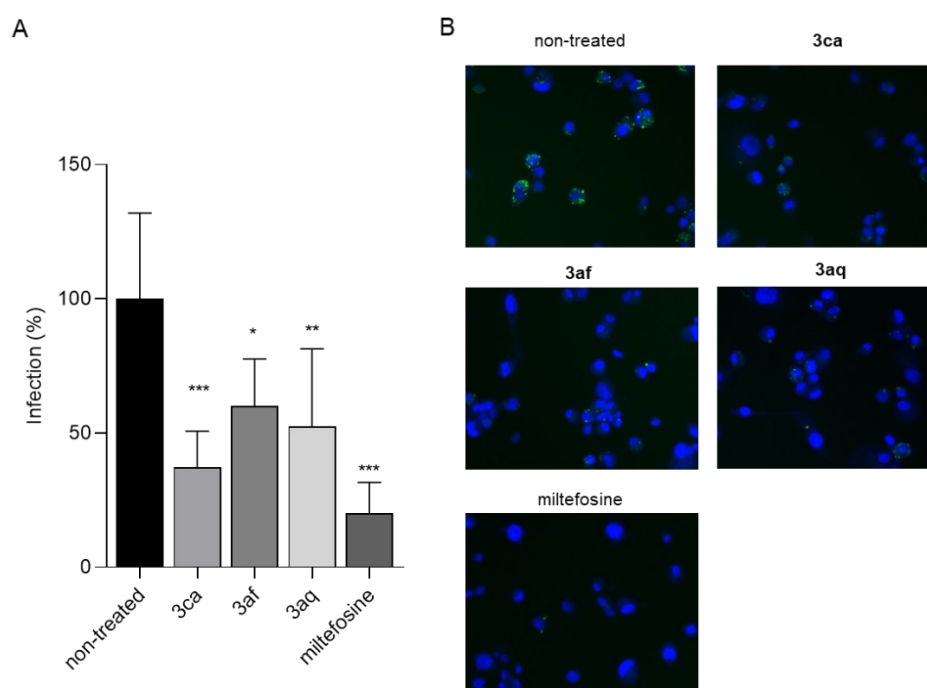


Figure 10. Effect of **3ca**, **3af**, and **3aq** on intracellular *L. infantum* amastigotes. Differentiated THP-1 cells were infected for 24 hours at 37 °C; then, the different compounds were added at 2 μ M, and the efficacy on intracellular amastigotes was calculated after 72 hours of treatment. Miltefosine was used as a positive control. (A) Percentages of infection are presented as mean $\pm$ SD. Ordinary one-way ANOVA with Dunnett's multiple comparisons test. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$. (B) Representative merged images showing THP-1 cells stained with Hoechst dye (blue) and Leishmania amastigotes stained with CFSE (green).

2.3 Conclusions

In this study, the late-stage functionalization of compound **1a** was successfully developed through Suzuki–Miyaura coupling using a novel, rapid, and robust methodology that tolerates the presence of both NH₂ and indole NH groups, affording diarylated compounds in isolated yields up to 93%. In particular, the optimal conditions involved Pd(dppf)Cl₂ as the catalyst, Cs₂CO₃ as the base, and dioxane/water (4:1) as the reaction medium. The reaction scope was demonstrated with good tolerance toward various functional groups. Moreover, tryptamine was coupled for the first time without the use of protecting groups.

From our new compound library, **3af** emerged as a promising candidate against *L. infantum* promastigotes, showing a selectivity index (SI) of 21.8 with an IC₅₀ of 1.1 μM. Compounds **3ca** and **3aq** also exhibited high activity, although with slightly lower SI values. In addition, all three compounds showed activity against intramacrophage amastigotes in *in vitro* models. Due to the synthetic strategy we developed, future studies will focus on improving the pharmacokinetic profiles of these compounds to enhance their potential as therapeutic agents against leishmaniasis.

2.4 Experimental Section

Material and Methods

Chemistry

All reactions were prepared under nitrogen atmosphere with dry solvents under anhydrous conditions, by using Schlenk technique. Reactions were monitored by ¹H NMR taking a direct sample of the crude mixture. ¹H NMR and ¹³C NMR were recorded on a Bruker Avance 400 spectrometer (¹H: 400 MHz, ¹³C: 101 MHz), or on a Bruker Avance Neo 600 spectrometer (¹H: 600 MHz, ¹³C: 151 MHz) using DMSO-*d*₆ or CDCl₃ as solvent and 1,3,5-trimethoxybenzene as standard. Chemical shifts are reported in the δ scale relative to residual CHCl₃ (s, 7.26 ppm) or DMSO (p, 2.50 ppm) for ¹H NMR and to the central line of CDCl₃ (77.16 ppm) or DMSO-*d*₆ (39.52 ppm) for ¹³C NMR. ¹³C

NMR were recorded with ^1H broadband decoupling. The following abbreviations were used to explain the multiplicities: br = broad, s = singlet, d = doublet, t = triplet, q = quartet, hept = heptet, dd = doublet of doublets, ddd = doublet of doublets of doublets, m = multiplet. Coupling constants (J) are reported in Hertz (Hz). ESI-MS spectra were recorded on Waters Micromass ZQ 4000, using electrospray ionisation techniques, with samples dissolved in MeOH. HRMS spectra were performed by slow direct infusion (5 $\mu\text{L}/\text{min}$) of $\approx 0.1 \mu\text{g}/\text{mL}$ solution (CH_3OH), using Orbitrap Exploris 240 mass spectrometer. Only molecular ions $[\text{M} + \text{H}]^+$ are given. For **Int-1c** and **Int-1d** molecular ions $[\text{M} + \text{Na}]^+$ are given.

1,4-dioxane was dried over molecular sieves (Alfa Aesar, 4 Å, 1–2 mm, beads). Both 1,4-dioxane and water were degassed (N_2 bubbling for 30 min).

Pure compounds **3** were isolated through flash column chromatography on silica gel 60 (40–60 μm , 230–400 mesh). Bromobisindole derivatives **1a** and **1b** have been synthesized as already described in the literature [31]. 6-bromotryptamine was synthesized as already described in the literature [32]. The new bromobisindoles **1c** and **1d** have been realized following a literature procedure [31], as reported in the Supporting Information. Boronic acids **2a**, **2c**, **2f** and boronic ester **2q** were purchased from BLDpharm. Boronic acids **2b**, **2i**, **2j** were purchased from Alfa Aesar - Thermo Fisher. Boronic acids **2d** and **2e** were purchased from TCI. Boronic acids **2g**, **2h**, **2l**, **2m**, **2r**, **2k** were purchased from Merck Sigma-Aldrich. Boronic acids **2n**, **2o**, **2p** were purchased from Fluorochem. Boronic ester **2a'** has been synthesized as already described in the literature [33]. All other chemicals were purchased from Merck Sigma-Aldrich and used without further purification. All solid reagents were weighed in an analytical balance without excluding moisture and air.

Parasite and cell cultures

L. infantum MHOM/TN/80/IPT1 (WHO international reference strain) was purchased from ATCC (ATCC[®] 50134TM) and was cultured in Evans's Modified Tobie Medium (EMTM) at 26–28 °C. To test the compounds, parasites were resuspended in RPMI-PY medium as described previously [14]. The human monocytic cell line THP-1 (ECACC 88081201) was cultured in RPMI-1640 medium supplemented with 10% heat-inactivated Fetal Bovine Serum (FBS), 100 $\mu\text{g}/\text{mL}$ streptomycin, 100 U/l penicillin and

maintained in a humidified incubator at 37 °C and 5% CO₂. All the cell culture reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA).

L. infantum promastigotes viability assay

Late log/stationary phase *L. infantum* promastigotes were resuspended in RPMI-PY medium at a density of 2.5×10^6 parasites/mL in 96-well plates (100 µL/well). The anti-parasitic activity of the eighteen compounds of the library was initially investigated at a single dose of 20 µM for 72 h at 26 °C. Three selected compounds were tested with scalar dilutions 2:3 (from 20 to 1.17 µM) on promastigotes to determine the exact IC₅₀ value. Not treated parasites were included as negative control. As positive control, the anti-leishmanial drug miltefosine (Sigma-Aldrich, Milan, Italy) was used. Each condition was carried out in duplicate. To evaluate the promastigotes viability, the CellTiter 96H Aqueous Non-Radioactive Cell Proliferation Assay (Promega), based on the ability of viable cells to convert a soluble tetrazolium salt [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxy-phenyl)-2-(4-sulfophenyl)-2H-tetrazolium. MTS] to a formazan product, was performed. To this end, 20 µL of MTS/PMS (phenazinemethosulfate, Sigma-Aldrich) was added to 100 µL of culture medium and incubated at 26 °C until formazan production. Absorbance was recorded on a Microplate Reader (Infinite® F50 Plus, Tecan) at 492 nm. The IC₅₀ values were calculated using nonlinear regression curves in GraphPad Prism 8.0 (GraphPad Software, Inc., San Diego, CA). The equation used for data fitting was $Y = 100 / \{1 + 10^{\Lambda[(\text{Log IC}_{50} - X) * \text{HillSlope}]}\}$ (hillslope not constrained), where X is the log of concentration and Y is the normalized response.

Evaluation of cytotoxicity on THP-1 cells

The cytotoxicity of the compounds was assessed on THP-1 cells seeded at a density of 5×10^5 cells per mL, 100µL/well in a 96-well plate and treated for 72 h with 20 ng/mL phorbol myristic acid (PMA) to induce differentiation into macrophages like cells. After cell adhesion to the plate, each molecule was tested at 4, 20 and 100 µM for 72 h at 37 °C.

Three selected compounds were further evaluated at different concentration, obtained from scalar dilutions 1:2 (from 100 µM to 3.12 µM) for 72 h at 37 °C. The negative control (untreated THP-1 cells) and the positive control (miltefosine) were included in each experiment. Each condition was carried out in duplicate. To evaluate the compounds cytotoxicity, the CellTiter 96H Aqueous Non-Radioactive Cell Proliferation

Assay (Promega), was used as described before. For each compound the Selectivity Index (SI) was calculated as the ratio between cytotoxicity in THP-1 (CC₅₀, 72 h) and activity against *L. infantum* promastigotes (IC₅₀, 72 h).

Anti-amastigote Assay on infected THP-1 cells

The activity of compounds **3af**, **3ca**, and **3aq** against intracellular amastigotes was evaluated in THP-1 infected cells. Briefly, THP-1 cells were seeded in a 12-well plate with a density of 3×10^5 cells/well and, to induce differentiation into macrophage-like cells, were treated with 20 ng/mL PMA for 48 h. After differentiation, cells were infected for 24 h with *L. infantum* MHOM/TN/80/IPT1 promastigotes stained with carboxyfluorescein succinimidyl ester (eBioscience™ CFSE, Invitrogen) as described in reference [15], with a parasite-to-cell ratio of 10:1. Non-internalized promastigotes were then removed through medium replacement, and infected cells were treated with **3af**, **3ca**, and **3aq** or with the positive control miltefosine for 72 h at concentrations of 2, 10, and 20 μ M. To monitor the infection, cells were washed, formaldehyde/methanol fixed, stained with Hoechst dye, and observed with a fluorescence microscope at 5x or 10x magnification. For each field, two images were acquired: one with excitation at 340-390 nm (for Hoechst dye) and the other with excitation at 460-495 nm (for CFSE). Automated cell counting of single-color images was performed with ImageJ software to monitor the rate of infection, which was determined by normalizing the amastigote count from each green (CFSE) image to the THP-1 cell count from the corresponding blue (Hoechst) image. At least 500 THP-1 cells were counted for each condition. The normalized infection rate for untreated infected cells was set as 100%.

Statistical Analysis

The evaluation of IC₅₀ in promastigotes and CC₅₀ in mammalian cells following the treatments was performed by nonlinear regression analysis and expressed as mean and 95% confidence interval. Statistical evaluation of treatment efficacy on amastigotes was performed using one-way ANOVA, followed by Dunnett's multiple comparisons test. All tests were performed using GraphPad Prism version 8 (GraphPad Software, Inc., La Jolla, CA).

Typical Procedure for the Suzuki-Miyaura coupling of unprotected bromo(bis)indole with boronic acids

Compound **1** (0.1 mmol, 43.3 mg), Pd(dppf)Cl₂ (8mol%, 5.9 mg), Cs₂CO₃ (0.5 mmol, 162.9 mg) and the selected boronic acid or pinacol derivative **2** (0.25 mmol) were added in a dried Schlenk tube, equipped with a magnetic stirring bar. Degassed dioxane (1.15 mL) and degassed water (0.28 mL) were added, and the reaction was vigorously stirred at 80 °C in an oil bath under balloon pressure of nitrogen, monitoring the progress of the reaction by TLC (silica gel; CH₂Cl₂/CH₃OH/NH₃, 90:10:1). The nitrogen was removed, and the reaction mixture was analyzed by ¹H NMR to determine the conversion of the bisindole. The crude was filtered on silica plug (CH₂Cl₂/CH₃OH/NH₃, 80:20:1) and the products were eventually obtained after column chromatography on silica gel. For the synthesis of compound **3ea**, 0.125 mmol of boronic acid **2a**, 4 mol% of Pd(dppf)Cl₂, and 0.25 mmol of base were utilized.

List of the utilized bromo(bis)indoles **1** and boronic acids **2**

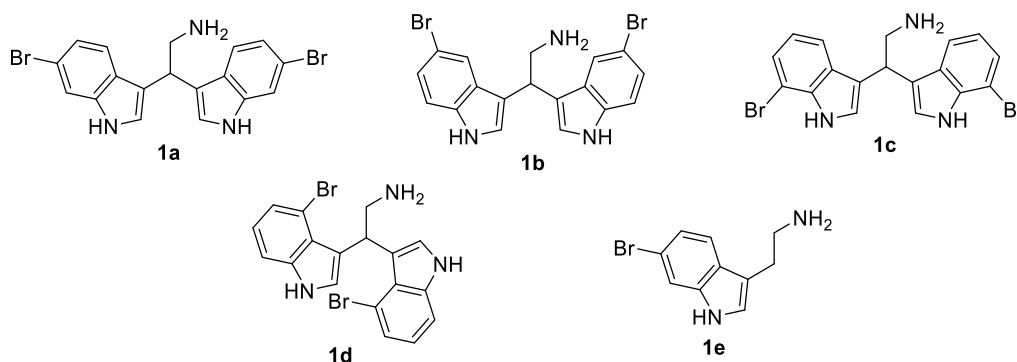


Figure 11. Complete list of the utilized Br-(bis)indoles **1**.

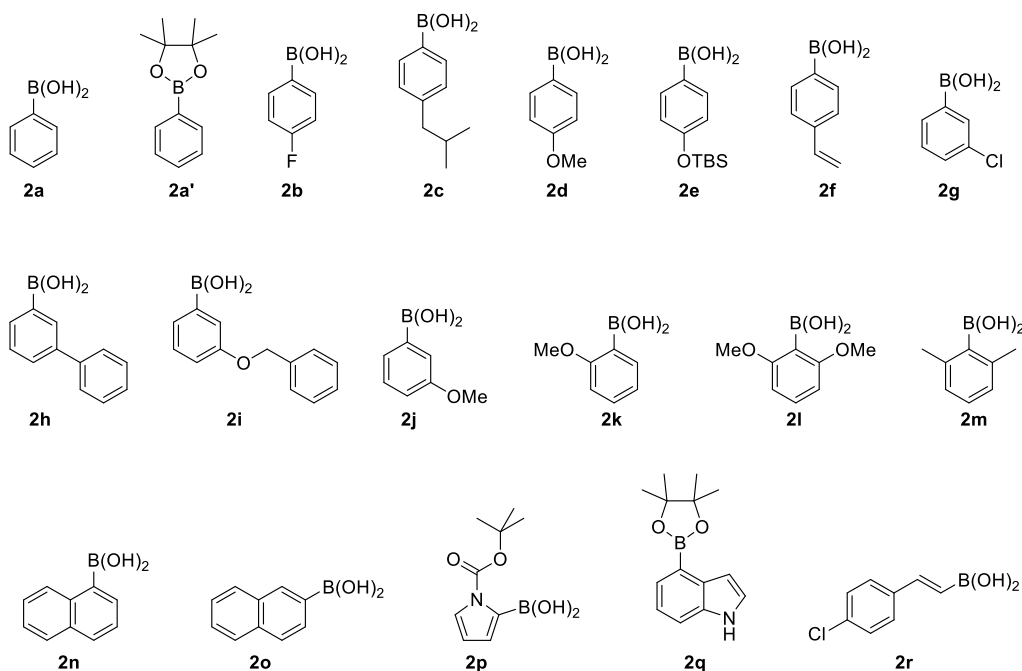
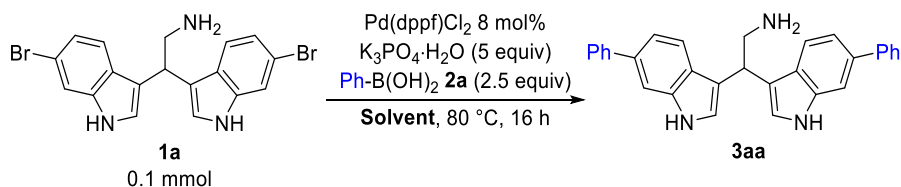


Figure 12 – Complete list of the utilized boronic acids or boronic esters **2**.

Complete Optimization Reactions for the Suzuki-Miyaura Coupling of Unprotected Bromobisindole.

Table 5. Screening of the solvent, the concentration and the ratio organic solvent:H₂O^[a]



Entry	Solvent ^[b]	Solvent:H ₂ O (v/v)	Concentration [M]	Yield 3aa [%] ^[c]
1	1,4-dioxane	4:1	0.07	61
2	THF	4:1	0.07	45
3	CH ₃ CN	4:1	0.07	18
4	<i>i</i> PrOH	4:1	0.07	40
5	1,4-dioxane	4:1	0.14	57
6	1,4-dioxane	3:1	0.07	35
7	1,4-dioxane	5:1	0.07	24

[a] Reaction performed under N₂ inert atmosphere using bisindole **1a** (0.1 mmol), boronic acid **2a** (0.25 mmol), Pd(dppf)Cl₂ (8 mol% of catalyst loading), K₃PO₄·H₂O (0.5 mmol), in the indicated reaction medium at 80 °C for 16 h. [b] Solvents dried over molecular sieves. [c] Isolated yields.

Table 6. Screening of the bases/additives.^[a]

Reaction scheme showing the conversion of bisindole **1a** (0.1 mmol) to product **3aa** using Pd(dppf)Cl₂ (8 mol%), Base (5 equiv), Ph-B(OH)₂ **2a** (2.5 equiv), dioxane/H₂O = 4:1 (0.07 M), 80 °C, 16 h.

Entry	Base	Yield 3aa [%] ^[b]
1	K ₂ CO ₃	16
2	Cs ₂ CO ₃	60
3	KOH	31
4	<i>n</i> Bu ₄ NOH	0
5	CsF	< 2

[a] Reaction performed under N₂ inert atmosphere using bisindole **1a** (0.1 mmol), boronic acid **2a** (0.25 mmol), 8 mol % of Pd(dppf)Cl₂, the indicated base (0.5 mmol), in dioxane/H₂O = 4:1 (0.07 M) at 80 °C for 16 h. [b] Isolated yields.

Table 7. Screening of various palladium sources and catalysts.^[a]

Reaction scheme showing the conversion of bisindole **1a** (0.1 mmol) to products **3aa** and **4aa** using [Pd] (8 mol%), Base (5 equiv), Ph-B(OH)₂ **2a** (2.5 equiv), dioxane/H₂O = 4:1 (0.07 M), 80 °C, 1 h.

Entry	[Pd]	Base	Conv. 1a [%] ^[b]	Yield 3aa [%] ^[b]	Yield 4aa [%] ^[b]
1 ^[c]	Pd(dppf)(OAc) ₂ ^[d]	K ₃ PO ₄ ·H ₂ O	> 98	21 ^[e]	n.d.
2 ^[c]	Pd(dppf)Cl ₂ ^[d]	K ₃ PO ₄ ·H ₂ O	> 98	21 ^[e]	n.d.
3	Pd(dppf)Cl ₂	K ₃ PO ₄ ·H ₂ O	100	70 (69 ^[e])	< 2
4	Pd(dppe)Cl ₂	K ₃ PO ₄ ·H ₂ O	95	43	25
5	Pd(dppp)Cl ₂	K ₃ PO ₄ ·H ₂ O	93	38	16
6	Pd(Xphos) ₂ Cl ₂	K ₃ PO ₄ ·H ₂ O	71	11	59
7	<i>t</i> BuXPhos Pd G1	K ₃ PO ₄ ·H ₂ O	90	7	0
8	Pd(PPh ₃) ₂ Cl ₂	K ₃ PO ₄ ·H ₂ O	100	59	20

9	(PPh ₃) ₄ Pd	K ₃ PO ₄ ·H ₂ O	85	18	38
10	Pd(dppf)Cl ₂	Cs ₂ CO ₃	100	79 (79 ^[e])	< 2
11	Pd(Binap)Cl ₂	Cs ₂ CO ₃	79	11	25
12	Pd(DPEPhos)Cl ₂	Cs ₂ CO ₃	95	52	16
13	Pd(dppf)(OAc) ₂ ^[f]	Cs ₂ CO ₃	90	29	32
14	Pd(JhonPhos) ₂ (OAc) ₂ ^[f]	Cs ₂ CO ₃	55	5	37

[a] Reaction performed under N₂ inert atmosphere using bisindole **1a** (0.1 mmol), boronic acid **2a** (0.25 mmol), the indicated Pd source (8 mol % of catalyst loading), the indicated base (0.5 mmol), in dioxane/H₂O = 4:1 (0.07 M) as the reaction medium at 80 °C for 1 h. [b] Determined by ¹H NMR analysis of the reaction crude using 1,3,5-trimethoxybenzene as standard. [c] Reaction time = 16 h. [d] Catalyst was formed by mixing Pd(OAc)₂ or Pd(cod)Cl₂ with dppf (ratio Pd:ligand = 1:1) [e] Isolated yield by means of chromatographic column. [f] Catalyst was formed in situ by mixing Pd(OAc)₂ and dppf or JhonPhos ligand (ratio Pd:ligand = 1:2). n.d. = not determinable.

Table 8. Screening of the effect of the amount of boronic acid **2a**, catalyst loading and base amount on the reaction.^[a]

Entry	Catalyst Loading [mol%]	Cs ₂ CO ₃ [equiv]	2a [equiv]	Conv. 1a [%] ^[b]	Yield 3aa [%] ^[b]	Yield 4aa [%] ^[b]
1	8	5	4	100	77	1
2	16	5	2.5	100	72	3
3	16	5	4	100	76	0
4	16	1	4	n.d.	16	24
5	4	5	2.5	>98	65	11

[a] Reaction performed under N₂ inert atmosphere using bisindole **1a** (0.1 mmol) and boronic acid **2a**, Pd(dppf)Cl₂ and Cs₂CO₃ in the indicated amount, in dioxane/H₂O = 4:1 (0.07 M) as the reaction medium at 80 °C for 1 h. [b] Determined by ¹H NMR analysis of the reaction crude using 1,3,5-trimethoxybenzene as standard.

¹H NMR and ESI-MS characterization of 2-(6-bromo-1*H*-indol-3-yl)-2-(6-phenyl-1*H*-indol-3-yl)ethan-1-amine (**4aa**).

Compound **4aa** was isolated from entry 3 of Table 6 in a mixture with compound **1a** and compound **3aa** using silica gel column chromatography (CH₂Cl₂/CH₃OH/NH₃,

99:1:1). ^1H NMR spectrum of the mixture (**4aa:1a:3aa** = 72:17:12) and ESI(+)-MS spectrum, confirming the formation of **4aa**, are reported below.

4aa: ^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ = 10.98 (br s, 1H, NH_{ind}), 10.93 (br s, 1H, NH_{ind}), 7.62-7.59 (m, 2H, Ar-H), 7.54 (d, J = 1.5 Hz, 1H, Ar-H), 7.52 (d, J = 8.5 Hz, 1H, Ar-H), 7.50 (d, J = 2.0 Hz, 1H, Ar-H), 7.44-7.40 (m, 3H, Ar-H), 7.31-7.26 (m, 3H, Ar-H), 7.18 (dd, J_1 = 1.5 Hz, J_2 = 8.5 Hz, 1H, Ar-H), 7.00 (dd, J_1 = 2.0 Hz, J_2 = 8.5 Hz, 1H, Ar-H), 4.39 (t, J = 7.0 Hz, 1H, CHCH_2NH_2), 3.27 (d, J = 7.0 Hz, 2H, CHCH_2NH_2) ppm. ESI-MS: m/z = 430 $[\text{M}+\text{H}]^+$.

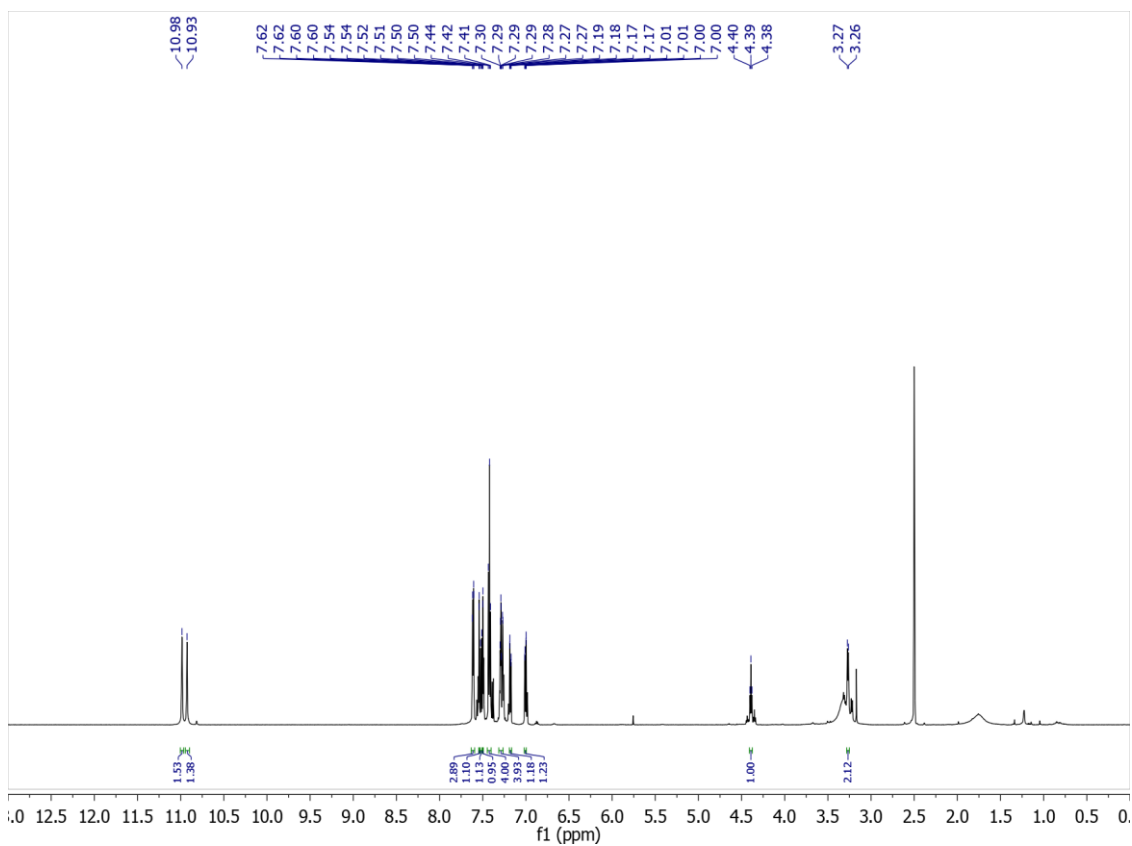
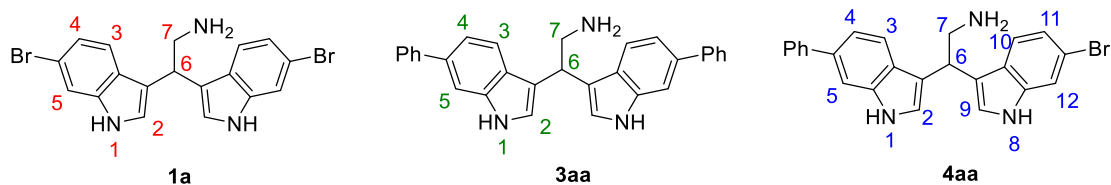


Figure 13. ^1H NMR of the isolated mixture.



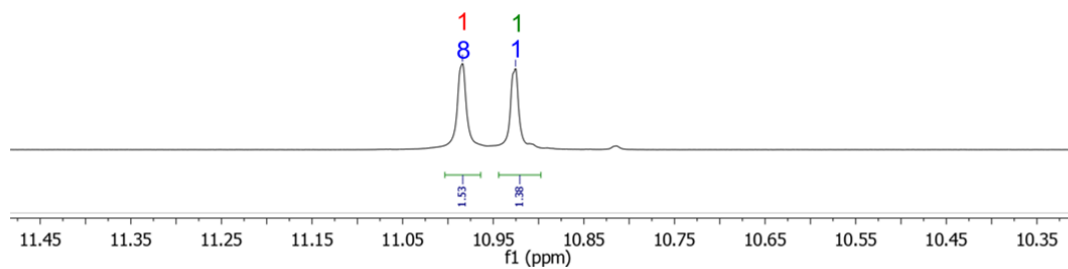


Figure 14. ^1H NMR of the isolated mixture. Zoom from 11.50 ppm to 10.40 ppm.

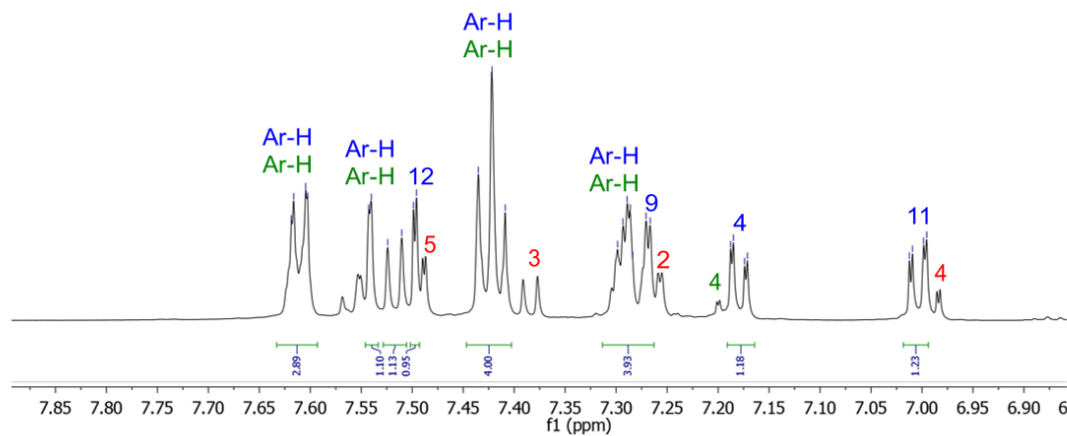


Figure 15. ^1H NMR of the isolated mixture. Zoom from 7.80 ppm to 6.85 ppm.

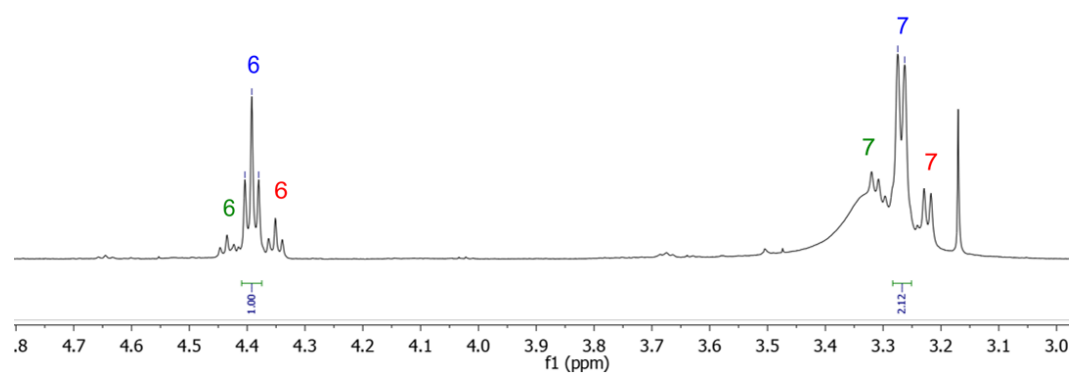


Figure 16. ^1H NMR of the isolated mixture. Zoom from 4.80 ppm to 2.90 ppm.

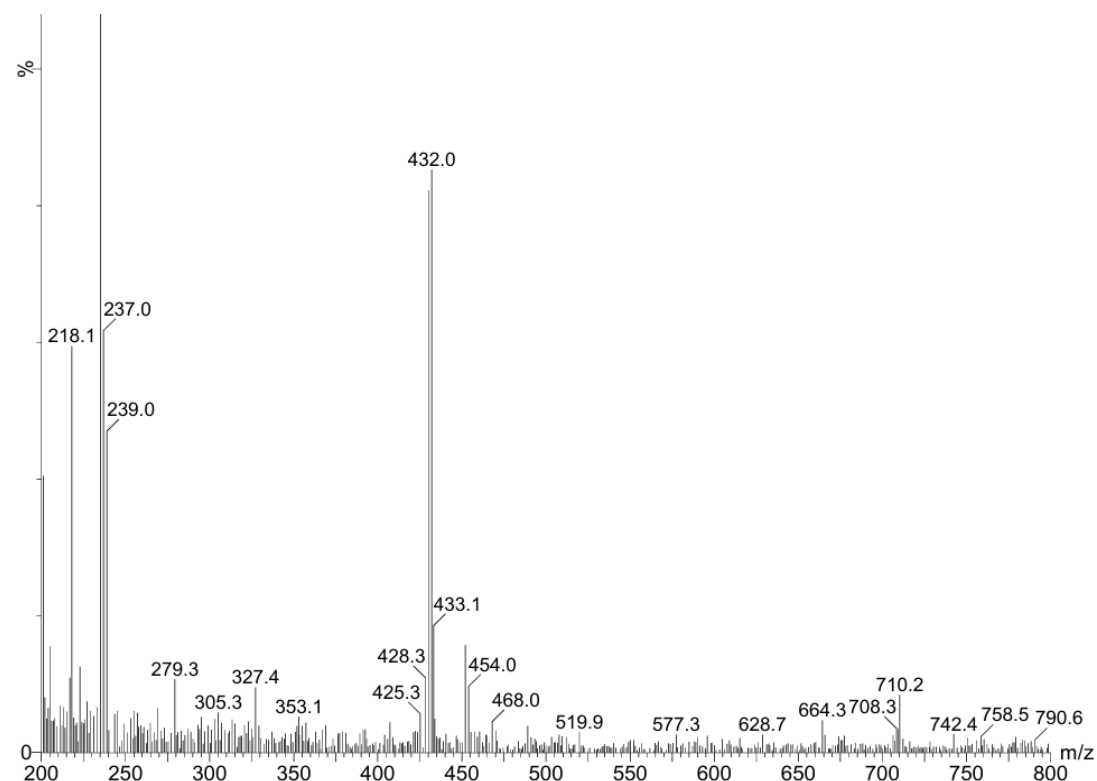
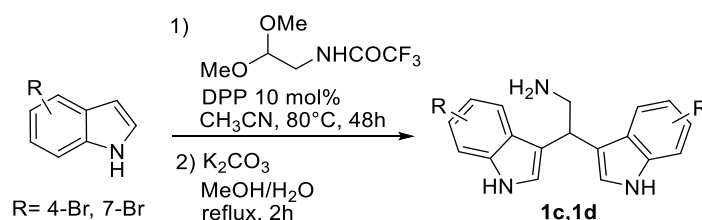


Figure 17. – ESI(+)-MS spectra compound **4aa**.

Although compound **4aa** was isolated in a mixture, the pure monoarylated compound **4am**, deriving from the coupling of the bisindole **1a** with the boronic acid **2m** was successfully isolated (*vide infra*).

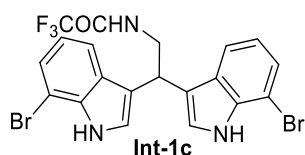
Synthesis of 2,2-bis(7-bromo-1*H*-indol-3-yl)ethan-1-amine (**1c**) and 2,2-bis(4-bromo-1*H*-indol-3-yl)ethan-1-amine (**1d**).

The synthesis of the new bromobisindoles **1c** and **1d** has been realized following a literature procedure [31].

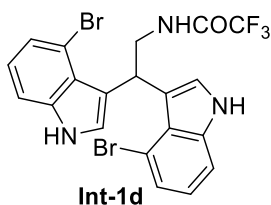


Synthesis of the N-trifluoroacetamide bisindoles **Int-1c** and **Int-1d**

A mixture of diphenyl phosphate (0.1 eq, 0.15 mmol, 37.5 mg), the appropriate bromoindole derivative (1.53 mmol, 300 mg) and (trifluoroacetyl amino)acetaldehyde dimethyl acetal (0.5 eq, 0.77 mmol, 154 mg) in an. CH₃CN (0.77 mL) was stirred at 80 °C for 48 h in a sealed tube. After cooling to room temperature, saturated aqueous NaHCO₃ solution (30 mL) and CH₂Cl₂ (30 mL) were added, and the two phases were separated. The aqueous solution was extracted with CH₂Cl₂ (3 x 20 mL). After drying over dry Na₂SO₄, the combined organic phases were concentrated in vacuo and the resulting crude product **Int-1c** or **Int-1d** was characterized and utilized without further purification.



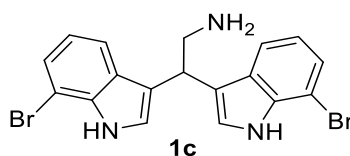
(**Int-1c**): R_f = 0.28 (cyclohexane/EtOAc, 1:1). ¹H NMR (400 MHz, CDCl₃): δ = 8.28 (br s, 2H, NH_{ind}), 7.49 (d, J = 8.0 Hz, 2H, H4), 7.36 (d, J = 8.0 Hz, 2H, H6), 7.11 (d, J = 2.5 Hz, 2H, H2), 6.96 (dd, $J_1 = J_2$ = 8.0 Hz, 2H, H5), 6.38 (br s, 1H, NHCOCF₃), 4.76 (t, J = 7.0 Hz, 1H, CHCH₂NH), 4.12 (t, J = 7.0 Hz, 2H, CHCH₂NH) ppm. ¹³C NMR (101 MHz, CDCl₃): δ = 157.5 (q, J = 37.0 Hz), 135.5, 127.7, 125.1, 122.7, 121.2, 118.7, 117.0, 115.9 (q, J = 289.0 Hz), 105.17, 43.8, 34.6 ppm. HRMS (m/z): [M+Na]⁺ calcd for C₂₀H₁₄Br₂F₃N₃NaO, 549.9348; found 549.9343 [M+Na]⁺.



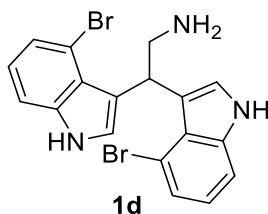
(**Int-1d**): R_f = 0.26 (cyclohexane/EtOAc, 1:1). ¹H NMR (400 MHz, CDCl₃): δ = 8.16 (br s, 2H, NH_{ind}), 7.30 (dd, J_1 = 1.0 Hz, J_2 = 8.0 Hz, 2H, H5), 7.27 (dd, J_1 = 1.0 Hz, J_2 = 8.0 Hz, 2H, H7), 7.02 (dd, $J_1 = J_2$ = 8.0 Hz, 2H, H6), 6.91 (d, J = 2.5 Hz, 2H, H2), 6.61 (br s, 1H, NHCOCF₃), 6.15 (t, J = 7.0 Hz, 1H, CHCH₂NH), 4.13 (t, J = 7.0 Hz, 2H, CHCH₂NH) ppm. ¹³C NMR (101 MHz, CDCl₃): δ = 157.4 (q, J = 37.0 Hz), 138.3, 124.7, 124.6, 124.0, 123.3, 117.8, 116.0 (q, J = 289.0 Hz), 114.3, 110.8, 45.0, 33.4 ppm. HRMS (m/z): [M+Na]⁺ calcd for C₂₀H₁₄Br₂F₃N₃NaO, 549.9348; found 549.9344 [M+Na]⁺.

Synthesis of bisindoles **1c** and **1d**

A mixture of crude trifluoroacetamide derivative **Int-1c** or **Int-1d** and potassium carbonate (2.5 eq, 3.8 mmol, 525 mg) in MeOH (7 mL) and H₂O (0.5 mL) was stirred and heated under reflux for 2 h. The solvent was removed under reduced pressure and water was added (30 mL). The aqueous solution was extracted with CH₂Cl₂ (3 x 30 mL) and the resulting solution was dried with Na₂SO₄ and concentrated in vacuo. The crude material was purified by flash chromatography.



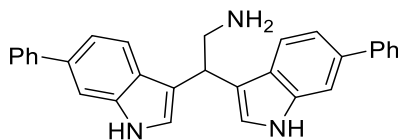
Compound **1c** has been purified by flash column chromatography on silica gel (CH₃Cl/CH₃OH/NH₃, 99:1:1), obtaining a white solid; yield: 55% (182 mg). *R*_f = 0.33 (silica gel; CH₃Cl/CH₃OH/NH₃, 90:10:1). ¹H NMR (400 MHz, DMSO-*d*₆): δ = 11.06 (br s, 2H, NH_{ind}), 7.50 (d, *J* = 8.0 Hz, 2H, H4), 7.29 (d, *J* = 2.0 Hz, 2H, H2), 7.23 (d, *J* = 8.0 Hz, 2H, H6), 6.84 (dd, *J*₁ = *J*₂ = 8.0 Hz, 2H, H5), 4.39 (t, *J* = 7.0 Hz, 1H, CHCH₂NH₂), 3.26 (d, *J* = 7.0 Hz, 2H, CHCH₂NH₂) ppm. ¹³C NMR (101 MHz, DMSO-*d*₆): δ = 134.6, 128.6, 123.7, 123.3, 119.6, 118.5, 118.1, 104.2, 46.5, 38.0 ppm. HRMS (*m/z*): [M+H]⁺ calcd for C₁₈H₁₆Br₂N₃, 431.9705; found 431.9713.



Compound **1d** has been purified by flash column chromatography on silica gel (CH₃Cl/CH₃OH/NH₃, 99:1:1), obtaining an off white solid; yield: 52% (171 mg). *R*_f = 0.23 (silica gel; CH₃Cl/CH₃OH/NH₃, 90:10:1). ¹H NMR (400 MHz, DMSO-*d*₆): δ = 11.15 (br s, 2H, NH_{ind}), 7.36 (dd, *J*₁ = 0.5 Hz, *J*₂ = 8.0 Hz, 2H, H5), 7.11 (dd, *J*₁ = 0.5 Hz, *J*₂ = 8.0 Hz, 2H, H7), 6.98 (br s, 2H, H2), 6.94 (dd, *J*₁ = *J*₂ = 8.0 Hz, H6), 5.71 (t, *J* = 7.0 Hz, 1H, CHCH₂NH₂), 3.16 (d, *J* = 7.0 Hz, 2H, CHCH₂NH₂) ppm. ¹³C NMR (101 MHz, DMSO-*d*₆): δ = 138.3, 124.8, 124.6, 122.8, 122.0, 118.2, 113.3, 111.3, 48.9, 37.0 ppm. HRMS (*m/z*): [M+H]⁺ calcd for C₁₈H₁₆Br₂N₃, 431.9705; found 431.9721.

Experimental data for compounds 3 and 4

2,2-Bis(6-phenyl-1*H*-indol-3-yl)ethan-1-amine (**3aa**).

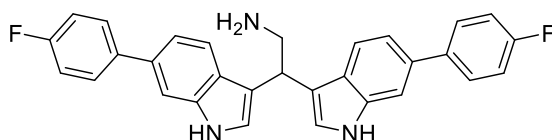


From boronic acid **2a**. Following the general procedure (reaction time = 1 h), compound **3aa** has been purified by flash column chromatography on silica gel ($\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}/\text{NH}_3$, 99:1:1), obtaining a white solid; yield: 79% (34 mg).

From phenylboronic acid pinacol ester **2a'**. Following the general procedure (reaction time = 4 h), compound **3aa** was obtained by flash column chromatography on silica gel ($\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}/\text{NH}_3$, 99:1:1) using phenylboronic acid pinacol ester in place of the corresponding boronic acid; yield: 74% (31 mg).

R_f = 0.38 (silica gel; $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}/\text{NH}_3$, 90:10:1). ^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ = 10.91 (br d, J = 2.5 Hz, 2H, NH_{ind}), 7.63-7.61 (m, 4H, Ar-H), 7.56 (d, J = 8.0 Hz, 2H Ar-H), 7.55 (s, 2H, Ar-H), 7.44-7.40 (m, 4H Ar-H), 7.33-7.25 (m, 4H, Ar-H), 7.19 (dd, J_1 = 1.5 Hz, J_2 = 8.0 Hz, 2H, Ar-H), 4.43 (t, J = 7.0 Hz, 1H, CHCH_2NH_2), 3.30 (d, J = 7.0 Hz, 2H, CHCH_2NH_2), 1.45 (br s, 2H, CHCH_2NH_2) ppm. ^{13}C NMR (101 MHz, $\text{DMSO}-d_6$): δ = 141.8, 137.1, 133.6, 128.8, 126.7, 126.4, 126.3, 123.2, 119.4, 117.5, 116.9, 109.4, 46.7, 38.1 ppm. HRMS (m/z): $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{30}\text{H}_{26}\text{N}_3$, 428.2121; found 428.2121.

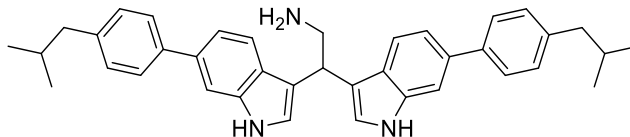
2,2-Bis(6-(4-fluorophenyl)-1*H*-indol-3-yl)ethan-1-amine (**3ab**).



Following the general procedure (reaction time = 45 min), compound **3ab** has been purified by flash column chromatography on silica gel ($\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}/\text{NH}_3$, 99/1/1), obtaining a white solid; yield: 82% (38 mg). R_f = 0.28 (silica gel; $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}/\text{NH}_3$, 90:10:1). ^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ = 10.94 (br s, 2H, NH_{ind}), 7.66-7.62 (m, 4H, Ar-H), 7.55 (d, J = 8.5 Hz, 2H, Ar-H), 7.53 (d, J = 1.5 Hz, 2H, Ar-H), 7.30 (d, J = 2.5 Hz, 2H, Ar-H), 7.26-7.22 (m, 4H, Ar-H), 7.16 (dd, J_1 = 1.5 Hz, J_2 = 8.5 Hz, 2H, Ar-H), 4.43 (t, J = 7.0 Hz, 1H, CHCH_2NH_2), 3.31 (d, J = 7.0 Hz, 2H, CHCH_2NH_2), 1.54 (br s, 2H, CHCH_2NH_2) ^{13}C NMR (101 MHz, $\text{DMSO}-d_6$): δ = 161.3 (d, J = 244.0), 138.3 (d, J = 3.0 Hz), 137.1, 132.3, 128.5 (d, J = 8.0 Hz), 126.3, 123.3,

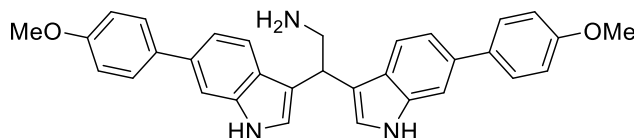
119.5, 117.5, 116.9, 115.5 (d, $J = 21.0$ Hz), 109.4, 46.7, 38.0 ppm. HRMS (m/z): $[M+H]^+$ calcd for $C_{30}H_{24}F_2N_3$, 464.1933; found 464.1937.

2,2-Bis(6-(4-isobutylphenyl)-1*H*-indol-3-yl)ethan-1-amine (3ac).



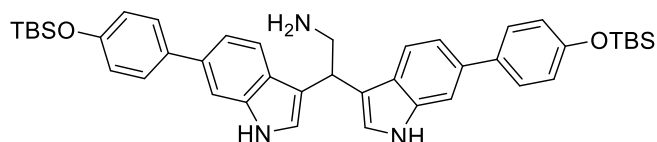
Following the general procedure (reaction time = 2 h), compound **3ac** has been purified by flash column chromatography on silica gel ($CH_2Cl_2/CH_3OH/NH_3$, 99:1:1), obtaining a white solid; yield: 76% (41 mg). $R_f = 0.39$ (silica gel; $CH_2Cl_2/CH_3OH/NH_3$, 90:10:1). ^1H NMR (400 MHz, $DMSO-d_6$): $\delta = 10.88$ (br d, $J = 2.5$ Hz, 2H, NH_{ind}), 7.56-7.50 (m, 8H, Ar-H), 7.28 (d, $J = 2.5$ Hz, 2H, H_{2ind}), 7.22-7.15 (m, 6H, Ar-H), 4.42 (t, $J = 7.0$ Hz, 1H, $CHCH_2NH_2$), 3.30 (d, $J = 6.5$ Hz, 2H, $CHCH_2NH_2$), 2.46 (d, $J = 7.0$ Hz, 4H, $CH_2CH(CH_3)_2$), 1.85 (hept, $J = 7.0$ Hz, 2H, $CH_2CH(CH_3)_2$), 0.89 (d, $J = 6.5$ Hz, 12H, $CH_2CH(CH_3)_2$) ppm. ^{13}C NMR (101 MHz, $DMSO-d_6$): $\delta = 139.3$, 139.2, 137.2, 133.3, 129.4, 126.4, 126.2, 123.1, 119.4, 117.4, 116.8, 109.1, 46.6, 44.2, 37.9, 29.6, 22.2 ppm. HRMS (m/z): $[M+H]^+$ calcd for $C_{38}H_{42}N_3$, 540.3373; found 540.3374.

2,2-Bis(6-(4-methoxyphenyl)-1*H*-indol-3-yl)ethan-1-amine (3ad).



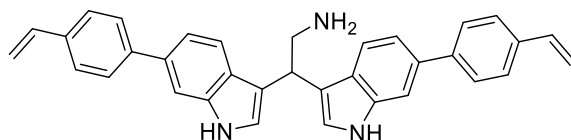
Following the general procedure (reaction time = 2 h), compound **3ad** has been purified by flash column chromatography on silica gel ($CH_2Cl_2/CH_3OH/NH_3$, 99:1:1), obtaining a white solid; yield: 86% (42 mg). $R_f = 0.33$ (silica gel; $CH_2Cl_2/CH_3OH/NH_3$, 90:10:1). ^1H NMR (400 MHz, $DMSO-d_6$): $\delta = 10.85$ (br d, $J = 2.5$ Hz, 2H, NH_{ind}), 7.56-7.50 (m, 6H, Ar-H), 7.48 (s, 2H, Ar-H), 7.25 (d, $J = 2.5$ Hz, 2H, H_{2ind}), 7.14 (dd, $J_1 = 1.5$ Hz, $J_2 = 8.5$ Hz, 2H, Ar-H), 7.01-6.97 (m, 4H, Ar-H), 4.41 (t, $J = 7.0$ Hz, 1H, $CHCH_2NH_2$), 3.77 (s, 6H, OCH_3), 3.30 (d, $J = 7.0$ Hz, 2H, $CHCH_2NH_2$), 1.85 (br s, 2H, $CHCH_2NH_2$) ppm. ^{13}C NMR (101 MHz, $DMSO-d_6$): $\delta = 158.2$, 137.2, 134.2, 133.1, 127.6, 125.9, 122.9, 119.3, 117.3, 116.7, 114.3, 108.8, 55.1, 46.5, 37.9 ppm. HRMS (m/z): $[M+H]^+$ calcd for $C_{32}H_{30}N_3O_2$, 488.2333; found 488.2336.

2,2-Bis(6-{4-[(*tert*-butyldimethylsilyl)oxy]phenyl}-1*H*-indol-3-yl)ethan-1-amine (3ae).



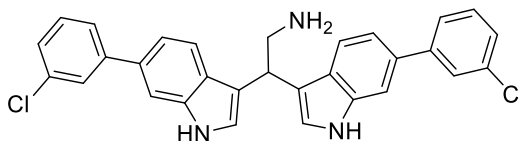
Following the general procedure (reaction time = 1 h), compound **3ae** has been purified by flash column chromatography on silica gel ($\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}/\text{NH}_3$, 99:1:1), obtaining a white solid; yield: 66% (45 mg). $R_f = 0.43$ (silica gel; $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}/\text{NH}_3$, 90:10:1). ^1H NMR (400 MHz, $\text{DMSO}-d_6$): $\delta = 10.86$ (br d, $J = 2.5$ Hz, 2H, NH_{ind}), 7.55–7.47 (m, 8H, Ar-H), 7.26 (d, $J = 2.5$ Hz, 2H, $\text{H}_{2\text{ind}}$), 7.14 (dd, $J_1 = 1.5$ Hz, $J_2 = 8.0$ Hz, 2H, Ar-H), 6.90 (m, 4H, Ar-H), 4.42 (t, $J = 7.0$ Hz, 1H, CHCH_2NH_2), 3.31 (d, $J = 7.0$ Hz, 2H, CHCH_2NH_2), 0.97 (s, 18H, Si-*t*-Bu), 0.21 (s, 12H, $\text{Si}(\text{CH}_3)_2$) ppm. ^{13}C NMR (101 MHz, $\text{DMSO}-d_6$): $\delta = 154.0, 137.2, 135.1, 133.0, 127.7, 125.9, 123.0, 120.1, 119.3, 117.3, 116.7, 108.9, 46.5, 37.7, 25.6, 18.0, -4.5$. HRMS (m/z): $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{42}\text{H}_{54}\text{N}_3\text{O}_2\text{Si}_2$, 688.3749; found 688.3740.

2,2-Bis[6-(4-vinylphenyl)-1H-indol-3-yl]ethan-1-amine (3af).



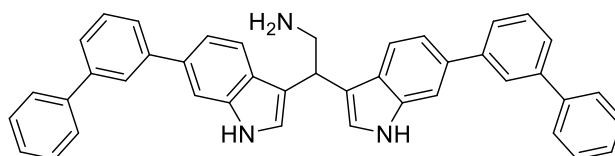
Following the general procedure (reaction time = 1h), compound **3af** has been purified by flash column chromatography on silica gel (dichloromethane/ammonia, 99/1 to $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}/\text{NH}_3$, 99:1:1), obtaining a yellow solid; yield: 29% (14 mg). $R_f = 0.32$ (silica gel; $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}/\text{NH}_3$, 90:10:1). ^1H NMR (400 MHz, CDCl_3): $\delta = 8.12$ (br s, 2H, NH_{ind}), 7.66 (d, $J = 8.5$ Hz, 2H, Ar-H), 7.61–7.55 (m, 6H, Ar-H), 7.48–7.46 (m, 4H, Ar-H), 7.33 (dd, $J_1 = 1.5$ Hz, $J_2 = 8.5$ Hz, 2H, Ar-H), 7.10 (br s, 2H, $\text{H}_{2\text{ind}}$), 6.76 (dd, $J_1 = 11.0$ Hz, $J_2 = 17.5$ Hz, 2H, $\text{CH}=\text{CH}_2$), 5.78 (dd, $J_1 = 1.0$ Hz, $J_2 = 17.5$ Hz, 2H, $\text{CH}=\text{CH}_2$), 5.26 (dd, $J_1 = 1.0$ Hz, $J_2 = 11.0$ Hz, 2H, $\text{CH}=\text{CH}_2$), 4.60 (t, $J = 7.0$ Hz, 1H, CHCH_2NH_2), 3.51 (d, $J = 7.0$ Hz, 2H, CHCH_2NH_2) ppm. ^{13}C NMR (101 MHz, CDCl_3): $\delta = 141.8, 137.4, 136.7, 136.1, 135.3, 127.5, 126.7, 126.6, 122.8, 120.0, 119.2, 117.9, 113.7, 109.8, 46.9, 38.5$ ppm. HRMS (m/z): $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{34}\text{H}_{30}\text{N}_3$, 480.2434; found 480.2434.

2,2-Bis[6-(3-chlorophenyl)-1H-indol-3-yl]ethan-1-amine (3ag).



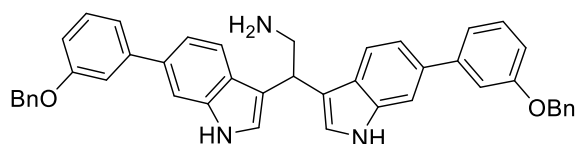
Following the general procedure (reaction time = 30 min), compound **3ag** has been purified by flash column chromatography on silica gel (CH₂Cl₂/CH₃OH/NH₃, 99:1:1), obtaining a white solid; yield: 73% (36 mg). *R*_f = 0.51 (silica gel; CH₂Cl₂/CH₃OH/NH₃, 90:10:1). ¹H NMR (400 MHz, DMSO-*d*₆): δ = 10.91 (br s, 2H, NH_{ind}), 7.65 (s, 2H, Ar-H), 7.61-7.54 (m, 6H, Ar-H), 7.44 (dd, *J*₁ = *J*₂ = 8.0 Hz, 2H, Ar-H), 7.36-7.31 (m, 4H, Ar-H), 7.21 (dd, *J*₁ = 1.5, *J*₂ = 7.5 Hz, 2H, Ar-H), 4.44 (t, *J* = 7.0 Hz, 1H, CHCH₂NH₂), 3.31 (d, *J* = 7.0 Hz, 2H, CHCH₂NH₂), 1.49 (br s, 2H, CHCH₂NH₂) ppm. ¹³C NMR (101 MHz, DMSO-*d*₆): δ = 144.0, 137.0, 133.5, 131.6, 130.6, 126.8, 126.3, 126.1, 125.3, 123.8, 119.5, 117.5, 116.9, 109.7, 46.7, 38.0 ppm. HRMS (*m/z*): [M+H]⁺ calcd for C₃₀H₂₄Cl₂N₃, 496.1342; found 496.1339.

2,2-Bis(6-([1,1'-biphenyl]-3-yl)-1*H*-indol-3-yl)ethan-1-amine (3ah).



Following the general procedure (reaction time = 1 h), compound **3ah** has been purified by flash column chromatography on silica gel (CH₂Cl₂/CH₃OH/NH₃, 99:1:1), obtaining a white solid; yield: 90% (52 mg). *R*_f = 0.37 (silica gel; CH₂Cl₂/CH₃OH/NH₃, 90:10:1). ¹H NMR (400 MHz, DMSO-*d*₆): δ = 10.93 (br d, *J* = 2.5 Hz, 2H, NH_{ind}), 7.65 (dd, *J*₁ = *J*₂ = 1.5 Hz, 2H, Ar-H), 7.64-7.55 (m, 4H, Ar-H), 7.65 (d, *J* = 1.5 Hz, 2H, Ar-H), 7.64-7.54 (m, 6H, Ar-H), 7.53 (d, *J* = 7.5 Hz, 2H, Ar-H), 7.50-7.45 (m, 4H, Ar-H), 7.41-7.35 (m, 2H, Ar-H), 7.32 (d, *J* = 2.5 Hz, 2H, H_{2ind}), 7.29 (dd, *J*₁ = 1.5 Hz, *J*₂ = 8.0 Hz, 2H, Ar-H), 4.47 (t, *J* = 7.0 Hz, 1H, CHCH₂NH₂), 3.34 (d, *J* = 7.0 Hz, 2H, CHCH₂NH₂) ppm. ¹³C NMR (101 MHz, DMSO-*d*₆): δ = 142.5, 140.8, 140.4, 137.1, 133.2, 129.4, 128.9, 127.5, 126.9, 126.5, 125.9, 125.1, 124.9, 123.4, 119.5, 117.7, 116.9, 109.6, 46.6, 37.9 ppm. HRMS (*m/z*): [M+H]⁺ calcd for C₄₂H₃₄N₃, 580.2747; found 580.2753.

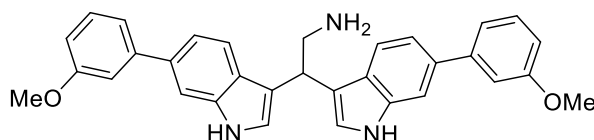
2,2-Bis{6-[3-(benzyloxy)phenyl]-1*H*-indol-3-yl}ethan-1-amine (3ai).



Following the general procedure (reaction time = 1 h), compound **3ai** has been purified by flash column chromatography on silica gel (CH₂Cl₂/CH₃OH/NH₃, 99:1:1),

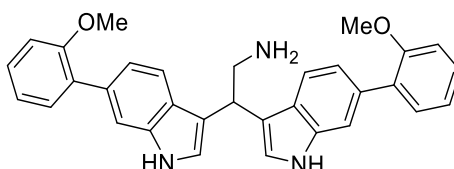
obtaining a white solid; yield: 80% (51 mg). $R_f = 0.37$ (silica gel; $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}/\text{NH}_3$, 90:10:1). ^1H NMR (400 MHz, $\text{DMSO}-d_6$): $\delta = 10.91$ (br d, $J = 2.5$ Hz, 2H, NH_{ind}), 7.56-7.52 (m, 4H, Ar-H), 7.50-7.45 (m, 4H, Ar-H), 7.42-7.37 (m, 4H, Ar-H), 7.35-7.30 (m, 4H, Ar-H), 7.30 (d, $J = 2.5$ Hz, 2H, $\text{H}_{2\text{ind}}$), 7.24-7.16 (m, 6H, Ar-H), 6.93 (ddd, $J_1 = 1.0$ Hz, $J_2 = 2.5$ Hz, $J_3 = 8.0$ Hz, 2H, Ar-H), 5.17 (s, 4H, OCH_2Ph), 4.42 (t, 7.0 Hz, 1H, CHCH_2NH_2), 3.30 (d, $J = 7.0$ Hz, 2H, CHCH_2NH_2), 1.48 (br s, 2H, CHCH_2NH_2) ppm. ^{13}C NMR (101 MHz, $\text{DMSO}-d_6$): $\delta = 158.8, 143.3, 137.2, 137.0, 133.1, 129.8, 128.4, 127.8, 127.7, 126.5, 123.3, 119.4, 119.3, 117.6, 116.9, 113.1, 112.9, 109.5, 69.2, 46.7, 38.1$ ppm. HRMS (m/z): $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{44}\text{H}_{38}\text{N}_3\text{O}_2$, 640.2959; found 640.2955.

2,2-Bis[6-(3-methoxyphenyl)-1H-indol-3-yl]ethan-1-amine (3aj).



Following the general procedure (reaction time = 2 h), compound **3aj** has been purified by flash column chromatography on silica gel ($\text{CH}_3\text{Cl}/\text{CH}_3\text{OH}/\text{NH}_3$, 99:1:1), obtaining a white solid; yield: 82% (40 mg). $R_f = 0.36$ (silica gel; $\text{CH}_3\text{Cl}/\text{CH}_3\text{OH}/\text{NH}_3$, 90:10:1). ^1H NMR (400 MHz, $\text{DMSO}-d_6$): $\delta = 10.91$ (br d, $J = 2.5$ Hz, 2H, NH_{ind}), 7.56-7.51 (m, 4H, Ar-H), 7.33 (dd, $J_1 = J_2 = 8.0$ Hz, 2H, Ar-H), 7.30 (d, $J = 2.5$ Hz, 2H, $\text{H}_{2\text{ind}}$), 7.21-7.16 (m, 4H, Ar-H), 7.13 (dd, $J_1 = J_2 = 2.0$ Hz, 2H, Ar-H), 6.86 (ddd, $J_1 = 1.0$ Hz, $J_2 = 2.5$ Hz, $J_3 = 8.0$ Hz, 2H, Ar-H), 4.42 (t, $J = 7.0$ Hz, 1H, CHCH_2NH_2), 3.80 (s, 6H, OCH_3), 3.30 (d, $J = 7.0$ Hz, 2H, CHCH_2NH_2) ppm. ^{13}C NMR (101 MHz, $\text{DMSO}-d_6$): $\delta = 159.6, 143.3, 137.0, 133.2, 129.8, 126.5, 123.3, 119.4, 119.1, 117.6, 116.9, 112.2, 111.9, 109.5, 55.0, 46.6, 38.0$ ppm. HRMS (m/z): $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{32}\text{H}_{30}\text{N}_3\text{O}_2$, 488.2333; found 488.2340.

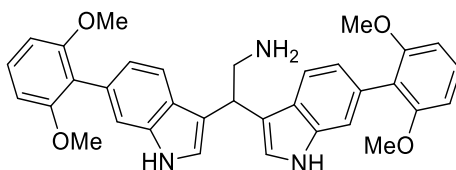
2,2-Bis[6-(2-methoxyphenyl)-1H-indol-3-yl]ethan-1-amine (3ak).



Following the general procedure (reaction time = 2 h), compound **3ak** has been purified by flash column chromatography on silica gel ($\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}/\text{NH}_3$, 99:1:1), obtaining a white solid; yield: 76% (37 mg). $R_f = 0.38$ (silica gel; $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}/\text{NH}_3$,

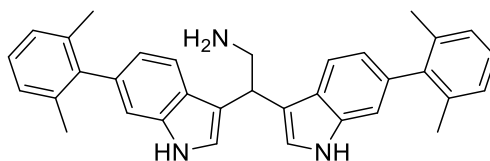
90:10:1). ^1H NMR (400 MHz, $\text{DMSO-}d_6$): δ = 10.82 (br d, J = 2.5 Hz, 2H, NH_{ind}), 7.50 (d, J = 8.5 Hz, 2H, Ar-H), 7.41 (dd, J_1 = 0.5 Hz, J_2 = 1.5 Hz, 2H, Ar-H), 7.30-7.25 (m, 6H, Ar-H), 7.07 (d, J = 8.0 Hz, 2H, Ar-H), 7.02-6.96 (m, 4H, Ar-H), 4.41 (t, J = 7.0 Hz, 1H, CHCH_2NH_2), 3.72 (s, 6H, OCH_3), 3.30 (d, J = 7.0 Hz, 2H, CHCH_2NH_2) ppm. ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$): δ = 156.2, 136.4, 131.3, 130.8, 130.6, 127.9, 125.8, 122.7, 120.7, 120.0, 118.3, 116.8, 112.1, 111.7, 55.4, 46.6, 38.1 ppm. HRMS (m/z): $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{32}\text{H}_{30}\text{N}_3\text{O}_2$, 488.2333; found 488.2333.

2,2-Bis[6-(2,6-dimethoxyphenyl)-1H-indol-3-yl]ethan-1-amine (3al).



Following the general procedure (reaction time = 20 min), compound **3al** has been purified by flash column chromatography on silica gel ($\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}/\text{NH}_3$, 99:1:1), obtaining a white solid; yield: 58% (32 mg). R_f = 0.44 silica gel; ($\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}/\text{NH}_3$, 90:10:1). ^1H NMR (400 MHz, $\text{DMSO-}d_6$): δ = 10.70 (br d, J = 2.5 Hz, 2H, NH_{ind}), 7.49 (d, J = 8.0 Hz, 2H, Ar-H), 7.26 (dd, $J_1 = J_2$ = 8.5 Hz, 2H, Ar-H), 7.24 (s, 2H, Ar-H), 7.11 (dd, J_1 = 0.5 Hz, J_2 = 1.5 Hz, 2H, Ar-H), 6.73 (dd, J_1 = 1.5 Hz, J_2 = 8.0 Hz, 2H, Ar-H), 6.72-6.69 (m, 4H, Ar-H), 4.41 (t, J = 7.0 Hz, 1H, CHCH_2NH_2), 3.62 (s, 12H, OCH_3), 3.31 (d, J = 7.0 Hz, 2H, CHCH_2NH_2) ppm. ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$): δ = 157.4, 136.3, 128.2, 126.5, 125.5, 122.1, 121.3, 120.1, 117.8, 116.8, 113.4, 104.3, 55.5, 46.6, 38.0 ppm. HRMS (m/z): $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{34}\text{H}_{34}\text{N}_3\text{O}_4$, 548.2544; found 548.2564.

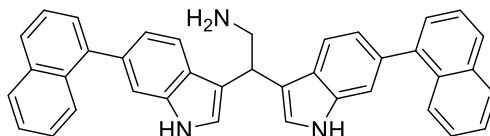
2,2-Bis(6-(2,6-dimethylphenyl)-1H-indol-3-yl)ethan-1-amine (3am).



Following the general procedure (reaction time = 4 h), compound **3am** has been purified by flash column chromatography on silica gel ($\text{CH}_2\text{Cl}_2/\text{NH}_3$, 99:1 to $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}/\text{NH}_3$, 99:1:1), obtaining an off white solid; yield: 23% (11 mg). R_f = 0.56 (silica gel; $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}/\text{NH}_3$, 90:10:1). ^1H NMR (400 MHz, $\text{DMSO-}d_6$): δ = 10.83 (br s, 2H, NH_{ind}), 7.66 (d, J = 8.0 Hz, 2H, Ar-H), 7.31 (d, J = 2.5 Hz, 2H, Ar-H), 7.14-7.07 (m, 6H, Ar-H), 7.05 (s, 2H, Ar-H), 6.67 (dd, J_1 = 1.5 Hz, J_2 = 8.0 Hz, 2H, Ar-H), 4.47 (t,

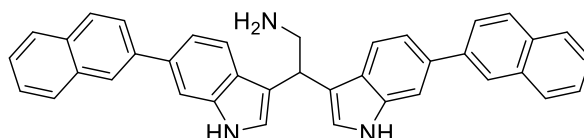
$J = 7.5$ Hz, 1H, CHCH_2NH_2), 3.34 (d, $J = 7.5$ Hz, 2H, CHCH_2NH_2), 1.96 (s, 6H, CH_3), 1.95 (s, 6H, CH_3) ppm. ^{13}C NMR (101 MHz, $\text{DMSO}-d_6$): $\delta = 142.6, 136.7, 135.6, 133.0, 127.1, 126.5, 125.6, 122.4, 119.2, 119.1, 117.0, 111.2, 46.8, 38.0, 20.8, 20.7$ ppm. HRMS (m/z): $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{34}\text{H}_{34}\text{N}_3$, 484.2747; found 484.2750.

2,2-Bis[6-(naphthalen-1-yl)-1H-indol-3-yl]ethan-1-amine (3an).



Following the general procedure (reaction time = 15 min), compound **3an** has been purified by flash column chromatography on silica gel ($\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}/\text{NH}_3$, 99:1:1), obtaining an off white solid; yield: 93% (49 mg). $R_f = 0.35$ (silica gel; $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}/\text{NH}_3$, 90:10:1). ^1H NMR (400 MHz, CDCl_3): $\delta = 8.19$ (br s, 2H, NH_{ind}), 8.00 (d, $J = 8.5$ Hz, 2H, Ar-H), 7.91 (d, $J = 8.0$ Hz, 2H, Ar-H), 7.85 (d, $J = 8.0$ Hz, 2H, Ar-H), 7.75 (d, $J = 8.0$ Hz, 2H, Ar-H), 7.54-7.44 (m, 8H, Ar-H), 7.38 (dd, $J_1 = J_2 = 8.0$ Hz, 2H, Ar-H), 7.23 (dd, $J_1 = 1.5$ Hz, $J_2 = 8.0$ Hz, 2H, Ar-H), 7.15 (d, $J = 2.0$ Hz, 2H, Ar-H), 4.69 (t, $J = 7.0$ Hz, 1H, CHCH_2NH_2), 3.58 (d, $J = 7.0$ Hz, 2H, CHCH_2NH_2) ppm. ^{13}C NMR (101 MHz, CDCl_3): $\delta = 141.3, 136.9, 134.9, 134.0, 132.2, 128.3, 127.3$ (2C), 126.6, 126.4, 126.0, 125.8, 125.5, 122.7, 122.2, 119.3, 117.8, 112.8, 46.9, 38.6 ppm. HRMS (m/z): $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{38}\text{H}_{30}\text{N}_3$, 528.2434; found 528.2445.

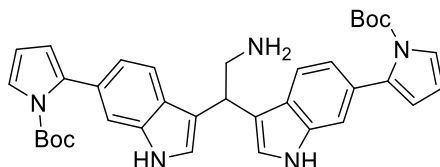
2,2-Bis[6-(naphthalen-2-yl)-1H-indol-3-yl]ethan-1-amine (3ao).



Following the general procedure (reaction time = 2 h), compound **3ao** has been purified by flash column chromatography on silica gel ($\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}/\text{NH}_3$, 99:1:1), obtaining a white solid; yield: 75% (40 mg). $R_f = 0.29$ (silica gel; $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}/\text{NH}_3$, 90:10:1). ^1H NMR (400 MHz, $\text{DMSO}-d_6$): $\delta = 10.99$ (br d, $J = 2.5$ Hz, 2H, NH_{ind}), 8.15 (d, $J = 2.0$ Hz, 2H, Ar-H), 7.99-7.95 (m, 4H, Ar-H), 7.91 (d, $J = 8.0$ Hz, 2H, Ar-H), 7.84 (dd, $J_1 = 2.0$ Hz, $J_2 = 8.5$ Hz, 2H, Ar-H), 7.72 (d, $J = 1.5$ Hz, 2H, Ar-H), 7.63 (d, $J = 8.5$ Hz, 2H, Ar-H), 7.54-7.45 (m, 4H, Ar-H), 7.38 (dd, $J_1 = 1.5$ Hz, $J_2 = 8.5$ Hz, 2H, Ar-H), 7.35 (d, $J = 2.5$ Hz, 2H, $\text{H}_{2\text{ind}}$), 4.51 (t, $J = 7.0$ Hz, 1H, CHCH_2NH_2), 3.33 (d, $J = 7.0$ Hz, 2H, CHCH_2NH_2) ppm. ^{13}C NMR (101 MHz, $\text{DMSO}-d_6$): $\delta = 139.6, 137.7, 134.0, 133.5,$

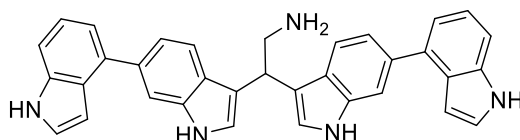
132.3, 128.7, 128.5, 127.9, 127.0, 126.7, 126.1 (2C), 125.1, 124.0, 120.0, 118.3, 117.2, 110.3, 47.0, 38.1 ppm. HRMS (m/z): $[M+H]^+$ calcd for $C_{38}H_{30}N_3$, 528.2434; found 528.2442.

Di-*tert*-butyl 2,2'-[(2-aminoethane-1,1-diyl)bis(1*H*-indole-3,6-diyl)]bis(1*H*-pyrrole-1-carboxylate) (3ap).



Following the general procedure (reaction time = 15 min), compound **3ap** has been purified by flash column chromatography on silica gel ($CH_3Cl/CH_3OH/NH_3$, 99:1:1), obtaining a white solid; yield: 91% (55 mg). R_f = 0.42 (silica gel; $CH_3Cl/CH_3OH/NH_3$, 90:10:1). ^1H NMR (400 MHz, $CDCl_3$): δ = 8.07 (br s, 2H, NH_{ind}), 7.49 (d, J = 8.5 Hz, 2H, H_{4ind}), 7.35 (dd, J_1 = 0.5 Hz, J_2 = 1.5 Hz, 2H, H_{7ind}), 7.33 (dd, J_1 = 2.0 Hz, J_2 = 3.5 Hz, 2H, H_{4pyr}), 7.03 (dd, J_1 = 1.5 Hz, J_2 = 8.5 Hz, 2H, H_{5ind}), 6.85 (d, J = 2.0 Hz, 2H, H_{2ind}), 6.22 (dd, $J_1 = J_2$ = 3.5 Hz, 2H, H_{3pyr}), 6.17 (dd, J_1 = 2.0, J_2 = 3.5 Hz, 2H, H_{2pyr}), 4.50 (t, J = 7.0 Hz, 1H, $CHCH_2NH_2$), 3.41 (d, J = 7.0 Hz, 2H, $CHCH_2NH_2$), 1.32 (s, 18H, Boc) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ = 149.6, 136.4, 136.3, 128.2, 126.4, 122.6, 122.3, 121.5, 118.6, 117.7, 114.3, 111.8, 110.6, 83.5, 46.9, 38.4, 27.8 ppm. HRMS (m/z): $[M+H]^+$ calcd for $C_{36}H_{40}N_5O_4$, 606.3075; found 606.3087.

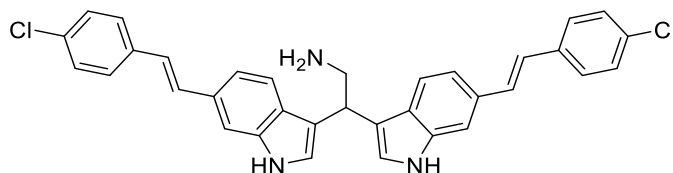
2,2-Di[1*H*,1'*H*-(4,6'-biindol)-3'-yl]ethan-1-amine (3aq).



Following the general procedure (reaction time = 4 h), compound **3aq** has been purified by flash column chromatography on silica gel ($CH_2Cl_2/CH_3OH/NH_3$, 99:1:1), obtaining a white solid; yield: 55% (28 mg). R_f = 0.37 (silica gel; $CH_2Cl_2/CH_3OH/NH_3$, 90:10:1). ^1H NMR (400 MHz, $DMSO-d_6$): δ = 11.18 (br s, 2H, NH_{ind}), 10.88 (br d, J = 2,5 Hz, 2H, NH_{ind}), 7.65 (d, J = 8.5 Hz, 2H, Ar-H), 7.62 (d, J = 1.5 Hz, 2H, Ar-H), 7.37 (dd, $J_1 = J_2$ = 3.0 Hz, 2H, Ar-H), 7.35 (ddd, $J_1 = J_2$ = 1.5 Hz, J_3 = 8.0 Hz, 2H, Ar-H), 7.32 (d, J = 2,5 Hz, 2H, Ar-H), 7.25 (dd, J_1 = 1.5 Hz, J_2 = 8.0 Hz, 2H, Ar-H), 7.15 (dd, $J_1 = J_2$ = 7.5 Hz, 2H, Ar-H), 7.06 (dd, J_1 = 1.0 Hz, J_2 = 7.5 Hz, 2H, Ar-H), 6.60-6.57 (m, 2H, Ar-

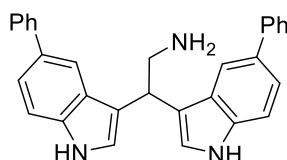
H), 4.48 (t, $J = 7.0$ Hz, 1H, CHCH_2NH_2), 3.35 (d, $J = 7.0$ Hz, 2H, CHCH_2NH_2) ppm. ^{13}C NMR (101 MHz, $\text{DMSO}-d_6$): $\delta = 137.0, 136.4, 134.6, 134.0, 126.0, 125.8, 125.4, 122.9, 121.3, 119.1, 119.0, 118.5, 116.9, 110.9, 109.9, 100.5, 46.7, 38.1$ ppm. HRMS (m/z): $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{34}\text{H}_{28}\text{N}_5$, 506.2339; found 506.2347.

2,2-Bis{6-[(*E*)-4-chlorostyryl]-1*H*-indol-3-yl}ethan-1-amine (3ar**).**



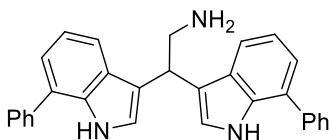
Following the general procedure (reaction time = 25 min), compound **3ar** has been purified by flash column chromatography on silica gel ($\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}/\text{NH}_3$, 99:1:1), obtaining a yellow solid; yield: 78% (43 mg). $R_f = 0.29$ (silica gel; $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}/\text{NH}_3$, 90:10:1). ^1H NMR (600 MHz, $\text{DMSO}-d_6$): $\delta = 10.96$ (br d, $J = 2.5$ Hz, 2H, NH_{ind}), 7.62-7.59 (m, 4H, Ar-H), 7.51-7.48 (m, 4H, Ar-H), 7.41-7.38 (m, 4H, Ar-H), 7.34 (d, $J = 16.5$ Hz, 2H, $\text{CH}=\text{CH}$), 7.28 (d, $J = 2.5$ Hz, 2H, $\text{H}_{2\text{ind}}$), 7.24 (dd, $J_1 = 1.5$ Hz, $J_2 = 8.5$ Hz, 2H, Ar-H), 7.12 (d, $J = 16.5$ Hz, 2H, $\text{CH}=\text{CH}$), 4.43 (t, $J = 7.0$ Hz, 1H, CHCH_2NH_2), 3.29 (d, $J = 7.0$ Hz, 2H, CHCH_2NH_2) ppm. ^{13}C NMR (151 MHz, $\text{DMSO}-d_6$): $\delta = 136.8, 136.4, 131.1, 131.0, 129.8, 128.9, 127.7, 127.0, 124.1, 123.7, 119.1, 117.1, 116.8, 110.5, 46.4, 37.3$ ppm. HRMS (m/z): $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{34}\text{H}_{28}\text{Cl}_2\text{N}_3$, 548.1655; found 548.1652.

2,2-Bis(5-phenyl-1*H*-indol-3-yl)ethan-1-amine (3ba**).**



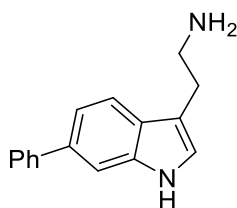
Following the general procedure (reaction time = 2 h), compound **3ba** has been purified by flash column chromatography on silica gel ($\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}/\text{NH}_3$, 99:1:1), obtaining a white solid; yield: 70% (30 mg). $R_f = 0.37$ (silica gel; $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}/\text{NH}_3$, 90:10:1). ^1H NMR (400 MHz, CDCl_3): $\delta = 8.10$ (br s, 2H, NH_{ind}), 7.83 (s, 2H, $\text{H}_{4\text{ind}}$), 7.60-7.55 (m, 4H, Ar-H), 7.43-7.36 (m, 8H, Ar-H), 7.29 (d, $J = 7.5$ Hz, 2H, Ar-H), 7.06 (br s, 2H, $\text{H}_{2\text{ind}}$), 4.66 (t, $J = 7.0$ Hz, 1H, CHCH_2NH_2), 3.51 (d, $J = 7.0$ Hz, 2H, CHCH_2NH_2) ppm. ^{13}C NMR (101 MHz, CDCl_3): $\delta = 142.6, 136.3, 133.1, 128.8, 127.6, 127.5, 126.5, 122.9, 122.1, 118.2, 117.9, 111.6, 46.5, 38.1$ ppm. HRMS (m/z): $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{30}\text{H}_{26}\text{N}_3$, 428.2121; found 428.2127.

2,2-Bis(7-phenyl-1*H*-indol-3-yl)ethan-1-amine (3ca).



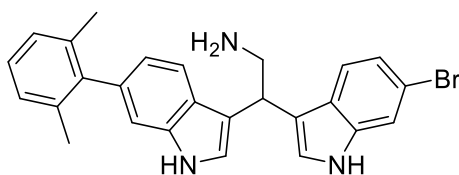
Following the general procedure (reaction time = 1.5 h), compound **3ca** has been purified by flash column chromatography on silica gel (CH₂Cl₂/CH₃OH/NH₃, 99:1:1), obtaining an off white solid; yield: 89% (38 mg). *R_f* = 0.39 (silica gel; CH₂Cl₂/CH₃OH/NH₃, 90:10:1). ¹H NMR (400 MHz, CDCl₃): δ = 8.27 (br s, 2H, NH_{ind}), 7.64-7.59 (m, 6H, Ar-H), 7.51-7.46 (m, 4H, Ar-H), 7.41-7.36 (m, 2H, Ar-H), 7.19 (dd, *J*₁ = 1.5 Hz, *J*₂ = 7.5 Hz, 2H, H_{4ind}), 7.14 (dd, *J*₁ = *J*₂ = 7.5 Hz, 2H, H_{5ind}), 7.06 (d, *J* = 2.5 Hz, 2H, H_{2ind}), 4.64 (t, *J* = 7.0 Hz, 1H, CHCH₂NH₂), 3.50 (d, *J* = 7.0 Hz, 2H, CHCH₂NH₂) ppm. ¹³C NMR (101 MHz, CDCl₃): δ = 139.3, 134.6, 129.3, 128.4, 127.6, 127.5, 125.8, 122.3, 122.1, 120.0, 119.0, 118.3, 46.9, 38.6 ppm. HRMS (*m/z*): [M+H]⁺ calcd for C₃₀H₂₆N₃, 428.2121; found 428.2121.

2-(6-Phenyl-1*H*-indol-3-yl)ethan-1-amine (3ea).



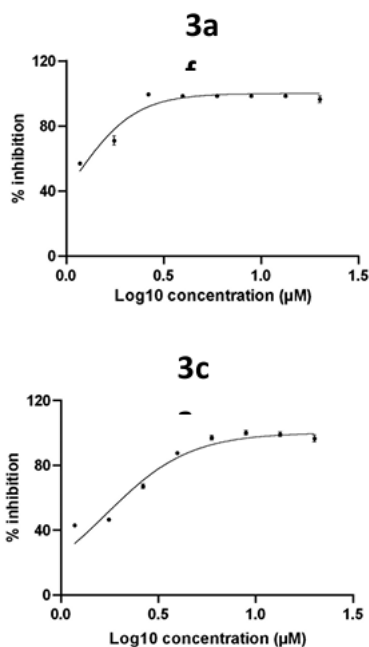
Following the general procedure (reaction time = 2 h), compound **3ea** has been purified by flash column chromatography on silica gel (CH₂Cl₂/CH₃OH/NH₃, 99:1:1), obtaining a white solid; yield: 51% (12 mg). *R_f* = 0.30 (silica gel; CH₂Cl₂/CH₃OH/NH₃, 90:10:1). ¹H NMR (400 MHz, DMSO-*d*₆): δ = 10.89 (br s, 1H, NH_{ind}), 7.67-7.63 (m, 2H, Ar-H), 7.59 (d, *J* = 8.5 Hz, 1H, H_{4ind}), 7.57 (d, *J* = 1.5 Hz, 1H, H_{7ind}), 7.47-7.41 (m, 2H, Ar-H), 7.33-7.28 (m, 1H), 7.28 (dd, *J*₁ = 8.0 Hz, *J*₂ = 1.5 Hz, 1H, H_{5ind}), 7.18 (d, *J* = 2.0 Hz, 1H, H_{2ind}), 2.87-2.80 (m, 2H, CH₂-CH₂-NH₂), 2.80-2.72 (m, 2H, CH₂-CH₂-NH₂) ppm. ¹³C NMR (101 MHz, DMSO-*d*₆): δ = 141.8, 136.9, 133.3, 128.8, 126.9, 126.7, 126.4, 123.6, 118.8, 117.6, 112.6, 109.4, 42.7, 29.5 ppm. HRMS (*m/z*): [M+H]⁺ calcd for C₁₆H₁₇N₂, 237.1386; found 237.1385.

2-(6-Bromo-1*H*-indol-3-yl)-2-[6-(2,6-dimethylphenyl)-1*H*-indol-3-yl]ethan-1-amine (4am).



Following the general procedure (reaction time = 4 h), compound **4am** has been purified by flash column chromatography on silica gel (CH₂Cl₂/NH₃, 99:1 to CH₂Cl₂/CH₃OH/NH₃, 99:1:1), obtaining a white solid; yield: 27% (13 mg). R_f = 0.44 (silica gel; CH₂Cl₂/CH₃OH/NH₃, 90:10:1). ¹H NMR (400 MHz, DMSO-*d*₆): δ = 11.00 (br s, 1H, NH_{ind}), 10.81 (br s, 1H, NH_{ind}), 7.55 (d, J = 8.0 Hz, 1H, H_{4ind}), 7.53 (d, J = 8.5 Hz, 1H, H_{4ind}), 7.51 (d, J = 2.0 Hz, 1H, H_{2ind}), 7.32 (d, J = 2.0 Hz, 1H, H_{7ind}), 7.24 (d, J = 2.5 Hz, 1H, H_{7ind}), 7.13-7.02 (m, 5H, Ar-H), 6.63 (dd, J_1 = 1.5 Hz, J_2 = 8.0 Hz, 1H, H_{5ind}), 4.41 (t, J = 7.0 Hz, 1H, CHCH₂NH₂), 3.28 (d, J = 7.0 Hz, 2H, CHCH₂NH₂), 1.95 (s, 3H, CH₃), 1.94 (s, 3H, CH₃) ppm. ¹³C NMR (101 MHz, DMSO-*d*₆): δ = 142.6, 137.3, 136.7, 135.7, 135.6, 133.1, 127.1, 126.5, 126.0, 125.5, 123.5, 122.4, 120.9, 120.7, 119.0, 117.3, 116.7, 113.9, 113.6, 111.2, 46.7, 37.8, 20.79, 20.76 ppm. HRMS (m/z): [M+H]⁺ calcd for C₂₆H₂₅BrN₃, 458.1226; found 458.1233.

Dose-response curves of **3af**, **3ca** and **3aq** on *L. infantum* promastigotes.



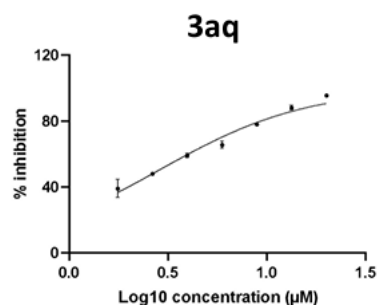


Figure 18. Dose-response curves of **3af**, **3ca** and **3aq** on *L. infantum* promastigotes. The curves represent the % inhibition of *L. infantum* parasites at increasing concentrations of each compound.

Dose-response curves of 3af, 3ca and 3aq on THP-1 cells.

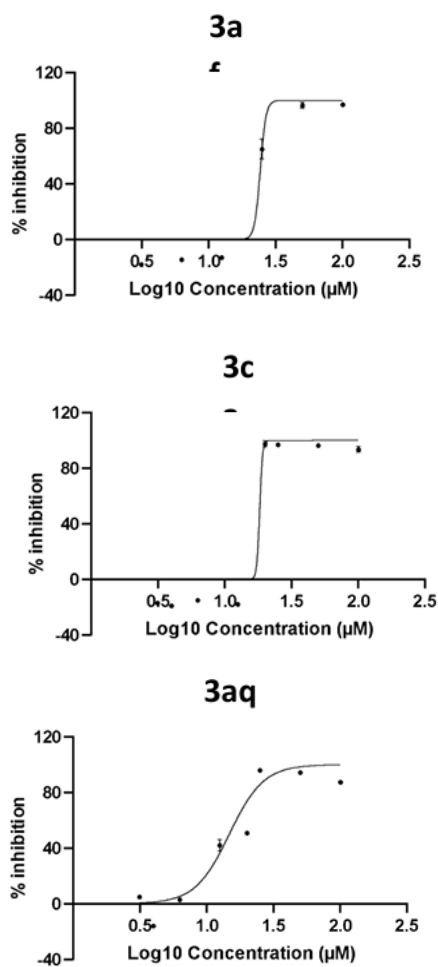


Figure 19. Dose-response curves of **3af**, **3ca** and **3aq** on THP-1 cells. The curves represent the % inhibition of THP-1 viability at increasing concentrations of each compound.

Representative phase-contrast images of THP-1 cells infected and treated with compounds 3af, 3aq, 3ca and miltefosine.

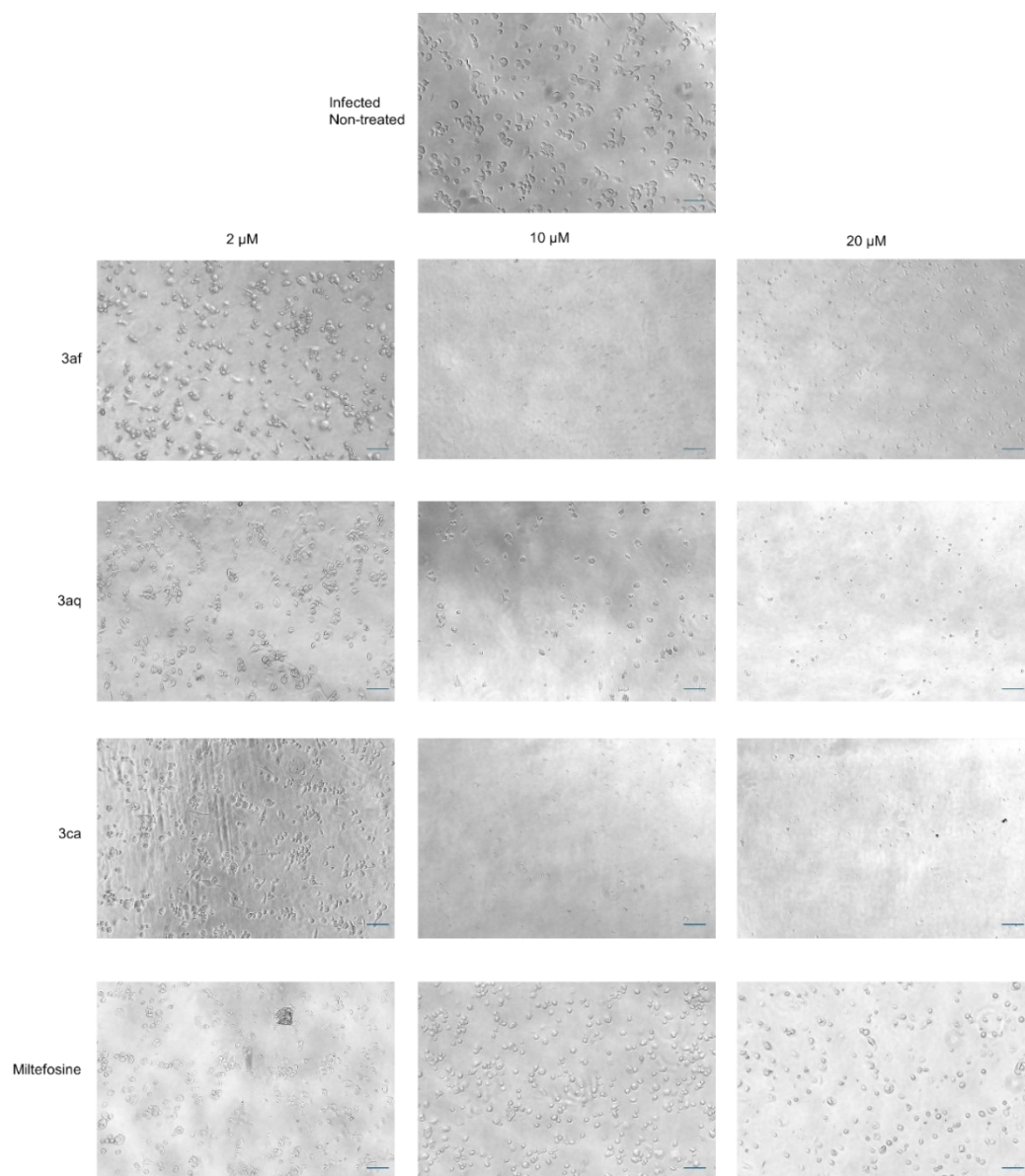


Figure 20. Representative phase-contrast images of THP-1 cells infected and treated with compounds **3af**, **3aq**, **3ca** and miltefosine at the indicated concentrations. Scale bar 100 μ m.

2.5 References

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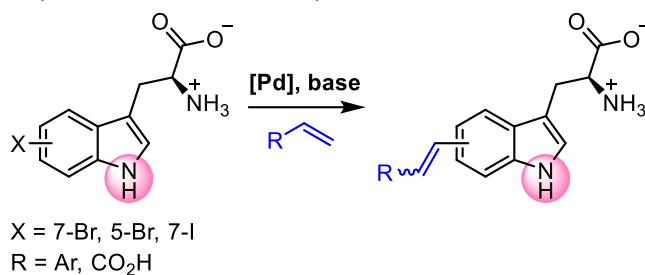
Chapter 3. Mizoroki-Heck Diversification of 2,2-Bis(6-bromo-1*H*-indol-3-yl)ethanamine for the Development of Antileishmanial Hit Compounds

3.1 Introduction

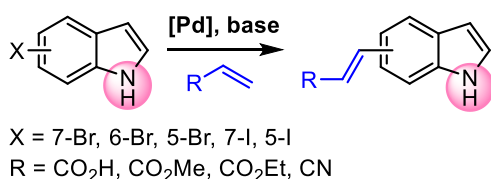
Cross-coupling reactions [1] have revolutionized the field of organic synthesis by enabling the formation of C–C bonds. Among these, the Mizoroki–Heck reaction (or simply the Heck reaction) is one of the most extensively studied and essentially consists of the arylation (or vinylation) of an alkene with an organic halide in the presence of transition metals [2]. A major challenge in the Heck reaction, as well as in other cross-coupling processes, is the development of new methodologies that tolerate a wide variety of functional groups. This is particularly relevant because many medicinal compounds contain nitrogen atoms [3], which has generated considerable interest in the pharmaceutical industry [4].

Typically, Heck reactions involving nitrogen-containing substrates are performed using protecting groups, and only a few examples have been reported with unprotected nitrogen functionalities [5]. The issue arises from the fact that free nitrogen atoms can coordinate to the metal catalyst, thereby negatively affecting both yield and selectivity, as observed with indoles and tryptamine derivatives [6]. Nevertheless, these scaffolds are highly valuable in medicinal chemistry [7]. Sewald and co-workers reported Heck couplings of 7-bromotryptophan with styrene derivatives, obtaining the corresponding products in yields ranging from 28% to 70% (**Scheme 3a**) [8]. Similarly, the Goss group described the coupling of haloindoles with acrylates (**Scheme 3b**), as well as bromo- and iodotryptophans with acrylates and substituted styrenes (**Scheme 3a**) [9]. These studies represent an important contribution to cross-coupling chemistry; however, it is worth noting that the nucleophilicity of the amino group in tryptophan is attenuated because the molecule exists in its zwitterionic form, preventing metal coordination [10].

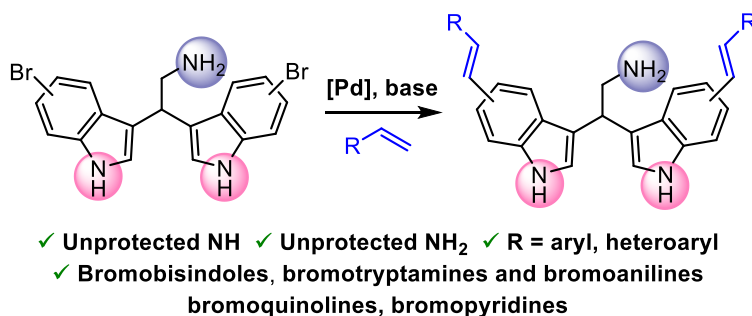
(a) Mizoroki-Heck coupling of unprotected halotryptophans
(Sewald 2019, Goss 2019)



(b) Mizoroki-Heck coupling of unprotected haloindoles
(Goss 2019)



(c) This work: Mizoroki-Heck coupling of unprotected bromobisindoles



Scheme 3. Mizoroki–Heck coupling of unprotected (a) halotryptophans, (b) haloindoles, and (c) bromobisindoles.

In recent years, our research group has built strong expertise in the synthesis of bisindole derivatives, as described in Chapter 1. These molecules form a fascinating class of compounds because of their wide range of potential biological activities. Among them, several have shown notable antileishmanial properties, offering a valuable starting point for the development of new therapeutic agents against leishmaniasis, a neglected tropical disease transmitted to humans through the bite of infected sandflies.

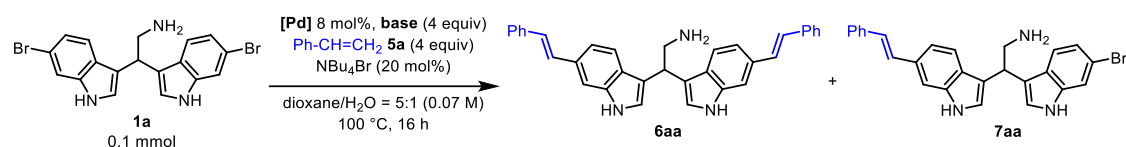
One of the most interesting compounds we studied, the natural marine alkaloid 2,2-bis(6-bromo-1*H*-indol-3-yl)ethanamine (**1a**), displayed significant activity against *L. infantum*. However, its moderate toxicity, likely linked to the presence of bromine atoms, encouraged us to explore new synthetic strategies aimed at improving its pharmacological profile.

As discussed in Chapter 2, this led us to develop a streamlined Suzuki–Miyaura coupling approach, using a range of aryl- and vinylboronic acids. This methodology allowed us to generate a small but diverse library of arylated bisindoles that retained promising biological activity. Notably, the entire process was carried out without the need for protecting groups on either the amine or the indole nitrogen, which made the synthesis both faster and more efficient.

Building on these encouraging results, we decided to expand our study by exploring the Mizoroki–Heck reaction as a complementary approach to functionalize unprotected brominated bisindole substrates (**Scheme 3c**). In parallel, we plan to test other electrophiles featuring free nitrogen atoms – such as bromotryptamines and bromoanilines – to identify reaction conditions capable of overcoming the typical challenges of these transformations. Through this work, we aim to establish a more general and practical strategy for the direct functionalization of indole-based systems with biological relevance.

3.2 Results and Discussion

Table 9. Screening of the reaction conditions^[a]



Entry	[Pd]	Base	Conversion 1a [%] ^[b]	Yield 6aa [%] ^[b]	Yield 7aa [%] ^[b]
1 ^[c,d]	$\text{Pd}(\text{dppf})\text{Cl}_2 \cdot \text{DCM}$	Cs_2CO_3	92	45	14
2 ^[c]	$\text{Pd}(\text{dppf})\text{Cl}_2 \cdot \text{DCM}$	Cs_2CO_3	100	60	10
3	$\text{Pd}(\text{dppf})\text{Cl}_2 \cdot \text{DCM}$	Cs_2CO_3	100	70 (67 ^[e])	< 2
4	$\text{Pd}(\text{dppf})\text{Cl}_2 \cdot \text{DCM}$	K_2CO_3	100	63	3
5	$\text{Pd}(\text{dppf})\text{Cl}_2 \cdot \text{DCM}$	KOAc	100	47	7
6	$\text{Pd}(\text{dppf})\text{Cl}_2 \cdot \text{DCM}$	CsF	100	70 (65 ^[e])	< 2
7	$\text{Pd}(\text{dppf})\text{Cl}_2 \cdot \text{DCM}$	-	50	20	21
8	$\text{Pd}(\text{dppp})\text{Cl}_2$	Cs_2CO_3	99	24	23

9	Pd(dppe)Cl ₂	Cs ₂ CO ₃	89	26	20
10	Pd(XantPhos)Cl ₂	Cs ₂ CO ₃	92	31	16
11	Pd(DPEPhos)Cl ₂	Cs ₂ CO ₃	100	55	< 2
12	Pd(BINAP)Cl ₂	Cs ₂ CO ₃	100	69 (66 ^[e])	< 2
13 ^[f]	Pd(BINAP)Cl ₂	Cs ₂ CO ₃	100	64	3
14 ^[g]	Pd(BINAP)Cl ₂	CsF	100	81 (80 ^[e])	3
15 ^[g,h]	Pd(BINAP)Cl ₂	CsF	100	81 (81 ^[e])	3
16 ^[g,d]	Pd(BINAP)Cl ₂	NBu ₄ F	100	61	12

[a] Reaction performed under N₂ inert atmosphere using bisindole **1a** (0.1 mmol), styrene **5a** (0.4 mmol), the indicated Pd source (8 mol % of catalyst loading), base (0.4 mmol) and NBu₄Br (0.02 mmol), in dioxane/H₂O = 5:1 (0.07 M) as the reaction medium at 100 °C for 16 h. [b] Determined by ¹H NMR analysis of the reaction crude using 1,3,5-trimethoxybenzene as standard. [c] dioxane/H₂O = 4:1 was utilized as reaction mixture. [d] No NBu₄Br was utilized. [e] Isolated yields by means chromatographic column. [f] Reaction time = 4 h. [g] Reaction time = 2 h. [h] 20 mol % of NBu₄F were utilized instead of NBu₄Br.

We began our investigation using the optimized reaction conditions reported in Chapter 2 clearly by replacing phenylboronic acid with styrene, employing Pd(dppf)Cl₂ as the catalyst (8 mol %), cesium carbonate (4 equiv) as the base, styrene (2.5 equiv), and dioxane/water (4:1) at 80 °C. Under these conditions, no Heck product was observed. Increasing the temperature to 100 °C, doubling the equivalents of the styrene and extending the reaction time afforded product **6aa** together with **7aa** in moderate yield (Table 9, entry 1). The reaction proved to be completely stereoselective and regioselective, as only the **E** isomer was detected, with no evidence of the terminal alkene or **Z** isomer. Since this newly developed reaction and the Suzuki coupling (Chapter 2) employ the same catalyst and electrophile, the difference in reaction times probably cannot be attributed to the oxidative addition step.

Upon addition of tetrabutylammonium bromide (NBu₄Br, 20 mol %), full conversion of **1a** was achieved, with higher yield and selectivity toward **6aa** (entry 2). Quaternary ammonium salts are known to facilitate the formation of Pd(0) from palladium–hydride species, to prevent palladium black formation, and to promote phase transfer of the various species between the organic and aqueous phases [11].

Additional bases were then tested (entries 4–6); in particular, CsF provided results similar to Cs₂CO₃ (entry 6), suggesting a possible beneficial role of the cesium cation in the reaction [12]. Notably, the reaction also proceeded in the absence of any added base, likely due to the amino group of **1a**, which acts as an internal base (entry 7).

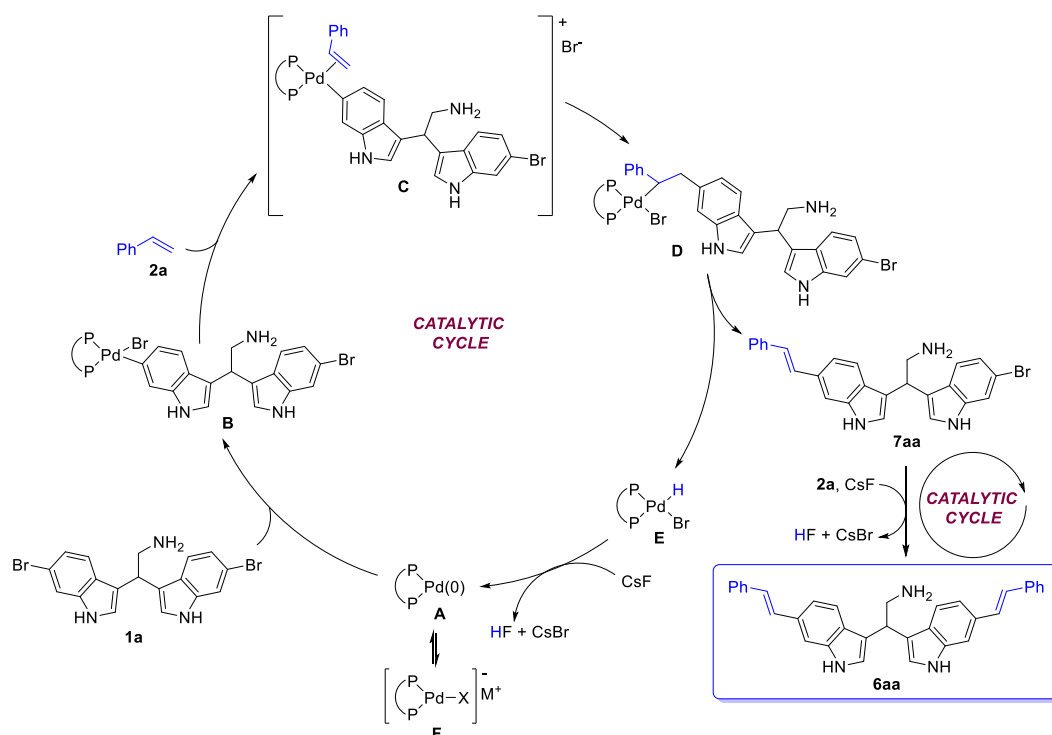
Several palladium catalysts were screened (see Experimental Section), but product **6aa** was obtained only with bidentate phosphine ligands (entries 8–12), likely because such ancillary ligands enforce a favorable *cis* orientation between the olefin and the sterically demanding substrate **1a**, which is essential for the reaction to proceed efficiently. In particular, Pd(BINAP)Cl₂ gave results comparable to Pd(dppf)Cl₂ (entry 12). However, a clear difference between the two catalysts emerged when the reaction time was reduced as with Pd(BINAP)Cl₂, the same conversion and yield of **6aa** were achieved in only 4 hours (entry 13), indicating a higher TOF [13].

Finally, using CsF as the base and Pd(BINAP)Cl₂ as the catalyst, **6aa** was obtained in 81% isolated yield in just 2 hours (entry 14). This suggests that the fluoride, besides being a weak base, can have additional positive effects on our reaction, such as stabilizing metal intermediates and facilitating hydrogen removal during the catalytic cycle, or assisting the initial Pd(0) formation from the Pd(II) precursor [14, 15].

With other ammonium salts (see Experimental Section), no significant beneficial effects were observed, except for NBu₄F, which provided essentially identical results of NBu₄Br (compare entries 14 and 15). However, when NBu₄F was used as the base, **6aa** was obtained in lower yields (entry 16).

Drawing on these findings, [2] a plausible catalytic cycle accounting for the formation of both **6aa** and **7aa** is illustrated in **Scheme 4**. The Pd(II) precatalyst (Pd(BINAP)Cl₂) is first reduced to the active Pd(0) species **A**, a transformation likely mediated by the phosphine ligand and facilitated by hard nucleophiles such as water, hydroxide, or fluoride ions.

Oxidative addition of bisindole **1a** to **A** furnishes intermediate **B**, which then coordinates styrene **5a** to generate complex **C**. Migratory insertion of the alkene forms **D**, from which β-hydride elimination produces the mono-Heck product **7aa** and a palladium–hydride intermediate **E**. Reductive elimination from **E**, assisted by the base acting as an HBr scavenger, regenerates the Pd(0) species **A**. Compound **7aa** can subsequently re-enter the catalytic cycle, undergoing the same sequence of steps to deliver the fully arylated product **6aa**. Palladium(0) complex **A** can be in equilibrium with the possibly more stable tricoordinated anionic complex [L₂PdX][−] **F**, in which X is a halide (i.e., bromide or fluoride), that is stabilized by free cations, such as NBu₄⁺ or Cs⁺ [2].



Scheme 4. Proposed catalytic cycle. X = F, Br; $M^+ = \text{NBu}_4^+, \text{Cs}^+$

With all this data in hand, we started the investigation of the generality of the reaction using various olefins and electrophiles bearing free amino groups. Testing other olefins, we observed that generally better results (i.e., higher yields with lower reaction times) were achieved with NBu_4F with respect to NBu_4Br , and therefore we decided to investigate the scope of the olefin employing the conditions of **Table 9**, entry 15.

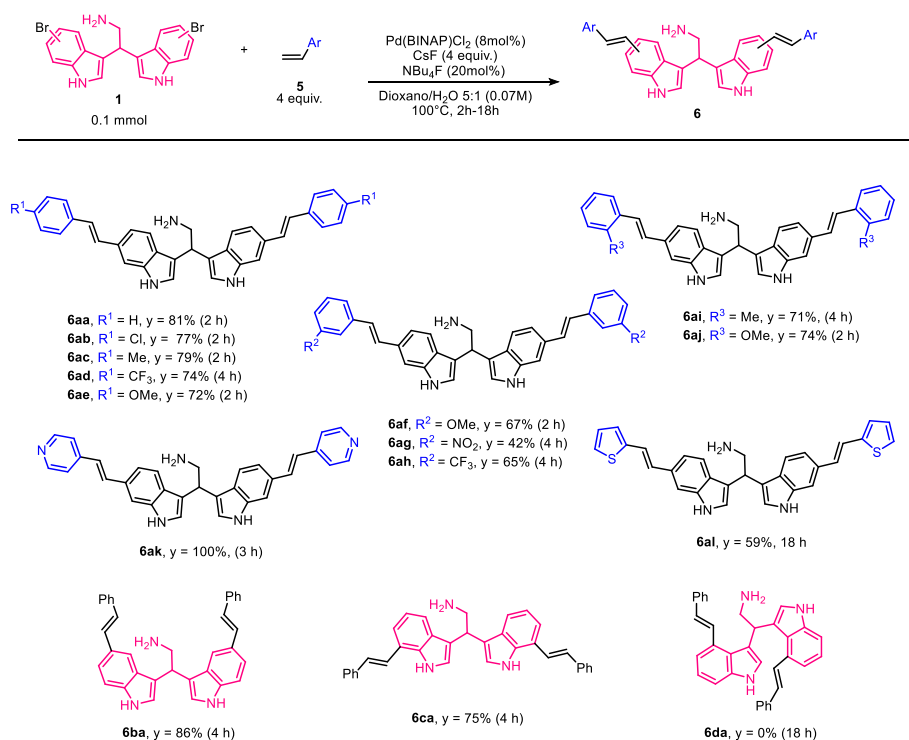
Using *para*-substituted styrene, high yields were obtained in the presence of chloride (**6ab**, $y = 77\%$), methyl (**6ac**, $y = 79\%$), or methoxy (**6ae**, $y = 72\%$) substituents. Using *p*-trifluoromethylstyrene, excellent results were obtained (**6ad**, 74%), but it was necessary to extend the reaction time to 4 hours. When *meta*-substituted styrenes were employed, yields ranging from acceptable to good were obtained from 42%–67% [3-nitrostyrene (**6ag**, $y = 42\%$), 3-methoxystyrene (**6af**, $y = 67\%$) and 3-trifluoromethylstyrene (**6ah**, $y = 65\%$), respectively]. When a methoxy group or methyl group was present in the *ortho* position, a good yield was achieved (**6aj**, $y = 74\%$, **6ai**, $y = 71\%$). We then tested heteroaryl olefins, 2-vinylthiophene and 4-vinylpyridine, obtaining good and excellent yields, respectively (**6al**, $y = 59\%$, **6ak**, $y = 100\%$). The outstanding performance of the Heck reaction with 4-vinylpyridine can be attributed to a combination of electronic and coordination effects. The strongly electronwithdrawing

nature of the pyridine ring renders the alkene electronpoor, thereby accelerating the migratory insertion step in the catalytic cycle. In addition, the nitrogen atom of pyridine can weakly coordinate to palladium, stabilizing key intermediates and preserving the catalyst in its active form, which helps suppress side reactions [16]. Together, these factors lead to exceptionally high reactivity and near-quantitative yields.

From the results reported above, a clear trend of reactivity based on the substituents on the styrenes does not emerge, and generally satisfying yields are obtained regardless of the utilized styrene.

Then, we tested other bromobisindoles in the presence of the styrene **5a**. With 5-bromobisindole and 7-bromobisindole high and good yields are respectively obtained (compare **6ba** and **6ca**), while 4-bromobisindole resulted to be completely unreactive, probably due to the presence of the bisindole ethylenimine group that sterically prevents the coupling, again excluding the possibility that the NH₂ group could act as a directing group, as discussed in Chapter 2.

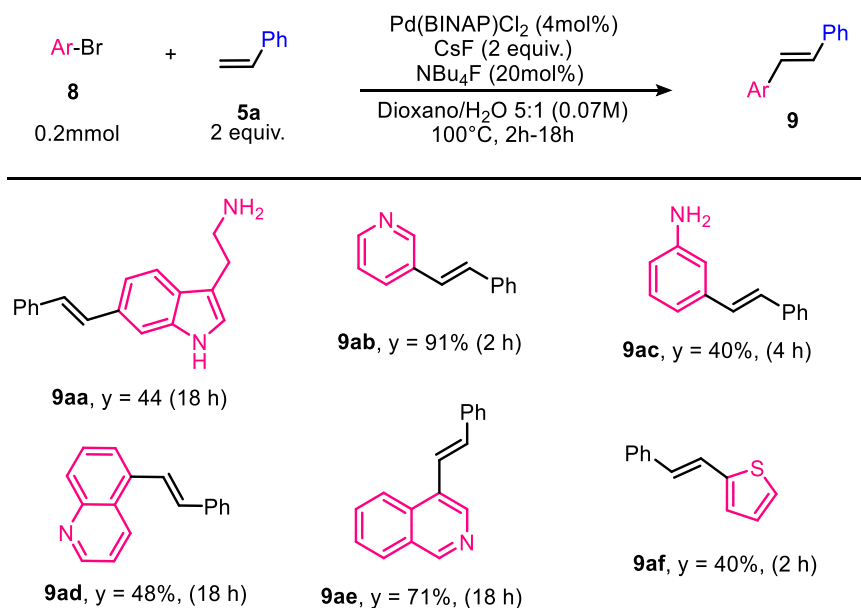
Table 10. Scope of the Pd-Catalyzed Mizoroki–Heck coupling of unprotected bisindoles.^[a]



[a] Reaction performed under N₂ inert atmosphere using bromobisindoles **1** (0.1 mmol), styrenes **5** (0.4 mmol), 8 mol% of Pd(BINAP)Cl₂, 0.4 mmol of CsF, NBu₄F (20 mol %) in dioxane/H₂O = 5:1 (0.07 M) at 100 °C for the indicated time. Isolated yields are reported.

Subsequently, to demonstrate the applicability of our reaction beyond indole-based substrates, we decided to test our conditions on different substrates, with the aim of developing a methodology that could overcome some of the limitations discussed in the introductory section (**Table 11**).

Table 11. Scope of the Pd-Catalyzed Mizoroki–Heck coupling of aryl bromide **8**.^[a]



[a] Reaction performed under N₂ inert atmosphere using the selected aryl bromides **8** (0.2 mmol), styrene **5a** (0.4 mmol), 4 mol % of Pd(BINAP)Cl₂, 0.4 mmol of CsF, NBU₄F (20 mol %) in dioxane/H₂O = 5:1 (0.07 M) at 100 °C for the indicated time. Isolated yields are reported.

To begin with, a noteworthy result was obtained: for the first time, the Mizoroki–Heck coupling was successfully applied to a completely NH- and NH₂-unprotected tryptamine. This achievement was far from trivial – given the known reactivity of such unprotected systems – yet, after 18 hours of careful reaction monitoring, 6-bromotryptamine finally yielded the desired product **9aa**, isolated in a satisfying 44% yield. This result marked a clear turning point, demonstrating that even a delicate substrate such as tryptamine could be engaged directly in the Heck process without the need for cumbersome protection strategies.

Encouraged by this success, the investigation was expanded to other nitrogen-containing aryl bromides. The reaction with 3-bromopyridine exceeded expectations and in just 2 hours, the coupling proceeded smoothly to give compound **9ab** in an excellent

91% yield, a result that confirmed both the robustness and the efficiency of the newly developed methodology.

The method also showed remarkable versatility, performing moderately well with other challenging substrates such as unprotected anilines (**9ac**, 40%) and quinoline derivatives (**9ad**, 48%; **9ae**, 71%). Even when the scope was extended to more electron-rich systems, such as 2-bromothiophene, the corresponding product **9af** was obtained in 40% yield.

Taken together, these results not only validate the feasibility of the method but also highlight its transformative potential, offering, for the first time, a straightforward and protection-free route to functionalized tryptamine and related heterocycles through the Mizoroki–Heck reaction. It is also worth noting that these results were achieved using a relatively low catalytic loading (4 mol % Pd(BINAP)Cl₂), highlighting both the intrinsic efficiency of the developed catalytic system and the overall robustness of the methodology.

3.3 Conclusion

In this study, we developed a robust, and efficient method for the Mizoroki–Heck coupling of NH₂-unprotected bromo-bisindole ethanamines, allowing the late-stage functionalization of these scaffolds with a variety of olefines. High yields (up to 100%) of diarylated products were obtained using Pd(BINAP)Cl₂ as the catalyst, CsF as the base, NBu₄F as a quaternary ammonium salt and dioxane/water = 5:1 as the reaction medium. A broad scope and excellent functional group tolerance are demonstrated. Remarkably, both the NH₂ and the indole NH of the starting bromobisindole ethanamines are unprotected, suggesting this methodology can be applied to late-stage diversification of unprotected halobisindoles. Indeed, to the best of our knowledge, using our procedure, an unprotected bromotryptamine (6-Br) was directly arylated for the first time achieving the corresponding product with good yield. Furthermore, to demonstrate the broad applicability of our methodology, we extended its use to a variety of heteroaryl electrophiles, including bromopyridines, bromoquinolines and bromoanilines. These

substrates were chosen to assess the reaction's tolerance toward nitrogen-containing heterocycles, which are highly relevant in pharmaceutical chemistry. The successful coupling of these challenging electrophiles highlights the robustness of the optimized conditions and their potential for late-stage functionalization. Overall, this study confirms that our strategy is not limited to indole scaffolds but can be effectively applied to a broader class of heteroaryl substrates.

3.4 Experimental Section

Material and methods

All reactions were prepared under nitrogen atmosphere with dry solvents under anhydrous conditions, by using Schlenk technique. Reactions were monitored by ^1H NMR taking a direct sample of the crude mixture. ^1H NMR and ^{13}C NMR were recorded on a Bruker Avance 400 spectrometer (^1H : 400 MHz, ^{13}C : 101 MHz), or on a Bruker Avance Neo 600 spectrometer (^1H : 600 MHz, ^{13}C : 151 MHz) using DMSO- d_6 or CDCl_3 as solvent and 1,3,5-trimethoxybenzene as standard. Chemical shifts are reported in the δ scale relative to residual CHCl_3 (s, 7.26 ppm) or DMSO (p, 2.50 ppm) for ^1H NMR and to the central line of CDCl_3 (77.16 ppm) or DMSO- d_6 (39.52 ppm) for ^{13}C NMR. ^{13}C NMR were recorded with ^1H broadband decoupling. The following abbreviations were used to explain the multiplicities: br = broad, s = singlet, d = doublet, t = triplet, q = quartet, hept = heptet, dd = doublet of doublets, ddd = doublet of doublets of doublets, m = multiplet. Coupling constants (J) are reported in Hertz (Hz). 1,4-dioxane was dried over molecular sieves (Alfa Aesar, 4 Å, 1–2 mm, beads). Both 1,4-dioxane and water were degassed (N_2 bubbling for 30 min). Liquid purchased olefins were filtered off a plug of neutral Al_2O_3 and used without further purification. Pure compounds **6** and **9** were isolated through flash column chromatography on silica gel 60 (40–60 μm , 230–400 mesh). Bromobisindole derivatives **1a** and **1b** have been synthesized as already described in the literature [17]. All other chemicals were purchased from Merck Sigma-Aldrich and used without further purification. All solid reagents were weighed in an analytical balance without excluding moisture and air.

General procedure 1 (GP1). Typical Procedure for the Mizoroki–Heck coupling of unprotected bromobisindole with styrenes.

Compound **1** (0.1 mmol), Pd(BINAP)Cl₂ (8mol%, 6.4 mg), CsF (0.4 mmol, 60.8 mg), NBu₄F·3H₂O (20mol%, 6.3mg) and the selected olefin **5** (0.4 mmol) were added in a dried Schlenk tube, equipped with a magnetic stirring bar. Degassed dioxane (1.19 mL) and degassed water (0.24 mL) were added, and the reaction was vigorously stirred at 100 °C in an oil bath under balloon pressure of N₂, monitoring the progress of the reaction by TLC. The nitrogen was removed, and the reaction mixture was analyzed by ¹H NMR to determine the conversion of the bisindole. The crude was filtered on silica plug (CH₂Cl₂/CH₃OH/NH₃, 80:20:1) and the products were eventually obtained after column chromatography on silica gel.

General procedure 2. (GP2). Typical procedure for the Mizoroki–Heck coupling of other nitrogen-containing aromatic electrophiles with styrenes.

Compound **8** (0.2 mmol), Pd(BINAP)Cl₂ (4mol%, 6.4 mg), CsF (0.4 mmol, 60.8 mg), NBu₄F·3H₂O (20 mol %, 12.6 mg) and the selected olefin **5** (0.4 mmol) were added in a dried Schlenk tube, equipped with a magnetic stirring bar. Degassed dioxane (2.38 mL) and degassed water (0.48 mL) were added, and the reaction was vigorously stirred at 100 °C in an oil bath under balloon pressure of N₂, monitoring the progress of the reaction by TLC. The nitrogen was removed, and the reaction mixture was analyzed by ¹H NMR to determine the conversion of the starting material. The crude was filtered on silica plug (EtOAc) and the products were eventually obtained after column chromatography on silica gel.

List of the utilized halide **1 and **8**, olefin **5****

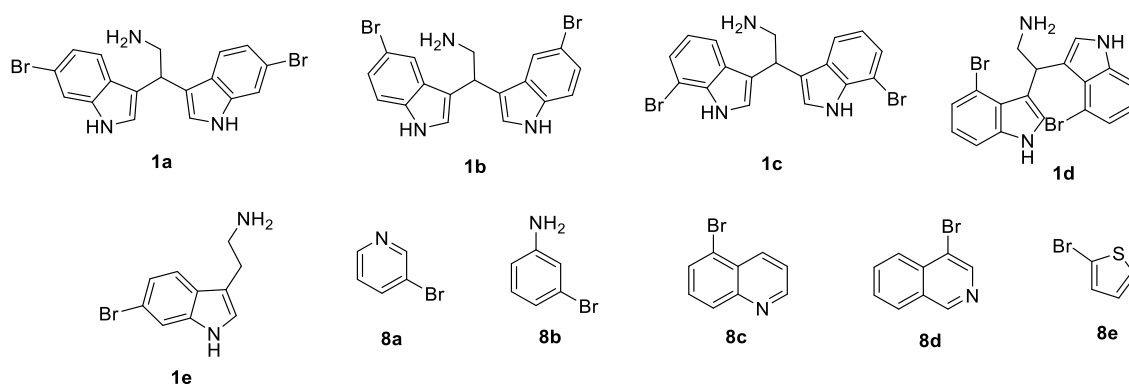


Figure 21. Complete list of the utilized Br-(bis)indoles **1** and halide **8**.

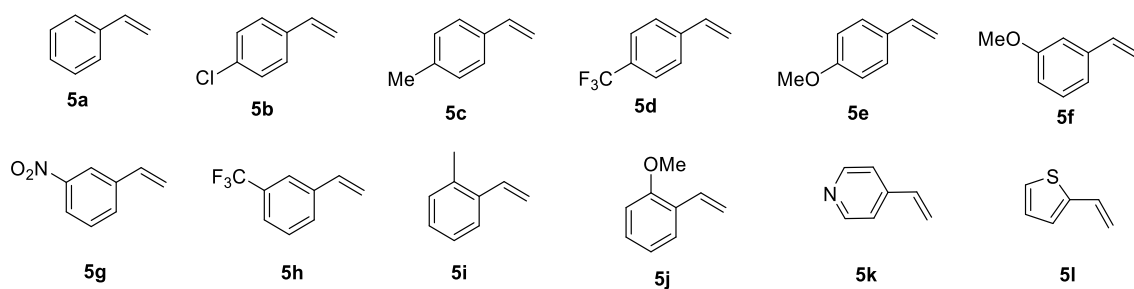


Figure 22. Complete list of the utilized styrenes **5**.

Complete Optimization Reactions for the Mizoroki–Heck Coupling of Unprotected Bromobisindole.

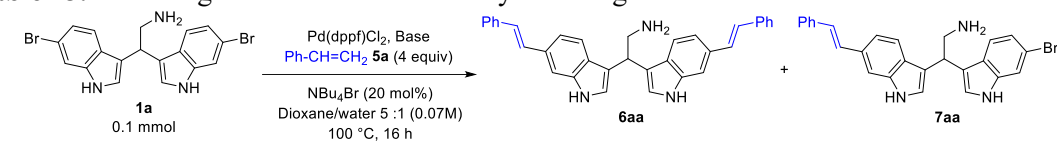
Table 12. Screening of the solvent, the concentration and the ratio solvent:co-solvent.^[a]

Entry	Solvent:Co-solvent (v/v)	Concentration [M]	Conversion 1a [%] ^[b]	Yield 6aa [%] ^[b]	Yield (7aa) [%] ^[b]
1	4 : 1	0.05	100	60	10
2	5 : 1	0.05	100	61	<2
3	5 : 1	0.07	100	70(67)	<2
4	6 : 1	0.07	84	40	18
5	7 : 1	0.07	90	36	15
6	5 : 1	0.1	100	60	6
7 ^[c]	5 : 1	0.07	40	Trace	11
8 ^[d]	5 : 1	0.07	50	Trace	10
9 ^[e]	5 : 1	0.07	60	0	15
10 ^[f]	5 : 1	0.07	92	15	25

[a] Reaction performed under N₂ inert atmosphere using bisindole **1a** (0.1 mmol), styrene **5a** (0.4 mmol), Pd(dppf)Cl₂ (8 mol% of catalyst loading), Cs₂CO₃ (0.4 mmol), in the indicated reaction medium at 100 °C for 16 h. [b] Determined by ¹H NMR analysis of the reaction crude using 1,3,5-

trimethoxybenzene as standard. [c] DMF instead of H₂O. [d] DMSO instead of H₂O [e] DMSO instead of dioxane. [f] DMF instead of dioxane.

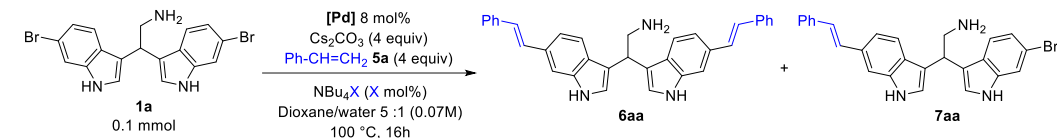
Table 13. Screening of the effect of the catalyst loading and base amount on the reaction.^[a]



Entry	Catalyst Loading [mol%]	Cs_2CO_3 [equiv]	Conversion 1a [%] ^[b]	Yield 6aa [%] ^[b]	Yield 7aa [%] ^[b]
1	4	4	87	39	20
2	16	4	100	51	3
3	8	2	100	60	6

[a] Reaction performed under N₂ inert atmosphere using bisindole **1a** (0.1 mmol) and styrene **5a**, Pd(dppf)Cl_2 and Cs_2CO_3 in the indicated amount, in dioxane/H₂O = 5:1 (0.07 M) as the reaction medium at 100 °C for 16 h. [b] Determined by ¹H NMR analysis of the reaction crude using 1,3,5-trimethoxybenzene as standard.

Table 14. Screening of the bases, ammonium salt, palladium source and reaction time.^[a]



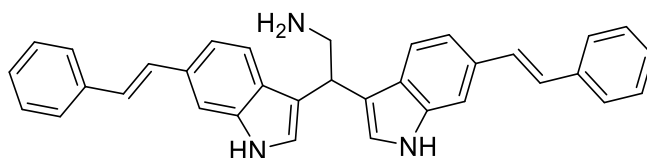
Entry	[Pd] 8mol%	Ammonium salt (mol%)	Conversion 1a [%] ^[b]	Yield 6aa [%] ^[b]	Yield 7aa [%] ^[b]
1	Pd(dppf)Cl_2	NBu_4Br (20)	100	70	<2
2	Pd(dppf)Cl_2	NBu_4Cl (20)	100	51	8
3	Pd(dppf)Cl_2	NBu_4I (20)	85	23	33
4	Pd(dppf)Cl_2	NBu_4HSO_4 (20)	95	42	20
5	Pd(dppf)Cl_2	NBu_4Br (10)	98	50	5
6	Pd(dppf)Cl_2	NBu_4Br (40)	100	60	4
7 ^[c]	Pd(dppf)Cl_2	NBu_4Br (20)	100	47	7
8 ^[d]	Pd(dppf)Cl_2	NBu_4Br (20)	100	63	3
9 ^[e]	Pd(dppf)Cl_2	NBu_4Br (20)	100	67	<2
10	Brettphos precatalyst	NBu_4Br (20)	75	8	N.D.
11	Ruphos precatalyst	NBu_4Br (20)	68	4	N.D.
12	$\text{PdCl}_2(\text{P(o-tol)})_3$	NBu_4Br (20)	43	0	15

13	PdCl ₂ BINAP	NBu ₄ Br (20)	100	64	<2
14	PdCl ₂ Xantphos	NBu ₄ Br (20)	92	31	16
15	PdCl ₂ DPEPhos	NBu ₄ Br (20)	100	55	<2
16	PdCl ₂ (dppp)	NBu ₄ Br (20)	99	24	23
17	PdCl ₂ (dppe)	NBu ₄ Br (20)	89	26	20
18 ^[f]	Pd(dppf)Cl ₂	NBu ₄ Br (20)	75	30	25
19 ^[f]	PdCl ₂ BINAP	NBu ₄ Br (20)	100	64	<2
20 ^[e,f]	PdCl ₂ BINAP	NBu ₄ Br (20)	100	81	3
21 ^[e,f]	PdCl ₂ BINAP	NBu ₄ F (20)	100	81	3

[a] Reaction performed under N₂ inert atmosphere using bisindole **1a** (0.1 mmol), styrene **5a** (0.4 mmol), 8 mol% of Palladium source, the indicated base (0.4 mmol), in dioxane/H₂O = 5:1 (0.07 M) at 100 °C for the indicate time. [b] Determined by ¹H NMR analysis of the reaction crude using 1,3,5-trimethoxybenzene as standard. [c] KOAc instead of Cs₂CO₃. [d] K₂CO₃ instead of Cs₂CO₃. [e] CsF instead of Cs₂CO₃. [f] Reaction time 2 h.

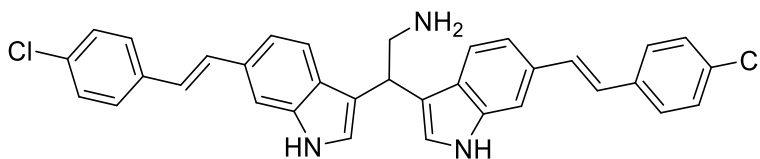
Experimental data for compounds 6 and 9

2,2'-Bis[(6-(*E*)-styryl)-1*H*-indol-3-yl]ethan-1-amine (**6aa**)



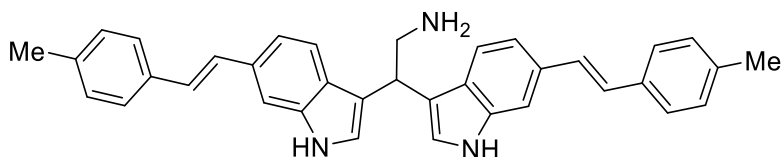
Following the **GP1** (reaction time = 2 h), compound **6aa** has been purified by flash column chromatography on silica gel (CH₂Cl₂/CH₃OH/NH₃, 99:1:1), obtaining a white solid; yield: 79% (38 mg). *R*_f = 0.38 (silica gel; CH₂Cl₂/CH₃OH/NH₃, 90:10:1). ¹H NMR (600 MHz, DMSO-*d*₆): δ = 10.91 (br d, *J* = 2.5 Hz, 2H), 7.59-7.56 (m, 4H), 7.50-7.47 (m, 4H), 7.36-7.32 (m, 4H), 7.31 (d, *J* = 16.5 Hz, 2H), 7.26-7.20 (m, 6H), 7.12 (d, *J* = 16.5 Hz, 2H), 4.39 (t, *J* = 7.0 Hz, 1H), 3.26 (d, *J* = 7.0 Hz, 2H) ppm. ¹³C NMR (151 MHz, DMSO-*d*₆): δ = 137.6, 136.8, 130.1, 130.0, 128.6, 126.9, 126.9, 126.1, 125.4, 123.5, 119.1, 117.4, 116.7, 110.2, 46.8, 37.9 ppm. HRMS (*m/z*): [M+H]⁺ calcd for C₃₄H₃₀N₃ 480.2434; found 480.2452.

2,2'-Bis[(6-(*E*)-4-chlorostyryl)-1*H*-indol-3-yl]ethan-1-amine (**6ab**)



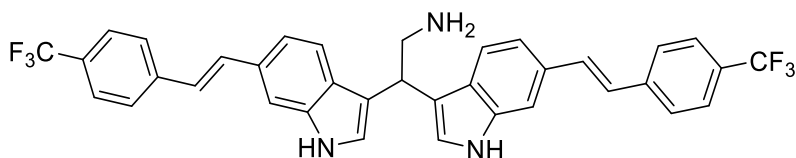
Following the **GP1** (reaction time = 2 h), compound **6ab** has been purified by flash column chromatography on silica gel (CH₂Cl₂/CH₃OH/NH₃, 99:1:1), obtaining a yellow solid; yield: 77% (42 mg). ¹H NMR (600 MHz, DMSO-*d*₆): δ = 10.95 (br d, 2H, *J* = 2.5 Hz), 7.60 (d, 2H, *J* = 8.5 Hz), 7.51-7.48 (m, 4H), 7.41-7.38 (m, 4H), 7.33 (d, 2H, *J* = 16.0 Hz), 7.28 (d, 2H, *J* = 2.5 Hz), 7.24 (dd, 2H, *J*₁ = 1.5 Hz, *J*₂ = 8.5 Hz), 7.11 (d, 2H, *J* = 16.0 Hz), 4.45 (t, 1H, *J* = 7.0 Hz), 3.32 (d, 2H, *J* = 7.0 Hz) ppm. ¹³C NMR (151 MHz, DMSO-*d*₆): δ = 136.8, 136.6, 131.1, 131.0, 129.8, 128.5, 127.7, 126.9, 124.1, 123.7, 119.1, 116.9, 116.8, 110.4, 46.2, 36.9 ppm. Spectroscopic data are in accordance with the literature [17].

2,2'-Bis[(6-(*E*)-4-methylstyryl)-1*H*-indol-3-yl]ethan-1-amine (6ac**)**



Following the **GP1** (reaction time = 2 h), compound **6ac** has been purified by flash column chromatography on silica gel (CH₂Cl₂/CH₃OH/NH₃, 99:1:1 to CH₂Cl₂/CH₃OH/NH₃, 96:4:1), obtaining a white solid; yield: 79% (40 mg). *R*_f = 0.30 (silica gel; CH₂Cl₂/CH₃OH/NH₃, 90:10:1). ¹H NMR (400 MHz, DMSO-*d*₆): δ = 10.89 (br d, *J* = 2.5 Hz, 2H), 7.50-7.44 (m, 8H), 7.26-7.19 (m, 6H), 7.17-7.13 (m, 4H), 7.07 (d, *J* = 16.5 Hz, 2H), 4.39 (t, *J* = 7.0 Hz, 1H), 3.26 (d, *J* = 7.0 Hz, 2 H), 2.30 (s, 6H) ppm. ¹³C NMR (101 MHz, DMSO-*d*₆): δ = 136.8, 136.2, 134.8, 130.2, 129.2, 129.1, 126.7, 126.0, 125.4, 123.4, 119.1, 117.3, 116.7, 110.0, 46.7, 37.8, 20.8 ppm. HRMS (*m/z*): [M+H]⁺ calcd for C₃₆H₃₄N₃ 508.2747; found 508.2763.

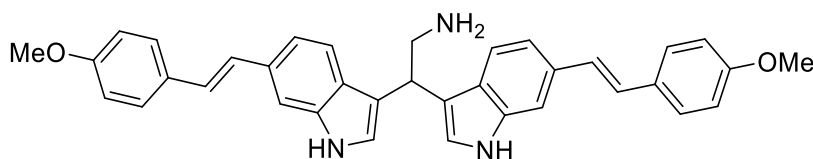
2,2'-Bis[(6-(*E*)-4-trifluoromethylstyryl)-1*H*-indol-3-yl]ethan-1-amine (6ad**)**



Following the **GP1** (reaction time = 4 h), compound **6ad** has been purified by flash column chromatography on silica gel (CH₂Cl₂/CH₃OH/NH₃, 99:1:1 to CH₂Cl₂/CH₃OH/NH₃, 97:3:1), obtaining a white solid; yield: 74% (46 mg). *R*_f = 0.22 (silica gel; CH₂Cl₂/CH₃OH/NH₃, 90:10:1). ¹H NMR (400 MHz, DMSO-*d*₆): δ = 10.99 (br d, *J* = 2.5 Hz, 2H), 7.81-7.77 (m, 4H), 7.71-7.66 (m, 4H), 7.54 (d, *J* = 1.5 Hz, 2H), 7.52

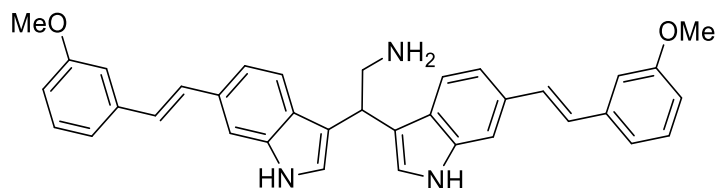
(d, $J = 8.5$ Hz, 2H), 7.49 (d, $J = 16.5$ Hz, 2H), 7.31-7.27 (m, 4H), 7.22 (d, $J = 16.5$ Hz, 2H), 4.41 (t, $J = 7.0$ Hz, 1H), 3.28 (d, $J = 7.0$ Hz, 2H) ppm. ^{19}F NMR (376 MHz, DMSO- d_6): $\delta = -60.69$ ppm. ^{13}C NMR (101 MHz, DMSO): $\delta = 141.9$ (q, $J = 1.0$ Hz), 136.7, 133.1, 129.5, 127.3, 126.8 (q, $J = 31.5$ Hz), 126.5, 125.5 (q, $J = 3.5$ Hz), 124.5 (q, $J = 271.5$ Hz), 124.0, 123.8, 119.2, 117.4, 116.9, 110.9, 46.7, 37.7 ppm. HRMS (m/z): $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{36}\text{H}_{28}\text{F}_6\text{N}_3$ 616.2182; found 616.2185.

2,2'-Bis[(6-(*E*)-4-methoxystyryl)-1*H*-indol-3-yl]ethan-1-amine (6ae)



Following the **GP1** (reaction time = 2 h), compound **6ae** has been purified by flash column chromatography on silica gel ($\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}/\text{NH}_3$, 99:1:1), obtaining a white solid; yield: 72% (39 mg). $R_f = 0.36$ (silica gel; $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}/\text{NH}_3$, 90:10:1). ^1H NMR (400 MHz, DMSO- d_6): $\delta = 10.87$ (br d, $J = 2.5$ Hz, 2H), 7.53-7.49 (m, 4H), 7.47 (d, $J = 8.5$ Hz, 2H), 7.44 (s, 2H), 7.23 (d, $J = 2.5$ Hz, 2H), 7.20 (dd, $J_1 = 1.5$ Hz, $J_2 = 8.5$ Hz, 2H), 7.14 (d, $J = 16.5$ Hz, 2H), 7.06 (d, $J = 16.5$ Hz, 2H), 6.95-6.89 (m, 4H), 4.39 (t, $J = 7.0$ Hz, 1H), 3.76 (s, 6H), 3.27 (d, $J = 7.0$ Hz, 2H) ppm. ^{13}C NMR (101 MHz, DMSO- d_6): $\delta = 158.5$, 136.9, 130.4, 130.3, 127.8, 127.3, 126.5, 125.2, 123.2, 119.1, 117.2, 116.6, 114.1, 109.8, 55.1, 46.7, 37.7 ppm. HRMS (m/z): $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{36}\text{H}_{34}\text{N}_3\text{O}_2$ 540.2646; found 540.2661.

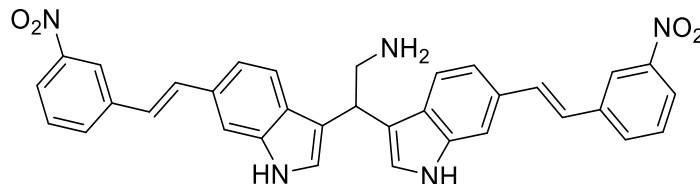
2,2'-Bis[(6-(*E*)-3-methoxystyryl)-1*H*-indol-3-yl]ethan-1-amine (6af)



Following the **GP1** (reaction time = 2 h), compound **6af** has been purified by flash column chromatography on silica gel ($\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}/\text{NH}_3$, 99:1:1) obtaining a white solid; yield: 67% (36 mg). $R_f = 0.28$ (silica gel; $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}/\text{NH}_3$, 90:10:1). ^1H NMR (400 MHz, DMSO- d_6): $\delta = 10.92$ (br s, 2H), 7.52-7.47 (m, 4H), 7.32 (d, $J = 16.5$ Hz, 2H), 7.29-7.21 (m, 6H), 7.18-7.13 (m, 4H), 7.09 (d, $J = 16.5$ Hz, 2H), 6.79 (dd, $J_1 = 2.5$ Hz, $J_2 = 8.0$ Hz, 2H), 4.39 (t, $J = 7.0$ Hz, 1H), 3.79 (s, 6H), 3.26 (d, $J = 7.0$ Hz, 2H) ppm. ^{13}C NMR (101 MHz, DMSO): $\delta = 159.6$, 139.1, 136.8, 130.4, 130.0, 129.6, 126.9, 125.4,

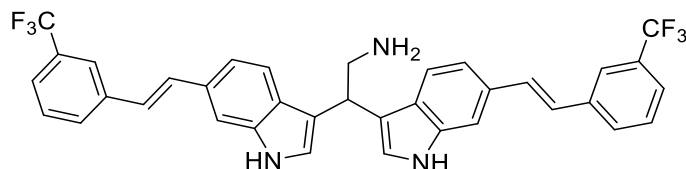
123.5, 119.2, 118.7, 117.3, 116.7, 112.8, 111.1, 110.3, 55.0, 46.8, 37.9 ppm. HRMS (m/z): $[M+H]^+$ calcd for $C_{36}H_{34}N_3O_2$ 540.2646; found 540.2666.

2,2'-Bis[(6-(*E*)-3-nitrostyryl)-1*H*-indol-3-yl]ethan-1-amine (6ag)



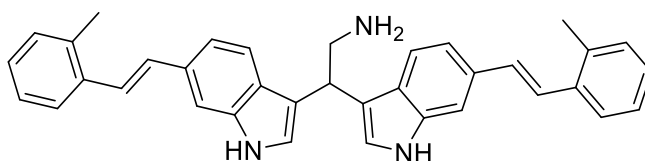
Following the **GP1** (reaction time = 4 h), compound **6ag** has been purified by flash column chromatography on silica gel ($CH_2Cl_2/CH_3OH/NH_3$, 99:1:1), obtaining a yellow solid; yield: 42% (24 mg). R_f = 0.41 (silica gel; $CH_2Cl_2/CH_3OH/NH_3$, 90:10:1). ^1H NMR (600 MHz, $DMSO-d_6$): δ = 11.00 (br d, 2H, J = 2.5 Hz), 8.40 (t, 2H, J = 2.0 Hz), 8.06-8.03 (m, 4H), 7.63 (dd, 2H, $J_1 \approx J_2$ = 8.0 Hz), 7.56 (d, 2H, J = 1.5 Hz), 7.54 (d, 2H, J = 16.5 Hz), 7.52 (d, 2H, J = 8.5 Hz), 7.31-7.27 (m, 6H), 4.43 (t, 1H, J = 7.0 Hz), 3.29 (d, 2H, J = 7.0 Hz) ppm. ^{13}C NMR (151 MHz, $DMSO$): δ = 148.4, 139.7, 136.7, 133.1, 132.1, 130.0, 129.4, 127.3, 123.9, 123.2, 121.2, 120.3, 119.2, 117.3, 117.0, 110.9, 46.7, 37.6 ppm. HRMS (m/z): $[M+H]^+$ calcd for $C_{34}H_{28}N_5O_4$ 570.2136; found 570.2139.

2,2'-Bis[(6-(*E*)-3-trifluoromethylstyryl)-1*H*-indol-3-yl]ethan-1-amine (6ah)



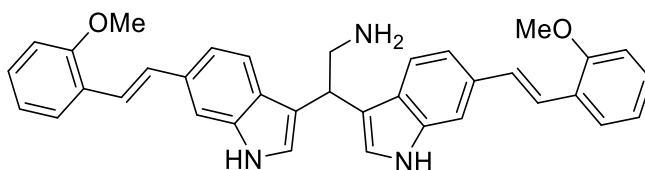
Following the **GP1** (reaction time = 4 h), compound **6ah** has been purified by flash column chromatography on silica gel ($CH_2Cl_2/CH_3OH/NH_3$, 99:1:1) obtaining a white solid; yield: 65% (40 mg). R_f = 0.23 (silica gel; $CH_2Cl_2/CH_3OH/NH_3$, 90:10:1). ^1H NMR (600 MHz, $CDCl_3$): δ = 8.24 (br s, 2H), 7.71 (s, 2H), 7.61 (d, 2H, J = 7.5 Hz), 7.56 (d, 2H, J = 8.0 Hz), 7.48-7.40 (m, 6H), 7.28 (d, 2H, J = 8.5 Hz), 7.21 (d, 2H, J = 16.5 Hz), 7.05 (d, 2H, J = 16.5 Hz), 7.03 (s, 2H), 4.54 (t, 1H, J = 7.0 Hz), 3.46 (d, 2H, J = 7.0 Hz) ppm. ^{19}F NMR (565 MHz, $CDCl_3-d$) δ -62.70. ^{13}C NMR (151 MHz, $CDCl_3$): δ = 138.7, 137.2, 131.7, 131.3, 129.5, 129.2, 127.4, 125.5, 124.4 (q, J = 272.5 Hz), 123.7 (q, J = 4.0 Hz), 123.3, 122.9 (q, J = 4.0 Hz), 119.9, 118.4, 118.1, 110.2, 46.8, 38.2 ppm. HRMS (m/z): $[M+H]^+$ calcd for $C_{36}H_{28}F_6N_3$ 616.2182; found 616.2186.

2,2'-Bis[(6-(*E*)-2-methylstyryl)-1*H*-indol-3-yl]ethan-1-amine (6ai)



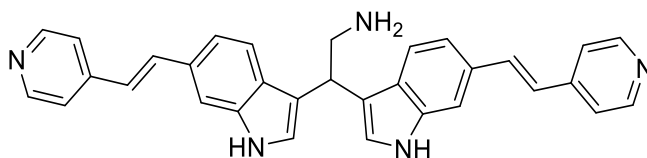
Following the **GP1** (reaction time = 4 h), compound **6ai** has been purified by flash column chromatography on silica gel ($\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}/\text{NH}_3$, 99:1:1 to $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}/\text{NH}_3$, 98:2:1), obtaining a white solid; yield: 71% (36 mg). $R_f = 0.32$ (silica gel; $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}/\text{NH}_3$, 90:10:1). ^1H NMR (600 MHz, CDCl_3): $\delta = 8.12$ (br s, 2H), 7.60 (d, 2H, $J = 7.5$ Hz), 7.57 (d, 2H, $J = 8.5$), 7.44 (s, 2H), 7.31 (d, 2H, $J = 16.0$ Hz), 7.31 (dd, 2H, $J_1 = 1.5$ Hz, $J_2 = 8.5$ Hz), 7.22-7.15 (m, 6H), 7.09 (d, 2H, $J = 16.0$ Hz), 7.02 (d, 2H, $J = 2.0$ Hz), 4.55 (t, 1H, $J = 7.0$ Hz), 3.48 (t, 2H, $J = 7.0$ Hz), 2.43 (s, 6H) ppm. ^{13}C NMR (151 MHz, CDCl_3): $\delta = 137.2, 137.0, 135.7, 132.3, 131.2, 130.5, 127.3, 127.1, 126.3, 125.3, 125.0, 123.0, 119.8, 118.3, 118.2, 109.9, 46.9, 38.4, 20.1$ ppm. HRMS (m/z): $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{36}\text{H}_{34}\text{N}_3$ 508.2747; found 508.2765.

2,2'-Bis[(6-(E)-2-methoxystyryl)-1H-indol-3-yl]ethan-1-amine (6aj)



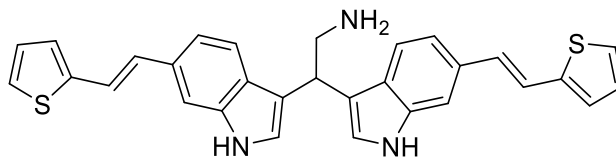
Following the **GP1** (reaction time = 2 h), compound **6aj** has been purified by flash column chromatography on silica gel ($\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}/\text{NH}_3$, 99:1:1 to $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}/\text{NH}_3$, 97:3:1), obtaining a white solid; yield: 74% (40 mg). $R_f = 0.28$ (silica gel; $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}/\text{NH}_3$, 90:10:1). ^1H NMR (400 MHz, CDCl_3): $\delta = 8.06$ (br s, 2H), 7.61 (dd, 2H, $J_1 = 1.5$ Hz, $J_2 = 7.5$ Hz), 7.56 (d, 2H, $J = 8.5$ Hz), 7.50-7.44 (m, 4H), 7.32 (dd, 2H, $J_1 = 1.5$ Hz, $J_2 = 8.5$ Hz), 7.25-7.17 (m, 4H), 7.02-6.94 (m, 4H), 6.90 (dd, 2H, $J_1 = 1.5$ Hz, $J_2 = 8.5$ Hz), 4.53 (t, 1H, $J = 7.0$ Hz), 3.89 (s, 6H), 3.45 (d, 2H, $J = 7.0$ Hz) ppm. ^{13}C NMR (101 MHz, CDCl_3): $\delta = 156.9, 137.2, 132.6, 130.3, 128.3, 127.1, 127.0, 126.3, 122.9, 121.7, 120.9, 119.7, 118.6, 118.1, 111.1, 109.7, 55.7, 46.9, 38.4$ ppm. HRMS (m/z): $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{36}\text{H}_{34}\text{N}_3\text{O}_2$ 540.2646; found 540.2644.

2,2'-Bis{[6-(E)-2-(pyridin-4-yl)vinyl]-1H-indol-3-yl}ethan-1-amine (6ak)



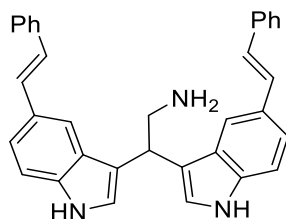
Following the **GP1** (reaction time = 3 h), compound **6ak** has been purified by flash column chromatography on silica gel (CH₂Cl₂/CH₃OH/NH₃, 98:2:1 to CH₂Cl₂/CH₃OH/NH₃, 82:18:1), obtaining a yellow solid; yield: 100% (50 mg). *R*_f = 0.15 (silica gel; CH₂Cl₂/CH₃OH/NH₃, 90:10:1). ¹H NMR (400 MHz, DMSO-*d*₆): δ = 11.17 (br d, *J* = 2.5 Hz, 2H), 8.52-8.47 (m, 4H), 7.62 (d, *J* = 16.5 Hz, 2H), 7.59-7.55 (m, 3H), 7.55-7.52 (m, 5H), 7.42 (d, *J* = 2.5 Hz, 2H), 7.33 (dd, *J*₁ = 1.5 Hz, *J*₂ = 8.5 Hz, 2H), 7.12 (d, *J* = 16.5 Hz, 2H), 4.69 (t, *J* = 7.5 Hz, 1H), 3.51 (d, *J* = 7.5 Hz, 2H) ppm. ¹³C NMR (101 MHz, DMSO): δ = 149.9, 144.8, 136.8, 134.7, 129.4, 127.0, 124.7, 123.1, 120.6, 119.0, 117.3, 115.2, 111.4, 43.7, 33.5 ppm. HRMS (*m/z*): [M+H]⁺ calcd for C₃₂H₂₈N₅ 482.2339; found 482.2334.

2,2'-Bis{6-[(*E*)-2-(thiophen-2-yl)vinyl]-1*H*-indol-3-yl}ethan-1-amine (6al)



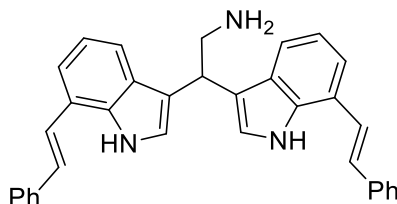
Following the **GP1** (reaction time = 18 h), compound **6al** has been purified by flash column chromatography on silica gel (CH₂Cl₂/NH₃, 99:1 to CH₂Cl₂/CH₃OH/NH₃, 96:4:1) obtaining a white solid; yield: 59% (29 mg). *R*_f = 0.29 (silica gel; CH₂Cl₂/CH₃OH/NH₃, 90:10:1). ¹H NMR (400 MHz, DMSO-*d*₆): δ = 10.92 (br d, 2H, *J* = 2.5 Hz), 7.47 (d, 2H, *J* = 8.5 Hz), 7.44 (s, 2H), 7.39 (dd, 2H, *J*₁ = 1.0, *J*₂ = 5.0 Hz), 7.31 (d, 2H, *J* = 16.0 Hz), 7.25 (d, 2H, *J* = 2.5 Hz), 7.19 (dd, 2H, *J*₁ = 1.5 Hz, *J*₂ = 8.5 Hz), 7.16 (d, 2H, *J* = 3.0 Hz), 7.04-7.02 (m, 2H), 6.99 (s, 2H, *J* = 16.0 Hz), 4.38 (t, 1H, *J* = 7.0 Hz), 3.26 (d, 2H, *J* = 7.0 Hz) ppm. ¹³C NMR (101 MHz, DMSO-*d*₆): δ = 143.0, 136.8, 129.6, 129.6, 127.9, 126.9, 125.6, 124.3, 123.6, 119.2, 119.1, 117.3, 116.6, 110.0, 46.7, 37.7 ppm. HRMS (*m/z*): [M+H]⁺ calcd for C₃₀H₂₆N₃S₂ 492.1563; found 492.1583.

2,2'-Bis[(5-(*E*)-styryl)-1*H*-indol-3-yl]ethan-1-amine (6ba)



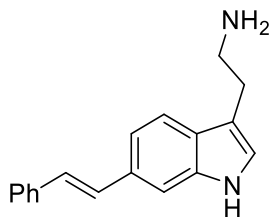
Following the **GP1** (reaction time = 4 h), compound **6ba** has been purified by flash column chromatography on silica gel ($\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}/\text{NH}_3$, 99:1:1 to $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}/\text{NH}_3$, 98:2:1), obtaining a white solid; yield: 86% (41 mg). R_f = 0.2 (silica gel; $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}/\text{NH}_3$, 90:10:1). ^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ = 10.94 (br d, J = 2.5 Hz, 2H), 7.81 (s, 2H), 7.58-7.54 (m, 4H), 7.38 (dd, J_1 = 1.5 Hz, J_2 = 8.5 Hz, 2H), 7.34-7.30 (m, 6H), 7.28 (dd, J_1 = 2.5 Hz, J_2 = 8.5 Hz, 4H), 7.21 (d, J = 7.0 Hz, 2H), 7.08 (d, J = 16.5 Hz, 2H), 4.55 (t, J = 7.0 Hz, 1H), 3.39 (d, J = 7.0 Hz, 2H) ppm. ^{13}C NMR (101 MHz, DMSO): δ = 137.7, 136.4, 13.3, 128.6, 127.4, 127.1, 126.8, 126.0, 124.7, 123.2, 119.6, 118.0, 117.1, 111.8, 46.0, 36.6 ppm. HRMS (m/z): $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{34}\text{H}_{30}\text{N}_3$ 480.2434; found 480.2435.

2,2'-Bis[(7-(*E*)-styryl)-1*H*-indol-3-yl]ethan-1-amine (**6ca**)



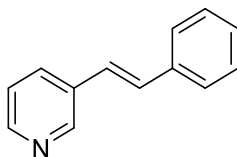
Following the **GP1** (reaction time = 4 h), compound **6ca** has been purified by flash column chromatography on silica gel ($\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}/\text{NH}_3$, 99:1:1), obtaining a white solid; yield: 75% (36 mg). R_f = 0.34 (silica gel; $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}/\text{NH}_3$, 90:10:1). ^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ = 11.16 (br d, J = 2.5 Hz, 2H), 7.75 (d, J = 16.5 Hz, 2H), 7.70-7.64 (m, 4H), 7.46 (d, J = 8.0 Hz, 2H), 7.44-7.37 (m, 6H), 7.33 (d, J = 2.5 Hz, 2H), 7.31-7.25 (m, 4H), 6.94 (dd, $J_1 \approx J_2$ = 7.5 Hz, 2H), 4.44 (t, J = 7.0 Hz, 1H), 3.30 (d, J = 7.0 Hz, 1H) ppm. ^{13}C NMR (101 MHz, DMSO): δ = 137.6, 134.7, 128.6, 127.8, 127.7, 127.3, 126.4, 124.3, 122.6, 120.8, 118.7, 118.6, 117.4, 117.2, 46.8, 37.9 ppm. HRMS (m/z): $[\text{M}+\text{K}]^+$ calcd for $\text{C}_{34}\text{H}_{29}\text{N}_3\text{K}$ 518.1993; found 518.2001.

2-[(*E*)-6-Styryl]-1*H*-indol-3-yl]ethan-1-amine (**9aa**)



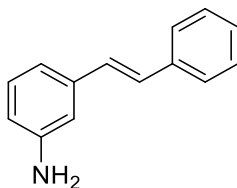
Following the **GP2** (reaction time = 18 h), compound **9aa** has been purified by flash column chromatography on silica gel ($\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}/\text{NH}_3$, 99:1:1 to $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}/\text{NH}_3$, 95:5:1), obtaining a white solid; yield: 44% (23 mg). $R_f = 0.29$ (silica gel; $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}/\text{NH}_3$, 90:10:1). ^1H NMR (400 MHz, $\text{DMSO}-d_6$): $\delta = 10.98$ (br d, $J = 2.5$ Hz, 1H), 7.62-7.58 (m, 2H), 7.55-7.51 (m, 2H), 7.39-7.31 (m, 4H), 7.26-7.21 (m, 2H), 7.17 (d, $J = 16.5$ Hz, 1H), 3.0-2.95 (m, 2H), 2.92-2.86 (m, 2H) ppm. ^{13}C NMR (101 MHz, $\text{DMSO}-d_6$): 137.6, 136.6, 130.4, 130.0, 128.6, 127.0, 126.1, 125.7, 124.2, 118.4, 117.0, 111.1, 110.4, 40.6, 25.5 ppm. HRMS (m/z): $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{18}\text{H}_{19}\text{N}_2$ 263.1543; found 263.1553.

(E)-3-styrylpyridine (9ab)



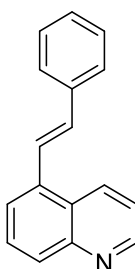
Following the **GP2** (reaction time = 2 h), compound **9ab** has been purified by flash column chromatography on silica gel (cyclohexane/EtOAc, 80:20) obtaining a white solid; yield: 91% (33 mg). ^1H NMR (400 MHz, CDCl_3): $\delta = 8.72$ (d, $J = 2.5$ Hz, 1H), 8.48 (dd, $J_1 = 1.5$ Hz, $J_2 = 5.0$ Hz, 1H), 7.82 (dt, $J_1 = 2.0$ Hz, $J_2 = 8.0$ Hz, 1H), 7.55-7.50 (m, 2H), 7.40-7.35 (m, 2H), 7.32-7.25 (m, 2H), 7.16 (d, $J = 16.5$ Hz, 1H), 7.06 (d, $J = 16.5$, 1H) ppm. ^{13}C NMR (101 MHz, CDCl_3): $\delta = 148.7$, 136.7, 133.1, 132.7, 130.9, 128.9, 128.3, 126.8, 125.0, 123.6 ppm. Spectroscopic data are in accordance with the literature [18].

(E)-3-Styrylaniline (9ac)



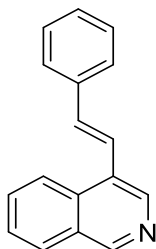
Following the **GP2** (reaction time = 4 h), compound **9ac** has been purified by flash column chromatography on silica gel (cyclohexane/EtOAc, 80:20) obtaining a white solid; yield: 40% (16 mg). ^1H NMR (400 MHz, CDCl_3): δ = 7.52-7.48 (m, 2H), 7.38-7.33 (m, 2H), 7.28-7.23 (m, 2H), 7.16 (dd, $J_1 \approx J_2 = 8.0$ Hz, 1H), 7.08 (d, $J = 16.5$ Hz, 1H), 7.02 (d, $J = 16.5$ Hz, 1H), 6.94 (d, $J = 7.5$ Hz, 1H), 6.86 (dd, $J_1 \approx J_2 = 2.0$ Hz, 1H), 6.61 (ddd, $J_1 = 1.0$ Hz, $J_2 = 2.0$ Hz, $J_3 = 8.0$ Hz, 1H), 3.69 (br s, 2H) ppm. ^{13}C NMR (101 MHz, CDCl_3): δ = 146.8, 138.6, 137.6, 129.7, 129.0, 128.8, 128.7, 127.7, 126.6, 117.5, 114.8, 113.0. ppm. Spectroscopic data are in accordance with the literature [19].

(E)-5-Styrylquinoline (9ad)



Following the **GP2** (reaction time = 18 h), compound **9ad** has been purified by flash column chromatography on silica gel (cyclohexane/EtOAc, 90:10 to cyclohexane/EtOAc, 85:15) obtaining a white solid; yield: 48% (22 mg). ^1H NMR (400 MHz, CDCl_3): δ = 8.95 (d, $J = 5.5$ Hz, 1H), 8.57 (d, $J = 8.5$ Hz, 1H), 8.08 (d, $J = 8.5$ Hz, 1H), 7.83-7.71 (m, 3H), 7.63-7.59 (m, 2H), 7.47-7.40 (m, 3H), 7.34 (d, $J = 7.5$ Hz, 1H), 7.19 (d, $J = 16.0$ Hz, 1H) ppm. ^{13}C NMR (101 MHz, CDCl_3): δ = 150.4, 148.7, 137.3, 135.5, 133.0, 132.3, 129.4, 129.4, 129.0, 128.3, 126.9, 124.3, 124.0, 121.1 ppm. Spectroscopic data are in accordance with the literature [20].

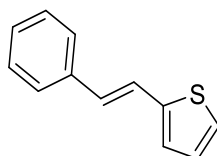
(E)-4-Styrylisoquinoline (9ae)



Following the **GP2** (reaction time = 18 h), compound **9ae** has been purified by flash column chromatography on silica gel (cyclohexane/EtOAc, 95:5 to cyclohexane/EtOAc 90:10) obtaining a white solid; yield: 71% (33 mg). ^1H NMR (400

MHz, CDCl₃): δ = 9.17 (s, 1H), 8.76 (s, 1H), 8.15 (dd, J_1 = 1.0 Hz, J_2 = 8.5 Hz, 1H), 7.98 (d, J = 8.0 Hz, 1H), 7.74 (ddd, J_1 = 1.0 Hz, J_2 = 7.0 Hz, J_3 = 8.5 Hz, 1H), 7.69 (d, J = 16.0 Hz, 1H), 7.64-7.58 (m, 3H), 7.45-7.39 (m, 2H), 7.36- 7.30 (m, 1H), 7.20 (d, J = 16.0 Hz, 1H) ppm. ¹³C NMR (101 MHz, CDCl₃): δ = 152.0, 140.6, 137.2, 133.9, 133.3, 130.6, 128.9, 128.7, 128.3, 128.3, 128.2, 127.3, 126.9, 123.0, 122.6 ppm. Spectroscopic data are in accordance with the literature [21].

(E)-2-Styrylthiophene (9af)



Following the **GP2** (reaction time = 2 h), compound **9af** has been purified by flash column chromatography on silica gel (cyclohexane/EtOAc 90:10) obtaining a yellowish solid; yield: 40% (15 mg). ¹H NMR (400 MHz, CDCl₃): δ = 7.49-7.46 (m, 2H), 7.38-7.33 (m, 2H), 7.28-7.23 (m, 2H), 7.20 (d, J = 5.0 Hz, 1H), 7.08 (d, J = 3.5 Hz, 1H), 7.01 (dd, J_1 = 3.5 Hz, J_2 = 5.0 Hz, 1H), 6.94 (d, J = 16.0 Hz, 1H) ppm. ¹³C NMR (101 MHz, CDCl₃): δ = 143.0, 137.1, 128.8, 128.5, 127.7, 126.4, 126.2, 124.5, 121.9 ppm. Spectroscopic data are in accordance with the literature [22].

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Chapter 4. Design and Synthesis of Stable I3C Analogues for Potential Biological Activity Evaluation

4.1 Introduction

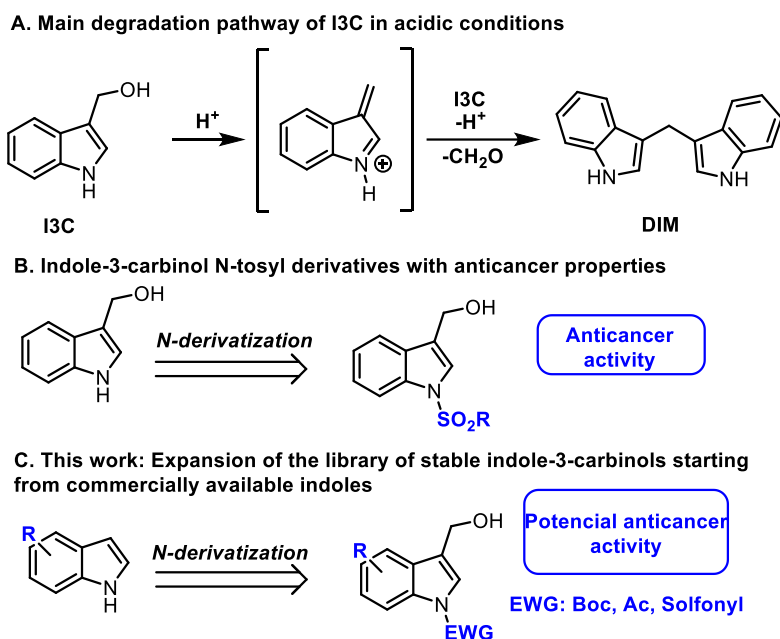
I3C is a natural compound found in vegetables of the Brassicaceae family, produced, as shown in **Figure 1** (Chapter 1), through the enzymatic cleavage of glucobrassicin [1, 2]. From a biomedical perspective, it has gained considerable interest over the years due to its antitumor properties, as discussed in Chapter 1 of this thesis [3]. Pioneering studies have demonstrated that dietary intake of I3C can significantly reduce the incidence of both spontaneous and chemically induced tumors in mammals, suggesting a potential protective effect against cancer [3]. However, the therapeutic use of I3C is limited by its chemical instability, particularly in acidic environments (such as the gastric milieu), where it rapidly polymerizes into oligomers such as 3,3'-DIM. This instability necessitates the administration of high concentrations of I3C to achieve anticancer effects, increasing the risk of exceeding the safe therapeutic window [4, 5].

On a molecular level, I3C is a “vinylogous” hemiaminal: under acidic conditions, it readily undergoes dehydration, forming a 3-methyleneindoleninium carbocation. This cation can react with nucleophiles, including another molecule of I3C, leading to condensation products such as diindolylmethane, thereby driving the rapid oligomerization of I3C in the gastric environment (**Scheme 5A**) [6].

Given the promising biological effects of I3C, yet its limited applicability for the reasons outlined above, various strategies of structural modification have been proposed to overcome these limitations. Efforts have focused on maintaining the core structure of I3C while introducing substituents on the indole ring to modulate electronegativity and lipophilicity. Another strategy involves introducing electronwithdrawing groups (EWGs) on the indole N–H to decrease the electron density on the nitrogen atom, thereby reducing its propensity to form the cationic intermediate. This approach aims to generate derivatives with improved acid stability while preserving biological activity [3].

In this regard, several SAR studies are reported in the literature [6]. For instance, N-substituted I3C analogues bearing acetyl or tosyl groups have been synthesized and evaluated. Notably, the study by Chen [7] and co-workers demonstrated that *N*-tosyl

derivatives exhibit anticancer properties even superior to those of I3C itself and possess unique stereoelectronic characteristics that enhance their interactions with biological targets (**Scheme 5B**). These findings suggest that the I3C core is the true pharmacophore, and that selective modifications at the indole nitrogen may even improve its activity [6, 7].



Scheme 5. **A** Degradation pathway of I3C in the acidic stomach environment, leading to the formation of **3,3'-DIM**. **B** Previously published work by Chen [7] and co-workers demonstrating the synthesis of stable I3C derivatives with anticancer activity. **C** Synthesis of stable I3C derivatives starting from commercially available indoles with diverse substitutions on the aromatic ring.

Therefore, this chapter is dedicated to the expansion of a focused library of I3C analogues, starting from commercially available indoles, through the introduction of electronwithdrawing substituents on the indole N–H, while deliberately preserving the remainder of the molecular scaffold (**Scheme 5C**). This rational design strategy is aimed at modulating the electronic properties of the indole core to improve the chemical stability of these derivatives under acidic conditions, without compromising their intrinsic biological potential. In the final stage of this project, these newly synthesized compounds will be subjected to biological evaluation to gain preliminary insights into the SAR associated with N-substitution in I3C analogues.

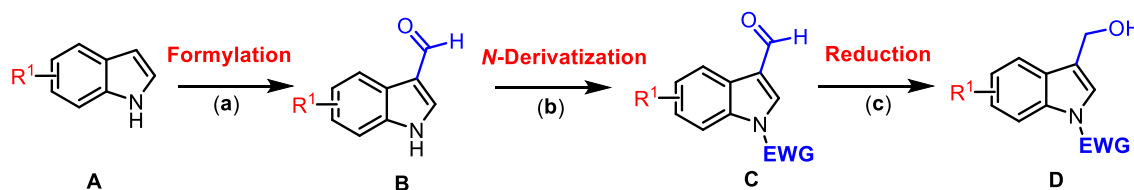
4.2 Results and Discussion

Craig's plot is a valuable tool for selecting substituents in QSAR studies, as it correlates physicochemical properties with biological activity. This approach allows for predicting the activity of new analogues, guiding synthesis in a more targeted manner, and reducing both time and cost. Substituents are distributed across four quadrants according to their hydrophobicity (π) and electronic effects (σ): for example, halogens exhibit positive π and σ values, hydroxyl groups are negative for both, alkyl groups have positive π and negative σ , while acyl groups display negative π and positive σ [8]. This plot was instrumental in selecting the substituents synthesized in this work.

The synthesis of stable analogues of I3C was developed starting from commercially available indoles. The strategy was inspired by literature procedures, which were then adapted and optimized to fit the goals of this work (see Experimental Section). The plan aimed to design a short, reliable route that would allow the preparation of stable intermediates suitable for further transformations and biological evaluation.

The synthetic sequence consisted of three main steps (**Scheme 6**).

1. Selective formylation of the indole ring
2. Protection of the indole nitrogen
3. Reduction of the aldehyde to the corresponding alcohol.



Scheme 6. Multistep synthesis employed in this work.

This order was chosen deliberately. Performing the formylation first takes advantage of the high reactivity at the C-3 position of the unprotected indole. When the nitrogen is not involved in resonance or coordination effects, its lone pair increases the electron density at C-3, facilitating electrophilic substitution. Once the aldehyde group was introduced, the protection of the indole nitrogen became essential to tune the electronic properties of the scaffold and to stabilize the intermediates, especially

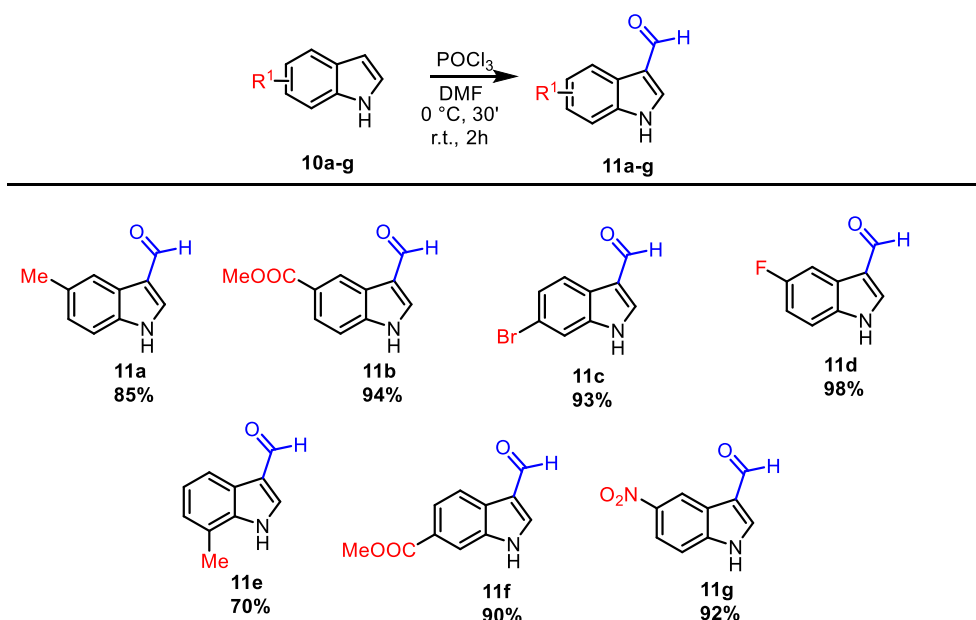
considering the known instability of free N–H analogues of I3C under acidic or physiological conditions.

The final reduction step was intentionally left for the end of the sequence. This choice minimized the handling of the hydroxymethyl group, which is particularly sensitive to acidic and moisture-induced transformations, leading to oligomerization or polymerization. Working with more stable intermediates in the earlier stages made the procedure safer, cleaner, and more reproducible.

This approach allowed the preparation of a small library of I3C analogues with finely adjusted electronic features, creating a solid basis for biological investigations.

Table 15 summarizes the synthesis of 1*H*-indole-3-carbaldehyde derivatives (**11a–g**). Seven indole scaffolds with different substituents were selected to evaluate how electronic and steric effects influence reactivity. The aldehyde group at C-3 was introduced through the classical Vilsmeier formylation. In the presence of phosphoryl chloride (POCl₃) and DMF, a reactive chloroiminium species is generated and readily attacked by the indole nucleus, affording the desired aldehydes (**11a–g**) after hydrolysis.

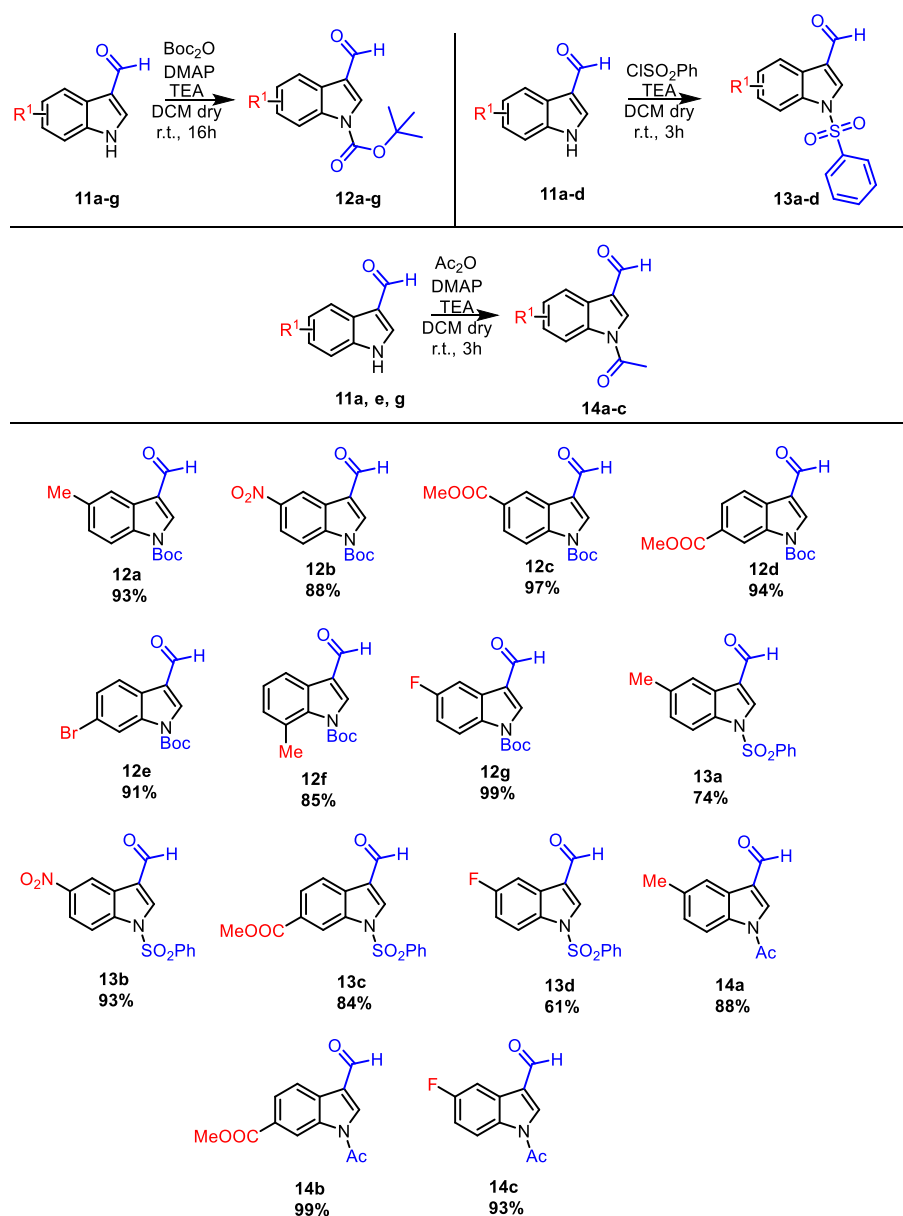
Table 15. Vilsmeier–Haack reaction on commercially available indoles.



To further tune the indole’s electronic environment and improve the chemical stability of the resulting I3C analogues, the indole NH was functionalized with electronwithdrawing groups through three standard reactions: *N*-Boc protection, *N*-

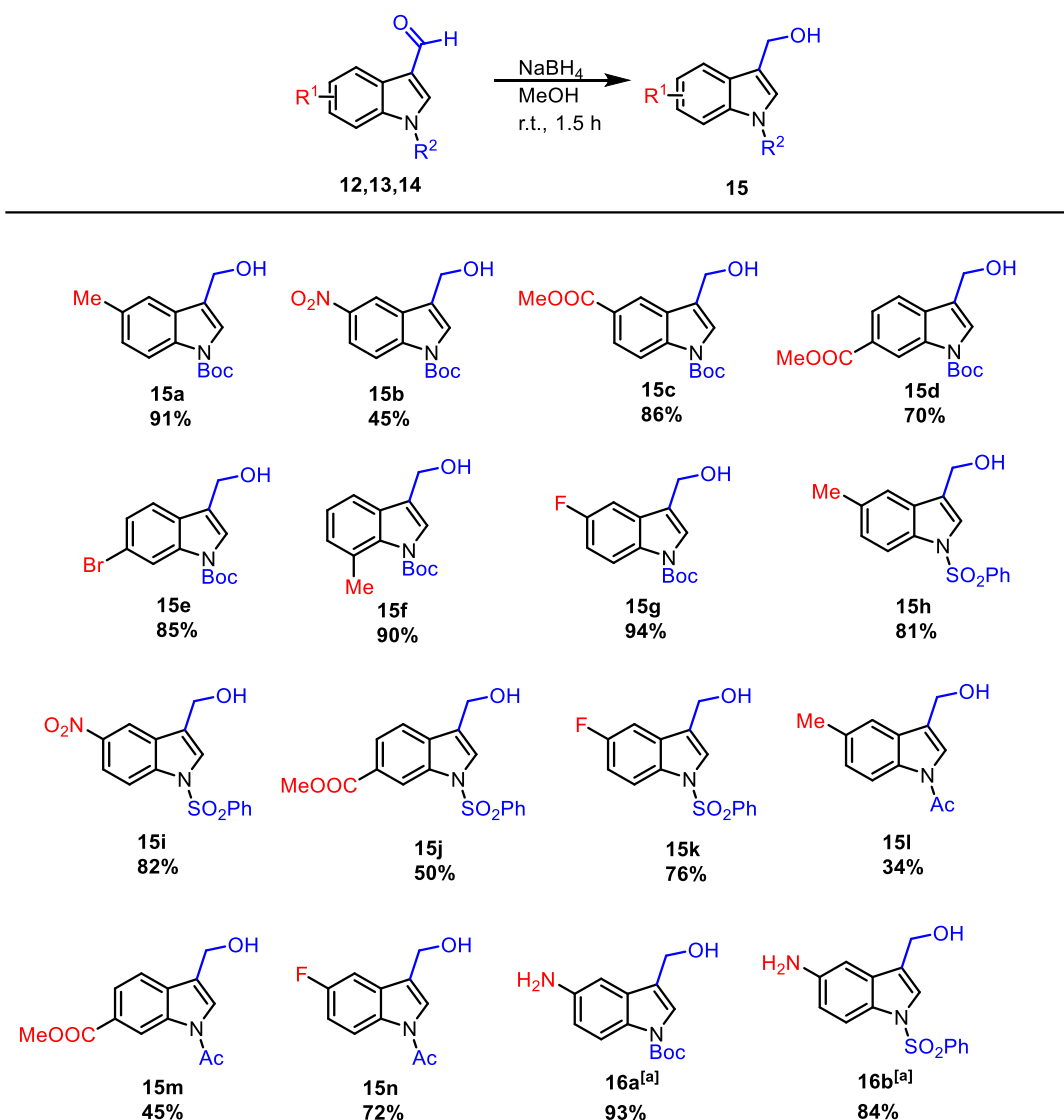
acetylation, and *N*-sulfonylation (Table 16). The Boc group was introduced using (Boc)₂O, dimethylaminopyridine (DMAP), and TEA under mild conditions, thereby suppressing the delocalization of the nitrogen lone pair and electronically deactivating the indole ring. Acetylation with acetic anhydride [(CH₃CO)₂O] provided simple amide derivatives that subtly modulate the ring's electron density, while sulfonylation with aromatic sulfonyl chlorides introduced strongly electronwithdrawing substituents. Together, these modifications reduced the nucleophilicity of the indole nitrogen and enhanced the overall stability of the molecules.

Table 16. Indole NH functionalization: *N*-Boc protection, *N*-acetylation, and *N*-sulfonylation.



Finally, the aldehyde derivatives were reduced with sodium borohydride (**Table 17**) to obtain the corresponding alcohols, reproducing the core structure of indole-3-carbinol while maintaining the previously introduced substituents. Compounds **15b** and **15i** were further hydrogenated under mild conditions using Pd/CaCO₃, affording the reduced analogues **16a** and **16b** in excellent yields (93% and 84%, respectively) without affecting other functional groups.

Table 17. Selective reduction of the aldehyde function using sodium borohydride to generate the corresponding alcohol.



[a] Obtained by reduction of compounds **15b** and **15i**.

In summary, this modular and straightforward route provided access to a small but diverse family of I3C analogues. The method proved reproducible and efficient, yielding compounds with balanced stability and tunable electronic properties, which will serve as valuable candidates for future structure–activity relationship and biological studies.

4.3 Conclusions

As part of this project, the research group focused on exploring the potential activity of I3C in the treatment of cancer across various cell lines, with particular emphasis on mammary gland cells. However, given that I3C undergoes rapid decomposition at gastric pH when administered orally, successful structural modifications were introduced to the I3C framework to retain its biological potential while simultaneously improving its pharmacokinetic and pharmacodynamic properties. A total of sixteen novel I3C analogues, each bearing diverse substituents on the indole scaffold, were synthesized. These substituents were selected based on their varying hydrophobic and electronic properties, following Craig's plot for rational substituent selection. These newly developed I3C analogues will be evaluated in the future as potential anticancer agents. The biological data will provide valuable insight to guide subsequent research, enabling the design and synthesis of additional analogues based on biological activity profiles, thereby continuing to explore new substituents on the indole scaffold to further enhance pharmacokinetic and pharmacodynamic properties.

4.4 Experimental Section

Materials and Methods

Sodium borohydride was purchased from TCI. 5-Fluoro-1*H*-indole, methyl 1*H*-indole-6-carboxylate, 5-methyl-1*H*-indole, and methyl 1*H*-indole-5-carboxylate were

obtained from BLD. 7-Methyl-1*H*-indole and di-*tert*-butyl dicarbonate [(Boc)₂O] were purchased from Fluorochem. 5-Nitro-1*H*-indole was obtained from Apollo Scientific. Palladium on calcium carbonate (5% Pd/CaCO₃) was purchased from Alfa Aesar. Trimethylamine, *N,N*-dimethylformamide (DMF), (CH₃CO)₂O, 4-dimethylaminopyridine, quinoline, POCl₃, benzenesulfonyl chloride, 6-bromo-1*H*-indole, as well as all organic solvents, were purchased from Sigma-Aldrich. Prior to use, dichloromethane and methanol were dried over 4 Å molecular sieves. ¹H NMR and ¹³C NMR spectra were recorded on a Bruker ULTRASHIELD PLUS 400 spectrometer (400 and 101 MHz, respectively) using CDCl₃ and DMSO-*d*₆ as solvents. Chemical shifts (δ) are reported in parts per million (ppm) relative to the residual solvent signal and coupling constants (*J* values) are expressed in Hertz (Hz). ESI-MS spectra were obtained using a Waters Micromass ZQ spectrometer. Column purifications were performed by flash column chromatography using Merck silica gel (230–400 mesh) as the stationary phase. TLC was carried out on silica gel 60 F254 plates, visualized by exposure to a solution of *p*-anisaldehyde in EtOH.

List of the utilized indole 10

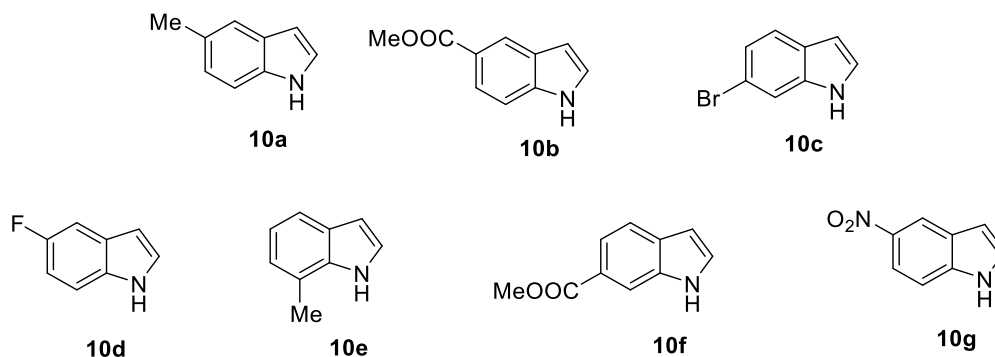


Figure 23. Complete list of the utilized indoles 10.

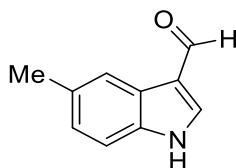
Experimental data for compounds 11,12,13,14,15

General Procedure 1 (GP1) for the Formylation of Indole. POCl₃ (1.1 equiv) was dissolved in DMF (0.84 M) at 0 °C and stirred for 30 min. Then the selected indole (1 equiv) dissolved in DMF (1.14 M) was added to the solution at 0 °C and stirred for 2 h at room temperature. The reaction was quenched by adding H₂O (25 mL) stirred at reflux for 15 min. The resulting solution was extract with EtOAc (3 times) and the

combined organic layers were dried over Na₂SO₄ and concentrated in vacuo to furnish the desired aldehyde that required no further purification [9].

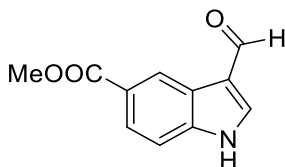
General Procedure 2 (GP2) for the Formylation of Indole. POCl₃ (2.5 equiv) was added dropwise to DMF (5 mL per 1 mL of POCl₃) with ice-bath cooling. The mixture was stirred for 5 minutes then the selected indole (1 equiv) was added as a dimethyl formamide solution (10 mL per 1 g of indole). The mixture was then allowed to warm to room temperature and stirred for 3 hours. The reaction became a heavy suspension that required vigorous stirring. 3.8 M aqueous KOH (10 equiv) was added via a dropping funnel and the mixture was heated to reflux overnight. It was cooled to room temperature before adding saturated aqueous NaHCO₃ and EtOAc until the mixture became clear and the organic layer separated. The aqueous layer was extracted with EtOAc, and the combined organic layers were dried over Na₂SO₄, filtered and concentrated in vacuo to furnish the desired aldehyde that required no further purification [10].

5-Methyl-1*H*-indole-3-carbaldehyde (11a)



11a was synthesized using **GP1**. Yield: 85 % as a red solid. ¹H NMR (400 MHz, DMSO-*d*₆): δ = 12.01 (br s, 1H), 9.90 (s, 1H), 8.20 (d, 1H, *J* = 3.0 Hz), 7.90 (d, 1H, *J* = 2.0 Hz), 7.38 (d, 1H, *J* = 8.5 Hz), 7.08 (dd, 1H, *J*₁ = 2.0, *J*₂ = 8.5 Hz), 2.40 (s, 3H) ppm. ESI-MS: 160 [M+1]⁺. The physicochemical data of compound agree with those reported in the literature [11].

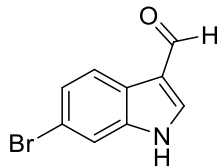
Methyl 3-formyl-1*H*-indole-5-carboxylate. (11b)



11b was synthesized using **GP1**. Yield: 94% as a pinkish solid. ¹H NMR (400 MHz, DMSO-*d*₆): δ = 12.43 (br s, 1H), 9.98 (s, 1H), 8.77 (d, 1H, *J* = 1.5 Hz), 8.43 (d, 1H, *J* = 3.0 Hz), 7.88 (dd, 1H, *J*₁ = 1.5 Hz, *J*₂ = 8.5 Hz), 7.61 (d, 1H, *J* = 8.5 Hz), 3.88 (s,

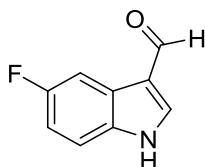
3H) ppm. ES-MS: 204 $[M+H]^+$. The physicochemical data of compound agree with those reported in the literature [9].

6-Bromo-1*H*-indole-3-carbaldehyde (11c)



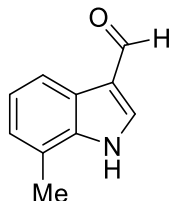
11c was synthesized using **GP2**. Yield: 93 % as a yellow solid. ^1H NMR (DMSO- d_6 , 400 MHz): δ = 12.21 (br s, 1H), 9.94 (s, 1H), 8.32 (s, 1H), 8.02 (dd, 1H, J_1 = 0.5 Hz, J_2 = 8.5 Hz), 7.71 (dd, 1H, J_1 = 0.5 Hz, J_2 = 2.0 Hz), 7.35 (dd, 1H, J_1 = 2.0 Hz, J_2 = 8.5 Hz) ppm. ESI-MS: 223 $[M+H]^+$, 225 $[M+H]^+$. The physicochemical data of compound agree with those reported in the literature [12].

5-Fluoro-1*H*-indole-3-carbaldehyde (11d)



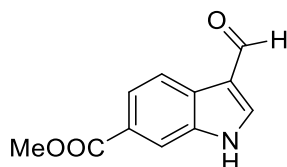
11d was synthesized using **GP2**. Yield: 98% as a colorless powder. ^1H NMR (400 MHz, DMSO- d_6): δ = 12.20 (br s, 1H), 9.92 (s, 1H), 8.35 (s, 1H), 7.76 (dd, 1H, J_1 = 2.5 Hz, J_2 = 9.0 Hz), 7.53 (dd, 1H, J_1 = 4.5 Hz, J_2 = 9.0 Hz), 7.12 (ddd, 1H, J_1 = 2.5 Hz, J_2 $\approx$ J_3 = 9.0 Hz) ppm. ES-MS: 164 $[M+H]^+$. The physicochemical data of compound agree with those reported in the literature [10].

7-methyl-1*H*-indole-3-carbaldehyde (11e)



11e was synthesized using **GP1**. Yield: 70 % as a red solid. ^1H -NMR (400 MHz, DMSO- d_6): δ = 12.14 (br s, 1H), 9.94 (s, 1H), 8.28 (d, 1H, J = 3.0 Hz), 7.91 (d, 1H, J = 7.5 Hz), 7.12 (dd, 1H, $J_1 \approx J_2$ = 7.5 Hz), 7.05 (d, 1H, J = 7.5 Hz), 2.50 (s, 3H) ppm. ESI-MS: 160 $[M+H]^+$. The physicochemical data of compound agree with those reported in the literature [11].

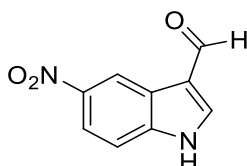
Methyl 3-formyl-1*H*-indole-6-carboxylate (**11f**)



11f was synthesized using **GP1**. Yield: 90% as a brown solid. ^1H NMR (400 MHz, DMSO- d_6): δ = 12.43 (br s, 1H), 9.98 (s, 1H), 8.50 (s, 1H), 8.18 (dd, 1H J_1 = 0.5 Hz, J_2 = 8.5 Hz), 8.14 (dd, 1H, J_1 = 0.5 Hz, J_2 = 1.5 Hz), 7.83 (dd, 1H, J_1 = 1.5 Hz, J_2 = 8.5 Hz), 3.87 (s, 3H) ppm. ESI-MS: 204 $[\text{M}+\text{H}]^+$. The physicochemical data of compound agree with those reported in the literature [13].

5-Nitro-1*H*-indole-3-carbaldehyde (**11g**)

DMF (3 mL) was cooled to 0 °C and POCl₃ (0.8 mL) was added slowly over 30 min. A solution of 5-nitro-1-*H*-indole **10g** (1.4 g, 8.64 mmol) in DMF (1.0 mL) was added slowly to the cooled solution over a period of 10 min and the resulting mixture was heated at 35 °C for 60 min until the clear yellow solution became a yellowish paste. The reaction mixture was poured into ice H₂O, basified with 10% aq. NaOH (3.5 mL) and refluxed for 30 min. After cooling, the precipitate was collected by filtration and dried in vacuum to give the product [14].



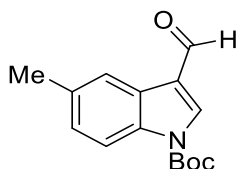
Yield: 92 % as a yellow solid. ^1H NMR (400 MHz, DMSO- d_6): δ = 12.69 (br s, 1H), 10.02 (s, 1H), 8.94 (d, 1H, J = 2.5 Hz), 8.57 (s, 1H), 8.14 (dd, 1H, J_1 = 2.5 Hz, J_2 = 9.0 Hz), 7.71 (dd, 1H, J = 9.0 Hz) ppm. ESI-MS: 191 $[\text{M}+\text{H}]^+$. The physicochemical data of compound agree with those reported in the literature [14].

General procedure for the synthesis of compounds **12**

The selected indole derivative **11** (1 equiv) was dissolved in CH₂Cl₂ to obtain a final concentration of 0.086 M, and the solution was stirred at room temperature. DMAP (0.1 equiv), Et₃N (1.3 equiv), and [(Boc)₂O, 1.3 equiv] were added sequentially. The reaction mixture was stirred at room temperature for 24 h. Upon completion, an aqueous solution of ammonium chloride (NH₄Cl) was added, and the organic layer was separated. The aqueous phase was extracted three times with CH₂Cl₂, and the combined organic

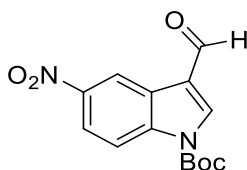
layers were washed with H₂O and brine, then dried over an. Na₂SO₄. Filtration and concentration under reduced pressure afforded a crude product, which was purified by flash column chromatography to yield the desired products **12**.

***Tert*-butyl 3-formyl-5-methyl-1*H*-indole-1-carboxylate (**12a**)**



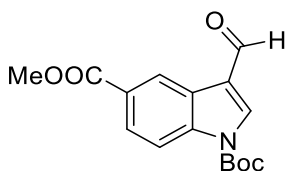
12a was isolated by column chromatography (cyclohexane/EtOAc 95:5) in 93% yield as a white solid. ¹H NMR (400 MHz, CDCl₃): δ = 10.08 (s, 1H), 8.19 (s, 1H), 8.09 (d, 1H, J = 2.0 Hz), 8.00 (d, 1H, J = 8.5 Hz), 7.23 (dd, 1H, J_1 = 2.0 Hz, J_2 = 8.5 Hz), 2.47 (s, 3H), 1.70 (s, 9H) ppm. ESI-MS: 260 [M+H]⁺. The physicochemical data of compound agree with those reported in the literature [15].

***Tert*-butyl 3-formyl-5-nitro-1*H*-indole-1-carboxylate (**12b**)**



After completion of the reaction, the precipitate was collected and washed with water and Et₂O and dried to obtain **12b** as a yellow solid. Yield = 88%. ¹H NMR (400 MHz, DMSO-*d*₆): δ = 10.14 (s, 1H), 8.94 (dd, 1H, J_1 = 1.0 Hz, J_2 = 2.0 Hz), 8.91 (s, 1H), 8.35 (dd, 1H, J_1 = 2.0 Hz, J_2 = 9.0 Hz), 8.32 (dd, 1H, J_1 = 1.0 Hz, J_2 = 9.0 Hz), 1.68 (s, 9H) ppm. ESI-MS: 291 [M+H]⁺. The physicochemical data of compound agree with those reported in the literature [16].

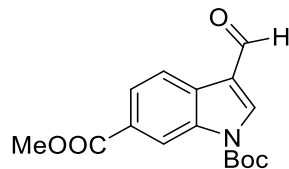
1-(*Tert*-butyl) 5-methyl 3-formyl-1*H*-indole-1,5-dicarboxylate (12c**).**



12c was isolated by column chromatography (cyclohexane/EtOAc 95:5) in 97% yield as a white solid. ¹H NMR (400 MHz, DMSO-*d*₆): δ = 10.12 (s, 1H), 8.79 (s, 1H), 8.77 (d, 1H, J = 2.0 Hz), 8.24 (d, 1H, J = 9.0 Hz), 8.06 (dd, 1H, J_1 = 2.0 Hz, J_2 = 9.0 Hz), 3.90 (s, 3H), 1.67 (s, 9H) ppm. ¹³C NMR (101 MHz, DMSO-*d*₆): δ = 187.2, 166.1, 148.0,

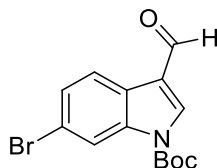
139.8, 137.9, 126.7, 125.8, 125.5, 122.9, 120.5, 115.2, 86.1, 52.2, 27.5 ppm. ESI-MS: 304 [M+H]⁺.

1-(*Tert*-butyl) 6-methyl 3-formyl-1*H*-indole-1,6-dicarboxylate (12d)



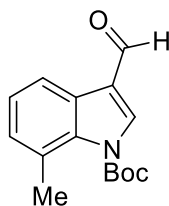
12d was isolated by column chromatography (cyclohexane/EtOAc 95:5) in 94% yield as a white solid. ¹H NMR (400 MHz, CDCl₃): δ = 10.12 (s, 1H), 8.87 (dd, 1H, *J*₁ = 0.5 Hz, *J*₂ = 1.5 Hz), 8.36 (s, 1H), 8.33 (dd, 1H, *J*₁ = 0.5 Hz, *J*₂ = 8.5 Hz), 8.07 (dd, 1H, *J*₁ = 1.5 Hz, *J*₂ = 8.5 Hz), 3.96 (s, 3H), 1.74 (s, 9H) ppm. ¹³C NMR (101 MHz, CDCl₃): δ = 185.5, 167.2, 148.5, 138.4, 135.6, 129.8, 127.9, 125.8, 121.9, 121.3, 117.2, 86.5, 52.4, 28.1 ppm. ESI-MS: 304 [M+H]⁺.

***Tert*-butyl 6-bromo-3-formyl-1*H*-indole-1-carboxylate (12e)**



12e was isolated by column chromatography (cyclohexane/EtOAc 95:5) in 91% yield as a white solid. ¹H NMR (400 MHz, CDCl₃): δ = 10.07 (s, 1H), 8.37 (d, 1H, *J* = 2.0 Hz), 8.19 (s, 1H), 8.15 (d, 1H, *J* = 8.5 Hz), 7.49 (dd, 1H, *J*₁ = 2.0 Hz, *J*₂ = 8.5 Hz), 1.71 (s, 9H) ppm. ESI-MS: 324 [M+H]⁺, 326 [M+H]⁺. The physicochemical data of compound agree with those reported in the literature [17].

***Tert*-butyl 3-formyl-7-methyl-1*H*-indole-1-carboxylate (12f)**



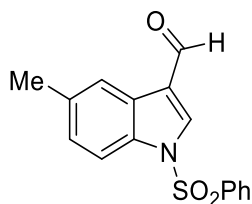
12f was isolated by column chromatography (cyclohexane/EtOAc 95:5) in 85% yield as a white solid. ¹H NMR (400 MHz, CDCl₃): δ = 10.08 (s, 1H), 8.16 (d, 1H, *J* = 7.5 Hz), 8.14 (s, 1H), 7.29 (dd, 1H, *J*₁ ≈ *J*₂ = 7.5 Hz), 7.21 (d, *J* = 7.5 Hz, 1H), 2.63 (s,

3H), 1.68 (s, 9H) ppm. ESI-MS: 260 [M+H]⁺. The physicochemical data of compound agree with those reported in the literature [15].

General procedure for the synthesis of compounds 13

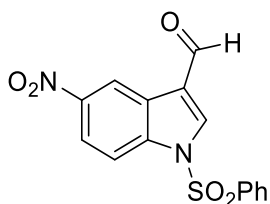
The selected indole derivative **11** was placed in a round-bottom flask equipped with a magnetic stir bar and cooled in an ice bath. CH₂Cl₂ was added to achieve a concentration of 0.145 M, followed by the addition of Et₃N (1.3 equiv). To this cold suspension, arylsulfonyl chloride (1.1 equiv) was added dropwise, and the reaction mixture was allowed to warm to room temperature and stirred for 3–4 hours. The progress of the reaction was monitored by TLC. Upon completion, the solvent was evaporated under reduced pressure to afford a crude residue. The resulting solid was washed with cyclohexane, filtered through a sintered glass funnel, then washed sequentially with warm H₂O and cyclohexane before being dried under reduced pressure. The residue obtained after evaporation of CH₂Cl₂ was purified by flash column chromatography on silica gel to afford the desired product **13** [18].

5-Methyl-1-(phenylsulfonyl)-1*H*-indole-3-carbaldehyde (**13a**)



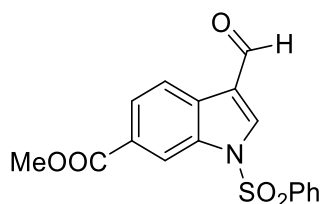
13a was isolated by column chromatography (cyclohexane/EtOAc 8:2) in 74% yield. ¹H NMR (400 MHz, CDCl₃): δ = 10.08 (s, 1H), 8.18 (s, 1H), 8.06 (d, 1H, *J* = 2.0 Hz), 7.98 – 7.93 (m, 2H), 7.83 (d, 1H, *J* = 8.5 Hz), 7.61 (dddd, 1H, *J*₁ ≈ *J*₂ = 1.0 Hz, *J*₃ ≈ *J*₄ = 7.5 Hz), 7.54 – 7.47 (m, 2H), 7.23 (dd, 1H, *J*₁ = 2.0 Hz, *J*₂ = 8.5 Hz), 2.44 (s, 3H) ppm. ESI-MS: 300 [M+H]⁺. The physicochemical data of compound agree with those reported in the literature [19].

5-Nitro-1-(phenylsulfonyl)-1*H*-indole-3-carbaldehyde (**13b**)



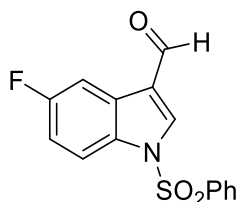
After completion of the reaction, the precipitate was collected and washed with water and Et₂O and dried to obtain the product **13b** as a yellow solid with 93%. ¹H NMR (400 MHz, DMSO-*d*₆): δ = 10.12 (s, 1H), 9.15 (s, 1H), 8.88 (d, 1H, J = 2.5 Hz), 8.23 (d, 1H, J = 9.0 Hz), 8.21 – 8.17 (m, 3H), 7.81 (dddd, 1H, $J_1 \approx J_2$ = 1.0 Hz, $J_3 \approx J_4$ = 7.5 Hz), 7.72 – 7.66 (m, 2H) ppm. ESI-MS: 331 [M+H]⁺. The physicochemical data of compound agree with those reported in the literature [18].

Methyl 3-formyl-1-(phenylsulfonyl)-1H-indole-6-carboxylate (13c)



13c was isolated by column chromatography (CH₂Cl₂/MeOH 97:3) in 84% yield. ¹H NMR (400 MHz, DMSO-*d*₆): δ = 10.09 (s, 1H), 9.11 (s, 1H), 8.51 (d, 1H, J = 1.5 Hz), 8.23 (d, 1H, J = 8.5 Hz), 8.10 – 8.06 (m, 2H), 7.99 (dd, 1H, J_1 = 1.5 Hz, J_2 = 8.5 Hz), 7.79 (dddd, 1H, $J_1 \approx J_2$ = 1.0 Hz, $J_3 \approx J_4$ = 7.5 Hz), 7.71 – 7.61 (m, 2H), 3.91 (s, 3H) ppm. ¹³C NMR (101 MHz, DMSO-*d*₆): δ = 186.7, 165.8, 141.0, 136.2, 135.7, 134.1, 130.4, 129.4, 127.4, 127.0, 125.8, 122.1, 121.4, 114.1, 52.5 ppm. ESI-MS: 344 [M+H]⁺.

5-Fluoro-1-(phenylsulfonyl)-1H-indole-3-carbaldehyde (13d)

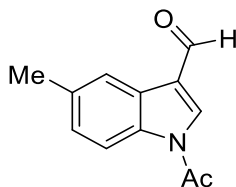


13d was isolated by column chromatography (cyclohexane/EtOAc 1:1) in 61% yield. ¹H NMR (400 MHz, CDCl₃): δ = 10.07 (s, 1H), 8.25 (s, 1H), 7.97-7.92 (m, 3H), 7.91 (dd, 1H, J_1 = 4.0 Hz, J_2 = 9.0 Hz), 7.65 (dddd, 1H, $J_1 \approx J_2$ = 1.0 Hz, $J_3 \approx J_4$ = 7.5 Hz), 7.56-7.50 (m, 2H), 7.15 (ddd, 1H, J_1 = 2.5, $J_2 \approx J_3$ = 9.0 Hz) ppm. ¹³C NMR (101 MHz, CDCl₃): δ = 185.1, 160.8 (d, J = 244.0 Hz), 137.4, 137.3, 135.1, 131.7, 130.0, 127.6 (d, J = 11.0 Hz), 127.3, 122.4 (d, J = 4.5 Hz), 114.8 (d, J = 26.0 Hz), 114.5 (d, J = 9.5 Hz), 108.7 (d, J = 25.5 Hz) ppm. ES-MS: 304 [M+H]⁺.

General procedure for the synthesis of compounds 14

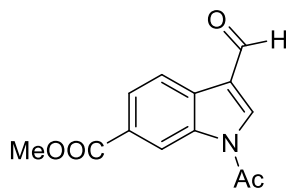
Following a literature-reported procedure [20], (AcO)₂O (1.5 equiv) was added dropwise to an ice-cooled, stirred solution of the selected indole derivative **11** (1 equiv) in dry CH₂Cl₂ (0.15 M) containing DMAP (0.1 equiv) and triethylamine (2.5 equiv). The reaction mixture was allowed to warm to room temperature and stirred for 3–5 hours, during which its progress was monitored until complete conversion was observed. The solvent was then removed under reduced pressure, affording a crude solid residue. This material was purified by flash column chromatography to yield the corresponding *N*-acetylated indole-3-carbaldehyde **14**.

1-Acetyl-5-methyl-1*H*-indole-3-carbaldehyde (**14a**)



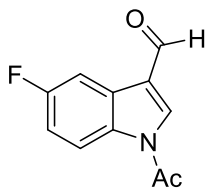
14a was isolated by column chromatography (cyclohexane/ethyl acetate 7:3) in 88% yield. ¹H NMR (400 MHz, CDCl₃): δ = 10.11 (s, 1H), 8.25 (d, 1H, *J* = 8.5 Hz), 8.06 (s, 1H), 8.03 (s, 1H), 7.26 (d, 1H, *J* = 8.5 Hz), 2.72 (s, 3H), 2.48 (s, 3H) ppm. ESI-MS: 202 [M+H]⁺. The physicochemical data of compound agree with those reported in the literature [21].

Methyl 1-acetyl-3-formyl-1*H*-indole-6-carboxylate (**14b**)



14b was isolated by column chromatography (CH₂Cl₂/MeOH 95:5) in 99% yield. ¹H NMR (400 MHz, CDCl₃): δ = 10.14 (s, 1H), 9.05 (s, 1H), 8.31 (d, 1H, *J* = 8.5 Hz), 8.21 (br s, 1H), 8.10 (dd, 1H, *J*₁ = 1.0 Hz, *J*₂ = 8.5 Hz), 3.96 (s, 3H), 2.78 (s, 3H) ppm. ¹³C NMR (101 MHz, CDCl₃): δ = 185.3, 168.4, 167.1, 137.1, 136.0, 129.8, 128.8, 126.7, 122.3, 121.8, 118.2, 52.5, 24.1 ppm. ESI-MS: 246 [M+H]⁺. The physicochemical data of compound agree with those reported in the literature [22].

1-Acetyl-5-fluoro-1*H*-indole-3-carbaldehyde. (14c)

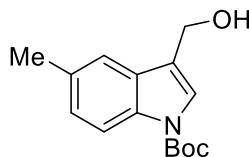


14c was isolated by column chromatography (cyclohexane/ethyl acetate 1:1) in 99% yield. ^1H NMR (400 MHz, CDCl_3): δ = 10.10 (s, 1H), 8.38 (dd, 1H, J_1 = 4.5 Hz, J_2 = 9.0 Hz), 8.09 (s, 1H), 7.95 (dd, 1H, J_1 = 2.5, J_2 = 9.0 Hz), 7.17 (ddd, 1H, J_1 = 2.5 Hz, $J_2 \approx J_3$ = 9.0 Hz), 2.74 (s, 3H) ppm. ^{13}C NMR (101 MHz, CDCl_3): δ = 185.3, 168.4, 160.7 (d, J = 244.0 Hz), 136.1, 132.8 (d, J = 1.5 Hz), 127.1 (d, J = 11.0 Hz), 122.4 (d, J = 4.0 Hz), 117.8 (d, J = 9.0 Hz), 114.9 (d, J = 25.0 Hz), 108.0 (d, J = 25.0 Hz), 23.7 ppm. ESI-MS: 206 [M+1]. The physicochemical data of compound agree with those reported in the literature [21].

General procedure for the synthesis of compounds 15

A solution of the selected N-functionalized indole-3-carboxaldehyde **14** (1 equiv) in CH_3OH (0.072 M) was prepared and stirred at room temperature. Sodium borohydride (NaBH_4 , 2 equiv) was added portion-wise, and the reaction mixture was allowed to stir for 1.5 hour at room temperature. After completion, the solvent was removed under reduced pressure, and the resulting residue was dissolved in a saturated aqueous solution of NH_4Cl . The aqueous phase was extracted three times with EtOAc, and the combined organic extracts were washed with H_2O and dried over an. Na_2SO_4 . Filtration and evaporation of the solvent under reduced pressure afforded a crude product, which was purified by flash column chromatography to provide the desired compound **15**.

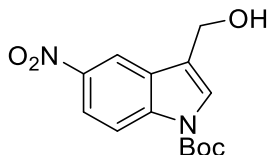
Tert-butyl 3-(hydroxymethyl)-5-methyl-1*H*-indole-1-carboxylate (**15a**)



15a was isolated by column chromatography (cyclohexane/ethyl acetate 7:3) in 91% yield as a white solid. ^1H NMR (400 MHz, CDCl_3): δ = 8.00 (br d, 1H, J = 8.5 Hz), 7.54 (br s, 1H), 7.44 (d, 1H, J = 1.5 Hz), 7.16 (dd, 1H, J_1 = 1.5 Hz, J_2 = 8.5 Hz), 4.85 (d, 1H, J = 19.5 Hz), 4.79 (d, 1H, J = 19.5 Hz), 2.45 (s, 3H), 1.66 (s, 9H) ppm. ESI-MS: 262

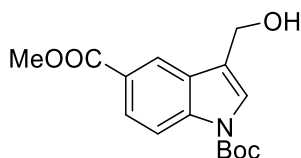
[M+H]⁺. The physicochemical data of compound agree with those reported in the literature [23].

***Tert*-butyl 3-(hydroxymethyl)-5-nitro-1*H*-indole-1-carboxylate (15b)**



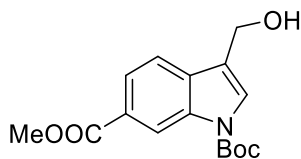
15b was isolated by column chromatography (cyclohexane/ethyl acetate 8:2) in 45% yield as a white solid. ¹H NMR (400 MHz, CDCl₃): δ = 8.58 (d, 1H, J = 2.5 Hz), 8.26 (br d, 1H, J = 9.0 Hz), 8.22 (dd, 1H, J_1 = 2.5 Hz, J_2 = 9.0 Hz), 7.72 (br s, 1H), 4.93 (d, 1H, J = 19.5 Hz), 4.88 (d, 1H, J = 19.5 Hz), 1.68 (s, 9H) ppm. ESI-MS: 293 [M+H]⁺. The physicochemical data of compound agree with those reported in the literature [24].

1-(*Tert*-butyl) 5-methyl 3-(hydroxymethyl)-1*H*-indole-1,5-dicarboxylate (15c)



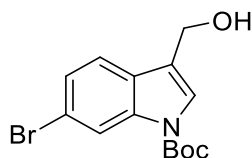
15c was isolated by column chromatography (cyclohexane/ethyl acetate 7:3) in 86% yield as a white solid. ¹H NMR (400 MHz, CDCl₃): δ = 8.36 (d, 1H, J = 1.0 Hz), 8.18 (br d, 1H, J = 8.5 Hz), 8.03 (dd, 1H, J_1 = 1.0, J_2 = 8.5 Hz), 7.64 (br s, 1H), 4.91 (d, 1H, J = 19.5 Hz), 4.86 (d, 1H, J = 19.5 Hz), 3.94 (s, 3H), 1.67 (s, 9H) ppm. ¹³C NMR (101 MHz, CDCl₃): δ = 167.6, 149.4, 138.6, 129.1, 126.1, 125.0, 124.7, 121.7, 121.1, 115.1, 84.6, 57.0, 52.2, 28.3 ppm. ESI-MS: 306 [M+H]⁺.

1-(*Tert*-butyl) 6-methyl 3-(hydroxymethyl)-1*H*-indole-1,6-dicarboxylate (15d)



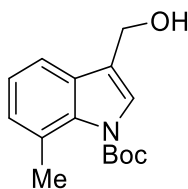
15d was isolated by column chromatography (cyclohexane/ethyl acetate 1:1) in 70% yield as a white solid. ¹H NMR (400 MHz, CDCl₃): δ = 8.85 (s, 1H), 7.95 (dd, 1H, J_1 = 1.5 Hz, J_2 = 8.5 Hz), 7.72 (br s, 1H), 7.67 (d, 1H, J = 8.5 Hz), 4.88 (d, 1H, J = 19.0 Hz), 4.83 (d, 1H, J = 19.0 Hz), 3.94 (s, 3H), 1.69 (s, 9H) ppm. ¹³C NMR (101 MHz, CDCl₃): δ = 167.8, 149.4, 135.4, 133.0, 126.7, 126.5, 124.1, 120.5, 119.2, 117.4, 84.6, 57.2, 52.3, 28.3 ppm. ESI-MS: 306 [M + H]⁺.

***Tert*-butyl 6-bromo-3-(hydroxymethyl)-1*H*-indole-1-carboxylate (15e)**



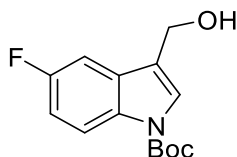
15e was isolated by column chromatography (cyclohexane/ethyl acetate 7:3) in 85% yield as a white solid. ¹H NMR (400 MHz, CDCl₃): δ = 8.34 (br s, 1H), 7.54 (br s, 1H), 7.50 (d, 1H, J = 8.5 Hz), 7.37 (dd, 1H, J_1 = 2.0 Hz, J_2 = 8.5 Hz), 4.85 (d, 1H, J = 19.5 Hz), 4.79 (d, 1H, J = 19.5 Hz), 1.66 (s, 9H) ppm. ESI-MS: 326 [M+H]⁺, 328 [M+H]⁺. The physicochemical data of compound agree with those reported in the literature [17].

***Tert*-butyl 3-(hydroxymethyl)-7-methyl-1*H*-indole-1-carboxylate (15f)**



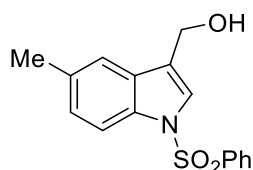
15f was isolated by column chromatography (cyclohexane/ethyl acetate 7:3) in 90% yield as a white solid. ¹H NMR (400 MHz, CDCl₃): δ = 7.52 (s, 1H), 7.47 (br d, 1H, J = 7.5 Hz), 7.19 (dd, 1H, $J_1 \approx J_2$ = 7.5 Hz), 7.13 (br d, 1H, J = 7.5 Hz), 4.85 (d, 1H, J = 9.5 Hz), 4.80 (d, 1H, J = 9.5 Hz), 2.63 (s, 3H), 1.63 (s, 9H) ppm. ESI-MS: 262 [M+H]⁺. The physicochemical data of compound agree with those reported in the literature [23].

***Tert*-butyl 5-fluoro-3-(hydroxymethyl)-1*H*-indole-1-carboxylate (15g)**



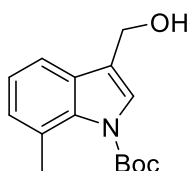
15g was isolated by column chromatography (cyclohexane/ethyl acetate 55:45) in 94% yield as a white solid. ¹H NMR (400 MHz, CDCl₃): δ = 8.08 (s, 1H), 7.61 (s, 1H), 7.31 (dd, 1H, J_1 = 2.5 Hz, J_2 = 9.0 Hz), 7.05 (ddd, 1H, J_1 = 2.5 Hz, J_2 = 9.0 Hz, J_3 = 11.5 Hz), 4.80 (s, 2H), 1.66 (s, 9H) ppm. ESI-MS: 266 [M + H]⁺. The physicochemical data of compound agree with those reported in the literature [25].

(5-Methyl-1-(phenylsulfonyl)-1*H*-indol-3-yl)methanol (15h)



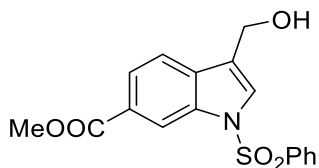
15h was isolated by column chromatography (cyclohexane/ethyl acetate 6:4) in 81% yield as a white solid. ^1H NMR (400 MHz, CDCl_3): δ = 7.90-7.85 (m, 3H), 7.55-7.49 (m, 2H), 7.46-7.38 (m, 3H), 7.16 (d, 1H, J = 8.5 Hz), 4.79 (s, 2H), 2.42 (s, 3H) ppm. ESI-MS: 302 $[\text{M}+\text{H}]^+$. The physicochemical data of compound agree with those reported in the literature [19].

***Tert*-butyl 3-(hydroxymethyl)-7-methyl-1*H*-indole-1-carboxylate (15f)**



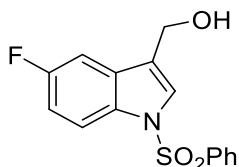
15f was isolated by column chromatography (cyclohexane/ethyl acetate 7:3) in 90% yield as a white solid. ^1H NMR (400 MHz, CDCl_3): δ = 7.52 (s, 1H), 7.47 (br d, 1H, J = 7.5 Hz), 7.19 (dd, 1H, $J_1 \approx J_2$ = 7.5 Hz), 7.13 (br d, 1H, J = 7.5 Hz), 4.85 (d, 1H, J = 9.5 Hz), 4.80 (d, 1H, J = 9.5 Hz), 2.63 (s, 3H), 1.63 (s, 9H) ppm. ESI-MS: 262 $[\text{M}+\text{H}]^+$. The physicochemical data of compound agree with those reported in the literature [23].

Methyl 3-(hydroxymethyl)-1-(phenylsulfonyl)-1*H*-indole-6-carboxylate (15j)



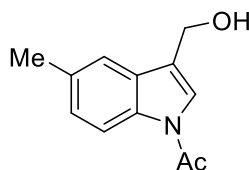
15j was isolated by column chromatography (cyclohexane/ethyl acetate 6:4) in 50% yield as a white solid. ^1H NMR (400 MHz, CDCl_3): δ = 8.67 (s, 1H), 7.94-7.89 (m, 3H), 7.67 (s, 1H), 7.62 (d, 1H, J = 8.0 Hz), 7.54 (dddd, 1H, $J_1 \approx J_2$ = 1.0 Hz, $J_3 \approx J_4$ = 8.0 Hz), 7.47-7.41 (m, 2H), 4.83 (s, 2H), 3.96 (s, 3H), 1.90 (br s, 1H) ppm. ^{13}C NMR (101 MHz, CDCl_3): δ = 167.3, 138.1, 135.0, 134.3, 133.2, 129.6, 127.0, 127.0, 126.6, 124.7, 122.5, 119.9, 115.5, 57.1, 52.4 ppm. ESI-MS: 346 $[\text{M} + \text{H}]^+$.

(5-Fluoro-1-(phenylsulfonyl)-1*H*-indol-3-yl)methanol (15k)



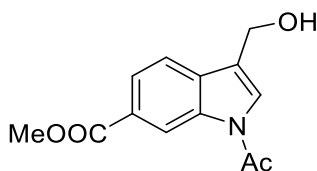
15k was isolated by column chromatography (cyclohexane/ethyl acetate 1:1) in 76% yield as a white solid. ^1H NMR (400 MHz, CDCl_3): δ = 7.94 (dd, 1H, J_1 = 4.5 Hz, J_2 = 9.0 Hz), 7.89-7.84 (m, 2H), 7.57 (s, 1H), 7.55 (dddd, 1H, $J_1 \approx J_2$ = 2.0 Hz, $J_3 \approx J_4$ = 7.5 Hz), 7.48-7.42 (m, 2H), 7.27 (dd, 1H, J_1 = 2.5 Hz, J_2 = 9.0 Hz), 7.07 (ddd, 1H, J_1 = 2.5 Hz, $J_2 \approx J_3$ = 9.0 Hz), 4.81 (d, 1H, J = 19.5 Hz), 4.76 (d, 1H, J = 19.5 Hz) ppm. ^{13}C NMR (101 MHz, CDCl_3): δ = 159.7 (d, J = 242.0 Hz), 138.1, 134.2, 131.9, 130.7 (d, J = 9.5 Hz), 129.5, 126.9, 125.4, 122.5 (d, J = 4.5 Hz), 114.9 (d, J = 9.5 Hz), 113.2 (d, J = 25.5 Hz), 105.9 (d, J = 24.5 Hz), 57.1 ppm. ESI-MS: 306 $[\text{M} + \text{H}]^+$.

1-(3-(Hydroxymethyl)-5-methyl-1*H*-indol-1-yl)ethan-1-one (15l)



15l was isolated by column chromatography (cyclohexane/ethyl acetate 7:3) in 34% yield as a white solid. ^1H NMR (400 MHz, CDCl_3): δ = 8.28 (d, 1H, J = 8.5 Hz), 7.42 (s, 1H), 7.35 (s, 1H), 7.19 (d, 1H, J = 8.5 Hz), 4.87 (d, 1H, J = 19.5 Hz), 4.82 (d, 1H, J = 19.5 Hz), 2.57 (s, 3H), 2.46 (s, 3H), 169 (br s, 1H) ppm. ^{13}C NMR (101 MHz, CDCl_3) δ = 168.6, 134.5, 133.5, 129.4, 127.0, 123.1, 122.2, 119.2, 116.5, 57.4, 24.0, 21.5 ppm. ESI-MS: 204 $[\text{M} + \text{H}]^+$.

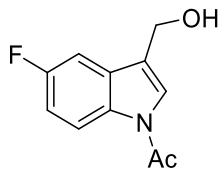
Methyl 1-acetyl-3-(hydroxymethyl)-1*H*-indole-6-carboxylate (15m)



15m was isolated by column chromatography (cyclohexane/ethyl acetate 6:4) in 45% yield as a white solid. ^1H NMR (400 MHz, CDCl_3): δ = 9.05 (s, 1H), 7.99 (d, 1H, J = 8.0 Hz), 7.64 (d, 1H, J = 8.0 Hz), 7.55 (s, 1H), 4.88 (s, 2H), 3.95 (s, 3H), 2.64 (s, 3H)

ppm. ^{13}C NMR (101 MHz, CDCl_3): δ = 168.5, 167.7, 135.7, 132.8, 127.4, 125.7, 125.1, 122.3, 119.1, 118.5, 57.2, 52.3, 24.1 ppm. ESI-MS: 248 $[\text{M}+\text{H}]^+$.

1-(5-Fluoro-3-(hydroxymethyl)-1*H*-indol-1-yl)ethan-1-one (15n)

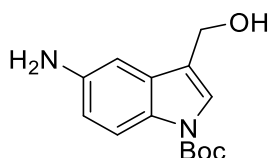


15n was isolated by column chromatography (cyclohexane/ethyl acetate 6:4) in 45% yield as a white solid. ^1H NMR (400 MHz, CDCl_3): δ = 8.39 (dd, 1H, J_1 = 5.0 Hz, J_2 = 9.5 Hz), 7.43 (s, 1H), 7.28 (dd, 1H, J_1 = 2.5 Hz, J_2 = 7.5 Hz), 7.08 (ddd, 1H, J_1 = 2.5 Hz, J_2 = 7.5 Hz, J_3 = 9.5 Hz), 4.83 (s, 2H), 2.60 (s, 3H) ppm. ^{13}C NMR (101 MHz, CDCl_3): δ = 168.5, 159.8 (d, J = 242.0 Hz), 132.7, 130.3 (d, J = 10.0 Hz), 124.4, 122.1 (d, J = 4.0 Hz), 118.0 (d, J = 9.0 Hz), 113.5 (d, J = 24.5 Hz), 105.2 (d, J = 24.0 Hz), 57.2, 23.8 ppm. ESI-MS: 208 $[\text{M}+\text{H}]^+$.

General procedure for the synthesis of compounds 16

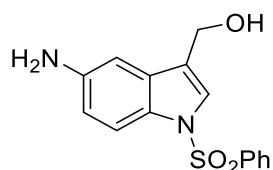
To a stirred solution of nitro compound **15b** or **15i** (1 equiv) in EtOAc (0.070 M) was added quinoline (0.5 equiv) and 5 mol % palladium on CaCO_3 and the reaction mixture stirred under 1 atm. H_2 for 24 h. The reaction mixture was filtered on celite, concentrated in vacuo, and purified by flash column chromatography [26].

***Tert*-butyl 5-amino-3-(hydroxymethyl)-1*H*-indole-1-carboxylate (16a)**



16a was isolated by column chromatography (cyclohexane/ethyl acetate 6:4) in 93% yield as a yellowish solid. ^1H NMR (400 MHz, CDCl_3): δ = 7.89 (br d, 1H, J = 8.5 Hz), 7.48 (br s, 1H), 6.94 (d, 1H, J = 2.0 Hz), 6.73 (dd, 1H, J_1 = 2.0 Hz, J_2 = 8.5 Hz), 4.78 (d, 1H, J = 19.5 Hz), 4.73 (d, 1H, J = 19.5 Hz), 2.69 (br s, 3H), 1.64 (s, 9H) ppm. ^{13}C NMR (101 MHz, CDCl_3): δ = 149.8, 142.2, 130.4, 129.9, 124.3, 120.0, 116.1, 114.1, 104.6, 83.5, 57.4, 28.3 ppm. ESI-MS: 263 $[\text{M}+\text{H}]^+$.

(5-Amino-1-(phenylsulfonyl)-1*H*-indol-3-yl)methanol (**16b**)



16b was isolated by column chromatography (cyclohexane/ethyl acetate 6:4) in 84% yield as a yellowish solid. ^1H NMR (400 MHz, CDCl_3): δ = 7.86–7.82 (m, 2H), 7.78 (d, 1H, J = 8.5 Hz), 7.52 (dddd, 1H, $J_1 \approx J_2$ = 1.0 Hz, $J_3 \approx J_4$ = 7.5 Hz), 7.44–7.39 (m, 3H), 6.85 (d, 1H, J = 2.5 Hz), 6.72 (dd, 1H, J_1 = 2.5 Hz, J_2 = 8.5 Hz), 4.76 (d, 1H, J = 19.0 Hz), 4.70 (d, 1H, J = 19.0 Hz), ppm. ^{13}C NMR (101 MHz, CDCl_3): δ = 143.0, 138.4, 133.8, 130.9, 129.6, 129.3, 126.9, 124.5, 122.4, 114.7, 114.6, 104.7, 57.4 ppm. ESI-MS: 303 $[\text{M}+\text{H}]^+$.

4.5 References

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Chapter 5. Design and Synthesis of 2,3'-Diindolylmethane Analogues for Potential Biological Activity Evaluation

5.1 Introduction

I3C has long been one of the most fascinating natural products derived from cruciferous vegetables. Beyond its simple structure, it conceals a complex chemical reactivity that becomes evident under acidic conditions, where it readily condenses to form a multitude of secondary metabolites [1]. Among these, DIM stands out as the most stable and biologically active product. Over the past decades, DIM has become a reference compound in the study of indole-based chemopreventive agents, known for its ability to inhibit cancer cell proliferation across a wide range of tissues, including breast, prostate, and pancreatic tumors. Its biological action is multifaceted: DIM promotes cell cycle arrest in the G1/S phase, induces apoptosis, modulates estrogen metabolism, and even influences immune pathways [2, 3, 4]. This versatility has made it one of the most studied small molecules of natural origin with promising anticancer and hormone-regulating properties.

However, the chemistry of I3C and its derivatives does not end with DIM. More complex structures, such as the CTet, obtained through the acidic degradation of I3C, have shown remarkable biological potential both *in vitro* and *in vivo*, displaying potent antiproliferative and proapoptotic effects. Yet, the practical limitations of CTet have proven significant. Its extremely low solubility, its instability under physiological conditions, and its challenging purification and low yields have made it unsuitable for detailed pharmacological studies. These issues have not only limited its application but also hindered a deeper understanding of the structural features responsible for its bioactivity [5].

In response to these challenges, researchers have sought simpler yet functionally rich analogues capable of retaining the essential structural relationships of CTet while overcoming its drawbacks [6]. One particularly rational simplification emerged from examining the type of linkage connecting the indole units in CTet (**Scheme 7B**): a methylene bridge that connects the C-2 position of one indole ring to the C-3' position of

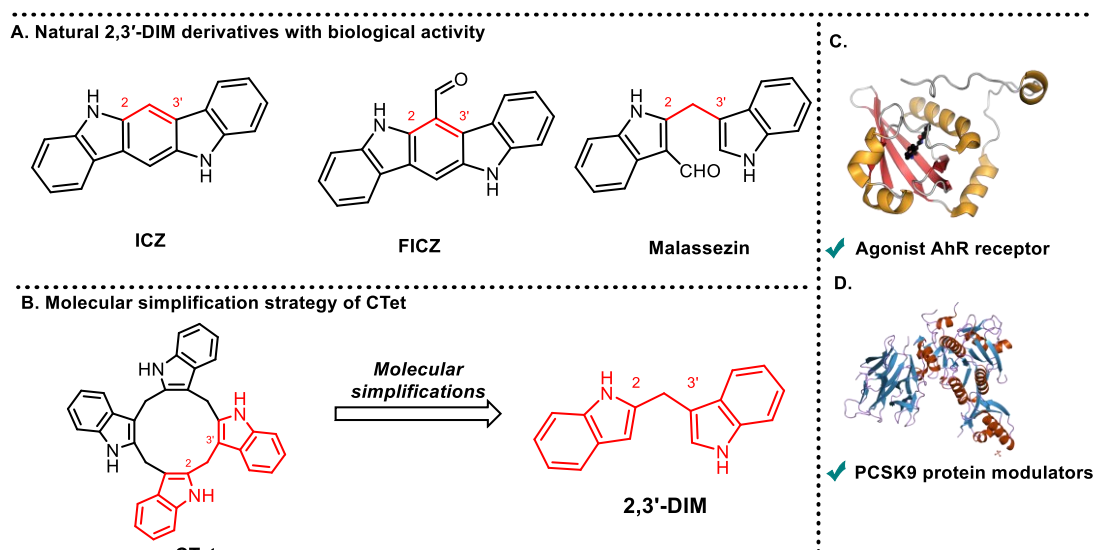
another. Replicating this connectivity gave rise to a family of structurally intriguing compounds known as 2,3'-DIMs. Though less studied than their more common 3,3'-isomers, these molecules represent a subtle yet profound shift in the architecture of indole dimers, one that reshapes their electronic properties, conformational flexibility, and, consequently, their biological potential.

The importance of this structural rearrangement cannot be overstated. Moving the methylene bridge from C-3 to C-2 alters the distribution of π -electrons across the indole system, changes the planarity of the molecule, and affects how the rings interact with biological macromolecules. This seemingly small modification can dramatically reshape the way these compounds fit into enzyme pockets, receptors, or DNA grooves. In essence, 2,3'-DIMs open a new window into the chemistry–biology interface of indole scaffolds, offering opportunities that the classical 3,3'-series could not.

A fascinating example of this is malassezin, a naturally occurring 2,3'-DIM isolated from the yeast *Malassezia furfur* [7] (**Scheme 7A**). Malassezin has been shown to act as an agonist of the AhR, a transcription factor that plays a pivotal role in xenobiotic metabolism, immune balance, and even tumor suppression. The discovery that a relatively simple bisindole structure can selectively activate AhR – similarly to much larger and more rigid indolocarbazoles like ICZ or FICZ [8] (**Scheme 7A**) – suggests that the 2,3'-linkage confers a highly favorable geometric and electronic arrangement for receptor binding. This finding has inspired renewed interest in exploring the AhR-modulating capacity of 2,3'-DIM derivatives, not just as basic chemical curiosities, but as potential therapeutic tools in oncology, immunology, and inflammation [9, 10].

Beyond their interaction with AhR, synthetic 2,3'-DIM analogues have also been shown to modulate proprotein convertase subtilisin/kexin type 9 (PCSK9), a key enzyme that regulates LDL receptor degradation and thus cholesterol homeostasis. Reducing PCSK9 levels represents one of the most effective strategies to control hypercholesterolemia, a goal currently achieved with costly monoclonal antibodies [11]. The idea that small, synthetically accessible molecules such as 2,3'-DIM derivatives could interfere with the same biological pathway is both scientifically and therapeutically exciting, as it opens the possibility of developing affordable small-molecule alternatives to biologics.

These findings hint at a much broader pharmacological landscape than initially anticipated. While the 3,3'-DIM family has been extensively studied for its anticancer effects, the 2,3'-isomers appear to embody a new chemical generation of bisindoles, which are compounds that combine synthetic simplicity with the ability to engage multiple molecular targets. Their modular architecture allows for systematic modification at different positions of the indole ring or at the nitrogen atom, providing fine control over lipophilicity, hydrogen bonding, and electronic balance. This makes them ideal scaffolds for medicinal chemistry campaigns, where small structural adjustments can be used to tune biological responses with remarkable precision.



Scheme 7. **A.** Molecular structures of naturally occurring 2,3'-DIM derivatives with biological activity **B.** Visual depiction of the molecular simplification strategy employed in this study **C.** Aryl hydrocarbon receptor image **D.** Crystal structure of PCSK9.

From a design perspective, the synthesis of 2,3'-DIMs offers an ideal platform for exploring SARs in a rational and predictive way. In the present work, a new library of 2,3'-DIM derivatives was synthesized with the aim of mapping how subtle variations in electronic and steric parameters can influence their physicochemical behavior. While biological testing is yet to be conducted, these compounds are intended to form the basis of a broader pharmacological investigation that will encompass both anticancer and metabolic targets. To guide the selection of substituents, a Craig plot approach was used to distribute them across diverse physicochemical quadrants, ensuring a balanced exploration of hydrophobicity and electronic character. This strategy not only provides a

logical framework for compound design but also lays the foundation for future quantitative correlations between structure and activity.

In summary, the 2,3'-DIM scaffold represents a promising and underexplored chemical space, one that bridges the simplicity of DIM with the potency of complex bisindole systems like CTet. Its versatility, both synthetic and biological, makes it an ideal candidate for the next generation of indole-based therapeutics. Through the systematic synthesis and characterization of these analogues, this study aims to contribute to the understanding and future development of multifunctional bisindoles capable of targeting cancer, metabolic, and immunological pathways with unprecedented selectivity and efficacy.

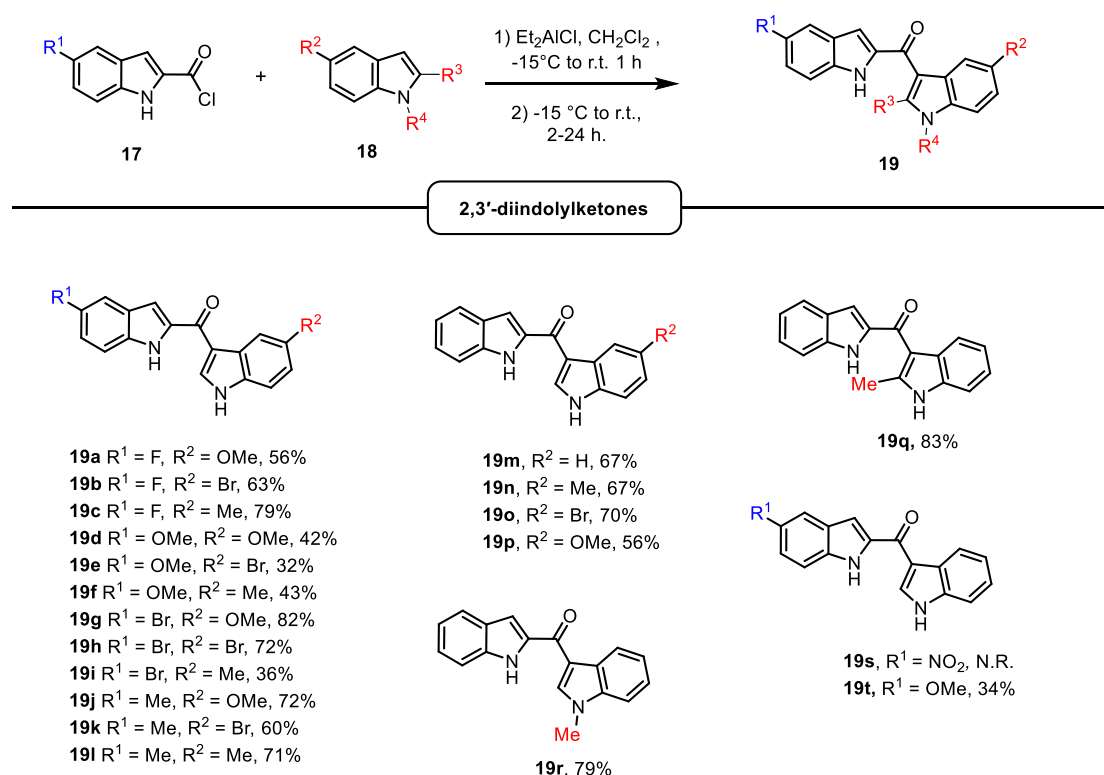
5.2 Results and Discussion

For the synthesis of 2,3'-DIM derivatives, we followed a procedure reported in the literature in 2004 by Wahlström and co-workers [12]. The strategy relies on the formation of a ketone intermediate generated through the coupling of a variably substituted indole with an appropriate indole-2-carbonyl chloride. This intermediate is subsequently reduced to afford the methylene bridge that links the two indole units at the 2,3'-positions. In this work, the starting indoles (**17** and **18**) were selected according to Craig's plot to explore the influence of substituent properties (see Chapter 4 for more details).

First, indoles **17** were synthesized from the corresponding carboxylic acids using thionyl chloride and were then employed directly in the next step. Indoles **18** were subjected to selective acylation at the C3 position, using diethylaluminum chloride (Et_2AlCl) as a key stoichiometric additive, starting from the corresponding acid chlorides **17** (**Table 19**). It is well established that the use of an acid chloride for the acylation of indoles inevitably releases HCl as a byproduct. Because indole is a highly nucleophilic molecule, it is also acid-sensitive: its aromatic system, particularly the C3 position, is extremely reactive toward strong acids. In the presence of free HCl, indole readily undergoes protonation, which can lead to degradation or polymerization, resulting in reduced yields [13].

To address these challenges, the reaction is typically carried out under nearly neutral conditions by employing trialkylaluminum reagents, such as Et₃Al, Me₃Al, or Et₂AlCl as HCl scavengers. These reagents rapidly react with the liberated HCl, effectively neutralizing it and protecting indole from acid-promoted decomposition. In particular, the use of Et₂AlCl enabled the reaction to proceed under tightly controlled conditions, preserving the integrity of the indole, ensuring excellent regioselectivity at C3, and minimizing the formation of undesired side products [13].

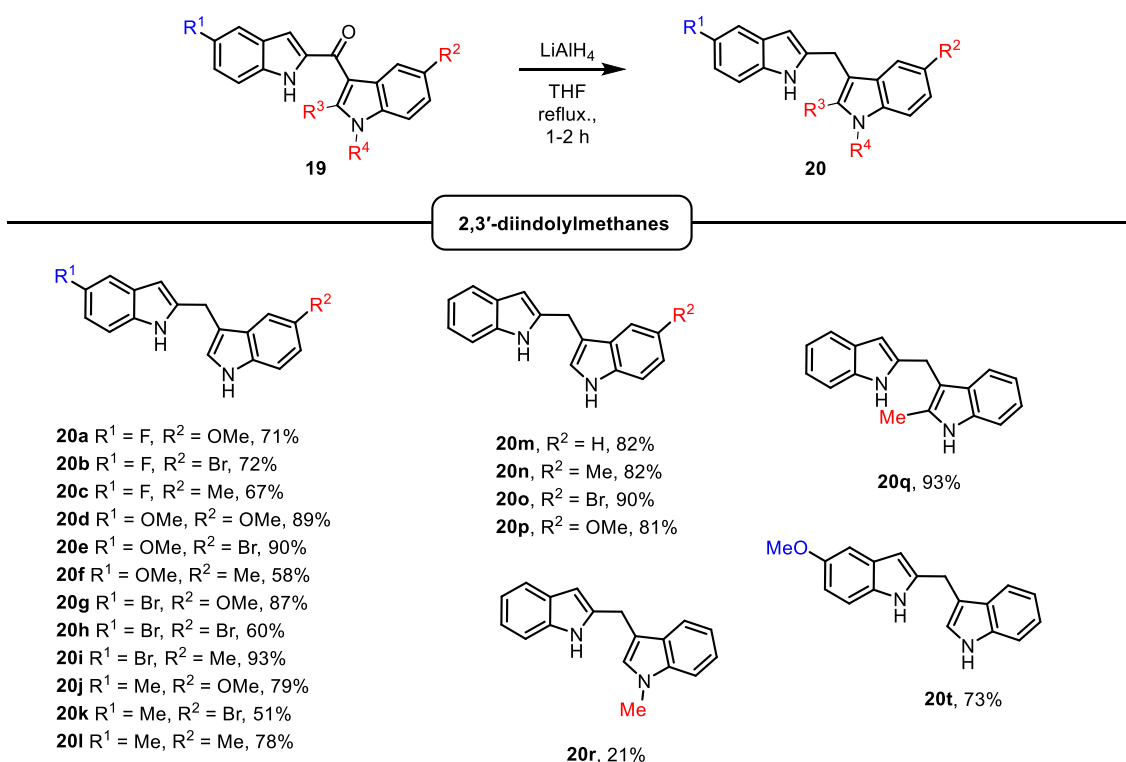
Table 19. Generation of the ketone intermediate promoted by Et₂AlCl.



The reduction was performed using a large excess of LiAlH₄ under reflux conditions. Under these conditions, complete deoxygenation of the substrate was observed instead of the usual reduction of the carbonyl group to the corresponding secondary alcohol. This atypical outcome can be attributed to the structural features of the molecule, in which the carbonyl carbon is directly bound to two indole rings. Compounds of this type are known to exhibit enhanced reactivity toward strong hydride donors, especially under forcing conditions, leading to the replacement of the carbonyl oxygen by hydrogen and the formation of a methylene bridge [14].

In order to achieve full conversion, a significant excess of the reducing agent and prolonged heating were required. The reaction was carried out under strictly anhydrous and inert conditions due to the high sensitivity and reactivity of LiAlH_4 . Quenching was performed with particular care to prevent exothermic decomposition and to ensure the safe removal of aluminum residues.

Table 20. Reduction of the ketone intermediate to the corresponding methylene bridge using LiAlH_4 .



5.3 Conclusions

A literature-reported procedure (Wahlström et al., 2004) was applied and optimized for the synthesis of 2,3'-diindolylmethane (2,3'-DIM) derivatives, designed according to the Craig plot to explore diverse electronic and steric properties of substituents. The strategy involved the chemoselective coupling of variously substituted indoles with the corresponding indole-2-carbonyl chlorides, followed by reduction of the

ketone intermediates to methylene bridges. The use of Et₂AlCl as a key additive enabled the acylations to be carried out under controlled conditions, ensuring high C3 regioselectivity and minimizing byproduct formation caused by the acidity generated *in situ*. This synthetic approach provided a structurally diverse set of 2,3'-DIM derivatives, which will serve as a foundation for future studies aimed at evaluating their biological activity and pharmacological potential.

5.4 Experimental Section

Materials and methods

All reactions were carried out under a nitrogen atmosphere, using anhydrous solvents and under strictly moisture-free conditions. ¹H NMR and ¹³C NMR spectra were recorded on a Bruker Avance 400 spectrometer (¹H: 400 MHz, ¹³C: 101 MHz), using DMSO-d₆ and acetone-d₆ as solvents. Chemical shifts (δ scale) are reported in parts per million (ppm), referenced to the residual solvent peak. Coupling constants (*J*) are given in Hertz (Hz). ¹³C NMR spectra were recorded with broadband ¹H decoupling. The following abbreviations were used to describe multiplicities: br = broad, s = singlet, d = doublet, t = triplet, q = quartet, dd = double doublet, dt = doublet of triplets, dq = doublet of quartets, ddd = doublet of double doublets, td = triplet of doublets, m = multiplet. ESI-MS spectra were obtained on a Waters Micromass ZQ 4000 instrument, using electrospray ionization techniques, with samples dissolved in CH₃OH or CH₃CN. Prior to use, THF and CH₂Cl₂ were dried over molecular sieves (Alfa Aesar, 4 Å, 1–2 mm, beads). Pure compounds **20** were isolated by flash column chromatography on neutral alumina (Brockmann activity, Fluka Analytical) as stationary phase. Thin-layer chromatography (TLC) was performed on silica gel 60 F254 plates, visualized by exposure to a solution of p-anisaldehyde in ethanol followed by heating. LiAlH₄ was purchased from TCI. 5-Methyl-1*H*-indole was purchased from BLD. 1*H*-Indole and 5-nitro-1*H*-indole-2-carboxylic acid were purchased from Fluorochem. 5-Methyl-1*H*-indole-2-carboxylic acid was purchased from Lancaster Synthesis. 5-fluoro-1*H*-indole-2-carboxylic acid, and 5-bromo-1*H*-indole were purchased from Alfa Aesar. Et₂AlCl (1 M in hexane), SOCl₂, an.

DMF, 5-methoxy-1*H*-indole, 5-bromo-1*H*-indole-2-carboxylic acid, 5-methoxy-1*H*-indole-2-carboxylic acid, and all organic solvents were obtained from Sigma Aldrich.

List of the utilized indoles 17 and 18

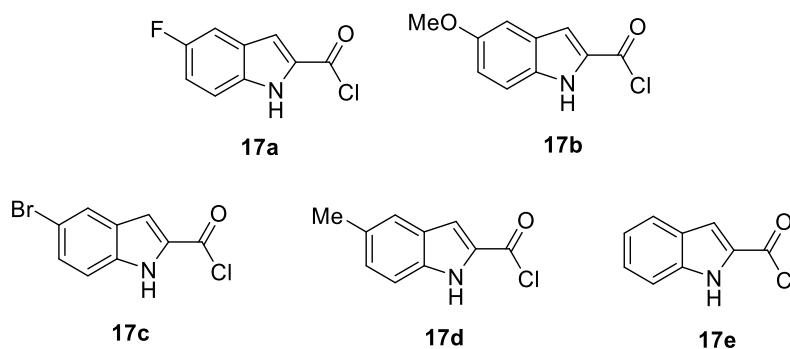


Figure 24. Complete list of the utilized indole-2-carbonyl chloride 17.

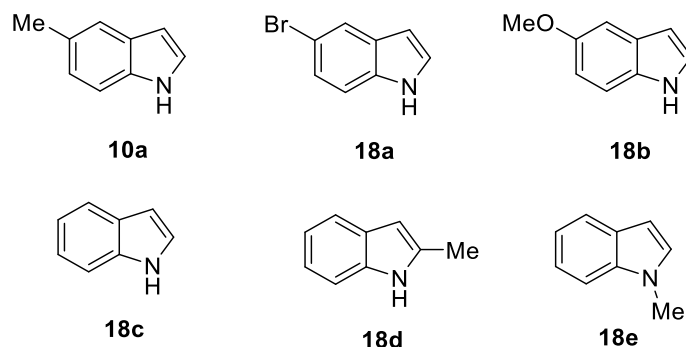


Figure 25. Complete list of the utilized indoles 10 and 18.

Experimental data for compounds 19 and 20.

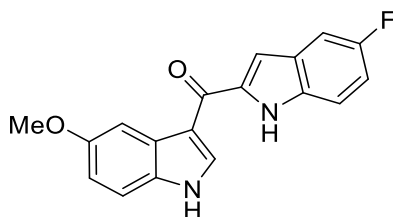
General procedure for the synthesis of compounds 19

The formation of the bisindoleketone was carried out using the procedure of Wahlström et al. [12]. A solution of Et_2AlCl (1 M in hexanes, 1.3 equiv) was added to a solution of chosen indole **18** or **10** (1 equiv) in an. CH_2Cl_2 (0.4 M) at $-15\text{ }^\circ\text{C}$. After the addition the temperature was allowed to increase during 1 h to $0\text{ }^\circ\text{C}$. After cooling again to $-15\text{ }^\circ\text{C}$, a suspension of the appropriate indole-2-carbonyl chloride **17** (1.3 equiv) in anhydrous CH_2Cl_2 (0.65 M) was added dropwise with a syringe. The temperature was then slowly raised to $0\text{ }^\circ\text{C}$, (in some cases to rt) for the reaction to be completed (as

followed by TLC). The reaction was quenched with ice and a saturated aq. solution of NH_4Cl to produce a solid that was filtered, washed with water and finally with CH_2Cl_2 to obtain the pure compounds **19** after oven-drying.

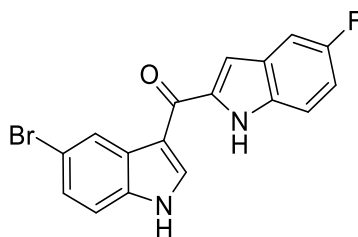
For compound **19r**, after quenching with H_2O , the reaction mixture was extracted three times with EtOAc. The obtained residue was purified by column chromatography.

(5-Fluoro-1*H*-indol-2-yl)(5-methoxy-1*H*-indol-3-yl)methanone (19a)



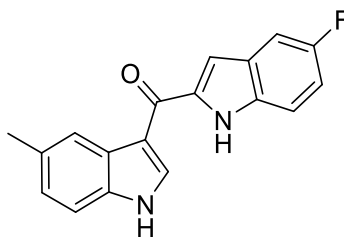
Following the general procedure compound **19a** was obtained as a brownish solid; yield: 56%. ^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ = 12.02 (br d, J = 3.0 Hz, 1H), 11.81 (br s, 1H), 8.39 (d, J = 3.0 Hz, 1H), 7.50 (dd, J_1 = 4.0 Hz, J_2 = 9.0 Hz, 1H), 7.44 (dd, J_1 = 2.5 Hz, J_2 = 9.0 Hz, 1H), 7.43 (d, J = 9.0 Hz, 1H), 7.31 (d, J = 3.0 Hz, 1H), 7.12 (ddd, J_1 = 2.5 Hz, J_2 = 9.0 Hz, J_3 = 9.0 Hz, 1H), 6.91 (dd, J_1 = 2.5 Hz, J_2 = 9.0 Hz, 1H), 3.82 (s, 3H) ppm. ^{13}C NMR (101 MHz, $\text{DMSO}-d_6$): δ = 180.1, 157.2 (d, J = 233.0 Hz), 155.5, 137.9, 134.2, 133.7, 131.4, 127.4 (d, J = 10.5 Hz), 127.2, 114.8, 113.7 (d, J = 9.5 Hz), 113.1, 112.8 (d, J = 4.0 Hz), 112.8, 106.7 (d, J = 5.0 Hz), 106.1 (d, J = 23.0 Hz), 103.4, 55.3 ppm. ESI-MS: 309 $[\text{M}+1]^+$, 307 $[\text{M}-1]^-$.

(5-Bromo-1*H*-indol-3-yl)(5-fluoro-1*H*-indol-2-yl)methanone (19b)



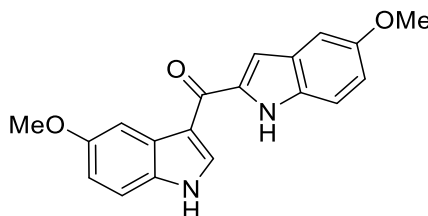
Following the general procedure compound **19b** was obtained as a yellow solid; yield: 63%. ^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ = 12.32 (br d, J = 3.0 Hz, 1H), 11.90 (br s, 1H), 7.54-7.48 (m, 2H), 7.45 (dd, J_1 = 9.5 Hz, J_2 = 2.5 Hz, 1H), 7.40 (dd, J_1 = 8.5 Hz, J_2 = 2.0 Hz, 1H), 7.37 (d, J = 2.0 Hz, 1H), 7.14 (ddd, J_1 = J_2 = 9.5 Hz, J_3 = 2.5 Hz, 1H) ppm. ESI-MS: 357 $[\text{M}+1]^+$, 359 $[\text{M}+1]^+$, 355 $[\text{M}-1]^-$, 357 $[\text{M}-1]^-$.

(5-Fluoro-1*H*-indol-2-yl)(5-methyl-1*H*-indol-3-yl)methanone (19c)



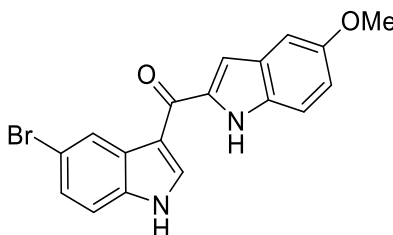
Following the general procedure compound **19c** was obtained as a greenish solid; yield: 79%. ^1H NMR (400 MHz, DMSO- d_6): δ = 12.02 (br d, J = 3.0 Hz, 1H), 11.84 (br s, 1H), 8.39 (dd, J_1 = 3.0 Hz, J_2 = 1.0 Hz, 1H), 8.11 (s, 1H), 7.50 (dd, J_1 = 9.0 Hz, J_2 = 4.5 Hz, 1H), 7.46-7.40 (m, 2H), 7.30 (d, J = 2.0 Hz, 1H), 7.12 (ddd, J_1 = J_2 = 9.5 Hz, J_3 = 2.5 Hz, 1H), 2.45 (s, 3H) ppm. ESI-MS: 293[M+1] $^+$, 291[M-1] $^-$.

(5-Methoxy-1H-indol-2-yl)(5-methoxy-1H-indol-3-yl)methanone (19d)



Following the general procedure compound **1d** was obtained as a brownish solid; yield: 42%. ^1H NMR (400 MHz, DMSO- d_6): δ = 11.98 (br d, J = 3.0 Hz, 1H), 11.57 (br d, J = 2.0 Hz, 1H), 8.36 (d, J = 3.0 Hz, 1H), 7.81 (d, J = 2.5 Hz, 1H), 7.42 (d, J = 9.0 Hz, 1H), 7.39 (d, J = 9.0 Hz, 1H), 7.23 (dd, J_1 = 1.0 Hz, J_2 = 2.5 Hz, 1H), 6.95-6.84 (m, 2H), 3.81 (s, 3H), 3.78 (s, 3H) ppm. The physicochemical data of compound agree with those reported in the literature [12].

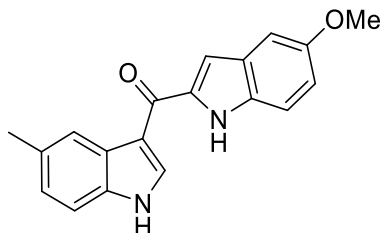
(5-Bromo-1H-indol-3-yl)(5-methoxy-1H-indol-2-yl)methanone (19e)



Following the general procedure compound **1e** was obtained as a brownish solid; yield: 32%. ^1H NMR (400 MHz, DMSO- d_6): δ = 12.28 (br d, J = 3.0 Hz, 1H), 11.65 (br d, J = 2.5 Hz, 1H), 8.49 (d, J = 3.0 Hz, 1H), 8.43 (d, J = 2.0 Hz, 1H), 7.51 (d, J = 8.5 Hz, 1H), 7.43-7.36 (m, 2H), 7.31-7.27 (m, 1H), 7.14 (d, J = 2.5 Hz, 1H), 6.93 (dd, J_1 = 2.5 Hz, J_2 = 9.0 Hz, 1H), 3.78 (s, 3H) ppm. ^{13}C NMR (101 MHz, DMSO- d_6): δ = 180.0,

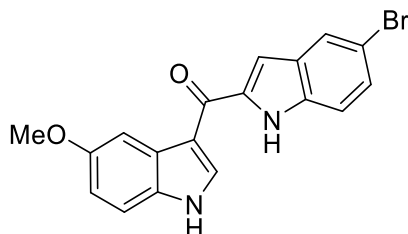
153.9, 136.2, 135.3, 134.7, 132.6, 128.2, 127.6, 125.6, 123.6, 116.0, 114.5, 114.4, 114.3, 113.4, 107.3, 102.3, 55.3 ppm. ESI-MS: 369[M+1]⁺, 371[M+1]⁺, 367[M-1]⁻.

(5-Methoxy-1*H*-indol-2-yl)(5-methyl-1*H*-indol-3-yl)methanone (19f)



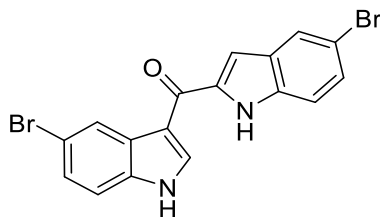
Following the general procedure compound **19f** was obtained as a brownish solid; yield: 43%. ¹H NMR (400 MHz, DMSO-*d*₆): δ = 11.97 (br d, *J* = 3.0 Hz, 1H), 11.58 (br d, *J* = 2.0 Hz, 1H), 8.35 (d, *J* = 3.0 Hz, 1H), 8.10 (s, 1H), 7.41 (d, *J* = 8.0 Hz, 1H), 7.40 (d, *J* = 9.0 Hz, 1H), 7.22 (d, *J* = 2.0 Hz, 1H), 7.14 (d, *J* = 2.5 Hz, 1H), 7.08 (dd, *J*₁ = 2.0 Hz, *J*₂ = 8.5 Hz, 1H), 6.92 (dd, *J*₁ = 2.5 Hz, *J*₂ = 9.0 Hz, 1H), 3.78 (s, 3H), 2.44 (s, 3H) ppm. ¹³C NMR (101 MHz, DMSO-*d*₆): δ = 180.3, 153.8, 136.8, 134.8, 133.6, 132.4, 130.4, 127.6, 126.7, 124.5, 121.2, 115.6, 114.8, 113.4, 111.8, 106.8, 102.3, 55.3, 21.4 ppm. ESI-MS: 305[M+1]⁺, 303[M-1]⁻, 349[M+45]⁻.

(5-Bromo-1*H*-indol-2-yl)(5-methoxy-1*H*-indol-3-yl)methanone (19g)



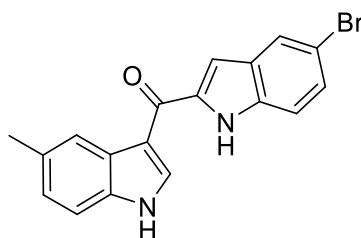
Following the general procedure compound **19g** was obtained as a yellow solid; yield: 82%. ¹H NMR (400 MHz, acetone-*d*₆): δ = 11.08 (br s, 2H), 8.43 (d, *J* = 3.0 Hz, 1H), 7.95 (d, *J* = 2.5 Hz, 1H), 7.89 (d, *J* = 2.0 Hz, 1H), 7.57 (d, *J* = 9.0 Hz, 1H), 7.47 (d, *J* = 9.0 Hz, 1H), 7.40 (dd, *J*₁ = 2.0 Hz, *J*₂ = 9.0 Hz, 1H), 7.32 (d, *J* = 2.0 Hz, 1H), 6.92 (dd, *J*₁ = 2.5 Hz, *J*₂ = 9.0 Hz, 1H), 3.86 (s, 3H) ppm. ¹³C NMR (101 MHz, acetone-*d*₆): δ = 181.0, 157.1, 138.7, 136.7, 134.4, 132.6, 130.7, 128.6, 128.0, 125.4, 116.6, 115.1, 114.3, 113.6, 113.6, 107.0, 104.5, 55.9 ppm. ESI-MS: 369[M+1]⁺, 371[M+1]⁺, 367[M-1]⁻, 369[M-1]⁻.

(5-Bromo-1*H*-indol-2-yl)(5-bromo-1*H*-indol-3-yl)methanone (19h)



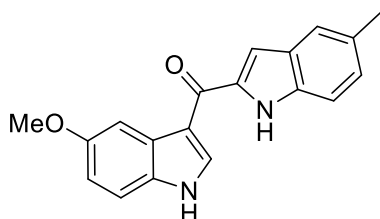
Following the general procedure compound **19h** was obtained as a yellow solid; yield: 72%. ^1H NMR (400 MHz, acetone- d_6): δ = 11.38 (br s, 1H), 11.14 (br s, 1H), 8.58 (d, J = 2.0 Hz, 1H), 8.54 (d, J = 3.0 Hz, 1H), 7.90 (d, J = 2.0 Hz, 1H), 7.61-7.53 (m, 2H), 7.42 (t, J = 2.0 Hz, 1H), 7.40 (t, J = 2.0 Hz, 1H), 7.37 (dd, J_1 = 1.0 Hz, J_2 = 2.5 Hz, 1H), ppm. ^{13}C NMR (101 MHz, acetone- d_6): δ = 180.8, 138.2, 136.8, 136.5, 135.2, 130.6, 129.5, 128.3, 127.0, 125.5, 125.2, 116.2, 115.9, 115.2, 114.8, 113.7, 107.5 ppm. ESI-MS: 417[M+1] $^+$, 419[M+1] $^+$, 421[M+1] $^+$, 415[M-1] $^-$, 417[M-1] $^-$.

(5-Bromo-1H-indol-2-yl)(5-methyl-1H-indol-3-yl)methanone (19i)



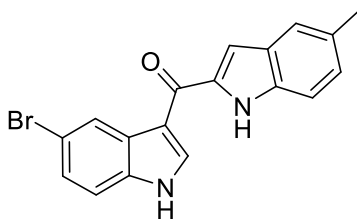
Following the general procedure compound **19i** was obtained as an orange solid; yield: 36%. ^1H NMR (400 MHz, acetone- d_6): δ = 11.08 (br s, 2H), 8.42 (d, J = 3.0 Hz, 1H), 8.22 (s, 1H), 7.89 (d, J = 2.0 Hz, 1H), 7.58 (d, J = 9.0 Hz, 1H), 7.45 (d, J = 8.5 Hz, 1H), 7.40 (dd, J_1 = 2.0 Hz, J_2 = 9.0 Hz, 1H), 7.32 (dd, J_1 = 1.0 Hz, J_2 = 2.5 Hz, 1H), 7.12 (dd, J_1 = 2.0 Hz, J_2 = 8.5 Hz, 1H), 2.47 (s, 3H) ppm. ^{13}C NMR (101 MHz, acetone- d_6): δ = 181.1, 138.8, 136.7, 136.1, 134.2, 132.1, 130.6, 128.0, 128.0, 125.8, 125.4, 122.6, 116.4, 115.1, 113.6, 112.5, 107.0, 21.7 ppm. ESI-MS: 353[M+1] $^+$, 355[M+1] $^+$, 351[M-1] $^-$, 353[M-1] $^-$, 397[M+45] $^-$.

(5-Methoxy-1H-indol-3-yl)(5-methyl-1H-indol-2-yl)methanone (19j)



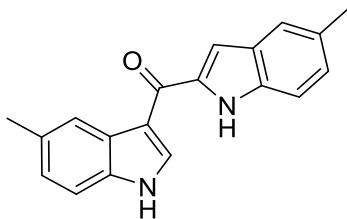
Following the general procedure compound **19j** was obtained as a brownish solid; yield: 72%. ¹H NMR (400 MHz, acetone-*d*₆): δ = 11.03 (br s, 1H), 10.76 (br s, 1H), 8.40 (d, *J* = 3.0 Hz, 1H), 7.96 (d, *J* = 2.5 Hz, 1H), 7.53-7.40 (m, 3H), 7.24 (dd, *J*₁ = 1.0 Hz, *J*₂ = 2.5 Hz, 1H), 7.15-7.10 (m, 1H), 6.91 (dd, *J*₁ = 2.5 Hz, *J*₂ = 9.0 Hz, 1H), 3.86 (s, 3H), 2.41 (s, 3H) ppm. ¹³C NMR (101 MHz, acetone-*d*₆): δ = 181.4, 157.0, 137.6, 136.6, 134.0, 132.6, 130.0, 129.2, 128.6, 127.3, 122.5, 116.8, 114.2, 113.5, 112.9, 107.4, 104.5, 55.9, 21.5 ppm. ESI-MS: 305[M+1]⁺, 303[M-1]⁻, 349[M+45]⁻.

(5-Bromo-1*H*-indol-3-yl)(5-methyl-1*H*-indol-2-yl)methanone (19k)



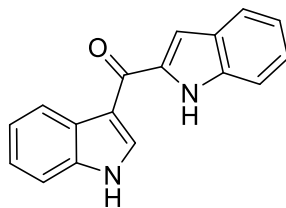
Following the general procedure compound **19k** was obtained as a yellow solid; yield: 60%. ¹H NMR (400 MHz, acetone-*d*₆): δ = 11.30 (br s, 1H), 10.82 (br s, 1H), 8.59 (s, *J* = 2.0 Hz, 1H), 8.51 (d, *J* = 2.0 Hz, 1H), 7.55 (d, *J* = 8.5 Hz, 1H), 7.49 (s, 1H), 7.40 (dd, *J*₁ = 2.0 Hz, *J*₂ = 8.5 Hz, 1H), 7.30-7.26 (m, 1H), 7.15 (dd, *J*₁ = 2.0 Hz, *J*₂ = 8.5 Hz, 1H), 2.41 (s, 3H) ppm. ¹³C NMR (101 MHz, acetone-*d*₆): δ = 180.2, 136.3, 135.9, 135.6, 133.8, 129.2, 128.7, 128.3, 126.7, 125.9, 124.4, 121.7, 115.5, 114.8, 113.8, 112.1, 107.0, 20.6 ppm. ESI-MS: 353[M+1]⁺, 355[M+1]⁺.

(5-Methyl-1*H*-indol-2-yl)(5-methyl-1*H*-indol-3-yl)methanone (19l)



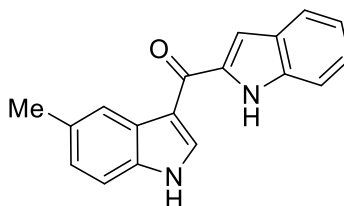
Following the general procedure compound **19l** was obtained as a brownish solid; yield: 71%. ¹H NMR (400 MHz, acetone-*d*₆): δ = 11.03 (br s, 1H), 10.81 (br s, 1H), 8.40 (d, *J* = 3.0 Hz, 1H), 8.24 (d, *J* = 1.0 Hz, 1H), 7.53-7.46 (m, 2H), 7.44 (d, *J* = 8.5 Hz, 1H), 7.23 (d, *J* = 1.0 Hz, 1H), 7.12 (td, *J*₁ = *J*₂ = 2.0 Hz, *J*₃ = 8.5 Hz, 2H), 2.47 (s, 3H), 2.41 (s, 3H) ppm. ¹³C NMR (101 MHz, acetone-*d*₆): δ = 180.5, 136.6, 135.6, 132.8, 132.7, 130.9, 129.0, 128.3, 127.2, 126.4, 124.7, 121.7, 121.6, 115.6, 112.0, 111.5, 106.5, 20.8, 20.6 ppm. ESI-MS: 289[M+1]⁺, 287[M-1]⁻.

(1*H*-indol-2-yl)(1*H*-indol-3-yl)methanone (19m)



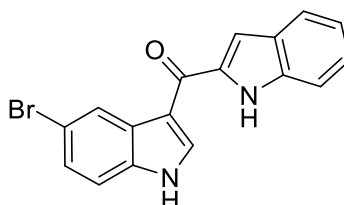
Following the general procedure compound **19m** was obtained as a greenish solid; Yield : 67%. (400 MHz, DMSO- d_6): δ = 12.10 (br s, 1H), 11.76 (br s, 1H), 8.46 (d, 1H, J = 3.0 Hz), 8.31 (dd, 1H, J_1 = 2.0 Hz, J_2 = 6.5 Hz), 7.71 (d, 1H, J = 8.0 Hz), 7.54 (dd, 1H, J_1 = 1.5 Hz, J_2 = 6.5 Hz), 7.50 (dd, 1H, J_1 = 0.5 Hz, J_2 = 8.0 Hz), 7.35 (d, 1H, J = 1.5 Hz), 7.29-7.23 (m, 3H), 7.09 (ddd, 1H, J_1 = 1.0 Hz, J_2 = 6.5 Hz, J_3 = 8.0 Hz) ppm. ESI-MS: 261[M+1]. The physicochemical data of compound agree with those reported in the literature [12].

(1*H*-indol-2-yl)(5-methyl-1*H*-indol-3-yl)methanone (19n)



Following the general procedure compound **19n** was obtained as a greenish solid; Yield: 67%. ^1H NMR (400 MHz, DMSO- d_6): δ = 11.97 (d, 1H, J = 3.0 Hz), 11.72 (br s, 1H), 8.39 (d, 1H, J = 3.0 Hz), 8.11 (s, 1H), 7.70 (d, 1H, J = 8.0 Hz), 7.50 (dd, 1H, J = 0.5, 8.0 Hz), 7.41 (d, 1H, J = 8.5 Hz), 7.32 (dd, 1H, J_1 = 0.5 Hz, J_2 = 2.0 Hz), 7.25 (ddd, 1H, J_1 = 1.0 Hz, J_2 = 7.0 Hz, J_3 = 8.0 Hz), 7.10-7.06 (m, 2H), 2.45 (s, 3H) ppm. ^{13}C NMR (101 MHz, DMSO- d_6): δ = 180.4, 137.0, 136.3, 134.8, 133.8, 130.5, 127.3, 126.7, 124.5, 124.2, 122.2, 121.2, 119.9, 114.7, 112.5, 111.8, 107.1, 21.4 ppm. ESI-MS: 275 [M+1].

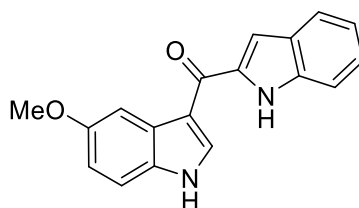
(5-Bromo-1*H*-indol-3-yl)(1*H*-indol-2-yl)methanone (19o)



Following the general procedure compound **19o** was obtained as a greenish solid; Yield: 70%. ^1H NMR (400 MHz, DMSO- d_6): δ = 12.31 (br d, 1H, J = 3.0 Hz), 11.78 (br

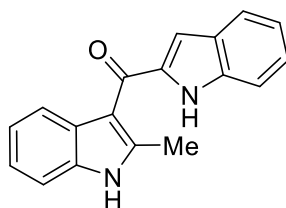
s, 1H), 8.53 (d, 1H $J = 3.0$ Hz), 8.44 (d, 1H, $J = 2.0$ Hz), 7.71 (d, 1H, $J = 8.0$ Hz), 7.52 (d, 1H, $J = 8.5$ Hz), 7.50 (d, 1H, $J = 8.0$ Hz), 7.42-7.38 (m, 2H), 7.27 (ddd, 1H, $J_1 = 0.5$, $J_2 = 7.0$, $J_3 = 8.0$ Hz), 7.11-7.07 (m, 1H) ppm. ^{13}C NMR (101 MHz, DMSO- d_6): $\delta = 180.2$, 137.2, 135.9, 135.3, 134.9, 128.2, 127.3, 125.6, 124.5, 123.6, 122.3, 120.0, 114.5 (2C), 114.3, 112.5, 107.6 ppm. ESI-MS: 339 $[\text{M}+\text{H}]^+$, 341 $[\text{M}+\text{H}]^+$, 337 $[\text{M}-\text{H}]^-$, 339 $[\text{M}-\text{H}]^-$. The physicochemical data of compound agree with those reported in the literature [15].

(1*H*-indol-2-yl)(5-methoxy-1*H*-indol-3-yl)methanone (19p)



Following the general procedure compound **19p** was obtained as a yellow solid; Yield: 56%. ^1H NMR (400 MHz, DMSO- d_6): $\delta = 11.98$ (d, 1H, $J = 3.0$ Hz), 11.70 (br s, 1H), 8.40 (d, 1H, $J = 3.0$ Hz), 7.83 (d, 1H, $J = 2.5$ Hz), 7.70 (d, 1H, $J = 8.0$ Hz), 7.50 (dd, 1H, $J_1 = 0.5$ Hz, $J_2 = 8.0$ Hz), 7.43 (d, 1H, $J = 9.0$ Hz), 7.33 (dd, 1H, $J_1 = 0.5$ Hz, $J_2 = 2.0$ Hz), 7.25 (ddd, 1H, $J_1 = 1.0$ Hz, $J_2 = 7.0$ Hz, $J_3 = 8.0$ Hz), 7.08 (ddd, 1H, $J_1 = 0.5$ Hz, $J_2 = 7.0$ Hz, $J_3 = 8.0$ Hz), 6.91 (dd, 1H, $J_1 = 2.5$ Hz, $J_2 = 9.0$ Hz), 3.82 (s, 3H) ppm. ESI-MS: 291 $[\text{M} + 1]^+$, 289 $[\text{M} - 1]^-$. The physicochemical data of compound agree with those reported in the literature [12].

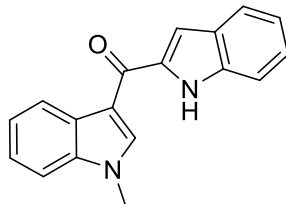
(1*H*-indol-2-yl)(2-methyl-1*H*-indol-3-yl)methanone (19q)



Following the general procedure compound **19q** was obtained as a brown solid; Yield: 83%. ^1H NMR (400 MHz, DMSO- d_6): $\delta = 11.85$ (br s, 1H), 11.77 (br s, 1H), 7.69 (d, 1H, $J = 8.0$ Hz), 7.65 (d, 1H, $J = 8.0$ Hz), 7.49 (dd, 1H, $J_1 = 0.5$ Hz, $J_2 = 8.0$ Hz), 7.40 (d, 1H, $J = 8.0$ Hz), 7.26 (ddd, 1H, $J_1 = 1.0$ Hz, $J_2 = 7.0$ Hz, $J_3 = 8.0$ Hz), 7.14 (ddd, 1H, $J_1 = 1.0$ Hz, $J_2 = 7.0$ Hz, $J_3 = 8.0$ Hz), 7.09-7.03 (m, 2H), 7.02 (d, 1H, $J = 1.5$ Hz), 2.56 (3H, s) ppm. ^{13}C NMR (101 MHz, DMSO- d_6): $\delta = 182.5$, 142.3, 137.3, 137.2, 135.0,

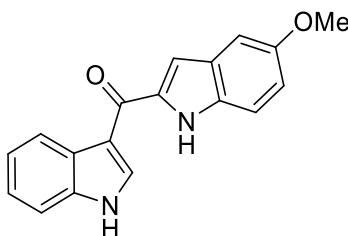
127.1, 126.9, 124.5, 122.3, 121.7, 120.6, 119.9 (2C), 113.2, 112.5, 111.2, 108.9, 13.9 ppm. ESI-MS: 275[M+1]; 273[M-1].

(1*H*-indol-2-yl)(1-methyl-1*H*-indol-3-yl)methanone (19r)



Following the general procedure compound **19r** was obtained by silica-gel flash column chromatography (cyclohexane/EtOAc 85:15) as a greenish solid; Yield: 79%. ¹H NMR (400 MHz, DMSO-*d*₆): δ = 11.76 (br s, 1H), 8.54 (s, 1H), 8.32 (d, 1H, *J* = 8.0 Hz), 7.71 (d, 1H, *J* = 8.0 Hz), 7.59 (d, 1H, *J* = 8.0 Hz), 7.50 (d, 1H, *J* = 8.5 Hz), 7.38 (d, 1H, *J* = 2.5 Hz), 7.36-7.24 (m, 3H), 7.09 (ddd, 1H, *J*₁ = 0.5 Hz, *J*₂ = 8.0 Hz, *J*₃ = 8.5 Hz), 3.95 (s, 3H) ppm. ¹³C NMR (101 MHz, DMSO-*d*₆): δ = 179.9, 137.5, 137.13, 137.07, 136.2, 127.3, 126.9, 124.3, 123.1, 122.2, 122.0, 121.6, 120.0, 113.8, 112.5, 110.6, 107.1, 33.2 ppm. ESI-MS: 275 [M+H]⁺. The physicochemical data of compound agree with those reported in the literature [16].

(1*H*-indol-3-yl)(5-methoxy-1*H*-indol-2-yl)methanone (19t)



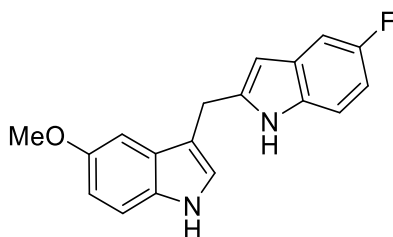
Following the general procedure compound **19t** was obtained as a greenish solid; Yield: 34%. ¹H NMR (400 MHz, DMSO-*d*₆): δ = 12.08 (br d, 1H, *J* = 3.0 Hz), 11.62 (br s, 1H), 8.41 (d, 1H, *J* = 3.0 Hz), 8.29 (dd, 1H, *J*₁ = 1.5 Hz, *J*₂ = 7.0 Hz), 7.55- 7.51 (m, 1H), 7.40 (d, 1H, *J* = 9.0 Hz), 7.29-7.20 (m, 3H), 7.15 (d, 1H, *J* = 2.5 Hz), 6.92 (dd, 1H, *J*₁ = 9.0 Hz, *J*₂ = 2.5 Hz), 3.78 (s, 3H) ppm. ESI-MS: 291 [M + H]⁺; 289 [M - H]⁻. The physicochemical data of compound agree with those reported in the literature [12].

General procedure for the synthesis of compound 2

The appropriate bisindolylketone (**19 a-t**) was reduced according to Jackson and co-workers' procedure [17] with 6 equiv of LiAlH₄ in an. THF (0.067 M) under N₂ at reflux. After 1-2 h the reaction mixture was cooled at room temperature and quenched

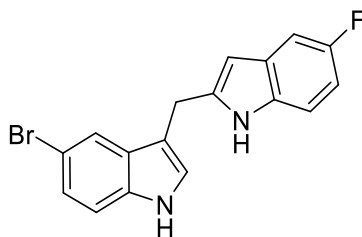
with a saturated aqueous solution of sodium potassium tartrate and extracted with EtOAc (3 x 20 mL). The combined organic layers were washed with H₂O and brine before drying over Na₂SO₄. The solvents were evaporated under reduced pressure, and the crude compound was purified by alumina flash column chromatography to produce the corresponding bisindolylmethane **20 a-t** as a solid.

5-Fluoro-2-[(5-methoxy-1*H*-indol-3-yl)methyl]-1*H*-indole (20a)



Following the general procedure, compound **20a** has been purified by flash column chromatography on alumina (cyclohexane/ethyl acetate, 8:2 to 75:25), obtaining an orange solid; yield: 71%. ¹H NMR (400 MHz, DMSO-*d*₆): δ = 10.98 (br s, 1H), 10.72 (br s, 1H), 7.25-7.20 (m, 2H), 7.16 (d, *J* = 2.5 Hz, 1H), 7.13 (dd, *J*₁ = 2.5 Hz, *J*₂ = 10.0 Hz, 1H), 6.96 (d, *J* = 2.5 Hz, 1H), 6.83-6.76 (m, 1H), 6.71 (dd, *J*₁ = 2.5 Hz, *J*₂ = 9.0 Hz, 1H), 6.16 (d, *J* = 1.0 Hz, 1H), 4.13 (s, 2H), 3.69 (s, 3H) ppm. ¹³C NMR (101 MHz, DMSO-*d*₆): δ = 156.8 (d, *J* = 231.0 Hz), 152.9, 141.9, 132.7, 131.4, 128.6 (d, *J* = 10.5 Hz), 127.3, 124.0, 112.0, 111.37, 111.36 (d, *J* = 10 Hz), 110.9, 107.7 (d, *J* = 26.0 Hz), 103.7 (d, *J* = 26 Hz), 100.5, 99.1 (d, *J* = 4.5 Hz), 55.3, 24.1 ppm. ESI-MS: 295[M+1]⁺, 293[M-1]⁻, 339[M+45]⁻.

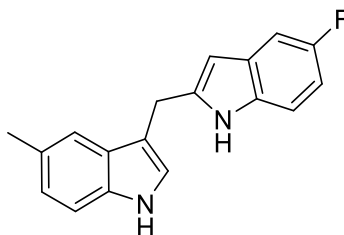
5-Bromo-3-[(5-fluoro-1*H*-indol-2-yl)methyl]-1*H*-indole (20b)



Following the general procedure, compound **2b** has been purified by flash column chromatography on alumina (cyclohexane/ethyl acetate, 9:1 to 8:2), obtaining a pinkish solid; yield: 72%. ¹H NMR (400 MHz, DMSO-*d*₆): δ = 11.12 (br s, 1H), 10.98 (br s, 1H), 7.64 (d, *J* = 2.0 Hz, 1H), 7.33 (dd, *J*₁ = 0.5 Hz, *J*₂ = 8.5 Hz, 1H), 7.30 (d, *J* = 2.5 Hz, 1H), 7.23 (dd, *J*₁ = 4.5 Hz, *J*₂ = 8.5 Hz, 1H), 7.17-7.11 (m, 2H), 6.80 (ddd, *J*₁ = 2.5 Hz, *J*₂ =

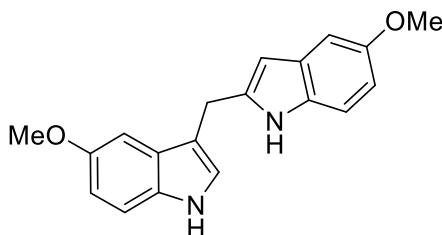
8.5 Hz, $J_3 = 9.5$ Hz, 1H), 6.14 (d, $J = 1.0$ Hz, 1H), 4.15 (s, 2H) ppm. ^{13}C NMR (101 MHz, DMSO- d_6): $\delta = 156.8$ (d, $J = 231.1$ Hz), 141.5, 135.0, 132.7, 128.8, 128.5 (d, $J = 10.5$ Hz), 125.1, 123.4, 121.0, 113.4, 111.6, 111.4 (d, $J = 10.0$ Hz), 111.0, 107.8 (d, $J = 26$ Hz), 103.8 (d, $J = 23.0$ Hz), 99.1 (d, $J = 4.5$ Hz), 23.8 ppm. ESI-MS: 343[M+1] $^+$, 345[M+1] $^+$, 341[M-1] $^-$, 343[M-1] $^-$.

5-Fluoro-2-[(5-methyl-1H-indol-3-yl)methyl]-1H-indole (20c)



Following the general procedure, compound **20c** has been purified by flash column chromatography on alumina (cyclohexane/ethyl acetate, 9:1 to 75:25), obtaining an orange solid; yield: 67%. ^1H NMR (400 MHz, DMSO- d_6): $\delta = 10.95$ (br s, 1H), 10.75 (br s, 1H), 7.27-7.20 (m, 3H), 7.16 (d, $J = 2.5$ Hz, 1H), 7.13 (dd, $J_1 = 2.5$ Hz, $J_2 = 10.0$ Hz, 1H), 6.88 (dd, $J_1 = 1.5$ Hz, $J_2 = 8.5$ Hz, 1H), 6.79 (ddd, $J_1 = 2.5$ Hz, $J_2 = 8.5$ Hz, $J_3 = 9.5$ Hz, 1H), 6.12 (d, $J = 1.5$ Hz, 1H), 4.13 (s, 2H), 2.33 (s, 3H) ppm. ^{13}C NMR (101 MHz, DMSO- d_6): $\delta = 156.8$ (d, $J = 231$ Hz), 142.0, 134.7, 132.7, 128.6 (d, $J = 10.5$ Hz), 127.2, 126.6, 123.4, 122.6, 118.0, 111.4 (d, $J = 10.0$ Hz), 111.1, 111.0, 107.7 (d, $J = 26$ Hz), 103.7 (d, $J = 23$ Hz), 99.0 (d, $J = 4.5$ Hz), 24.0, 21.3 ppm. ESI-MS: 279[M+1] $^+$, 277[M-1] $^-$, 323[M+45] $^-$.

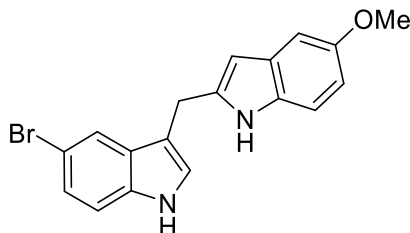
5-Methoxy-3-[(5-methoxy-1H-indol-2-yl)methyl]-1H-indole (20d)



Following the general procedure, compound **20d** has been purified by flash column chromatography on alumina (cyclohexane/ethyl acetate, 8:2), obtaining a brownish solid; yield: 89%. ^1H NMR (400 MHz, DMSO- d_6): $\delta = 10.69$ (br s, 2H), 7.24 (d, $J = 8.5$ Hz), 7.17-7.11 (m, 2H), 6.97 (d, $J = 2.5$ Hz, 1H), 6.90 (d, $J = 2.5$ Hz, 1H), 6.71 (dd, $J_1 = 2.5$ Hz, $J_2 = 8.5$ Hz, 1H), 6.61 (dd, $J_1 = 2.5$ Hz, $J_2 = 8.5$ Hz, 1H), 6.11-6.06 (m,

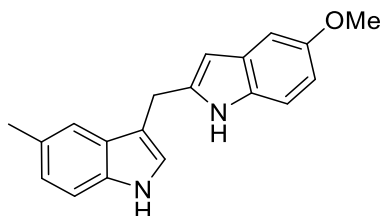
1H), 4.10 (s, 2H), 3.70 (s, 3H), 3.69 (s, 3H) ppm. The physicochemical data of compound agree with those reported in the literature [12].

5-Bromo-3-[(5-methoxy-1H-indol-2-yl)methyl]-1H-indole (20e)



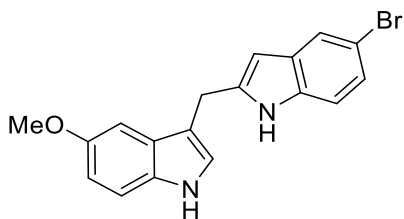
Following the general procedure, compound **20e** has been purified by flash column chromatography on alumina (cyclohexane/ethyl acetate, 8:2), obtaining an orange solid; yield: 90%. ¹H NMR (400 MHz, acetone-*d*₆): δ = 10.27 (br s, 1H), 9.80 (br s, 1H), 7.67 (d, *J* = 2.0 Hz, 1H), 7.36 (dd, *J*₁ = 0.5 Hz, *J*₂ = 8.5 Hz, 1H), 7.29 (d, *J* = 2.5 Hz, 1H), 7.19 (dd, *J*₁ = 2.0 Hz, *J*₂ = 8.5 Hz, 1H), 7.14 (d, *J* = 8.5 Hz, 1H), 6.96 (d, *J* = 2.5 Hz, 1H), 6.64 (dd, *J*₁ = 2.5, *J*₂ = 8.5 Hz, 1H), 6.19 (dd, *J*₁ = 1.0, *J*₂ = 2.0 Hz, 1H), 4.23 (s, 2H), 3.75 (s, 3H) ppm. ¹³C NMR (101 MHz, Acetone-*d*₆): δ = 154.9, 140.5, 136.5, 132.7, 130.4, 130.3, 125.8, 124.8, 122.1, 114.0, 113.4, 112.4, 112.0, 111.1, 102.5, 100.3, 55.8, 24.9 ppm. ESI-MS: 355[M+1]⁺, 357[M+1]⁺, 353[M-1]⁻, 355[M-1]⁻.

5-Methoxy-2-[(5-methyl-1H-indol-3-yl)methyl]-1H-indole (20f)



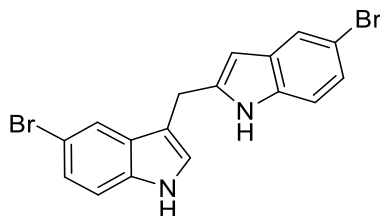
Following the general procedure, compound **20f** has been purified by flash column chromatography on alumina (cyclohexane/ethyl acetate, 8:2), obtaining a brownish solid; yield: 58%. ¹H NMR (400 MHz, Acetone-*d*₆): δ = 9.91 (br s, 1H), 9.73 (br s, 1H), 7.31 (dd, *J*₁ = 1.0 Hz, *J*₂ = 1.5 Hz, 1H), 7.27 (dd, *J*₁ = 1.0 Hz, *J*₂ = 8.5 Hz, 1H), 7.17-7.12 (m, 2H), 6.95 (d, *J* = 2.5 Hz, 1H), 6.92 (*J*₁ = 2.0 Hz, *J*₂ = 8.5 Hz, 1H), 6.63 (*J*₁ = 2.5 Hz, *J*₂ = 8.5 Hz, 1H), 6.18 (*J*₁ = 1.0 Hz, *J*₂ = 2.0 Hz, 1H), 4.21 (s, 2H), 3.75 (s, 3H), 2.34 (s, 3H) ppm. ¹³C NMR (101 MHz, Acetone-*d*₆): δ = 154.8, 141.1, 136.2, 132.6, 130.4, 128.7, 128.2, 124.1, 123.8, 119.2, 112.9, 112.0, 111.9, 110.9, 102.5, 100.2, 55.8, 25.1, 21.6 ppm. ESI-MS: 291[M+1]⁺.

5-Bromo-2-[(5-methoxy-1*H*-indol-3-yl)methyl]-1*H*-indole (20g)



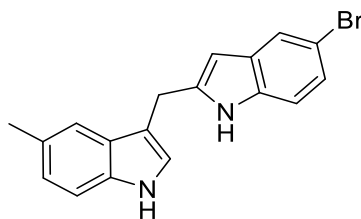
Following the general procedure, compound **20g** has been purified by flash column chromatography on alumina (cyclohexane/ethyl acetate, 8:2), obtaining a white solid; yield: 87%. ¹H NMR (400 MHz, Acetone-*d*₆): δ = 10.15 (br s, 1H), 9.94 (br s, 1H), 7.60 (d, *J* = 2.0 Hz, 1H), 7.28 (dd, *J*₁ = 0.5 Hz, *J*₂ = 8.5 Hz, 1H), 7.22 (dd, *J* = 1.0 Hz, *J*₂ = 8.5 Hz, 1H), 7.18 (d, *J* = 2.0 Hz, 1H), 7.09 (dd, *J*₁ = 2.0 Hz, *J*₂ = 8.5 Hz, 1H), 7.00 (d, *J* = 2.5 Hz, 1H), 6.75 (ddd, *J*₁ = 0.5 Hz, *J*₂ = 2.5 Hz, *J*₃ = 9.0 Hz, 1H), 6.28 (dd, *J*₁ = 1.0 Hz, *J*₂ = 2.0 Hz, 1H), 4.24 (s, 2H), 3.71 (s, 3H) ppm. ¹³C NMR (101 MHz, Acetone-*d*₆): δ = 154.8, 142.3, 136.2, 132.9, 131.9, 128.8, 124.8, 123.7, 122.6, 113.2, 112.8, 112.7, 112.5, 112.4, 101.4, 100.0, 55.8, 25.0 ppm. ESI-MS: 355[M+1]⁺, 357[M+1]⁺, 353[M-1]⁻, 355[M+1]⁻, 399[M+45]⁻.

5-Bromo-3-[(5-bromo-1*H*-indol-2-yl)methyl]-1*H*-indole (20h)



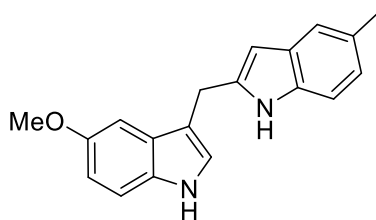
Following the general procedure, compound **20h** has been purified by flash column chromatography on alumina (cyclohexane/CH₂Cl₂ 1:1), obtaining a white solid; yield: 60%. ¹H NMR (400 MHz, Acetone-*d*₆): δ = 10.31 (br s, 1H), 10.20 (br s, 1H), 7.66 (d, *J* = 2.0 Hz, 1H), 7.61 (d, *J* = 2.0 Hz, 1H), 7.37 (dd, *J*₁ = 0.5, *J*₂ = 8.5 Hz, 1H), 7.31 (d, *J* = 2.5 Hz, 1H), 7.27-7.15 (m, 2H), 7.10 (dd, *J*₁ = 2.0, *J*₂ = 8.5 Hz, 1H), 6.27 (dd, *J*₁ = 1.0 Hz, *J*₂ = 2.0 Hz, 1H), 4.28 (s, 2H) ppm. ¹³C NMR (101 MHz, Acetone-*d*₆): δ = 141.9, 136.5, 136.2, 131.8, 130.2, 125.9, 124.9, 123.8, 122.7, 122.0, 114.1, 113.3, 112.9, 112.6, 112.5, 100.1, 24.8 ppm. ESI-MS: 403[M+1]⁺, 405[M+1]⁺, 401[M-1]⁻, 403[M+1]⁻, 447[M+45]⁻.

5-Bromo-2-[(5-methyl-1*H*-indol-3-yl)methyl]-1*H*-indole (20i)



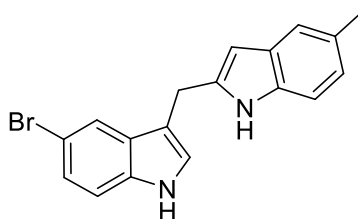
Following the general procedure, compound **20i** has been purified by flash column chromatography on alumina (cyclohexane/CH₂Cl₂ 1:1), obtaining a pink solid; yield: 93%. ¹H NMR (400 MHz, Acetone-*d*₆): δ = 10.13 (br s, 1H), 9.95 (br s, 2H), 7.60 (d, J = 2.0 Hz, 1H), 7.33-7.25 (m, 2H), 7.22 (d, J = 8.5 Hz, 1H), 7.18-7.16 (m, 1H), 7.09 (dd, J_1 = 2.0 Hz, J_2 = 8.5 Hz, 1H), 6.93 (dd, J = 2.0 Hz, J_2 = 8.0 Hz, 1H), 6.28-6.24 (m, 1H), 4.25 (s, 1H), 2.33 (s, 3H) ppm. ¹³C NMR (101 MHz, Acetone-*d*₆): δ = 141.5, 135.3, 135.3, 131.0, 127.7, 127.4, 123.4, 123.0, 122.7, 121.7, 118.2, 112.3, 111.6, 111.5, 111.0, 99.0, 24.1, 20.7 ppm. ESI-MS: 339[M+1]⁺, 341[M+1]⁺, 337[M-1]⁻, 339[M+1]⁻, 383[M+45]⁻.

5-Methoxy-3-[(5-methyl-1*H*-indol-2-yl)methyl]-1*H*-indole (20j)



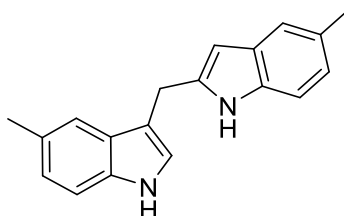
Following the general procedure, compound **20j** has been purified by flash column chromatography on alumina (cyclohexane/ethyl acetate 1:1), obtaining an orange solid; yield: 79%. ¹H NMR (400 MHz, Acetone-*d*₆): δ = 9.89 (br s, 1H), 9.77 (br s, 1H), 7.27 (dd, J_1 = 0.5 Hz, J_2 = 8.5 Hz, 1H), 7.22 (d, J = 1 Hz, 1H), 7.18-7.11 (m, 2H), 7.01 (d, J = 2.5 Hz, 1H), 6.85-6.78 (m, 1H), 6.74 (dd, J_1 = 2.5 Hz, J_2 = 8.5 Hz, 1H), 6.18 (dd, J_1 = 1.0 Hz, J_2 = 2.0 Hz, 1H), 4.21 (s, 1H), 3.71 (s, 3H), 2.34 (s, 2H) ppm. ¹³C NMR (101 MHz, Acetone-*d*₆): δ = 154.7, 140.3, 135.9, 132.9, 130.3, 128.8, 128.3, 124.7, 122.6, 120.0, 113.2, 112.7, 112.3, 111.1, 101.5, 99.8, 55.8, 25.1, 21.6 ppm. ESI-MS: 291[M+1]⁺.

5-Bromo-3-[(5-methyl-1*H*-indol-2-yl)methyl]-1*H*-indole (20k)



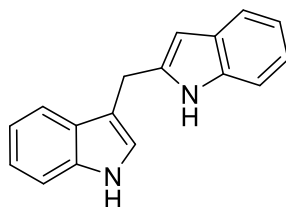
Following the general procedure, compound **20k** has been purified by flash column chromatography on alumina (cyclohexane/ethyl acetate 8:2), obtaining a yellow solid; yield: 51%. ¹H NMR (400 MHz, Acetone-*d*₆): δ = 10.26 (br s, 1H), 9.83 (br s, 1H), 7.67 (d, *J* = 2.0 Hz, 1H), 7.36 (d, *J* = 8.5 Hz, 1H), 7.29 (d, *J* = 2.5 Hz, 1H), 7.22 (s, 1H), 7.19 (dd, *J*₁ = 2.0 Hz, *J*₂ = 8.5 Hz, 1H), 7.14 (d, *J* = 8.0 Hz, 1H), 6.82 (dd, *J*₁ = 2.0 Hz, *J*₂ = 8.0 Hz, 1H), 6.20-6.15 (m, 1H), 4.24 (s, 1H), 2.34 (s, 3H) ppm. ¹³C NMR (101 MHz, Acetone-*d*₆): δ = 139.8, 136.5, 136.0, 130.27, 130.21, 128.4, 125.8, 124.8, 122.7, 122.1, 120.1, 114.0, 113.4, 112.4, 111.2, 99.9, 24.9, 21.6 ppm. ESI-MS: 339[M+1]⁺, 341[M+1]⁺, 337[M-1]⁻, 339[M-1]⁻.

5-Methyl-3-[(5-methyl-1*H*-indol-2-yl)methyl]-1*H*-indole (20l)



Following the general procedure, compound **20l** has been purified by flash column chromatography on alumina (cyclohexane/ethyl acetate 8:2), obtaining an orange solid; yield: 78%. ¹H NMR (400 MHz, Acetone-*d*₆): δ = 9.92 (br s, 1H), 9.76 (br s, 1H), 7.32-7.29 (m, 1H), 7.27 (d, *J* = 8.5 Hz, 1H), 7.21 (s, 1H), 7.16-7.10 (m, 2H), 6.92 (dd, *J*₁ = 1.5 Hz, *J*₂ = 8.5 Hz, 1H), 6.81 (dd, *J*₁ = 2.0 Hz, *J*₂ = 8.5 Hz, 1H), 6.18-6.12 (m, 1H), 4.21 (s, 2H), 2.34 (s, 3H), 2.34 (s, 3H) ppm. ¹³C NMR (101 MHz, Acetone-*d*₆): δ = 140.4, 136.2, 135.9, 130.3, 128.7, 128.3, 128.2, 124.1, 123.8, 122.6, 120.0, 119.2, 112.9, 111.9, 111.1, 99.8, 25.1, 21.6, 21.6 ppm. ESI-MS: 275[M+1]⁺, 273[M-1]⁻.

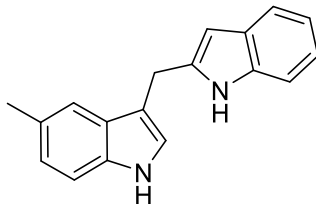
3-[(1*H*-indol-2-yl)methyl]-1*H*-indole (20m)



Following the general procedure, compound **20m** has been purified by flash column chromatography on alumina (cyclohexane/EtOAc 8:2) as a white solid; yield: 82%. ¹H NMR (400 MHz, DMSO-*d*₆): δ = 10.87 (br s, 2H), 7.48 (d, *J* = 8.0 Hz, 1H), 7.39-7.33 (m, 2H), 7.26 (d, *J* = 8.0 Hz, 1H), 7.22 (d, *J* = 2.5 Hz, 1H), 7.05 (dd, *J*₁ $\approx$ *J*₂ =

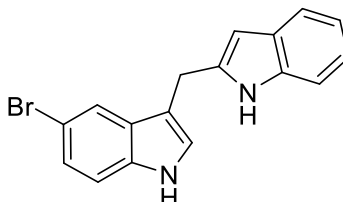
7.0 Hz, 1H), 6.98-6.87 (m, 3H), 6.15 (d, $J = 1.5$ Hz, 1H), 4.17 (s, 2H) ppm. The physicochemical data of compound agree with those reported in the literature [12].

3-[(1*H*-indol-2-yl)methyl]-5-methyl-1*H*-indole (20n)



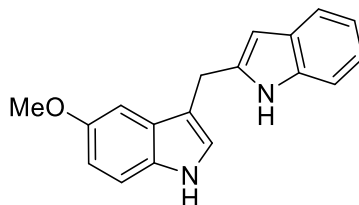
Following the general procedure, compound **20n** has been purified by flash column chromatography on alumina (cyclohexane/EtOAc 8:2) as a white solid; yield: 82%. ^1H NMR (400 MHz, DMSO- d_6): $\delta = 10.85$ (br s, 1H), 10.73 (br s, 1H), 7.37 (d, 1H, $J = 8.0$ Hz), 7.28-7.21 (m, 3H), 7.16 (d, 1H, $J = 2.5$), 6.96 (ddd, 1H, $J_1 = 1.5$ Hz, $J_2 = 7.0$ Hz, $J_3 = 8.0$ Hz), 6.91-6.86 (m, 2H), 6.11 (d, 1H, $J = 2.0$ Hz), 4.13 (s, 2H), 2.33 (s, 3H) ppm. ^{13}C NMR (101 MHz, DMSO- d_6): $\delta = 139.7, 136.1, 134.7, 128.3, 127.2, 126.6, 123.4, 122.5, 119.9, 119.1, 118.5, 118.0, 111.3, 111.1, 110.7, 98.7, 24.0, 21.3$ ppm. ES-MS: 261 $[\text{M}+\text{H}]^+$; 259 $[\text{M}-\text{H}]^-$. The physicochemical data of compound agree with those reported in the literature [11].

3-[(1*H*-indol-2-yl)methyl]-5-bromo-1*H*-indole (20o)



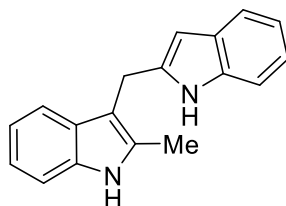
Following the general procedure, compound **20o** has been purified by flash column chromatography on alumina (cyclohexane/EtOAc 8:2) as a white solid; yield: 90%. ^1H NMR (400 MHz, DMSO- d_6): $\delta = 11.11$ (br s, 1H), 10.88 (br s, 1H), 7.65 (d, 1H, $J = 2.0$ Hz), 7.38 (d, 1H, $J = 8.0$ Hz), 7.33 (d, 1H, $J = 8.5$ Hz), 7.30 (d, 1H, $J = 2.0$ Hz), 7.26 (dd, 1H, $J_1 = 1.0$, $J_2 = 8.0$ Hz), 7.16 (dd, 1H, $J_1 = 2.0$, $J_2 = 8.5$ Hz), 6.97 (ddd, 1H, $J_1 = 1.0$ Hz, $J_2 = 7.0$ Hz, $J_3 = 8.0$ Hz), 6.90 (ddd, 1H, $J_1 = 1.0$ Hz, $J_2 = 7.0$ Hz, $J_3 = 8.0$ Hz), 6.14 (d, 1H, $J = 1.0$ Hz), 4.15 (s, 2H) ppm. ^{13}C NMR (101 MHz, DMSO- d_6) $\delta = 139.3, 136.1, 135.0, 128.8, 128.3, 125.1, 123.4, 120.7, 120.0, 119.1, 118.5, 113.4, 111.8, 110.9, 110.7, 98.8, 23.8$ ppm. ES-MS: 325 $[\text{M}+\text{H}]^+$; 327 $[\text{M}+\text{H}]^+$; 323 $[\text{M}-\text{H}]^-$; 325 $[\text{M}-\text{H}]^-$. The physicochemical data of compound agree with those reported in the literature [15].

3-[(1*H*-indol-2-yl)methyl]-5-methoxy-1*H*-indole (20p)



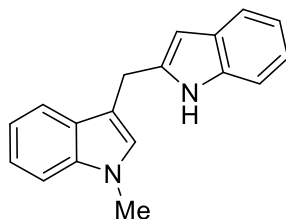
Following the general procedure, compound **20p** has been purified by flash column chromatography on alumina (cyclohexane/EtOAc 8:2) as a white solid; yield: 81%. ¹H NMR (400 MHz, DMSO-*d*₆): δ = 10.87 (br s, 1H), 10.71 (br s, 1H), 7.38 (d, 1H, *J* = 7.5 Hz), 7.26 (d, 1H, *J* = 7.5 Hz), 7.22 (d, 1H, *J* = 9.0 Hz), 7.16 (d, 1H, *J* = 2.5 Hz), 6.99-6.94 (m, 1H), 6.97 (d, 1H, *J* = 2.5 Hz), 6.89 (ddd, 1H, *J*₁ = 1.0 Hz, *J*₂ = 7.5 Hz, *J*₃ = 8.0 Hz), 6.71 (dd, 1H, *J*₁ = 2.5 Hz, *J*₂ = 9.0 Hz), 6.16 (d, 1H, *J* = 1.0 Hz), 4.13 (s, 2H), 3.69 (s, 3H) ppm. ES-MS: 277 [M+H]⁺. The physicochemical data of compound agree with those reported in the literature [12].

3-[(1*H*-indol-2-yl)methyl]-2-methyl-1*H*-indole (20q)



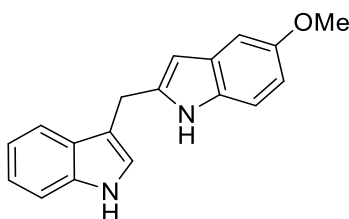
Following the general procedure, compound **20q** has been purified by flash column chromatography on alumina (cyclohexane/EtOAc 8:2) as a white solid; yield: 93%. ¹H NMR (400 MHz, DMSO-*d*₆): δ = 10.79 (br s, 1H), 10.75 (br s, 1H), 7.35 (d, 1H, *J* = 7.5 Hz), 7.34 (d, 1H, *J* = 8.0 Hz), 7.25 (dd, 1H, *J*₁ = 1.0 Hz, *J*₂ = 8.0 Hz), 7.24 (d, 1H, *J* = 8.0 Hz), 6.98-6.92 (m, 2H), 6.90-6.83 (m, 2H), 6.04 (d, 1H, *J* = 1.0 Hz), 4.10 (s, 2H), 2.39 (s, 3H) ppm. ¹³C NMR (101 MHz, DMSO-*d*₆): δ = 139.9, 136.1, 135.2, 132.2, 128.4, 128.3, 119.9, 119.8, 119.0, 118.5, 118.1, 117.7, 110.7, 110.3, 107.4, 98.6, 22.9, 11.4 ppm. ES-MS: 261 [M+H]⁺, 259 [M-H]⁻.

3-[(1*H*-indol-2-yl)methyl]-1-methyl-1*H*-indole (20r)



Following the general procedure, compound **20r** has been purified by flash column chromatography on alumina (cyclohexane/EtOAc 95:5) as a red solid; yield: 21%. ^1H NMR (400 MHz, DMSO- d_6): δ = 10.87 (br s, 1H), 7.49 (d, 1H, J = 8.0 Hz), 7.38 (d, 1H, J = 8.0 Hz), 7.37 (d, 1H, J = 7.5 Hz), 7.25 (d, 1H, J = 8.0 Hz), 7.18 (s, 1H), 7.12 (ddd, 1H, J_1 = 1.0 Hz, J_2 = 7.0 Hz, J_3 = 8.0 Hz), 7.00-6.93 (m, 2H), 6.89 (ddd, 1H, J_1 = 1.0 Hz, J_2 = 7.0 Hz, J_3 = 7.5 Hz), 6.16 (d, 1H, J = 1.0 Hz), 4.16 (s, 2H), 3.75 (s, 3H) ppm. ^{13}C NMR (101 MHz, DMSO- d_6): δ = 139.4, 136.7, 136.1, 128.3, 127.6, 127.3, 121.1, 119.9, 119.1, 118.7, 118.5, 118.3, 111.3, 110.7, 109.5, 98.8, 32.2, 23.9 ppm. ES-MS: 261 $[\text{M}+\text{H}]^+$. The physicochemical data of compound agree with those reported in the literature [9].

2-(Indolin-3-ylmethyl)-5-methoxy-1H-indole (20t)



Following the general procedure, compound **20t** has been purified by flash column chromatography on alumina (cyclohexane/EtOAc 95:5) as a greenish solid; yield: 73%. ^1H NMR (400 MHz, DMSO- d_6): δ = 10.86 (br s, 1H), 10.68 (br s, 1H), 7.47 (d, 1H, J = 8.0 Hz), 7.34 (d, 1H, J = 8.0 Hz), 7.20 (d, 1H, J = 2.5 Hz), 7.13 (d, 1H, J = 8.5 Hz), 7.05 (ddd, 1H, J_1 = 1.0 Hz, J_2 = 7.0 Hz, J_3 = 8.0 Hz), 6.92 (ddd, 1H, J_1 = 1.0 Hz, J_2 = 7.0 Hz, J_3 = 8.0 Hz), 6.89 (d, 1H, J = 2.5 Hz), 6.60 (dd, 1H, J_1 = 2.5 Hz, J_2 = 8.5 Hz), 4.13 (s, 2H), 3.70 (s, 3H) ppm. ES-MS: 277 $[\text{M}+\text{H}]^+$. The physicochemical data of compound agree with those reported in the literature [12].

5.5 References

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Chapter 6. HITL Bayesian-optimization for the Development of a Photochemical *B*-Alkyl-Suzuki Alkenylation of Vinyl Triflates

6.1 Introduction

Over the past few decades, transition metal-catalyzed $\text{sp}^2\text{--sp}^2$ cross-coupling reactions have completely transformed the way chemists build complex molecules. They have made it remarkably easy to access a wide range of bioactive scaffolds, and their impact on pharmaceutical and agrochemical research is hard to overstate. However, this very success has also introduced a kind of structural bias: most of the molecules accessible through these couplings tend to be flat and structurally similar, limiting the three-dimensional diversity that often plays a crucial role in biological activity [1]. As a result, the chemical community has gradually shifted its attention toward new strategies that can incorporate sp^3 -rich fragments, aiming to bring more shape and complexity into small-molecule design [2].

In this context, the Suzuki–Miyaura cross-coupling reaction with alkyl boranes (*B*-alkyl SMC) allows the coupling of alkyl boranes with aryl or vinyl (pseudo)halides [3, 4]. This methodology offers several advantages, such as (i) the generation of the alkyl partner via hydroboration of alkenes, (ii) the possibility to conduct the reaction under non-rigorously anhydrous conditions [4], and (iii) the easy management of boron-derived by-products [5]. Typically, these couplings rely on palladium-based catalysts, which, however, require harsh reaction conditions, posing a significant limitation to reaction scalability [6].

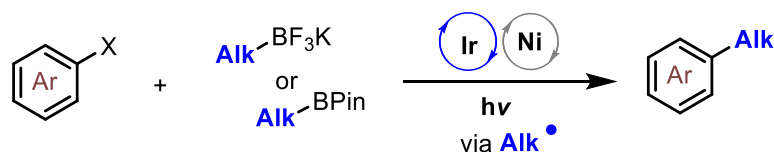
In recent years, light-mediated synthesis has gained tremendous interest for $\text{C}(\text{sp}^3)\text{--C}(\text{sp}^2)$ bond formation within the framework of *B*-alkyl SMC, leading to the emergence of metallaphotoredox catalysis as a key methodology. In this approach, a photocatalyst generates carbon-centered radicals from boron derivatives (such as trifluoroborate potassium salts and boronic esters), which are then typically intercepted by a nickel-based complex (**Scheme 8A**) [7, 8, 9]. Although these methods are widely used for the functionalization of benzylic, allylic, and α -to-oxygen positions, they still face a major limitation: the efficient generation of unstabilized primary carbon radicals

remains challenging [8, 10, 11, 12]. Moreover, accessing more complex derivatives is often difficult, as their preparation typically requires harsh conditions or metal catalysts that are not always compatible with multifunctional molecules.

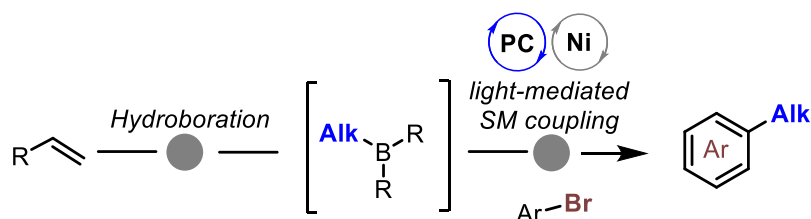
In 2024, Noël and co-workers reported an outstanding photocatalytic protocol for the coupling of alkyl boronates with aryl bromides [13], using nickel as the metal catalyst and 4CzIPN as the photocatalyst, leveraging the facile hydroboration of commercially available olefins, which proceeds with anti-Markovnikov regioselectivity (**Scheme 8B**).

Encouraged by these results, during my visiting period at the University of Amsterdam, we decided to develop a *B*-alkyl SMC aimed at the alkylation of vinyl triflates to obtain highly substituted olefins (**Scheme 8C**). The synthesis of such highly substituted olefins is well-documented in the literature using methodologies such as allene hydrofunctionalization [14], carbonyl olefination [15], cross-metathesis [15], and carbometallation [16]. However, these approaches often require multistep preparation of starting materials or strongly basic and cryogenic conditions. A *B*-alkyl SMC offers a remarkably practical way to build C(sp³)–C(sp²) bonds, combining two simple yet powerful advantages. On one hand, the alkyl borane partner can be quickly obtained through a mild and highly regioselective hydroboration of readily available olefins. On the other, the vinyl triflate counterpart can be easily prepared from common ketones or aldehydes. Together, these features make the method not only efficient and versatile, but also genuinely accessible for the synthesis of highly substituted olefins.

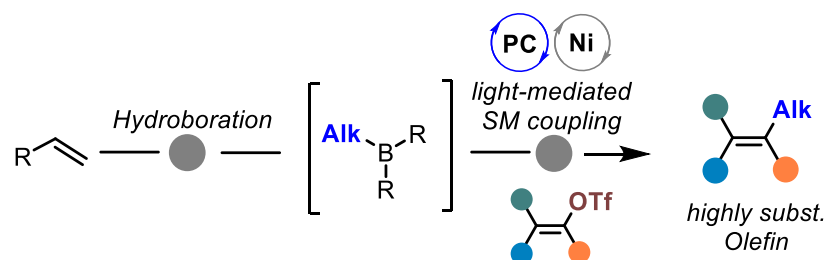
A. Light-mediated *B*-alkyl Suzuki–Miyaura cross-coupling reaction via single-electron transmetalation



B. Photocatalytic C(sp²)–C(sp³) Suzuki–Miyaura cross-coupling of aryl bromides with alkyl boranes



C. This work: photochemical *B*-Alkyl-Suzuki alkenylation of vinyl triflates



- ✓ Machine learning optimization
- ✓ Machine learning flow optimization
- ✓ Mild conditions
- ✓ Easy synthesis of the starting materials
- ✓ Highly diversified scope
- ✓ Yield up to 95%

Scheme 8. **A** Light-mediated *B*-alkyl Suzuki–Miyaura cross-coupling reaction via single-electron transmetalation: this approach is mainly restricted to the generation of stabilized radicals. **B** Photocatalyzed *B*-alkyl Suzuki–Miyaura cross-coupling with alkyl borane to append primary fragments onto arenes. **C** Photochemical *B*-Alkyl-Suzuki alkenylation of vinyl triflates to obtain highly substituted olefins.

However, turning this synthetic potential into a reaction that consistently delivers high yields, and broad applicability was anything but simple. Even when working with an elegant and well-behaved system, getting everything aligned just right is rarely straightforward. Finding the perfect balance between catalyst, base, solvent, and all the other moving parts often feels less like following a recipe and more like navigating a maze, one where intuition alone is rarely enough.

In practice, optimization in reaction development means dealing with a complex, high-dimensional landscape that combines categorical variables, such as catalyst, base,

solvent, or ligand, with continuous ones like concentration or reaction time. The interplay between these parameters can be subtle and unpredictable, making the identification of optimal conditions one of the most intricate and time-consuming stages of research.

Traditional “one-factor-at-a-time” (OFAT) strategies fail to capture nonlinear interactions between parameters, while design-of-experiments (DoE) approaches scale poorly as dimensionality increases [17]. This creates a bottleneck in which promising chemical transformations often stall, since exhaustive condition screening is experimentally prohibitive [18].

Machine learning (ML) provides a compelling solution. Active-learning strategies, such as Bayesian Optimization (BO), can efficiently navigate complex parameter spaces by balancing exploration and exploitation, drastically reducing the number of experiments required to identify high-performing regions [19]. When applied to reaction development, ML-guided workflows can accelerate the discovery of general and scalable conditions while minimizing experimental cost [20]. In this work, we set out to explore the machine learning–guided optimization of a photocatalytic Suzuki–Miyaura coupling between trialkylboranes and vinyl triflates, a reaction that, while powerful, had remained relatively underexplored. By letting the machine learning model learn from each experiment, we were able to rapidly identify conditions that perform exceptionally well, something that would have taken an enormous amount of time using traditional trial-and-error approaches. Moreover, we then applied transfer learning to adapt the optimized conditions to a continuous flow setup, making the process more scalable and practical. Finally, we put the method to the test, demonstrating a broad and versatile substrate scope in batch, highlighting not just the efficiency of chemistry, but also the real-world potential of combining modern data-driven approaches with synthetic innovation.

6.2 Results and Discussion

The substrates selected for the initial reaction were **21a** and **22a**, aiming to obtain compound **23aa**. By applying the conditions reported in entry 1 of **Table 24** (experimental section), we were pleased to immediately observe clear reactivity of our catalytic system

(60% NMR yield), an encouraging result that confirmed the validity of our chosen approach.

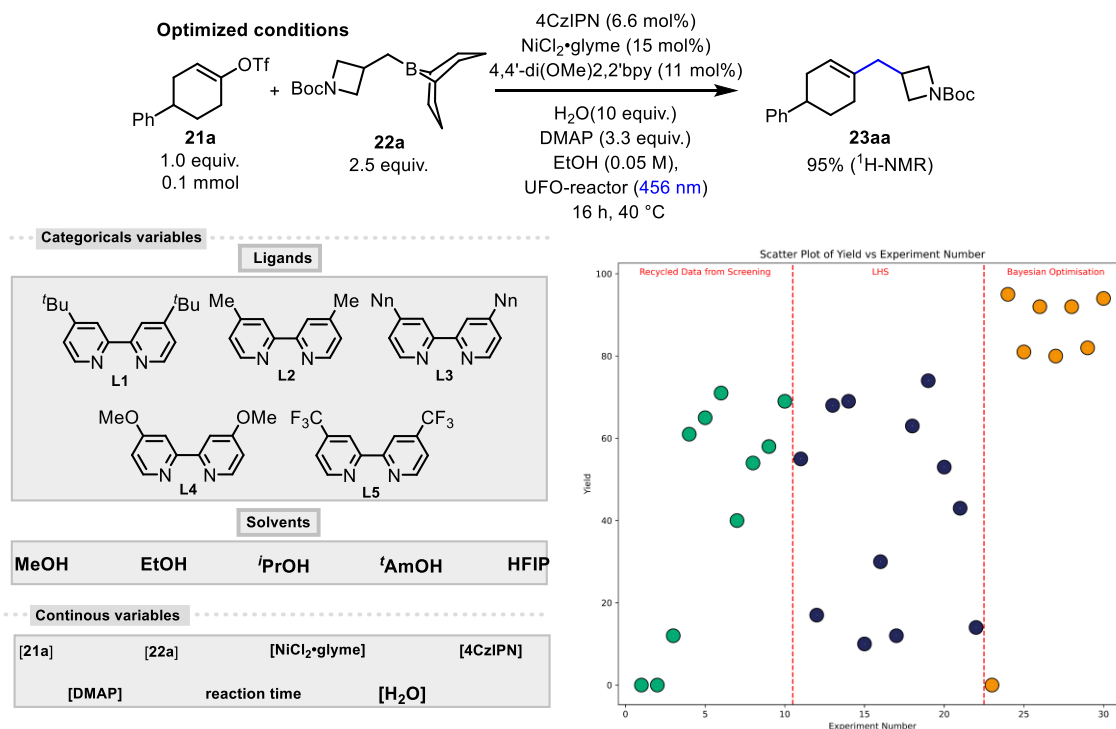
The optimization of the reaction was carried out using a machine learning-guided approach, which turned out to be both efficient and surprisingly intuitive once the workflow was established. At first, we ran a preliminary screening of 27 experiments (see **Table 24**, Experimental Section) to get a rough idea of how the reaction behaved under different conditions. The goal at this stage was not to find the perfect setup, but simply to explore the parameter space and collect enough data to start training the model. The variables we considered included both categorical factors, such as the choice of ligand and solvent, and continuous parameters, like the mol % Ni, ligand, and 4CzIPN, reaction time, the equivalents of DMAP and H₂O, and overall reaction concentration.

Once the first batch of data was collected, we moved on to a Latin Hypercube Sampling (LHS) stage with 12 experiments, designed to systematically explore the continuous variables. This method allowed us to cover a wide range of conditions without running an unnecessarily large number of reactions, which saved a lot of time and materials.

With these two sets of data in hand, the next step was to feed them into a BO process. This part was particularly interesting as the algorithm started suggesting new conditions by alternating between exploration, testing areas of the parameter space that had not been well characterized, and exploitation, where it refined the most promising conditions already identified. After just 8 experiments, the model had already converged towards a set of reaction conditions that were close to ideal.

Following this iterative process, we were able to pinpoint a combination of parameters that were not only highly efficient but also simple and reproducible, leading to yields of up to 95%. The final optimized setup involved 6.6 mol % of 4CzIPN, 15 mol % of NiCl₂·glyme, 11 mol % of 4,4'-dimethoxy-2,2'-bipyridine, 10 equivalents of H₂O, 2.5 equivalents of borane, and 3.3 equivalents of DMAP, in EtOH (0.05 M), with 456 nm irradiation for 16 hours (**Table 21**).

Table 21. *B*-Alkyl-Suzuki alkenylation campaign: Yield optimization.



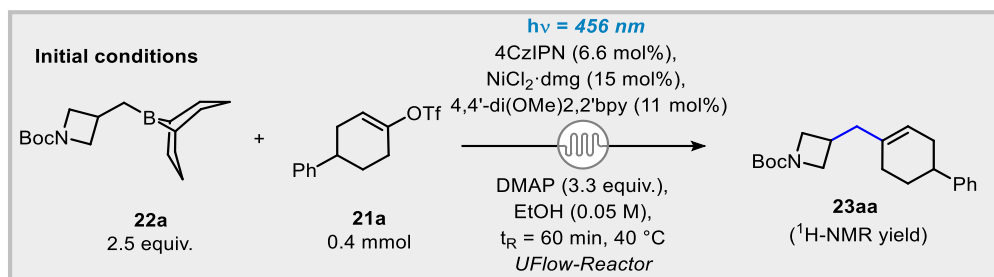
In the next stage of the work, machine learning was employed to support the optimization of the reaction conditions under flow. The main idea was to explore whether an algorithm could truly accelerate the optimization process, reducing the number of required experiments while improving the overall quality of the results.

At first, five exploratory flow reactions were carried out without the intervention of the ML model, with the aim of collecting preliminary data and observing the behavior of the system under different conditions. These experiments provided a useful experimental basis for understanding how key variables, such as temperature, flow rate, and reagent ratio, affected the course of the reaction.

Subsequently, the machine learning algorithm processed all the available information, combining the data obtained from the batch experiments with those from the five exploratory flow runs. Through a correlation matrix, the model identified the most significant relationships between the experimental parameters and the reaction yield and then constructed an overlap matrix that allowed the two data sets to be compared and integrated coherently (Table 22).

Due to this integrated approach, the algorithm was able to progressively and efficiently propose new sets of conditions, optimizing the flow system in just eight generations. In practice, within only a few cycles, the model identified a set of parameters very close to the optimal ones, considerably reducing the time and experimental effort compared to a traditional manual optimization process.

Table 22. Transfer learning flow optimization.

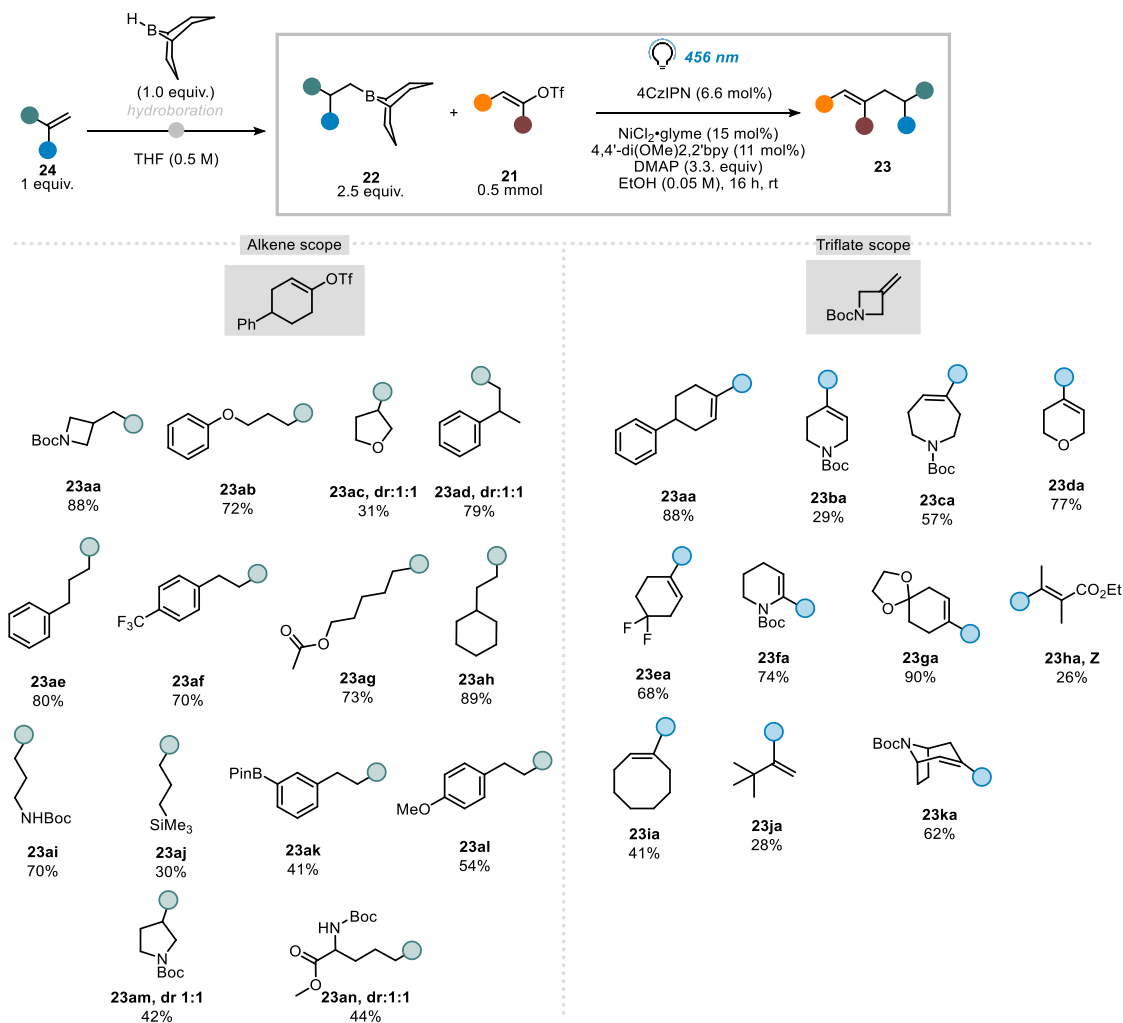


Entry	Variation from initial conditions	NMR yield [%] (Conversion%)
1	None	52 (100)
Exploration (5 reactions)		
2	22a (1.4 equiv), DMAP (2.3 equiv), 4CzIPN (1.7 mol %), Ni (18.7 mol %), L4 (14 mol %), MeOH (0.77 M), H ₂ O (3.3 equiv), $t_R = 14\text{ min}$	33 (35)
1°, 2°, 3° Generation (9 reactions)		
3	22a (2.1 equiv), DMAP (2.8 equiv), 4CzIPN (6.1 mol %), Ni (16.3 mol %), L4 (12.5 mol %), EtOH (0.05 M), H ₂ O (5.5 equiv), $t_R = 12\text{ min}$	64 (86)
4°- 7° Generation (12 reactions)		
4	22a (2 equiv), DMAP (2.9 equiv), 4CzIPN (6.1 mol %), Ni (18.2 mol %), L4 (13.6 mol %), EtOH (0.05 M), H ₂ O (4.6 equiv), $t_R = 18\text{ min}$	70 (90)
8° Generation (3 reactions)		
5	22a (2.13 equiv), DMAP (2.5 equiv), 4CzIPN (3.0 mol %), Ni (15.7 mol %), L5 (11 mol %), MeOH (0.16 M), H ₂ O (5.0 equiv), $t_R = 60\text{ min}$	71 (94)

t_R = Residence Time

At this point, having established the optimized batch and flow conditions for our photocatalytic coupling, we decided to demonstrate the generality of the reaction under batch (**Table 23**) conditions by performing the experiments on a 0.5 mmol scale.

Table 23. Batch scope of the photocatalytic *B*-alkyl Suzuki reaction.



We began investigating the reaction scope by testing a variety of olefins, which underwent rapid hydroboration with 9-BBN to generate the corresponding alkyl borane partners. In all cases, the desired regioselectivity was successfully achieved.

To start, we successfully synthesized compounds **23ab** and **23ae**, both allylic olefins. Similarly, a series of long-chain alkenes incorporating multiple functional groups proved to be well-suited to these conditions, delivering the expected products as single regioisomers with excellent yields (**23ag**, **23ai**, **23aj**). Moreover, the transformation

performed efficiently with vinylcyclohexene, affording compound **23ah** in an excellent 89% yield.

Interestingly, when phenylsubstituted alkenes were used as substrates, the formation of alkylarenes (**23ad**, **23af**, **23ak**, **23al**) was observed. Once again, this highlights the potential of our methodology for the functionalization of alkyl terminal chains.

Five-membered heterocycles also delivered the expected products **23ac** and **23am**, with yields of 31% and 42%, respectively. Finally, the reaction also gave good results when employing a protected amino acid substrate, affording compound **23an**.

Next, our focus shifted to the vinyl triflate coupling partner, which can be conveniently synthesized starting from commercially available ketones or aldehydes (see experimental section). The reaction performs remarkably well with cyclic vinyl triflates, both heteroatom-containing (**23ba**, **23ca**, **23da**, **23fa**) and purely aliphatic ones (**23ea**, **23ia**). The desired products were also obtained when spirocyclic (**23ga**) and bicyclic (**23ka**) systems were employed as substrates. Moreover, to our great satisfaction, we were able to demonstrate the applicability of the newly developed conditions to acyclic vinyl triflates, successfully affording the corresponding products **23ha** and **23ja**.

6.3 Conclusions

In summary, we have developed a photocatalytic *B*-alkyl Suzuki–alkenylation of vinyl triflate that efficiently enables the construction of C(sp³)–C(sp²) bonds under mild and operationally simple conditions. By integrating machine learning-guided optimization, we rapidly identified optimal reaction parameters in batch, significantly reducing experimental workload while maintaining high yields and reproducibility.

Subsequently, by applying a transfer learning approach, we successfully translated the optimized conditions to continuous flow, highlighting the adaptability and scalability of the methodology. Finally, we demonstrated the generality of the reaction across a broad substrate scope in batch, encompassing a wide variety of alkenes and vinyl triflates, including cyclic, acyclic, and heteroatom-containing systems.

Overall, this work showcases the power of data-driven optimization in accelerating the development of new photocatalytic transformations and establishes a versatile, scalable platform for light-mediated *B*-alkyl Suzuki–Miyaura couplings, paving the way for future applications in complex molecule synthesis and automated reaction discovery.

6.4 Experimental Section

Materials and methods

Chemistry

All reagents and solvents were bought from Sigma Aldrich, TCI, Flurochem, VWR International and Biosolv and used as received. Disposable syringes were purchased from Laboratory Glass Specialist. Syringe pumps were purchased from Chemix Inc. model Fusion 200 Touch. All capillary tubing, microfluidic fittings and Back Pressure Regulator (BPR) were purchased from IDEX Health & Science. Product isolation was performed manually, using silica (P60, SILICYCLE), or automatically, by a Biotage[®] Isolation Four, with Biotage[®] SNAP KP-Sil 20 or 50 g flash chromatography cartridges. TLC analysis was performed using Silica on aluminum foils TLC plates (F254, SILICYCLE) with visualization under ultraviolet light (254 nm and 365 nm) or appropriate TLC staining (potassium permanganate or cerium ammonium molybdate). ¹H (500 MHz, 400 MHz or 300 MHz), ¹³C (125 MHz, 101 MHz or 75 MHz), ¹⁹F NMR (282 MHz, CDCl₃), and ¹¹B (96 MHz) spectra were recorded unless stated otherwise on ambient temperature using a Bruker AV500, Bruker AV400 or a Bruker AV300. ¹H NMR spectra are reported in parts per million (ppm) downfield relative to CDCl₃ (7.26 ppm) and all ¹³C NMR spectra are reported in ppm relative to CDCl₃ (77.2 ppm) unless stated otherwise. The multiplicities of signals are designated by the following abbreviations: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), dd (doublet of doublets), dt (doublet of triplets), td (triplet of doublets), tt (triplets of triplets), ddd (doublet of doublet of doublets), qd (quartet of doublet). Coupling constants (*J*) are reported in hertz (Hz). NMR data was processed using the MestReNova 15 software package. Known products

were characterized by comparing to the corresponding ^1H NMR, ^{13}C NMR, ^{19}F NMR and ^{11}B NMR with those available in the literature.

Machine learning strategies

The machine learning workflow was divided into three sequential stages. First, chemists performed an initial scoping of the chemical space to identify promising reaction conditions and additives. Once critical parameters and a defined set of reagents had been selected, a data curation step was performed in which only reaction data falling within the selected chemical space from the pre-screening stage were retained for subsequent optimization. In the second stage, a set of LHS experiments was generated. These experiments were designed in awareness of the existing data and served to provide space-filling coverage, ensuring that the Gaussian process (GP) model would be trained on a well-distributed dataset. A first round of batched Bayesian optimization was then performed using a batch size of four and a qUCB acquisition function with a GP regressor (radial basis function kernel, $\beta = 2$). Finally, to extend the workflow to flow chemistry, the same transformation was subjected to a second optimization stage. To avoid discarding the information collected in the initial campaign, transfer learning was implemented through multi-task BO. This stage began with a limited number of LHS seed experiments, after which the optimization was continued until convergence.

UFO reactor

For all batch experiments a homemade, 3D-printed reactor was adopted. The reactor was designed to fit reaction vials and to be equipped with a Kessil lamp PR160L series ($\lambda_{\text{em}} = 390$ or 456 nm). The reactor was designed in Adobe Inventor 2021 with 4 different parts. The lid ($100\text{ mm} \times 12\text{ mm}$) is designed to host up to 8 reactions vials and holds the Kessil lamp in the center (**Figure 26A**); a fan (SUNON DCLüfter 24 V; 50x50x15 Vapo RoHS) is mounted on the bottom of the reactor for cooling. The box is designed with holes to allow the air flow to escape the reactor and keep the temperature stable around $30\text{--}33\text{ }^{\circ}\text{C}$ (**Figure 26B**), as measured by an external thermometer. A reflector is situated underneath the lamp and reflects the photons inside the box to have homogeneous light distribution (**Figure 26C**). Finally, the stirring plate adapter (**Figure 26D**) was added to fix the system on a stirring plate and provide homogeneous stirring (**Figure 26E**). It also spaces the reflector from the plate to ensure a continuous air flow

from the top to the bottom of the system. All the inside surfaces were covered with reflective tape. An overview of the assembled reactor is shown in **Figure 27**.

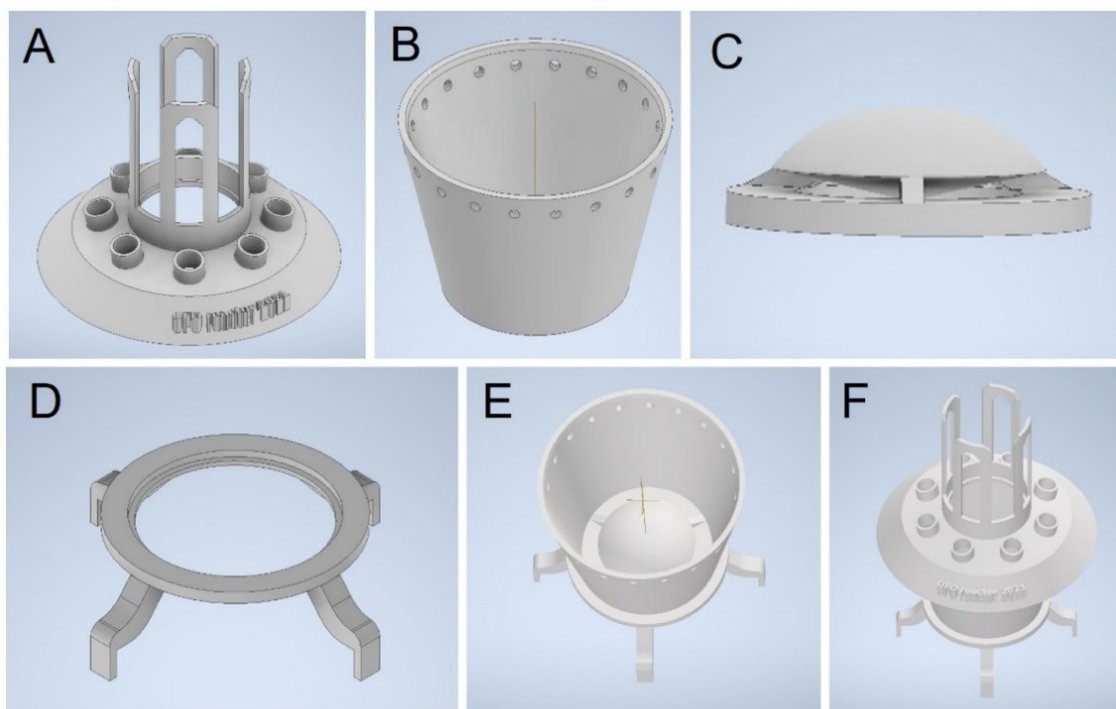


Figure 26. Overview of the 3D-printed reactor: A) lid designed to host up to 8 reactions vials and hold the Kessil lamp in the center; B) body of the reactor; C) light reflector: it is coated with reflective tape; D) adapter for stirring plate; E) inside of the reactor; G) overall reactor.



Figure 27. Picture of the assembled Ufo reactor equipped with a Kessil lamp ($\lambda_{em} = 456 \text{ nm}$).

Uflow reactor

For all flow experiments a homemade 3D-printed reactor was adopted (**Figure 28**, left). The reactor consists of a lid to host the Kessil lamp and a support around which a PFA tubing (0.8 mm inner diameter) is coiled (**Figure 28**, middle); a fan (SUNON DCLüfter 24 V; 50x50x15 Vapo RoHS) is mounted on the bottom of the reactor for cooling. The coil is inserted in a cylindrical plastic body (**Figure 28**, right) with holes to allow the air flow to escape the reactor and keep the temperature stable around 30–33 °C. An overview of the assembled reactor is shown in **Figure 29**.

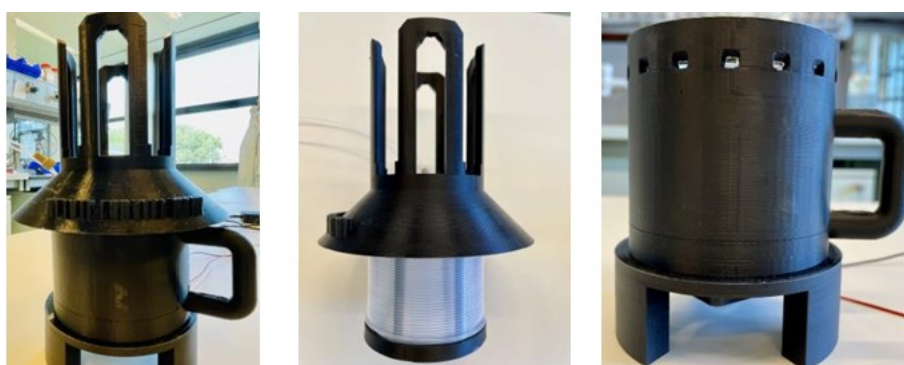


Figure 28. Overview of the 3D-printed flow reactor. Left: overall reactor; middle: inside of the reactor (flow loop) and holder for the Kessil lamp in the center; right: main body, internally coated with reflective tape. A fan is mounted on the bottom to keep the temperature stable around 30–33 °C.

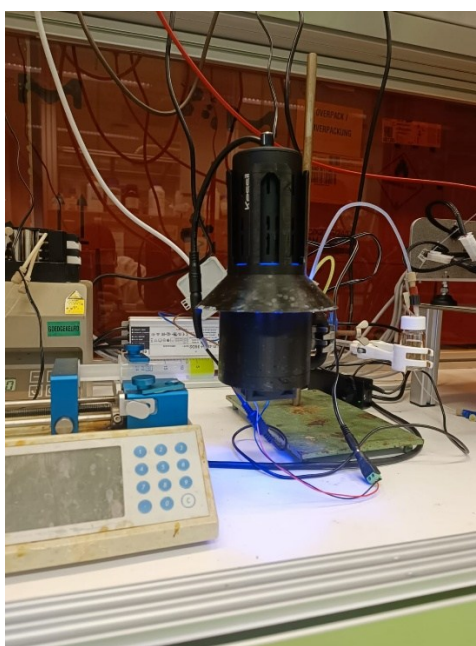


Figure 29. Picture of the assembled Uflow reactor equipped with a Kessil lamp ($\lambda_{\text{em}} = 456 \text{ nm}$).

Charts of starting materials

Olefins

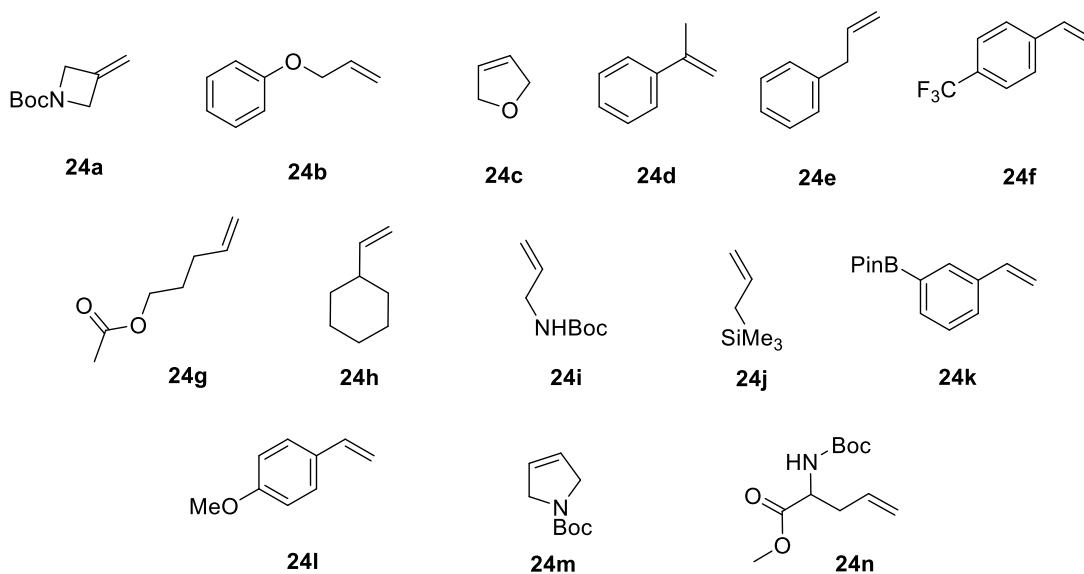


Figure 28. Olefins **24** used as starting materials for hydroboration.

Vinyl triflate

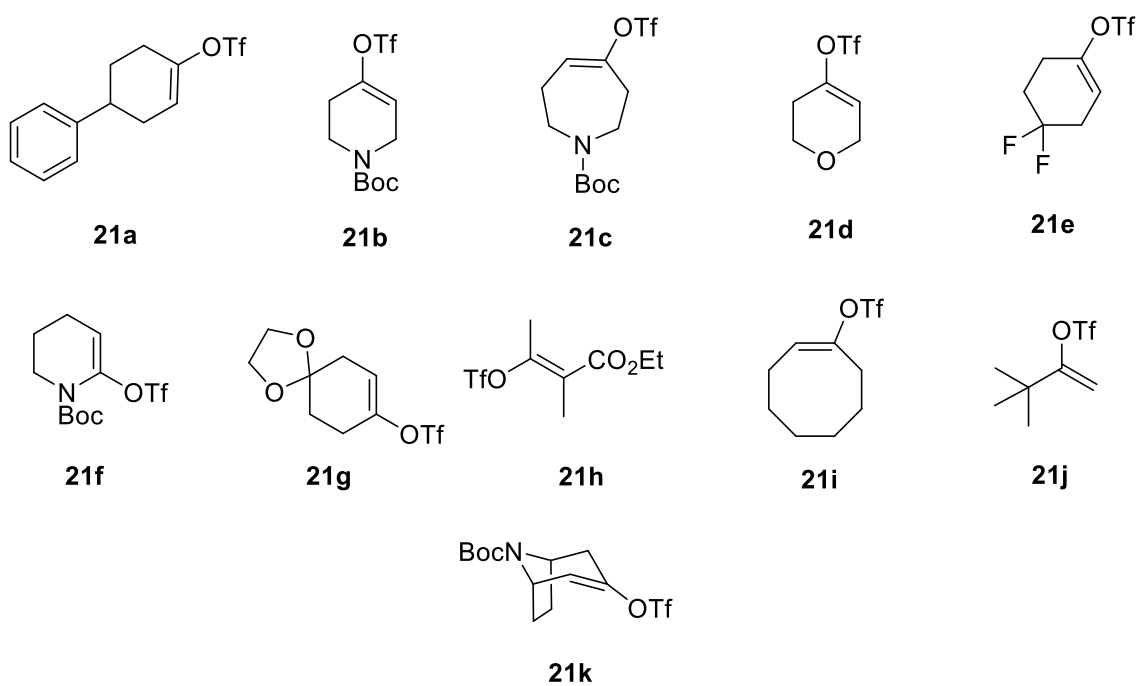
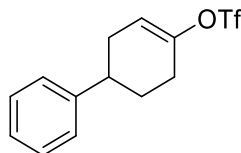


Figure 29. Vinyl triflate **21** used in this work.

Starting materials

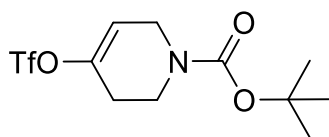
Synthesis of vinyl triflate

1,2,3,6-Tetrahydro-[1,1'-biphenyl]-4-yl trifluoromethanesulfonate (21a)



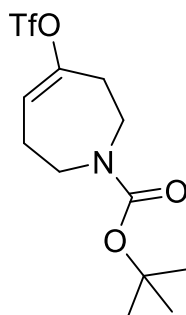
Compound **21a** was synthesized following a procedure reported in the literature [21]. ^1H NMR (300 MHz, CDCl_3): δ = 7.38-7.31 (m, 2H), 7.29-7.21 (m, 3H), 5.91-5.85 (m, 1H), 2.95-2.81 (m, 1H), 2.66-2.29 (m, 4H), 2.16-2.91 (m, 2H) ppm. ^{19}F NMR (282 MHz, CDCl_3): δ = -73.97 ppm. ^{13}C NMR (75 MHz, CDCl_3): δ = 149.1, 144.7, 128.8, 126.9, 126.8, 118.7 (q, J = 318.0 Hz), 118.2, 38.9, 31.7, 29.8, 28.0 ppm.

Tert-butyl 4-[[[(trifluoromethyl)sulfonyl]oxy]-3,6-dihydropyridine-1(2*H*)-carboxylate (21b)



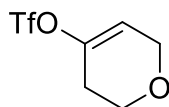
The triflate **21b** was synthesized following a procedure reported in the literature [22]. ^1H (300 MHz, CDCl_3): δ = 5.75 (s, 1H), 4.03 (q, J = 3.0 Hz, 2H), 3.62 (t, J = 5.5 Hz, 2H), 2.46-2.39 (m, 2H), 1.46 (s, 9H) ppm. ^{19}F (282 MHz, CDCl_3): δ = -73.86 ppm. ^{13}C (75 MHz, CDCl_3): δ = 154.4, 147.0, 118.6 (q, J = 318.0 Hz), 115.8, 80.7, 41.9, 31.0, 28.5, 28.2 ppm.

Tert-butyl 4-[[[(trifluoromethyl)sulfonyl]oxy]-2,3,6,7-tetrahydro-1*H*-azepine-1-carboxylate (21c)



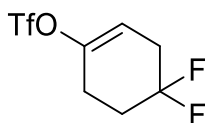
The triflate **21c** was synthesized following a procedure reported in the literature [23]. ^1H (300 MHz, CDCl_3): δ = 5.95-5.81 (m, 1H), 4.07-3.82 (m, 2H), 3.62-3.49 (m, 2H), 2.60-2.50 (m, 2H), 2.00-1.89 (m, 2H), 1.45 (s, 9H) ppm. ^{19}F (282 MHz, CDCl_3): δ = -73.75 ppm. ^{13}C (75 MHz, CDCl_3): δ = 155.2, 152.7, 151.8, 121.1, 118.6 (q, J = 319.0 Hz), 80.3, 47.5, 46.7, 41.7, 41.5, 31.2, 31.0, 28.4, 28.3, 24.7 ppm. Additional signals arise from the presence of inseparable structural isomers, whose occurrence was further confirmed by GC–MS analysis.

3,6-Dihydro-2H-pyran-4-yl trifluoromethanesulfonate (21d)



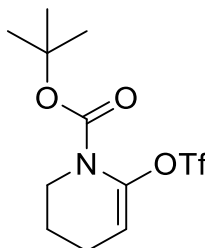
The triflate **21d** was synthesized following a procedure reported in the literature [24]. ^1H (300 MHz, CDCl_3): δ = 5.82-5.79 (m, 1H), 4.24 (q, J = 3.0 Hz, 2H), 3.87 (t, J = 5.5 Hz, 2H), 2.48-2.41 (m, 2H) ppm. ^{19}F (282 MHz, CDCl_3): δ = -73.96 ppm. ^{13}C (75 MHz, CDCl_3): δ = 145.9, 118.6 (q, J = 319.0 Hz), 117.1, 64.3, 64.0, 28.4 ppm.

4,4-Difluorocyclohex-1-en-1-yl trifluoromethanesulfonate (21e)



The triflate **21e** was synthesized following a procedure reported in the literature [25]. ^1H (300 MHz, CDCl_3): δ = 5.57-5.50 (m, 1H), 2.61-2.41 (m, 5H), 2.05 (tt, J_1 = 6.5 Hz, J_2 = 13.0 Hz, 1H) ppm. ^{19}F (282 MHz, CDCl_3): -74.41, -99.15 ppm. ^{13}C (75 MHz, CDCl_3): δ = 147.5, 120.9 (t, J = 239.5 Hz), 118.5 (q, J = 320.5 Hz), 114.1 (t, J = 5.0 Hz), 33.1 (t, J = 29.0 Hz), 30.1 (t, J = 25.5 Hz), 25.4 (t, J = 6.0 Hz) ppm.

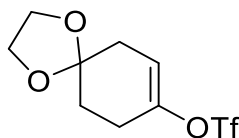
***Tert*-butyl 6-[[[(trifluoromethyl)sulfonyl]oxy]-3,4-dihydropyridine-1(2H)-carboxylate (21f)**



The triflate **21f** was synthesized following a procedure reported in the literature [26]. ^1H (300 MHz, CDCl_3): δ = 5.28-5.24 (m, 1H), 3.60-3.53 (m, 2H), 2.27-2.19 (m,

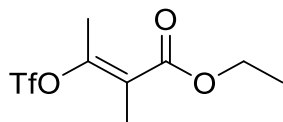
2H), 1.77-1.69 (m, 2H), 1.46 (s, 9H) ppm. ^{19}F (282 MHz, CDCl_3): $\delta = -74.32$ ppm. ^{13}C (75 MHz, CDCl_3): $\delta = 152.7, 140.3, 118.5$ (q, $J = 318.5$ Hz) 107.1, 82.8, 45.6, 28.0, 22.4, 22.2 ppm.

1,4-Dioxaspiro[4.5]dec-7-en-8-yl trifluoromethanesulfonate (21g)



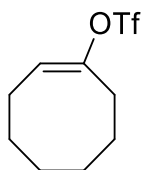
The triflate **21g** was synthesized following a procedure reported in the literature [27]. ^1H (300 MHz, CDCl_3): $\delta = 5.66$ -5.60 (m, 1H), 3.96 (s, 4H), 2.55-2.47 (m, 2H), 2.40-2.45 (m, 2H), 1.87 (t, $J = 6.5$ Hz, 2H) ppm. ^{19}F (282 MHz, CDCl_3): $\delta = -74.09$ ppm. ^{13}C (75 MHz, CDCl_3): $\delta = 148.30, 118.6$ (q, $J = 318.0$ Hz), 115.9, 106.2, 64.7, 34.2, 31.1, 26.4 ppm.

Ethyl (E)-2-methyl-3-[[trifluoromethyl)sulfonyl]oxy}but-2-enoate (21h)



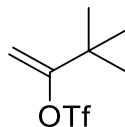
The triflate **21h** was synthesized following a procedure reported in the literature [28]. The product was isolated by silica gel flash chromatography (pentane/ Et_2O , 94:6 $\rightarrow$ 93:7). ^1H (300 MHz, CDCl_3): $\delta = 4.24$ (q, $J = 7.0$ Hz, 2H), 2.41 (q, $J = 2.0$ Hz, 3H), 1.98 (q, $J = 2.0$ Hz, 3H), 1.31 (t, $J = 7.0$ Hz, 3H) ppm. ^{19}F (282 MHz, CDCl_3): $\delta = -74.60$ ppm. ^{13}C (75 MHz, CDCl_3): $\delta = 166.4, 154.6, 122.8, 118.3$ (q, $J = 318.0$ Hz), 61.5, 19.0, 14.0, 13.8 ppm.

(E)-Cyclooct-1-en-1-yl trifluoromethanesulfonate (21i)



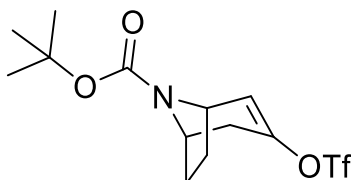
The triflate **21i** was synthesized following a procedure reported in the literature [29]. The product was isolated by silica gel flash chromatography (pentane). ^1H (300 MHz, CDCl_3): $\delta = 5.69$ (t, $J = 8.5$ Hz, 1H), 2.56-2.41 (m, 2H), 2.28-2.07 (m, 2H), 1.77-1.66 (m, 2H), 1.65-1.53 (m, 6H) ppm. ^{19}F (282 MHz, CDCl_3): $\delta = -74.31$ ppm. ^{13}C (75 MHz, CDCl_3): $\delta = 151.09, 120.71, 118.7$ (q, $J = 318.0$ Hz), 29.7, 29.2, 27.3, 25.9, 25.6, 25.1 ppm.

3,3-Dimethylbut-1-en-2-yl trifluoromethanesulfonate (**21j**)



The triflate **21j** was synthesized following a procedure reported in the literature [29]. The product was isolated by silica gel flash chromatography (pentane). ^1H (300 MHz, CDCl_3): δ = 5.05 (d, J = 4.0 Hz, 1H), 4.96 (d, J = 4.0 Hz, 1H), 1.18 (s, 9H) ppm. ^{19}F (282 MHz, CDCl_3): δ = -74.82 ppm. ^{13}C (75 MHz, CDCl_3): δ = 164.6, 118.6 (q, J = 317.5 Hz), 99.9, 36.6, 27.6 ppm.

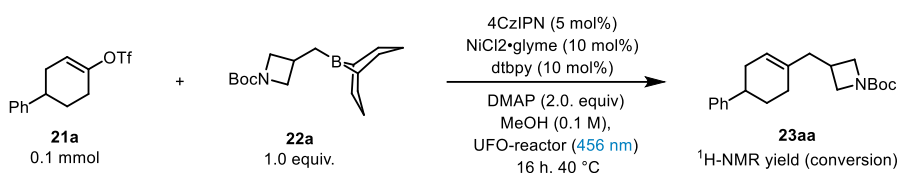
Tert-butyl (1*R*,5*S*)-3-[[trifluoromethyl)sulfonyl]oxy]-8-azabicyclo[3.2.1]oct-2-ene-8-carboxylate (**21k**)



The triflate **21k** was synthesized following a procedure reported in the [30]. ^1H (400 MHz, CDCl_3): δ = 6.07 (d, J = 5.5 Hz, 1H), 4.58-4.31 (m, 2H), 3.04 (s, 1H), 2.22 (s, 1H), 2.13-1.93 (m, 3H), 1.84-1.65 (m, 1H), 1.44 (s, 9H) ppm. ^{19}F (282 MHz, CDCl_3): δ = -73.65 ppm. ^{13}C (101 MHz, CDCl_3): δ = 153.8, 147.4 (d, J = 60.0 Hz), 123.7 (d, J = 60.0 Hz), 118.6 (q, J = 239.0 Hz), 80.4, 52.3 (d, J = 30.5 Hz), 51.8, 36.7 (d, J = 75.0 Hz), 34.6 (d, J = 67 Hz), 29.5 (d, J = 85.0 Hz), 29.1 (t, J = 5.0 Hz) ppm.

Preliminary screening

Table 24.



Entry	Variation from standard conditions	H ₂ O	NMR	yield
		equiv	(conversion)	[%]
1	None	0	60 (94)	
2	No photocatalyst	0	<i>n.d.</i> (0)	

3	No Ni nor dtbbpy	0	<i>n.d.</i> (0)
4	No DMAP	0	12 (17)
5	No light, T: 80 °C	0	<i>n.d.</i> (0)
6	dry MeOH	0	58 (95)
7	dry MeOH	5	71 (96)
8	dry MeOH, 2 equiv 9-BBN	5	57 (97)
9	dry MeOH, ligand load 15mol%	5	53 (97)
10	<i>i</i> -PrOH instead of MeOH	5	61 (97)
11	dry MeOH, 4,4'-di-Me-2,2'-bpy as L	5	65 (94)
12	dry MeOH, 2 equiv vinyl triflate	5	71 (<i>n.d.</i>)
13	dry MeOH, 4 equiv DMAP	5	51 (97)
14	dry MeOH, 2.5 mol % 4CzIPN	5	40 (86)
15	dry MeOH	10	49 (96)
16	dry MeOH, 7.5 mol % 4CzIPN	5	54 (95)
17	dry MeOH, 2 equiv 9-BBN, 4 equiv DMAP	5	71 (100)
18	dry MeOH, 4,4',4''-tri- <i>tert</i> -butyl-2,2':6',2''-terpyridine as L	5	59 (100)
19	dry MeOH, Xantphos as L	5	65 (95)
20	EtOH instead of MeOH	5	52 (93)
21	HFIP instead of MeOH	5	<i>n.d.</i> (10)
22	TAmylOH instead of MeOH	5	58 (98)
23	dry MeOH, 5 mol % Ni	5	54 (100)
24	dry MeOH, 0.25 M	5	62 (95)
25	dry MeOH, 0.0625 M	5	63 (93)
26	dry MeOH, 2,5-Bis(2,6-diisopropylphenyl)imidazolium chloride as L	5	69 (89)
27	dry MeOH, 2,2'-Bis[(4 <i>R</i>)-4-Benzyl-2-Oxazoline] as L	5	65 (94)

General procedures (GPs) for preparative experiments

GPI: batch conditions - via hydroboration of olefins with 9-BBN

Step 1: Hydroboration. 3 mL of 9-BBN solution in THF (0.5 M, 1.5 mmol, 1.0 equiv) were added to a flame-dried Schlenk flask equipped with a stirring bar at 0 °C (ice bath). The olefin (1.5 mmol, 1.0 equiv) was added neat dropwise. After addition, the ice bath was removed, and the reaction was stirred at room temperature for 1 hour. The reaction was monitored via ¹H NMR without any purification. (In the case of solid olefins, the substrate was added to a flame dried Schlenk flask under a nitrogen atmosphere and dissolved using the minimum amount of anhydrous THF. Hence, 9-BBN was added dropwise at 0 °C, after which the cold bath was removed. The solution was left stirring for 1 h at room temperature).

Step 2: Photoreaction solution. Vinyl triflate (0.5 mmol, 1.0 equiv), NiCl₂glyme (15 mol %, 16.5 mg), 4,4'-dimethoxy-2,2'-bipyridine (11 mol %, 11.9 mg), 4CzIPN (6.6 mol %, 26 mg), 4-DMAP (3.3 equiv, 202 mg) and H₂O (10 equiv, 90.4 μL) were prepared in a tube equipped with a septum cap and a stirring bar. EtOH (0.05 M, 10 mL) and alkyl borane from Step 1 (in THF) (2.5 equiv, 2.5 mL) were added subsequently under nitrogen atmosphere. The solution was bubbled with N₂ (3 min) and then sonicated (2 min). Then the solution was irradiated by adopting the setup shown in **Figure 27** equipped with a 40 W Kessil lamp (λ = 456 nm, full intensity) for 16 h. The solvent was removed under reduced pressure. The residue was filtered over a silica plug using EtOAc, and the solvent was removed again. The reaction mixture was analyzed by ¹H NMR to determine the conversion of the vinyl triflate and the products were eventually obtained after column chromatography on silica gel.

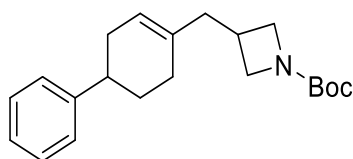
GP2: flow conditions blue light 456 nm - via hydroboration of olefins with 9-BBN (optimal conditions)

21a (122 mg, 0.4 mmol, 1.0 equiv), NiCl₂glyme (15.7 mol %, 13.15 mg), 4,4'-bis(trifluoromethyl)-2,2'-bipyridine (11 mol %, 12.84 mg), 4CzIPN (3 mol %, 9.5 mg), 4-DMAP (2.5 equiv, 122 mg) and water (5 equiv, 36 μL) were prepared in a 10 mL volumetric flask, then dissolved in MeOH (2.5 mL). Alkyl borane **22a** from Step 1 (in THF) (2.13 equiv, 1.7 mL) was added under N₂ atmosphere. The solution was bubbled with N₂ (3 min) and then sonicated (2 min). Next, the solution was taken up with plastic syringes and mounted on a syringe pump set at 0.118 mL/min before entering an UFlow reactor equipped with a 1.6 mm ID PTFE coil (7.1 mL internal volume, corresponding to

60 minutes residence time) (setup shown in **Figure 29**). When the syringe was fully empty, again MeOH was loaded into a syringe and injected to collect all product at the end of the reactor in a flask. The solvent was removed under reduced pressure. The residue was filtered over a silica plug using EtOAc, and the solvent was removed again. The reaction mixture was analyzed by ^1H NMR to determine the conversion of the vinyl triflate **21a**.

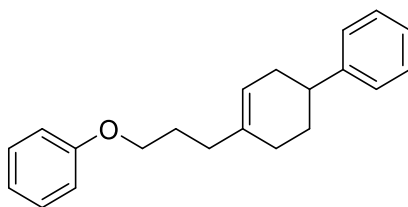
Characterization data

***Tert*-butyl 3-{[1,2,3,6-tetrahydro-(1,1'-biphenyl)-4-yl]methyl}azetidine-1-carboxylate (**23aa**)**



Prepared according to GP1. Purified by flash column chromatography on silica gel (pentane/EtOAc 98:2 $\rightarrow$ 97:3) to afford the product as a colorless sticky oil (144 mg, 88%). R_f = 0.35 (silica gel; pentane/EtOAc 85:15). ^1H NMR (300 MHz, CDCl_3): δ = 7.37-7.29 (m, 3H), 7.25-7.19 (m, 2H), 5.47 (d, J = 4.0 Hz, 1H), 4.05 (t, J = 8.5 Hz, 2 H), 3.61 (d, J = 5.5 Hz, 1H), 3.58 (d, J = 5.5 Hz, 1H), 2.84-2.66 (m, 2H), 2.30 (d, J = 7.5 Hz, 3 H), 2.23-2.06 (m, 2H), 2.05-1.93 (m, 2H), 1.87-1.77 (m, 1H), 1.48 (s, 9H) ppm. ^{13}C NMR (75 MHz, CDCl_3): δ = 156.6, 147.1, 135.2, 128.5, 127.0, 126.1, 121.8, 79.3, 54.6, 42.4, 40.1, 33.5, 30.0, 29.2, 28.6, 27.1 ppm. HRMS (EI) m/z calcd for $\text{C}_{21}\text{H}_{30}\text{NO}_2$ 328.2271; found: 328.2219.

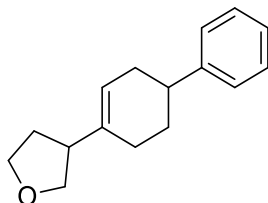
4-(3-Phenoxypropyl)-1,2,3,6-tetrahydro-1,1'-biphenyl (23ab**)**



Prepared according to GP1, step 1: 3 hours room temperature. Purified by flash column chromatography on silica gel (pentane) to afford the product as a colorless oil (105 mg, 80 %). R_f = 0.61 (silica gel; pentane/EtOAc 98:2). ^1H NMR (300 MHz, CDCl_3): δ = 7.36-7.27 (m, 4H), 7.24-7.17 (m, 3H), 6.99-6.88 (m, 3H) 5.61-5.53 (m, 1H), 3.99 (t,

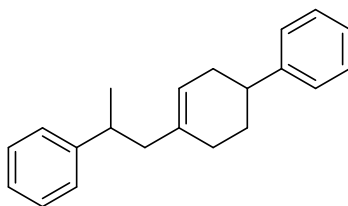
$J = 6.5$ Hz, 2H), 2.90-2.66 (m, 1H), 2.37-2.24 (m, 1H), 22.22-2.03 (m, 5H), 2.02-1.90 (m, 3H), 1.88-1.72 (m, 1H) ppm. ^{13}C NMR (75 MHz, CDCl_3): $\delta = 159.2, 147.3, 136.9, 129.6, 128.5, 127.0, 126.1, 121.2, 120.7, 114.7, 67.6, 40.3, 34.0, 33.6, 30.2, 29.1, 27.5$ ppm. HRMS (EI) m/z calcd for $\text{C}_{21}\text{H}_{25}\text{O}$: 293.1900; found: 293.1851.

3-[1,2,3,6-Tetrahydro-(1,1'-biphenyl)-4-yl]tetrahydrofuran (23ac)



Prepared according to GP1, step 1: 1 hour 50 °C. Purified by flash column chromatography on silica gel (pentane/EtOAc 98:2 $\rightarrow$ 97:3) to afford the product as a colorless oil (35 mg, 31 %). $R_f = 0.58$ (silica gel; pentane/EtOAc 95:5). ^1H NMR (300 MHz, CDCl_3): $\delta = 7.35$ -7.28 (m, 2H), 7.25-7.16 (m, 3H), 5.60 (d, $J = 1.5$, 1H), 3.97-3.88 (m, 2H), 3.83 (ddd, $J_1 = 1.5$ Hz, $J_2 = 7.0$ Hz, $J_3 = 8.0$ Hz, 1H), 3.58 (dt, $J_1 = 8.0$ Hz, $J_2 = 13.0$ Hz, 1H), 2.89-2.71 (m, 2H), 2.39-2.27 (m, 1H), 2.24-2.09 (m, 3H), 2.07-1.96 (m, 2H), 1.93-1.69 (m, 2H) ppm. ^{13}C NMR (75 MHz, CDCl_3): $\delta = 147.1, 137.2, 137.1, 128.5, 127.0, 126.2, 121.2, 121.1, 72.0, 71.7, 68.5$ (0), 68.4 (5), 46.3, 46.2, 40.3 (0), 40.2 (6), 33.7, 33.6, 31.2, 30.7, 30.1 (4), 30.1 (2), 27.5 (3), 27.4 (9) ppm. Additional signals in the ^{13}C NMR spectrum arise from the presence of two diastereoisomers with identical ^1H NMR spectra in a 1:1 ratio (confirmed by GC-MS analysis). HRMS (EI) m/z calcd for $\text{C}_{16}\text{H}_{21}\text{O}$: 229.1587; found: 229.1524.

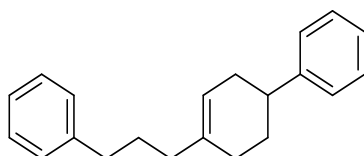
4-(2-Phenylpropyl)-1,2,3,6-tetrahydro-1,1'-biphenyl (23ad)



Prepared according to GP1. Purified by flash column chromatography on silica gel (pentane) to afford the product as a white solid (109 mg, 79 %). $R_f = 0.35$ (silica gel; pentane). ^1H NMR (300 MHz, CDCl_3): $\delta = 7.35$ -7.27 (m, 4H), 7.25-7.15 (m, 6H), 5.53-5.43 (m, 1H), 2.98-2.88 (m, 1H), 2.80-2.64 (m, 1H), 2.38-1.89 (m, 7H), 1.88-1.67 (m, 1H), 1.25 (dd, $J_1 = 1.5$ Hz, $J_2 = 7.0$ Hz, 3H) ppm. ^{13}C NMR (75 MHz, CDCl_3): $\delta = 147.9,$

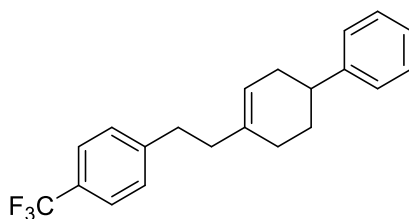
147.8, 147.4, 147.3, 136.1 (8), 136.1 (5), 128.5, 128.4, 128.3 (9), 128.3 (7), 127.1 (1), 127.1 (0), 127.0 (4), 126.0, 125.9 (6), 122.8 (4), 122.7 (8), 47.3, 46.8, 40.2, 40.1, 38.2, 38.0, 33.6, 33.5, 30.3, 30.2, 29.0, 28.9, 21.8, 21.5 ppm. Additional signals in the ^{13}C NMR spectrum arise from the presence of two diastereoisomers with identical ^1H NMR spectra in a 1:1 ratio (confirmed by GC–MS analysis). HRMS (EI) m/z calcd for $\text{C}_{21}\text{H}_{25}$: 277.1951; found: 277.1979.

4-(3-Phenylpropyl)-1,2,3,6-tetrahydro-1,1'-biphenyl (23ae)



Prepared according to GP1. Purified by flash column chromatography on silica gel (pentane) to afford the product as a colorless oil (111 mg, 80 %). R_f = 0.31 (silica gel; pentane). ^1H NMR (300 MHz, CDCl_3): δ = 7.37-7.27 (m, 6H), 7.25-7.19 (m, 4H), 5.61-5.51 (m, 1H), 2.79 (dddd, J_1 = 3.0 Hz, J_2 = 5.5 Hz, J_3 = 10.0 Hz, J_4 = 12.0 Hz, 1H), 2.70-2.62 (m, 2H), 2.41-2.27 (m, 1H), 2.26-2.14 (m, 2H), 2.13-2.05 (m, 3H), 2.04-1.94 (m, 1H), 1.88-1.73 (m, 3H) ppm. ^{13}C NMR (75 MHz, CDCl_3): δ = 147.4, 142.8, 137.6, 128.6, 128.5, 128.4, 127.0, 126.1, 125.8, 120.9, 40.4, 37.4, 35.7, 33.7, 30.2, 29.6, 29.0 ppm. HRMS (EI) m/z calcd for $\text{C}_{21}\text{H}_{25}$: 277.1951; found: 277.1943.

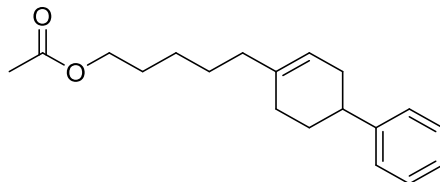
4-(4-(Trifluoromethyl)phenethyl)-1,2,3,6-tetrahydro-1,1'-biphenyl (23af)



Prepared according to GP1, step 1: 3 hours room temperature. Purified by flash column chromatography on silica gel (pentane) to afford the product as a white solid (116 mg, 70 %). R_f = 0.31 (silica gel; pentane). ^1H NMR (300 MHz, CDCl_3): δ = 7.58 (d, J = 8.0 Hz, 2H), 7.38-7.31 (m, 4H), 7.29-7.20 (m, 3H), 5.58-5.53 (m, 1H), 2.90-2.75 (m, 3H), 2.39-2.28 (m, 3H), 2.26-1.97 (m, 4H), 1.94-1.73 (m, 1H) ppm. ^{19}F NMR (282 MHz, CDCl_3): δ = -62.18 ppm. ^{13}C NMR (75 MHz, CDCl_3): δ = 147.2, 146.7 (q, J = 1.5 Hz), 136.7, 128.8, 128.5, 127.6, 127.2 (q, J = 144.0 Hz), 127.0, 126.1, 125.0 (q, J = 4.0 Hz),

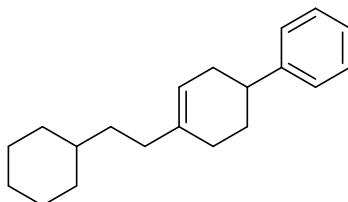
121.6, 40.2, 39.2, 34.3, 33.6, 30.2, 29.2 ppm. HRMS (EI) m/z calcd for $C_{21}H_{22}F_3$: 331.1668; found: 331.1633.

Methyl {6-[1,2,3,6-tetrahydro-(1,1'-biphenyl)-4-yl]hexyl} carbonate (23ag)



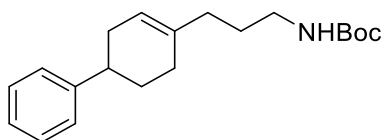
Prepared according to GP1. Purified by flash column chromatography on silica gel (pentane/EtOAc 100:0 $\rightarrow$ 98:2) to afford the product as a colorless oil (104 mg, 73 %). R_f = 0.61 (silica gel; pentane/EtOAc 98:2). ^1H NMR (300 MHz, $CDCl_3$): 7.34-7.27 (m, 2H), 7.25-7.16 (m, 3H), 5.49 (d, J = 4.0 Hz, 1H), 4.08 (t, J = 7.0 Hz, 2H), 2.81-2.69 (m, 1H), 2.36-2.23 (m, 1H), 2.22-2.09 (m, 2H), 2.06 (s, 3H), 2.05-1.91 (m, 4H), 1.85-1.72 (m, 1H), 1.71-1.60 (m, 2H), 1.52-1.32 (m, 4H) ppm. δ = ^{13}C NMR (75 MHz, $CDCl_3$): δ = 171.4, 147.4, 137.7, 128.5, 127.0, 126.1, 120.7, 64.7, 40.4, 37.6, 33.7, 30.2, 29.0, 28.7, 27.5, 25.8, 21.2 ppm. HRMS (EI) m/z calcd for $C_{19}H_{27}O_2$: 287.2006; found: 287.2009.

4-(2-Cyclohexylethyl)-1,2,3,6-tetrahydro-1,1'-biphenyl (23ah)



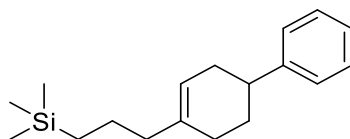
Prepared according to GP1. Purified by flash column chromatography on silica gel (pentane) to afford the product as a colorless oil (120 mg, 89 %). R_f = 0.38 (silica gel; pentane). ^1H NMR (300 MHz, $CDCl_3$): δ = 7.34-7.27 (m, 2H), 7.25-7.16 (m, 3H), 5.48 (d, J = 4.0 Hz, 1H), 2.75 (ddt, J_1 = 3.0 Hz, J_2 = 8.5 Hz, J_3 = 17.5 Hz, 1H), 2.36-2.09 (m, 3H), 2.08-1.91 (m, 4H), 1.85-1.60 (m, 6H), 1.37-1.10 (m, 6H), 0.99-0.82 (m, 2H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 147.5, 138.5, 128.5, 127.1, 126.0, 120.1, 40.4, 37.6, 35.8, 35.1, 33.7, 33.6, 30.3, 29.2, 26.9, 26.6 ppm. HRMS (EI) m/z calcd for $C_{20}H_{29}$: 269.2264; found: 269.2230.

***Tert*-butyl {3-[1,2,3,6-tetrahydro-(1,1'-biphenyl)-4-yl]propyl}carbamate (23ai)**



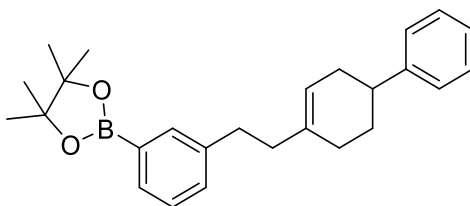
Prepared according to GP1. Purified by flash column chromatography on silica gel (pentane/EtOAc 99:1 $\rightarrow$ 97:3) to afford the product as a colorless oil (111 mg, 70 %). R_f = 0.8 (silica gel; pentane/EtOAc 8:2). ^1H NMR (300 MHz, CDCl_3): δ = 7.36-7.29 (m, 2H), 7.27-7.19 (m, 3H), 5.54 (d, J = 4.0 Hz, 1H), 4.62 (br s, 1H), 3.15 (d, J = 6.5 Hz, 2H), 2.77 (tdd, J_1 = 3.0 Hz, J_2 = 5.5 Hz, J_3 = 12.0 Hz, 1H), 2.38-2.12 (m, 3H), 2.11-1.93 (m, 4H), 1.87-1.75 (m, 1H), 1.70-1.58 (m, 2H), 1.49 (s, 9H) ppm. ^{13}C NMR (75 MHz, CDCl_3): δ = 156.1, 147.3, 137.0, 128.4, 127.0, 126.0, 121.2, 79.1, 40.5, 40.2, 35.0, 33.6, 30.1, 28.9, 28.6, 28.1 ppm. HRMS (EI) m/z calcd for $\text{C}_{20}\text{H}_{30}\text{NO}_2$: 316.2271; found: 316.2217.

Trimethyl{3-[1,2,3,6-tetrahydro-(1,1'-biphenyl)-4-yl]propyl}silane (23aj)



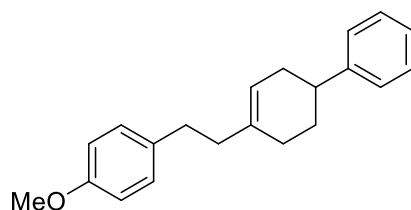
Prepared according to GP1, step 1: 3 hours room temperature. Purified by flash column chromatography on silica gel (pentane) to afford the product as a colorless solid (41 mg, 30 %). R_f = 0.8 (silica gel; pentane). ^1H NMR (300 MHz, CDCl_3): δ = 7.27-7.35 (m, 3H), 7.25-7.17 (m, 2H), 5.53-5.47 (m, 1H), 2.76 (ddt, J_1 = 2.5 Hz, J_2 = 5.0 Hz, J_3 = 6.5 Hz, 1H), 2.44-2.24 (m, 1H), 2.26-2.08 (m, 2H), 2.15-1.91 (m, 3H), 1.95 (dq, J_1 = 1.5 Hz, J_2 = 4.5 Hz, 1H), 1.89-1.69 (m, 1H), 1.54-1.32 (m, 2H), 0.79-0.38 (m, 2H), 0.01 (s, 9H) ppm. ^{13}C NMR (75 MHz, CDCl_3): δ = 147.5, 138.0, 128.5, 127.1, 126.0, 120.7, 41.7, 40.4, 33.7, 30.3, 29.0, 22.3, 16.6, -1.5 ppm. HRMS (EI) m/z calcd for $\text{C}_{18}\text{H}_{29}\text{Si}$: 273.2033; found: 273.1975.

3,3,4,4-Tetramethyl-1-{3-{2-[1,2,3,6-tetrahydro-(1,1'-biphenyl)-4-yl]ethyl}phenyl}-1-boro-2,5-dioxolane (23ak)



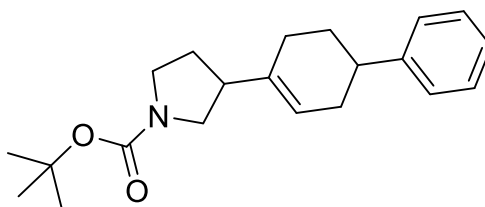
Prepared according to GP1, step 1: 2 hours room temperature. Purified by flash column chromatography on silica gel (pentane → pentane:EtOAc 99:1) to afford the product as a white solid (79 mg, 41 %). R_f = 0.47 (silica gel; pentane). ^1H NMR (300 MHz, CDCl_3): δ = 7.73-7.76 (m, 2H), 7.38-7.31 (m, 5H), 7.30-7.24 (m, 2H), 5.58-5.52 (m, 1H), 2.86-2.69 (m, 3H), 2.40-2.26 (m, 4H), 2.25-2.13 (m, 2H), 2.07-2.91 (m, 1H), 1.82 (tdd, J_1 = 5.5 Hz, J_2 = 11.0 Hz, J_3 = 12.5 Hz, 1H), 1.40 (s, 12H) ppm. ^{11}B NMR (96 MHz, CDCl_3): δ = 33.21 ppm. ^{13}C NMR (75 MHz, CDCl_3): δ = 147.4, 141.9, 137.4, 134.9, 132.4, 132.3, 131.5, 128.4, 127.9, 127.0, 126.0, 121.1, 83.8, 40.3, 39.9, 34.6, 33.6, 30.2, 29.2, 25.0 ppm. HRMS (EI) m/z calcd for $\text{C}_{26}\text{H}_{34}\text{BO}_2$: 389.2647; found: 389.2602.

4-(4-Methoxyphenethyl)-1,2,3,6-tetrahydro-1,1'-biphenyl (23al)



Prepared according to GP1. Purified by flash column chromatography on silica gel (pentane → pentane/toluene 9:1) to afford the product as a white solid (79 mg, 54 %). R_f = 0.34 (silica gel; heptane:EtOAc 8:2). ^1H NMR (300 MHz, CDCl_3): δ = 7.36-7.29 (m, 2H), 7.27-7.18 (m, 3H), 7.14 (d, J = 8.5 Hz, 2H), 6.86 (d, J = 8.5, 2H), 5.71-5.39 (m, 1H), 3.81 (s, 3H), 2.83-2.67 (m, 3H), 2.34-2.24 (m, 3H), 2.23-1.94 (m, 4H), 1.92-1.67 (m, 1H) ppm. ^{13}C NMR (75 MHz, CDCl_3): δ = 157.8, 147.4, 137.4, 134.7, 129.4, 128.5, 127.0, 126.1, 121.1, 113.8, 55.4, 40.3, 39.9, 33.7, 33.6, 30.2, 29.3 ppm. HRMS (EI) m/z calcd for $\text{C}_{21}\text{H}_{25}\text{O}$: 293.1900; found: 293.1848.

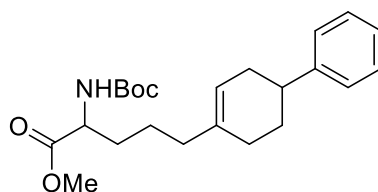
***Tert*-butyl 3-[1,2,3,6-tetrahydro-(1,1'-biphenyl)-4-yl]pyrrolidine-1-carboxylate (23am)**



Prepared according to GP1, step 1: 2 hours at room temperature and 1 hour at 50 °C. Purified by flash column chromatography on silica gel (heptane → heptane:EtOAc 95:5) to afford the product as a yellowish oil (68 mg, 42%). R_f = 0.47 (silica gel;

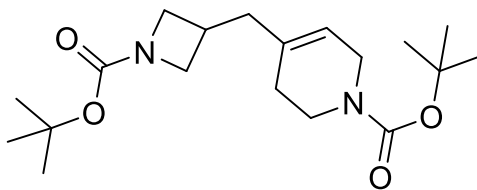
pentane/EtOAc 9:1). ^1H NMR (400 MHz, CDCl_3): δ = 7.33-7.27 (m, 2H), 7.24-7.17 (m, 3H), 5.57 (d, J = 5.0 Hz, 1H), 3.65-3.42 (m, 2H), 3.36-3.24 (m, 1H), 3.19-3.04 (m, 1H), 2.81-2.63 (m, 2H), 2.17 (d, J = 17.0 Hz, 1H), 2.21-2.07 (m, 3H), 2.03-1.92 (m, 2H), 1.85-1.71 (m, 2H), 1.47 (s, 9H) ppm. ^{13}C NMR (101 MHz, CDCl_3): δ = 154.9, 147.1, 136.8, 128.5, 127.0, 126.2, 121.0, 79.2, 50.2, 49.9, 49.8, 49.4, 46.0, 45.6 (0), 45.5 (7), 44.7, 40.2 (4), 40.1 (8), 33.6, 33.5, 30.1, 30.0, 28.7 (4), 28.6 (9), 28.7, 27.7 ppm. Additional signals in the ^{13}C NMR spectrum arise from the presence of two diastereoisomers with identical ^1H NMR spectra in a 1:1 ratio (confirmed by GC–MS analysis). HRMS (EI) m/z calcd for $\text{C}_{21}\text{H}_{29}\text{NO}_2$: 328.2271; found: 328.2218.

Methyl 2-[(*tert*-butoxycarbonyl)amino]-5-[1,2,3,6-tetrahydro-(1,1'-biphenyl)-4-yl]pentanoate (23an)



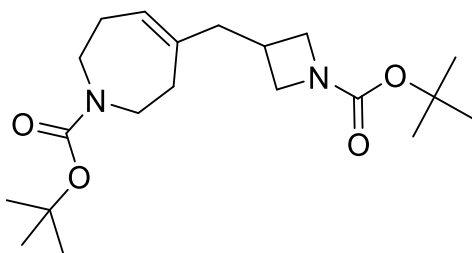
Prepared according to GP1. Purified by flash column chromatography on silica gel (pentane/EtOAc 96:4 $\rightarrow$ 9:1), second column with reverse phase C18, ($\text{H}_2\text{O}/\text{CH}_3\text{CN}$ 3:7) to afford the product as a colorless oil (85 mg, 44 %). R_f = 0.5 (silica gel; pentane/EtOAc 9:1). ^1H NMR (300 MHz, CDCl_3): δ = 7.34-7.27 (m, 2H), 7.24-7.16 (m, 3H), 5.49 (d, J = 4.5 Hz, 1H), 5.01 (d, J = 7.5 Hz, 1H), 4.32 (d, J = 7.0 Hz, 1H), 3.75 (s, 3H), 2.85-2.67 (m, 1H), 2.35-2.22 (m, 1H), 2.20-2.06 (m, 2H), 2.05-1.90 (m, 5H), 1.84-1.71 (m, 2H), 1.69-1.57 (m, 2H), 1.45 (s, 9H) ppm. ^{13}C NMR (75 MHz, CDCl_3): δ = 173.6, 155.5, 147.3, 137.0, 128.5, 127.0, 126.1, 121.3, 80.0, 53.5, 52.4, 40.3, 40.2, 37.2, 37.1, 33.7, 33.6, 32.5 (1), 32.4 (7), 31.1, 30.2, 28.9, 28.8, 28.5, 23.4 (2), 23.3 (7) ppm. Additional signals in the ^{13}C NMR spectrum arise from the presence of two diastereoisomers with identical ^1H NMR spectra in a 1:1 ratio (confirmed by GC–MS analysis). HRMS (EI) m/z calcd for $\text{C}_{23}\text{H}_{34}\text{NO}_4$: 388.2483; found: 388.2380.

***Tert*-butyl 4-{[1-(*tert*-butoxycarbonyl)azetidin-3-yl]methyl}-3,6-dihydropyridine-1(2*H*)-carboxylate (23ba)**



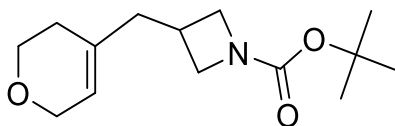
Prepared according to GP1. Purified by flash column chromatography on silica gel (pentane/EtOAc 90:10 $\rightarrow$ 87:13) to afford the product as a colorless oil (51 mg, 29 %). R_f = 0.45 (silica gel; pentane/EtOAc 75:25). ^1H NMR (400 MHz, CDCl_3): δ = 5.25 (d, J = 5.5 Hz, 1H), 3.96 (t, J = 8.5 Hz, 2H), 3.80 (d, J = 4.0 Hz, 2H), 3.49 (dd, J_1 = 5.5 Hz, J_2 = 8.5 Hz, 2H), 3.42 (t, J = 5.5 Hz, 2H), 2.67-2.56 (m, 1H), 2.23 (d, J = 8.0 Hz, 2H), 1.95 (s, 2H), 1.42 (s, 9H), 1.39 (s, 9H) ppm. ^{13}C NMR (101 MHz, CDCl_3): δ = 156.4, 154.9, 134.1, 119.0, 79.6, 79.3, 54.6, 54.0, 43.3, 41.9, 39.6, 28.5, 28.5, 26.8 ppm. HRMS (EI) m/z calcd for $\text{C}_{19}\text{H}_{33}\text{N}_2\text{O}_4$: 353.2435; found: 353.2511.

***Tert*-butyl 4-([1-(*tert*-butoxycarbonyl)azetidin-3-yl]methyl)-2,3,6,7-tetrahydro-1H-azepine-1-carboxylate (23ca)**



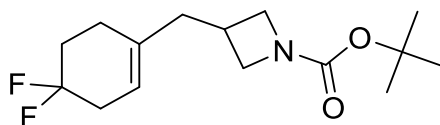
Prepared according to GP1. Purified by flash column chromatography on silica gel (heptane/EtOAc 100:0 $\rightarrow$ 88:12) to afford the product as a yellowish oil (104 mg, 57 %). R_f = 0.38 (silica gel; heptane/EtOAc 7:3). ^1H NMR (400 MHz, CDCl_3): δ = 5.48 (d, J = 5.5 Hz, 1H), 3.89 (t, J = 8.5 Hz, 2H), 3.81-3.69 (m, 2H), (3.44, dd, J_1 = 5.5 Hz, J_2 = 8.5 Hz, 4 H), 2.70-2.42 (m, 1H), 2.19 (d, J = 8.0 Hz, 2H), 2.12-1.99 (m, 2H), 1.69-1.56 (m, 2H), 1.38 (s, 9H), 1.36 (s, 9H) ppm. ^{13}C NMR (101 MHz, CDCl_3): δ = 156.4, 155.4 (2), 155.3 (5), 141.9, 140.9, 124.1, 123.8, 79.2, 54.2, 48.4, 48.3, 44.8, 44.2, 31.1, 30.8, 28.5, 28.4, 26.8 ppm. Additional signals arise from the presence of inseparable structural isomers, whose occurrence was further confirmed by GC-MS analysis. HRMS (EI) m/z calcd for $\text{C}_{20}\text{H}_{35}\text{N}_2\text{O}_4$: 367.2592; found: 367.2566.

***Tert*-butyl 3-[(3,6-dihydro-2H-pyran-4-yl)methyl]azetidine-1-carboxylate (23da)**



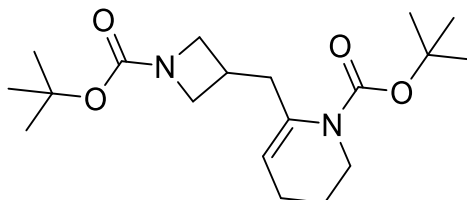
Prepared according to GP1. Purified by flash column chromatography on silica gel (heptane/EtOAc 98:2 $\rightarrow$ 88:12) to afford the product as a yellow oil (95 mg, 75 %). R_f = 0.31 (silica gel; pentane/EtOAc Pentane: Ethyl Acetate 75:25). ^1H NMR (400 MHz, CDCl_3): δ = 5.33-5.28 (m, 1H), 4.05 (d, J = 2.5 Hz, 2H), 3.96 (t, J = 8.5 Hz, 2H), 3.71 (t, J = 5.5 Hz, 2H), 3.50 (dd, J_1 = 5.5 Hz, J_2 = 8.5 Hz, 2H), 2.69-2.58 (m, 1H), 2.27-2.18 (m, 2H), 2.00-1.88 (m, 2H), 1.39 (s, 9H) ppm. ^{13}C NMR (101 MHz, CDCl_3): δ = 156.3, 133.1, 120.7, 79.2, 65.3, 64.1, 54.3, 41.7, 28.6, 28.4, 26.6 ppm. HRMS (EI) m/z calcd for $\text{C}_{14}\text{H}_{24}\text{NO}_3$: 254.1751; found: 254.1702.

***Tert*-butyl 3-[(4,4-difluorocyclohex-1-en-1-yl)methyl]azetidine-1-carboxylate (23ea)**



Prepared according to GP1. Purified by flash column chromatography on silica gel (Pentane/diethyl ether 95:5 $\rightarrow$ 87:13) to afford the product as a yellowish oil (98 mg, 68 %). R_f = 0.32 (silica gel; pentane/EtOAc 9:1). ^1H NMR (300 MHz, CDCl_3): δ = 5.15 (s, 1H), 3.95 (t, J = 8.5 Hz, 2H), 3.48 (dd, J_1 = 5.5 Hz, J_2 = 8.5 Hz, 2H), 2.78-2.51 (m, 1H), 2.43 (t, J = 14.0 Hz, 2H), 2.22 (d, J = 7.5 Hz, 2H), 2.17-2.06 (m, 2H), 1.95 (tt, J_1 = 6.5 Hz, J_2 = 14.0 Hz, 2H), 1.38 (s, 9H) ppm. ^{19}F (282 MHz, CDCl_3) δ = -96.87 ppm. ^{13}C NMR (75 MHz, CDCl_3): δ = 156.3, 134.7, 122.7 (t, J = 238.0), 116.8, 79.2, 54.3, 41.3, 34.5 (d, J = 26.5 Hz), 30.3, 30.1 (d, J = 24.5 Hz), 28.4, 26.8 ppm. HRMS (EI) m/z calcd for $\text{C}_{15}\text{H}_{24}\text{F}_2\text{NO}_2$: 288.1770; found: 288.1689.

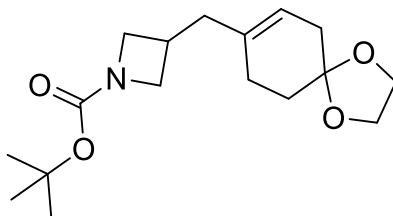
***Tert*-butyl 6-[[1-(*tert*-butoxycarbonyl)azetidin-3-yl]methyl]-3,4-dihydropyridine-1(2*H*)-carboxylate (23fa)**



Prepared according to GP1. Purified by flash column chromatography on silica gel (pentane/EtOAc + 1% Et_3N 100:0 $\rightarrow$ 94:6) to afford the product as a colorless oil (130

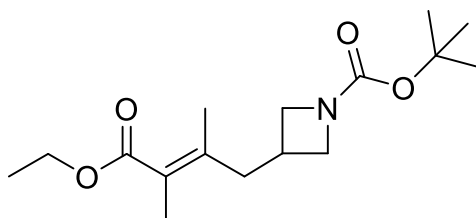
mg, 74 %). R_f = 0.27 (silica gel; pentane/EtOAc 9:1). ^1H NMR (400 MHz, CDCl_3): δ = 4.88 (t, J = 3.5 Hz, 1H), 3.87 (t, J = 8.5 Hz, 2H), 3.45 (dd, J_1 = 5.5 Hz, J_2 = 8.5 Hz, 2H), 3.40-3.31 (m, 2H), 2.67 (d, J = 8.0 Hz, 2H), 2.60-2.48 (m, 1H), 1.98 (dddd, J_1 = 2.0 Hz, J_2 = 4.0 Hz, J_3 = 5.5 Hz, J_4 = 6.5 Hz, 2H), 1.66 (dt, J_1 = 6.5 Hz, J_2 = 11.0 Hz, 2H), 1.40 (s, 9H), 1.35 (s, 9H) ppm. ^{13}C NMR (101 MHz, CDCl_3): δ = 156.37, 153.49, 137.53, 113.28, 80.47, 79.04, 54.3, 44.91, 39.87, 28.38, 28.34, 26.90, 23.28, 22.93 ppm. HRMS (EI) m/z calcd for $\text{C}_{19}\text{H}_{33}\text{N}_2\text{O}_4$: 353.2435; found: 353.2417.

***Tert*-butyl 3-[(1,4-dioxaspiro[4.5]dec-7-en-8-yl)methyl]azetidine-1-carboxylate (23ga)**



Prepared according to GP1. Purified by flash column chromatography on silica gel (pentane/EtOAc 82:18 $\rightarrow$ 80:20) to afford the product as a yellow oil (140 mg, 90 %). R_f = 0.43 (silica gel; pentane/EtOAc 75:25). ^1H NMR (400 MHz, CDCl_3): δ = 5.14 (dt, J_1 = 2.0 Hz, J_2 = 3.5 Hz, 1H), 3.91 (t, J = 8.5 Hz, 2H), 3.88 (s, 4H), 3.45 (dd, J_1 = 5.5 Hz, J_2 = 8.5 Hz, 2H), 2.58 (tt, J_1 = 5.5 Hz, J_2 = 8.0 Hz, 1H), 2.23-2.12 (m, 4H), 2.09-1.95 (m, 2H), 1.66 (t, J = 6.5 Hz, 2H), 1.35 (s, 9H) ppm. ^{13}C NMR (101 MHz, CDCl_3): δ = 156.3, 134.8, 119.0, 107.8, 79.0, 64.3, 54.6, 41.6, 35.5, 31.0, 28.4, 27.8, 26.9 ppm. HRMS (EI) m/z calcd for $\text{C}_{17}\text{H}_{28}\text{NO}_4$: 310.2013; found: 310.1865.

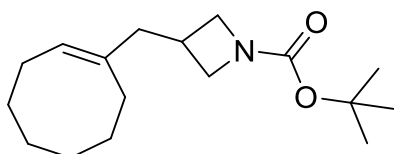
***Tert*-butyl (*E*)-3-(4-ethoxy-2,3-dimethyl-4-oxobut-2-en-1-yl)azetidine-1-carboxylate (23ha)**



Prepared according to GP1. Purified by flash column chromatography on silica gel [pentane/(Et) $_2$ O 90:10 $\rightarrow$ 80:20], and then by Preparative TLC [pentane/(Et) $_2$ O 8:2] to afford the product as a yellow oil (39 mg, 26 %). R_f = 0.44 (silica gel; [pentane/(Et) $_2$ O

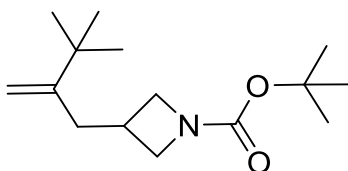
75:25]. ^1H NMR (400 MHz, CDCl_3): δ = 4.16 (qd, J_1 = 4.0 Hz, J_2 = 7.0 Hz, 2H), 3.98 (t, J = 8.5 Hz, 1H), 3.93 (t, J = 8.0 Hz, 1H), 3.61 (dd, J_1 = 5.5 Hz, J_2 = 8.5 Hz, 1H), 3.56 (dd, J_1 = 5.5 Hz, J_2 = 8.5 Hz, 1H), 2.75-2.62 (m, 2H), 2.43 (d, J = 8.0 Hz, 1H), 1.88 (s, 3H), 1.82 (s, 1H), 1.71 (s, 1H), 1.40 (s, 9H), td (J_1 = 3.0 Hz, J_2 = 7.0 Hz, 3H) ppm. ^{13}C NMR (101 MHz, CDCl_3): 169.9, 156.5, 142.2, 142.0, 125.0, 79.4, 60.4, 54.5, 40.0, 28.5, 20.6, 16.0, 14.4 ppm. HRMS (EI) m/z calcd for $\text{C}_{16}\text{H}_{28}\text{NO}_4$: 298.2013; found: 298.1979. The presence of (*Z*)-isomer was confirmed by GC-MS.

***Tert*-butyl (*E*)-3-(cyclooct-1-en-1-ylmethyl)azetidine-1-carboxylate (23ia)**



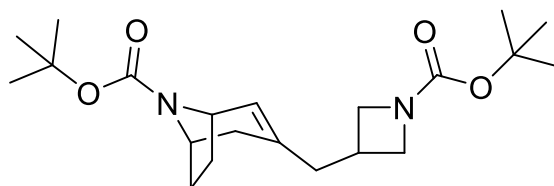
Prepared according to GP1. Purified by flash column chromatography on silica gel [pentane/(Et) $_2\text{O}$ 95:5 $\rightarrow$ 92:8] to afford the product as a colorless oil (57 mg, 41%). R_f = 0.44 [silica gel; pentane/(Et) $_2\text{O}$ 9:1]. ^1H NMR (400 MHz, CDCl_3): δ = 5.22 (t, J = 8.0 Hz, 1H), 3.96 (t, J = 8.5 Hz, 2H), 3.49 (dd, J_1 = 5.5 Hz, J_2 = 8.5 Hz, 2H), 2.70-2.59 (m, 1H), 2.23 (d, J = 8.0 Hz, 2H), 2.10-2.01 (m, 4H), 1.48-1.37 (m, 17H) ppm. ^{13}C NMR (101 MHz, CDCl_3): δ = 156.5, 138.1, 124.9, 79.2, 54.5, 42.4, 29.9, 29.3, 29.0, 28.5, 27.1, 26.6, 26.3, 26.3 ppm. HRMS (EI) m/z calcd for $\text{C}_{17}\text{H}_{30}\text{NO}_2$: 280.2271; found: 280.2227.

***Tert*-butyl 3-(3,3-dimethyl-2-methylenebutyl)azetidine-1-carboxylate (23ja)**



Prepared according to GP1. Purified by flash column chromatography on silica gel (pentane/ EtOAc 95:5 $\rightarrow$ 93:7), and then by Preparative TLC (pentane/ EtOAc 9:1) to afford the product as a colorless oil (36 mg, 28%). R_f = 0.68 (silica gel; pentane/ EtOAc 9:1). ^1H NMR (500 MHz, CDCl_3): δ = 4.83 (s, 1H), 4.40 (s, 1H), 4.04 (t, J = 8.5 Hz, 2H), 3.51 (dd, J_1 = 5.5 Hz, J_2 = 8.5 Hz, 2H), 2.78-2.68 (m, 1H), 2.32 (d, J = 8.0 Hz, 2H), 1.43 (s, 9H), 1.05 (s, 9H) ppm. ^{13}C NMR (125 MHz, CDCl_3): δ = 156.5, 155.4, 106.1, 79.3, 55.3, 54.2, 36.0, 29.4, 28.6, 27.3 ppm. HRMS (EI) m/z calcd for $\text{C}_{15}\text{H}_{28}\text{NO}_2$: 254.2116; found: 254.2078.

Tert-butyl (1*R*,5*S*)-3-{{1-(*tert*-butoxycarbonyl)azetidin-3-yl}methyl}-8-azabicyclo[3.2.1]oct-2-ene-8-carboxylate (23ka)



Prepared according to GP1. Purified by flash column chromatography on silica gel (heptane/EtOAc 94:6 → 88:12) to afford the product as a colorless oil (117 mg, 62 %). R_f = 0.27 (silica gel; heptane/EtOAc 75:25). ^1H NMR (500 MHz, CDCl_3): δ = 5.60 (s, 1H), 4.27–4.11 (m, 2H), 3.90 (t, J = 8.5 Hz, 1H), 3.86 (t, J = 8.5 Hz, 1H), 3.42 (ddd, J_1 = 2.5 Hz, J_2 = 5.5 Hz, J_3 = 8.5 Hz, 2H), 2.69–2.46 (m, 2H), 2.12–2.05 (m, 3H), 1.83 (tt, J_1 = 6.5 Hz, J_2 = 11.61 Hz, 1H), 1.78–1.70 (m, 1H), 1.59–1.45 (m, 2H), 1.36 (s, 9H), 1.35 (s, 9H) ppm. ^{13}C NMR (125 MHz, CDCl_3): δ = 156.3, 154.1, 132.9, 132.4, 127.4, 79.1, 54.6, 53.4, 52.6, 52.2, 51.6, 40.9, 37.8, 37.0, 34.8, 30.1, 29.4, 28.4, 26.7 ppm. HRMS (EI) m/z calcd for $\text{C}_{21}\text{H}_{35}\text{N}_2\text{O}_4$: 379.2592; found: 379.2608.

6.5 References

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