UNIVERSITÀ DEGLI STUDI DI MILANO

DIPARTIMENTO DI
SCIENZE FARMACEUTICHE

SCUOLA DI DOTTORATO IN SCIENZE E TECNOLOGIE CHIMICHE

DOTTORATO DI RICERCA IN CHIMICA DEL FARMACO
CICLO XXVIII

Investigation of the role of alpha7 nicotinic Acetylcholine receptors in inflammatory diseases through the design, synthesis and biological evaluation of new heterocyclic derivatives

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ANNO ACCADEMICO2014/2015

Table of contents

CHAPTER I

Introduction

1.1 The nicotinic acetylcholine receptors

The nicotinic acetylcholine receptors (nAChRs) are transmembrane excitatory receptors physiologically activated by the neurotransmitter acetylcholine (ACh). [1, 2] They are widely distributed in the central and the peripheral nervous system, [2] in the immune system and in several peripheral tissues, where they play critical roles [3] in the maintenance of cognitive, autonomic and immune homeostasis. [4] Neuronal nAChRs present on the postsynaptic neuronal terminals or acting as presynaptic receptors indirectly modulate the release of a variety of key neurotransmitters including serotonin, dopamine, γ-aminobutyric acid (GABA), glutamate, histamine, and norepinephrine. [5] Thus, nAChRs represent a class of validated therapeutic targets for a variety of pathologies and disorders, including Alzheimer's and Parkinson's diseases, addiction disorders, schizophrenia, pain management and inflammation-mediated processes, in which significant unmet medical needs still remain. [6] Moreover, nicotinic receptors are involved in the modulation of nociceptive neurotransmission, thus representing a promising target for non-opioid analgesics. [4]

The nAChRs are members of the large superfamily of pentameric transmembrane ligand-gated ion channels, [7] and are formed by combinations of five subunits, symmetrically arranged around a central aqueous pore that is permeable to cations. The high degree of complexity is conferred by 16 different alpha (alpha1-alpha10) and non-alpha (beta1-beta4, gamma, delta and epsilon) subunits which may assemble to form homomeric or heteromeric receptors (**Figure 1**). The former consist of only one type of subunit, i.e. alpha7 nAChRs, while the latter are formed by a combinations of various alpha and beta subunits. [8] Depending on the different association of the subunits, the resulting nAChR possesses distinct structural and functional properties, including different agonist affinity and different kinetics of activation, closure, desensitization, resensitization and internalization. [9] Structurally, each subunit of the pentameric neuronal nAChR is composed of a number of distinct functional domains (**Figure 1**): [10] an extended *N*-terminal extracellular domain, which represents the binding domain for endogenous neurotransmitter (ACh) or exogenous ligands, a transmembrane domain, constituted by four highly hydrophobic α-helices (M1 - M4), a long cytoplasmic loop between the M3 and M4 domains and a short extracellular *C*-terminal region.

Each subunit is encoded by a single gene, thus generating a cluster of 16 different genes widely distributed in the 23 human chromosomes. [11, 12] These encoding genes have progressively diverged from a common ancestor by mechanisms of gene duplications and mutations, leading to the gene structures currently present in humans. [13] In this respect, the ionotropic channels present in bacteria are characterized by a high degree of structural homology with the human nAChRs and therefore are useful prototypes in the elucidation of the tridimensional structure of the nAChRs. [14, 15]

The orthosteric binding sites of the receptor are situated at the alpha/beta or alpha/alpha subunits interface respectively in the heteromeric or homopentameric subtypes. The homopentameric subtypes can host and bind up to five different molecules of ACh, while in the heteromeric receptors, the ratio of alpha and beta subunits determines

the number of ligands that can be bound. Consequently the binding of the endogenous neurotransmitter ACh or exogenous orthosteric ligands, the nAChR undergoes a conformational change in its structure, resulting in rapid channel opening and a net influx of cations (usually Na^+, Ca^{2+} and K^+) through the membrane. The cation influx causes channel depolarization of the cell membrane and increase of neuronal excitability, thereby an excitatory postsynaptic potential is generated.
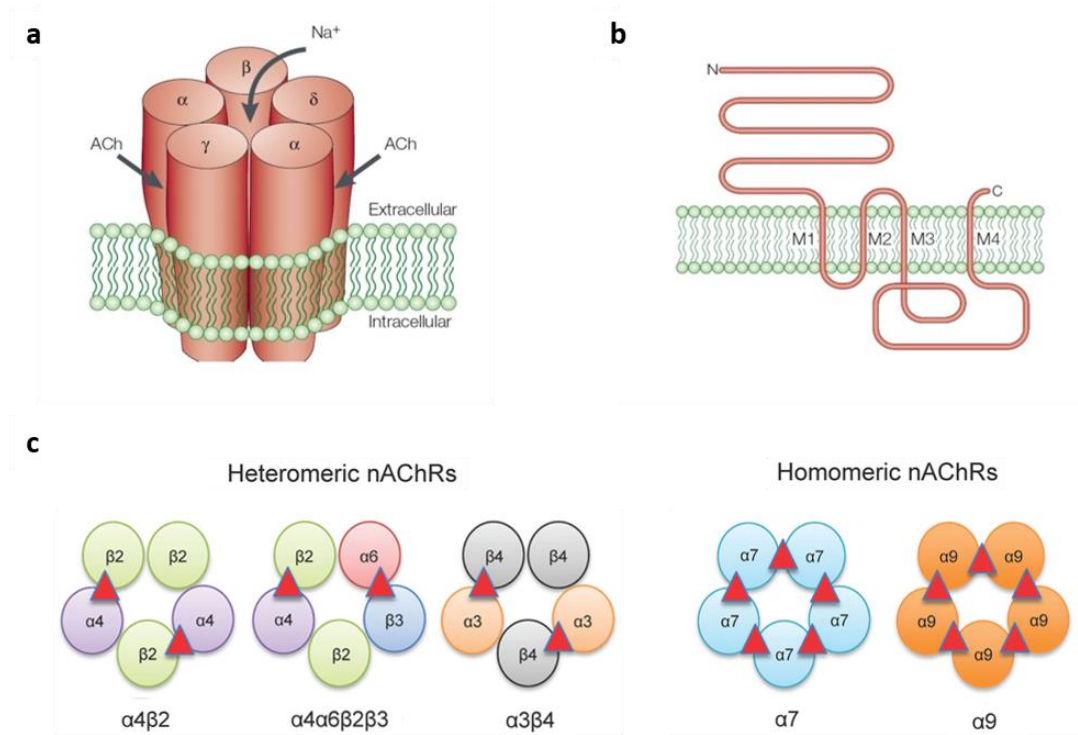
*Figure 1. Structure of the nicotinic acetylcholine receptors. **a.** A schematic representation of the quaternary structure of the receptor, showing the arrangement of the subunits in the muscle-type receptor, the location of the two acetylcholine (ACh)-binding sites the axial cation-conducting channel. **b.** The threading pattern of receptor subunits through the membrane. **c.** Structures of heteropentameric and homopentameric nAChRs. ACh binding sites are depicted as red triangles. (adapted from Nature Reviews Neuroscience 2010, 11, 389-401 and Frontiers in Phychiatry 2013, 4, 1-16).*

1.2 The alpha7 nAChR subtype

The alpha7 receptor is one of the two most abundant nAChR subtype in the central nervous system (CNS), where is mainly localized in the cortex, hippocampus and auditory cortex regions. Because of its wide distribution, the alpha7 subtype is involved in a broad range of central disorders, i.e. Alzheimer's and Parkinson's diseases, attention deficit hyperactivity disorder (ADHD), schizophrenia, anxiety and depression, which can be addressed by alpha7 subtype-selective ligands. [5, 12, 16, 17, 18, 19] Besides its neuroprotective potential, the alpha7 nAChR displays sensory gating and regulation of inflammatory properties, thus representing a promising pharmacological target for the treatment of chronic pain and inflammatory diseases.

The alpha7 nAChR is characterized by a number of unique physiological and pharmacological properties. [20] By virtue of its unique high permeability to calcium, [21] besides electrical signaling, alpha7 receptors may play a role also in direct activation of calcium-dependent processes, such as interneuron excitability, release of excitatory and inhibitory neurotransmitters and neuroprotection. As previously introduced, in this pentameric receptor structure five potential ligand-binding domains (*LBDs*) are hosted and located at the interface between two alpha subunits (**Figure 1**). [20] In addition to these orthosteric binding sites, the alpha7 nAChR subtype hosts multiple allosteric sites, [21] structurally distinct from the orthosteric one and bound by the allosteric modulators. Compounds like these are usually not able to evoke channel opening by their own, but they influence the ability of orthosteric ligands to effect channel gating, by increasing (positive allosteric modulator, PAMs) or decreasing (negative allosteric modulators, NAMs) the response evoked.

The alpha7 subtype is characterized by a lower affinity for ACh compared to other nicotinic subtypes and a very low probability of channel opening during agonist evoked currents, [22] which creates a peculiar challenge for its pharmacological characterization. In respect of its unique kinetic properties, [20] this subtype shows a unique form of concentration-dependent rapid reversible desensitization, [23] by which application of an agonist in concentrations sufficiently high to produce saturation of the binding sites induces the maximal synchronous transient activation when only a fraction of the agonist binding site are occupied. [24] The observed phenomenon relies on two main effects: non-conducting conformations of the receptor promoted by application of high agonist concentration [20] and receptor responses faster than solution exchange kinetics. [23] Alpha7 maximal channel activation induced by ACh occurs at a concentration of 300 μM. Application of greater concentrations produce larger peak currents, but not larger response as a result of progressive increment in synchronization of channel activation. For alpha7 receptor, peak currents take place at relatively low agonist concentration, resulting in extremely sharp macroscopic responses with minimal area and in an over estimation of the agonist concentrations responsible for maximum channel opening. [25] Conversely, net charges better estimate the agonist concentration-response relationship for the alpha7 receptor, since they are not affected by the amount of synchronization of channel opening induced by rapid change in agonist concentration.

1.2.1 Different conformational states of the alpha7 nAChR subtype

The nAChRs are protein dynamic structures which can exists in multiple discrete functional conformations or states (**Figure 2**). [26] The equilibria and the rate of transition between two conformational states are determined by the differences in the free energy values of the different states and by the chemical nature and the amount of ligand bound to the receptor. [20] Ligand binding at the *LBD* influences the equilibria by reversibly and differentially stabilizing the receptor conformation to which the ligand has the greatest affinity. [26]
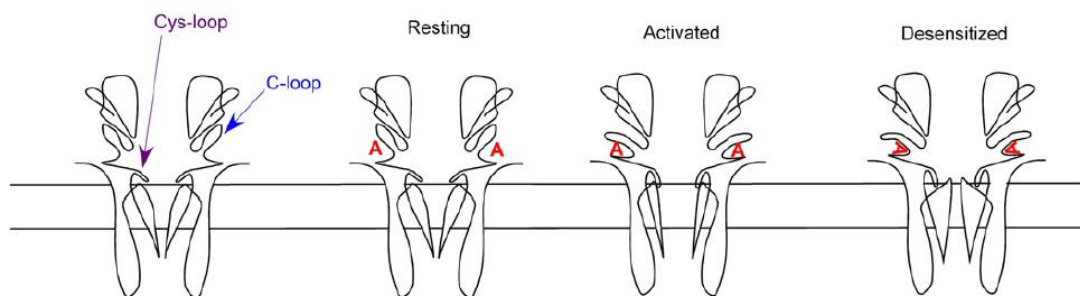

Figure 2. Representation of the different conformational states of the nAChRs (adapted from Biochemical pharmacology 2014). [20]

The alpha7 nAChR can be present in at least four interconvertible and functionally distinct states (**Figure 3**): [27]
- a resting closed state (C), populated in absence of agonists, in which no ion influx through the channel can be detected;
- an active open state (O*), transiently stabilized by the presence of agonists; it is a very short-lasting cation-permeable open state characterized by detectable ion fluxes through the receptor channel;
- desensitized closed states (D), stabilized by the sustained presence of the agonist but with no ion fluxes detected.

Upon complete washout and depending on the nature of the agonist, the nAChR can rapidly recover from the desensitization, thus converting again to a close resting state, or show a residual inhibition or desensitization. [28]

In **Figure 3**, the models depicted in the first panel represent the energy situation and state transitions for the alpha7 depending on various levels of agonist occupancy. Under all conditions, this receptor has a very low open probability and very brief open times, which are usually less than 100 μs, thus reflecting in a really steep energy barrier to enter the open state. [26] Under resting conditions, in absence of a ligand, the resting closed state (C) is the most stable receptor conformation, with some equilibration between the closed and the desensitized (D) states, and negligible probability of channel opening. [20, 26] Specific hydrophobic residues (leucine and valine) form a gate in the central portion of the receptor, acting as a block for ions permeation through the channel. Upon

interaction between a ligand and the receptor *LDB*, there is an increased probability for the receptor to enter the active open state (O*), undergoing conformational changes that lead channel opening and the subsequent permeation of ions through it. In particular, the O* state is a short-lived open-state characteristic of the alpha7 receptor subtype: [24] transition from the closed to the open state is a rapid and transient process, in a range of micro- to milli-seconds, and occurs before saturation of the agonist binding sites. In the heteromeric nAChRs, channel opening is induced by a single agonist binding site occupied and further agonist bindings exert a cooperative effect, promoting the stability of a long-lived open state. [26] Conversely, in homomeric alpha7 nAChRs, this positive cooperativity does not occur, since high agonist concentrations promote a rapid desensitization of the receptor. For the alpha7 nAChRs, the energy difference between the desensitized and the resting states are very small. Binding of an agonist to the nAChR orthosteric sites stabilizes the active open state of the channel, whereas a competitive antagonist promotes the resting closed state. Allosteric modulators can affect the transition rates between different conformational states as well, by interfering in the resting-open equilibria or in the desensitization kinetics.
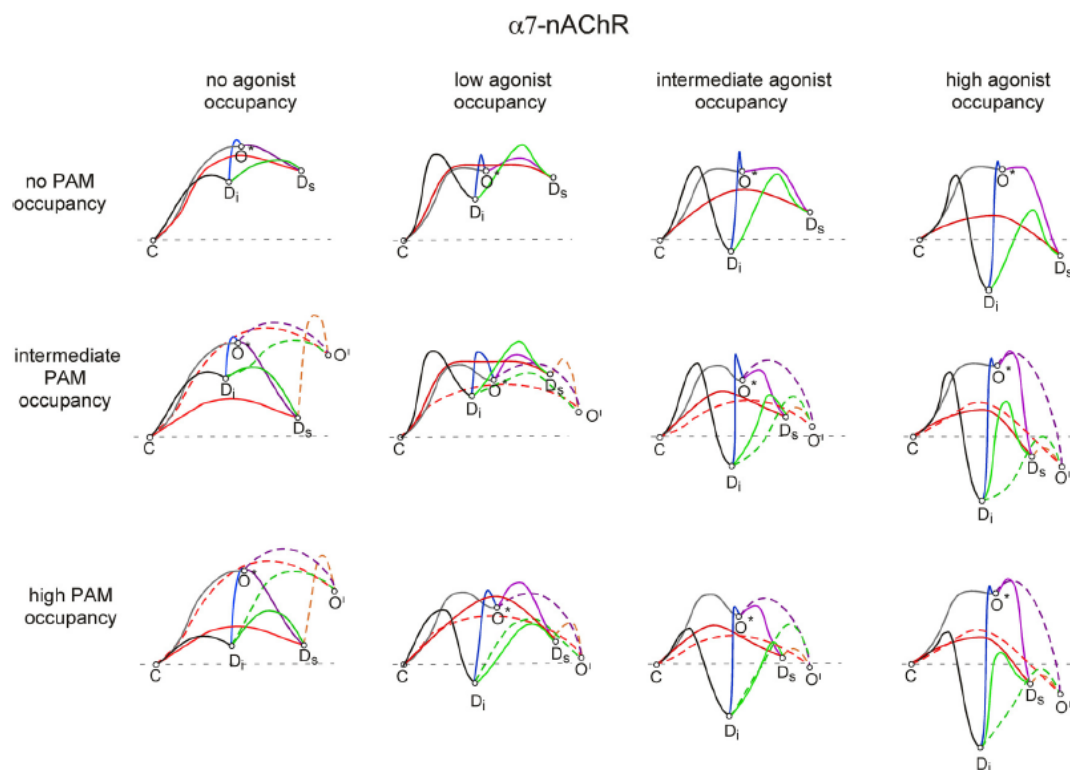
Figure 3. *Proposed models for the activation, desensitization, and modulation of alpha7 nAChR. Qualitative representations of minimal models for 7 nAChR conformational states, their relative energy levels, and transition rates are represented (adapted from Molecular Pharmacology 2011).* [24]

Similarly to other nAChRs, protracted agonist stimulation of the alpha7 subtype induces receptor desensitization, thus promoting a specific receptor conformation in which, even though the agonist is bound, the receptor is a closed state and no activation can

occur. [28] Two distinct forms of alpha7 desensitization, namely D_s and D_i, have been identified and distinguished for being sensitive or insensitive to be converted to open states by a type II PAM of the alpha7 receptor, such as the PNU-120596 (**Figure 3**, second and third rows). The D_s state represents a rapid form of desensitization unique to the alpha7 receptor, similar to the long-lived open state observed for the heteromeric nAChRs and occurs as a long burst or as groups of openings separated by very short closures. [26] Sensitive to and destabilized by the presence of the PAM PNU-120596, the D_s state has a greater stability compared to the closed O* state and it is preferentially entered in presence of high levels of agonist occupancy. The second desensitized conformational state of the alpha7 nAChR is called D_i, because it is insensitive to the PAM PNU-120596. The D_i state is promoted by high levels of agonist or PAM PNU-120596 occupancy [24] and represents the true intrinsic desensitized state of the alpha7 receptor. [26] Compared to the heteromeric desensitized states, the alpha7 D_i has a lower stability that results in a lower free energy difference between the D_i and the close state in presence of high agonist occupancy. Because of the different effects induced in combination with type II PAM, D_s and D_i might involve different intracellular signaling pathways. [28] Although lacking of ability to conduct net ion-currents through the receptor, alpha7 desensitized states are functionally relevant and a signal transduction pathway mediated by the alpha7 receptor independent from ion channel activation, and rather more similar to a metabotropic transmission, has been hypothesized. [29, 30] Therefore, identification of compounds able to preferentially stabilize the desensitized states, and in particular the D_s state, may represent an innovative approach to investigate phenomena modulated by the alpha7 receptor and to develop novel therapeutics.

1.2.2 The human alpha7 nAChR genes CHRNA7 and CHRFAM7A

Phylogenetically, the alpha7 gene represents the closest form to the ancestor nAChR, a protein evolved millions of years ago in organism not relying on fast chemical neurotransmission. [20] The human gene encoding for the alpha7 subunit is the CHRNA7 gene and is located on the human chromosome 15, locus q13,14 (**Figure 4**). [31, 32] The CHRNA7 gene possesses 10 exons localized to highly conserved splice-junction positions. [32] The alpha7 receptor locus is unusually large compared to other species and this evidence led to the discovery of duplicated alpha7 nAChR sequence (**Figure 4**). Six duplicated exons (5-10) of the human alpha7 nAChR gene were identified to be located more than 1.6 Mb 5' upstream from the original alpha7 nAChR gene and, in addition, four completely novel exons have been identified, which create a new open reading frame (ORF). This ORF and the duplicated 5-10 exons form a new human alpha7 nAChR gene called CHRFAM7A or dupalpha7nAChR. [33] The CHRFAM7A is structurally related to the classical alpha7 receptor and retains a significant portion of the original *LBD*, so that it can successfully take part to the pentameric arrangement. [33] It is able to change cell responsiveness to the classical alpha7 nAChR or CHRN7A. It is a human specific and evolutionarily new gene and therefore, not investigable in animal models.

The CHRFAM7A is low expressed in human brain, but is overexpressed in human leukocytes. The vagus nerve regulates systemic and local anti-inflammatory responses through the alpha7 receptor on leukocytes activated by the ACh and the identification of unique human alpha7-like gene CHRFAM7A and its preferential expression in peripheral tissues suggests the possibility of specific mechanisms in anti-inflammatory responses regulated by the alpha7 receptor, thus affecting the therapeutic development of new drugs targeting this receptor subtype for injury, infection and inflammation. [33] The impact of dupalpha7 gene expression in peripheral tissues in human inflammatory responses needs to be further investigated.
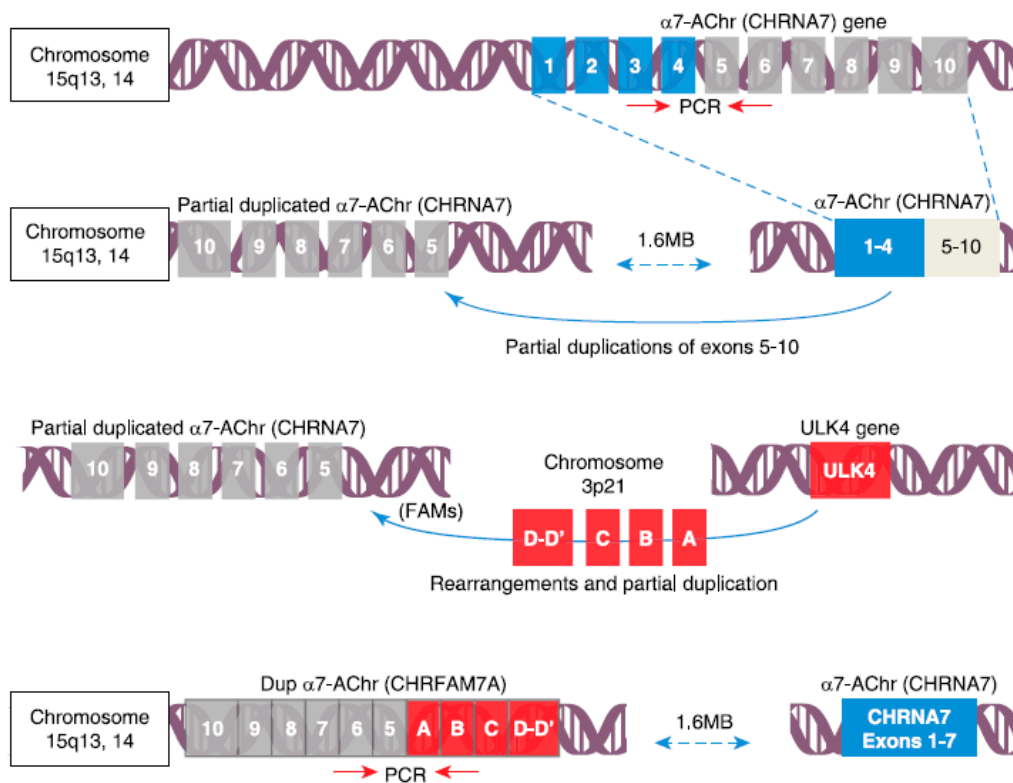

Figure 4. The human genes encoding for the alpha7 nAChRs. The human CHRNA7 gene (top row) and the partial duplication process (from top to bottom) of CHRNA7 that leads to the human-specific duplicated CHRFAM7A gene (adapted from Journal of Leukocyte Biology 2015). [33]

1.3 Involvement of the alpha7 nAChR in the cholinergic anti-inflammatory pathway

Recent evidences report the involvement of alpha7 receptors in modulation of cellular functions beyond the synaptic transmission. [21] Despite the great abundancy in the CNS, the alpha7 subtype is expressed in non-neuronal tissues, like microglia and B-cells, monocytes, dendritic cells, lymphocytes, macrophages, intestinal/lung endothelial and epithelial cells. [34, 35] In these cells the alpha7 receptor is an essential regulator of pro-inflammatory responses mediated by the cholinergic anti-inflammatory pathway, a signaling transduction pathway that involves both the nervous and immune systems. Consequently, [36] this receptor represents a potential therapeutic target for various inflammatory-related diseases and conditions in which systemic inflammation is strongly present, like asthma, sepsis, osteoarthritis, obesity, type 2 diabetes and chronic pain, *via* pro-inflammatory cytokines modulation. Although a well-controlled inflammation constitutes the physiological responses to noxious stimuli and helps restoring homeostatic conditions, excessive inflammation may induce serious health problems, including tissue injury, organ dysfunction and death, and is one of the principal causes of human morbidity and mortality. [35]

Acetylcholine was proven to inhibit the release of pro-inflammatory cytokines, such as TNF-α, through a post-transcriptional mechanism dependent on the alpha7 nAChR subtype. [37] To assess the critical role of the alpha7 receptor in the cholinergic inhibition of TNF release, antisense oligonucleotides specific for this subunit were synthesized. Macrophages exposed to these antisense oligonucleotides showed a lower sensitivity towards inhibition of TNF release after nicotine administration. [37] Moreover, alpha7 knockout mice showed higher serum levels of TNF-α, IL-1β and IL-6 after administration of bacterial endotoxin compared to wild-type animals, confirming the role of the alpha7 subunit in the cholinergic anti-inflammatory pathway (**Figure 5**) [33, 38]

The afferent vagus nerve terminals are activated by an inflammatory stimulus and act by means of spleen-dependent or spleen-independent mechanisms. [33, 38, 39] In both cases, the inhibition of inflammation occurs *via* release of the major vagal neurotransmitter ACh in organs of the reticuloendothelial system, including lungs, spleen, liver, kidneys, and the gastrointestinal tract. [40, 41] Upon release, ACh interacts with the alpha7 nAChRs expressed on cells of both innate and adaptive immunity, including macrophages neutrophils, monocytes, B cells and T cells, and other cytokine-producing cells, thus suppressing the release of TNF-α, down-regulating pro-inflammatory cytokines synthesis and preventing tissue damage. The proposed mechanisms involve NF-κB (nuclear factor kappa) and JAK2-STAT3 (janus kinase–signal transducers and activator of transcription) signaling pathways, which may converge to suppress inflammatory responses. More recent insights suggest the involvement in this modulation of other signaling cascades, such as PI3K (phosphatidylinositol 3-kinase). [39] Upon pro-inflammatory stimulation, alpha7 receptors in macrophages inhibit nuclear translocation of NF-κB which regulates the expression of pro-inflammatory cytokines (**Figure 5**). [39, 40] In the cellular cytoplasm, NF-κB is present as latent and inactive complex bound to I-κB. Inflammatory injury induces

phosphorylation and degradation of I-κB and therefore release and translocation of NF-κB subunits to the cell nucleus where transcription of pro-inflammatory cytokines is evoked. Activation of the alpha7 receptor prevents NF-κB nuclear translocation in macrophages and contrasts inflammation. To date, the exact mechanism by which activation of alpha7 in macrophages causes the blockade of NF-κB nuclear translocation is still unclear. [42]
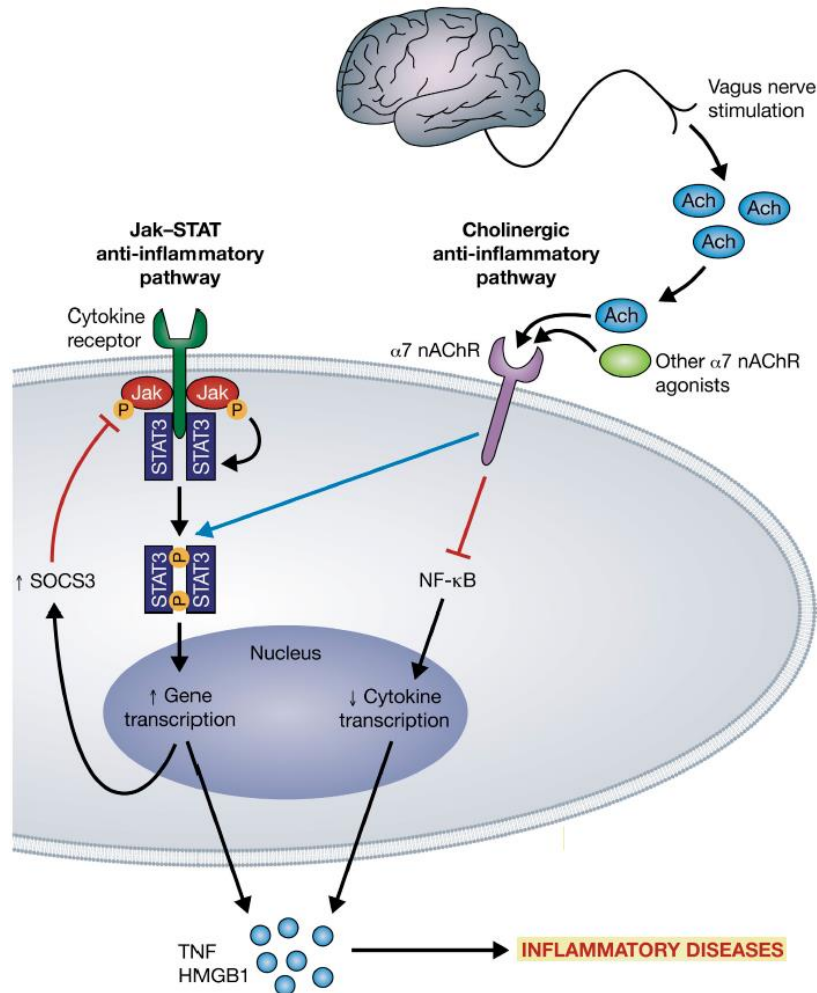

Figure 5. *The cholinergic anti-inflammatory pathway mediated by the alpha7 nAChR. Activation of the vagus nerve leads to ACh release and binding to alpha7 receptors on cytokine producing cells, affecting different intracellular signaling pathways involved in the anti-inflammatory responses (adapted from The Journal of Experimental Medicine 2005, 202(8), 1017-1021).*

The JAK2–STAT3 signaling cascade mediated by the alpha7 in macrophages regulates cytokine responses (**Figure 5**) [39, 43] by phosphorylating transcription factors and activating other kinases. These mechanisms influence the transcription of several genes involved in immunity, inflammation, and controlled cell death. Alpha7 activation determines JAK2 recruitment and auto-phosphorylation, followed by recruitment and phosphorylation of STAT3 by JAK2. The phosphorylated STAT3 dimerize and translocate to the nucleus where they negatively regulate production and release of pro-inflammatory cytokines. Alternatively, unphosphorylated STAT3 may form a complex with the NF-κB and

prevent NF-κB activation. Mice lacking the STAT3 showed reduced anti-inflammatory response to vagus nerve stimulation, thus confirming the role for STAT3 in the cholinergic anti-inflammatory pathway. [40] Alpha7 agonist administration prevents STAT3 phosphorylation and inhibits TNF-α release in wild-type but not in alpha7 knockout mice, and these effects are inhibited by alpha7 selective antagonists and by selective inhibitors of JAK2 phosphorylation. [44, 45]

Although traditionally considered as a ligand-gated ion channel, very recently alpha7 receptor presence in non-neuronal tissues and in particular in not excitable cells of the immune system raised the hypothesis of an alternative ion-independent and metabotropic-like mechanism of signal transduction mediated by this subtype. [33] This metabotropic mechanism is associated with non-conducting states of the receptor and is involved in the modulation of the cholinergic anti-inflammatory pathway. [25, 46, 39] The absence of alpha7 macroscopic currents in the immune cells may be due to the abundant expression of the duplicated alpha7 nAChR gene CHRFAM7A in non-neuronal tissues.

1.4 Alpha7 nAChR Positive Allosteric Modulators (PAMs)

Allosteric modulators are ligands able to bind nAChRs in sites topographically distinct from the orthosteric binding sites of acetylcholine, including sites located in the transmembrane region of the receptor [2] and represent an alternative strategy to investigate the activation of the alpha 7 nAChR. They usually lack intrinsic activity and enhance receptor function through endogenous cholinergic neurotransmission, thus potentiating the binding and/or signaling of an orthosteric ligand. [47]

According to the current classification, two different generations of alpha7 PAMs can be identified. The first generation includes calcium ions, which were the very first alpha7 PAMs described, the anthelminthic agent *ivermectin*, genistein and 5-hydroxyindole and lacks of potency, selectivity and efficacy. [26] On the other hand, in the second generation two different profiles for allosteric modulation, type I and type II (**Figure 6**), are included and they differ in the ability to impact the equilibrium between active and desensitized state of the alpha7 receptor. According to the energy landscapes depicted in **Figure 7**, PAMs influence the conformational states equilibria in two distinct ways. The first model matches both type I and type II PAMs mode of action and claims for a decrease in the energy barrier between the close and the long-lived open state, together with an increase in the energetic difference between the open and the desensitized state. Therefore, the probability of channel openings overcomes the desensitization and increases immediately after agonist application. In this case, the absolute energy differences between the states are not influenced. [24] Alternatively, the increase of open probability induced by PAMs may be due to destabilization of the desensitized state, altering the equilibrium between the active and the desensitized states of the alpha7 receptor, providing prolonged activations of previously desensitized receptors and exerting large effects under equilibrium conditions. The second model illustrated is possessed only by type II PAM.
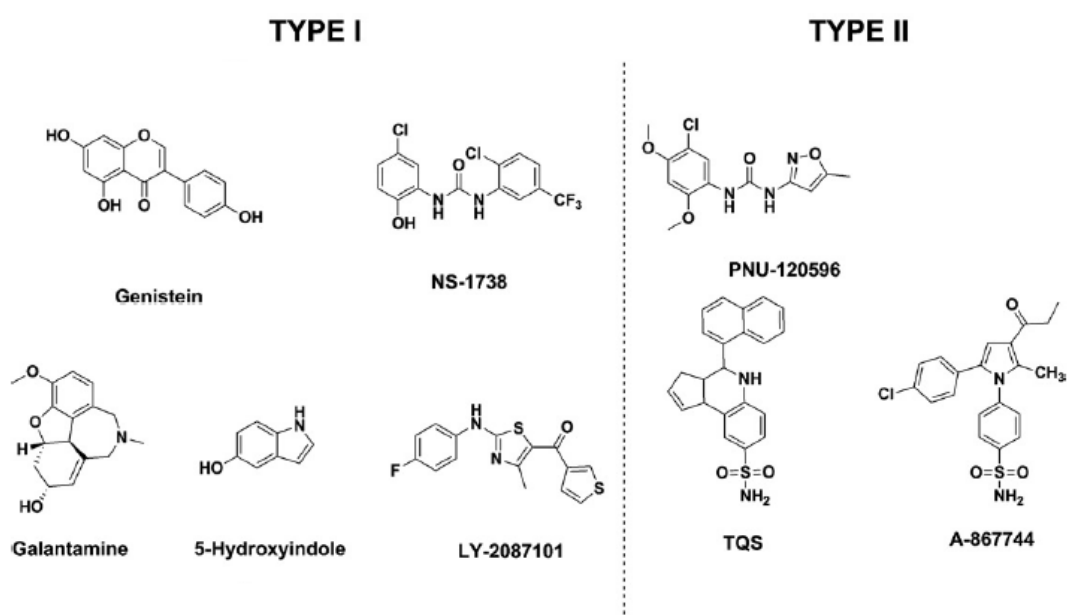
Figure 6. *Structure of known type I and type II positive allosteric modulators (PAMs) of the alpha7 nAChR (adapted from Biochemical Pharmacology 2011).* [26]

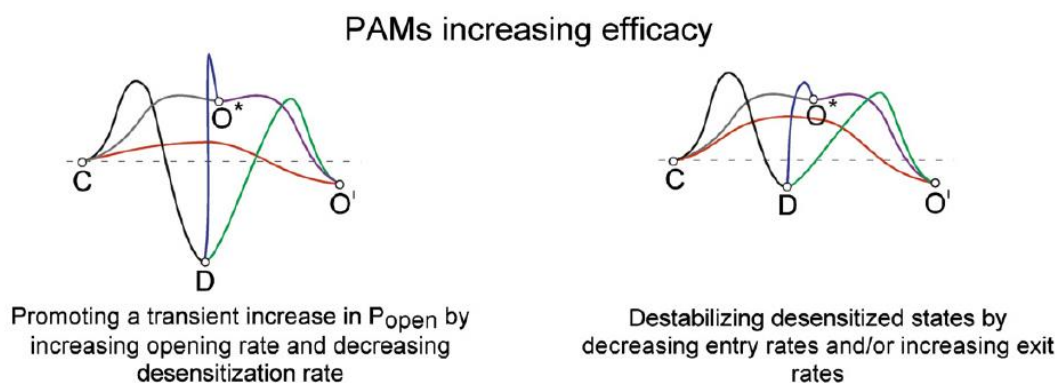**Figure 7.** *Mechanisms of PAMs influence in the equilibria between different conformational states of the alpha7 nAChR subtype (adapted from Biochemical Pharmacology 2011).* [26]

In details, type II PAMs revert the D_s state into a conductive long-lived open state in a manner dependent on both agonist and modulator concentrations. At fixed PAM amount, increasing agonist concentration generate responses characterized by a greatest peak at intermediate and lower at higher agonist concentrations, so that the efficacy does not reach a true plateau. A similar effect is observed when modulator concentrations are increased in presence of fixed agonist concentration. Relatively low occupancy of agonist and PAM binding sites results in an equilibrium between the different open, D_s and D_i states and in large prolonged steady-state currents, while at high occupancy the D_i state is preferentially induced, possibly to protect the cell from toxicity. [26]

Positive allosteric modulators offer a wide range of advantages compared to conventional agonists and represent a relevant alternative to the development of receptor agonists, limited in therapeutic benefits by the low probability of channel opening and the rapid desensitization of the alpha7 receptor: [26]

- PAMs act synergizing and enhancing the responses evoked by endogenous ligands, thus preserving a more physiological spatial and temporal selectivity [26] and restricting the drug effects to regions where ACh was is being released; [21, 26, 48]
- the magnitude of alpha7 potentiation induced by PAMs depends on agonist concentration, enhancing lower agonist concentration with minimal effects on higher concentrations; [2]
- the "ceiling effect": allosteric modulation is a saturable process and after occupancy of the allosteric binding sites no additional effects are observed, resulting in reduced toxicity compared to agonists. [2, 47] The sustained activation and desensitization of the nAChR occurring with subtype selective agonists chronic treatment are not observed with PAMs;
- subtype selectivity may be easily achieving with allosteric ligands because their binding sites are distinct from the well-conserved orthosteric binding domains.

On the contrary, PAMs presents also some limitations as therapeutic approach to target the alpha7 receptor. These limitations are linked to the need of the endogenous agonist in sufficient concentrations to evoke potentiated responses. [26, 21] Therefore, pathological conditions affecting the ACh concentrations may influence potentiation induced by PAMs. Indeed, lacking of intrinsic receptor activation, PAMs may exert a reduced effect in patients with decreased levels of endogenous agonist, i.e. advanced stages of neurodegeneration. Conversely, under trauma, ACh concentrations may become unregulated, resulting in an over-stimulation in presence of PAMs. Other pathological events are associated with undesired alpha7 activation (cell proliferation, angiogenesis and inhibition of apoptosis in some cancers) and in these cases PAM potentiation would exacerbate them. [26] A further limitation to PAM stimulation is due to the Di state of the alpha7 receptor, which is known to be insensitive to conversion to an open state by PAMs. At the same time, however, the Di state represents a safety mechanism to avoid dramatic potentiation induced by PAMs.

Interestingly, a competition between type I and type II PAMs has been observed and co-application of 5-HI (type I) with PNU-120596 (type II) caused a significant reduction in the potentiation compared with the effects of PNU-120596 alone. The present competition may be ascribed to a common binding site of the receptor or to mechanistic interference at sites required for the effects of the compounds. [24] Competitive binding assays between a different type I PAM (NS1738) and PNU-120596 on mutated alpha7 nAChR confirmed the hypothesis of a similar site of interaction for them, since co-application of NS1738 with PNU-120596 resulted in a dose-dependent inhibition of recovery from desensitization. The common binding site for allosteric modulators has been identified and located between the four transmembrane helices of a single alpha7 subunit. [2] Both type I and type II PAMs showed therapeutic efficacy in *in vivo* studies, for example in reverting learning impairment induced by scopolamine [27] or improving short-term recognition memory and sensory gating deficits induced by amphetamine in rat models.

1.4.1 Alpha7 PAMs in inflammatory processes

Several experimental evidences, performed in *in vitro* and *in vivo* models with type I and type II PAMs, support the therapeutic potential of alpha7 allosteric modulation in the treatment of inflammation and chronic pain. [49] For instance, in literature is reported that the type I PAM genistein inhibits I-kB phosphorylation and significantly reduces translocation of NF-kB to the nucleus, [50] thus preventing binding of NF-kB to its target DNA and blocking transcription of NF-kB downstream genes. Additionally, in inflammation models induced by LPS stimulation, *in vitro* administration of genistein attenuates pro-inflammatory cytokines overproduction in macrophages resulting in decrease of both TNF-α and IL-6 levels (**Figure 8**). [51]
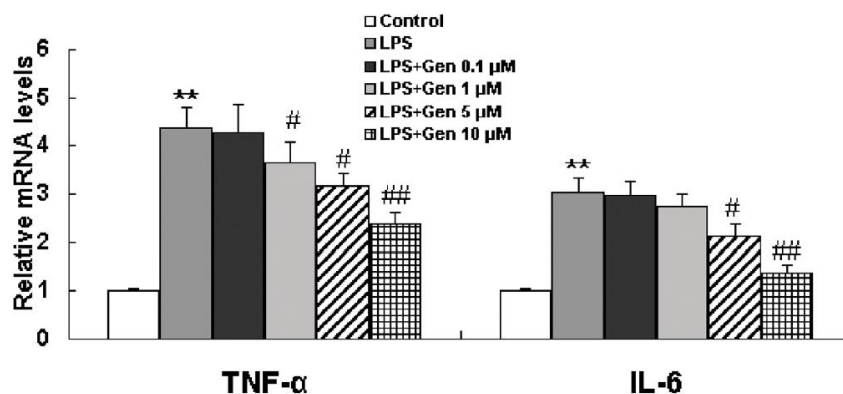

Figure 8. *Inhibitory effect of genistein on TNF-α and IL-6 mRNA levels in LPS-treated macrophages. Cells were pretreated with genistein (0.1-10 μM) for 1h and then incubated with LPS (1 mg/mL) for 24h, IL-6 and TNF-α mRNA levels were quantified (adapted from PLOS ONE 2012).* [51]

Furthermore, the alpha7 nAChR is involved in the chronic low grade inflammation associated with obesity and modulates inflammatory gene expression in human adipocytes. Compared to normal weight subjects, obese patients showed appreciably decreased expression of the alpha7 nAChR levels. [52] Stimulation of differentiated primary human adipocytes with 10 μM of genistein induced relevant up-regulation of alpha7 receptor expression, together with significant modulation of pro-inflammatory genes (TNF-α and IL-6) (**Figure 9**). Two different types of PAMs, NS-1738 and PNU-120596, were investigated in neuropathic pain models represented by carrageenan-induced inflammatory pain and chronic constriction injury (CCI) (**Figure 10**). [49] Both of them significantly reduced thermal hyperalgesia at doses of 10 and 30 mg/kg and PNU-120596 significantly attenuated the paw edema after carrageenan infusion. These effects were observed only in pain conditions, since in sham-operated mice did not occurred and the involvement of the alpha7 receptor was proved by systemic pre-treatment with mecamylamine (MLA), a selective alpha7 antagonist, which completely reverted the observed anti-inflammatory effects.
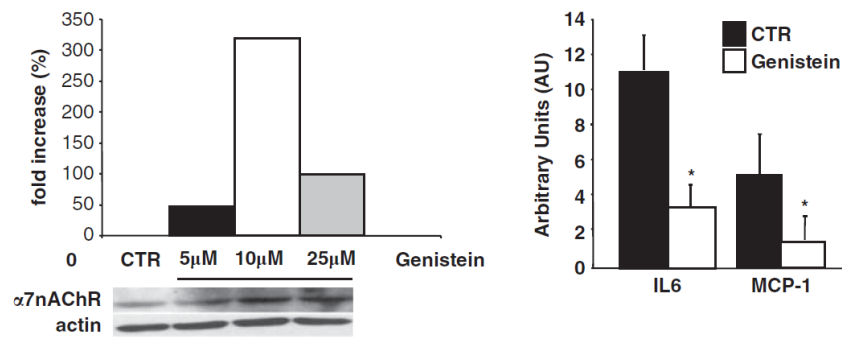
Figure 9. *Effects of genistein stimulation in in vitro differentiated human adipocytes. Expression levels of alpha7 nAChR were increased in percentage after 3h stimulation with 5, 10 and 25 µM genistein (left panel). 10 µM genistein stimulation of in in vitro differentiated human adipocytes reduced the expression levels of inflammatory genes (IL6 and MCP-1) compared to the control (CTR) (right panel) (adapted from International Journal of Obesity 2012).* [52]
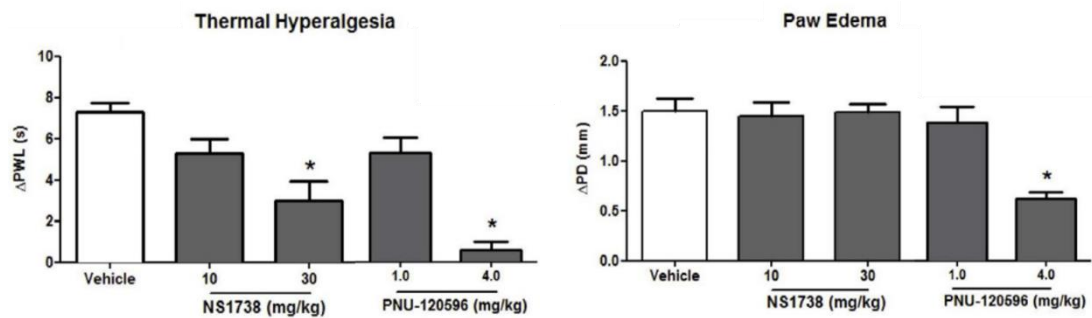

Figure 10. *Effects of alpha7 PAMs NS-1738 and PNU-120596 on carrageenan-induced hyperalgesia and edema in mice. NS1738 (30mg/kg) or PNU-120596 (4mg/kg) were administrated 15 min before carrageenan intraplantar injection and hyperalgesia and paw edema were assessed 6h later (adapted from Neuropharmacology 2013).* [49]

In particular, PNU-120596 showed long-lasting and dose-dependent anti-hyperalgesic and anti-allodynic effects in the chronic constriction injury (CCI) neuropathic pain model. [49, 53, 54] The differences in the observed anti-inflammatory effects of the two PAMs may be ascribable to their distinct mechanism of alpha7 modulation. To explain the antinociceptive effects of PNU-120596 different mechanisms have been hypothesized, including potentiation of ACh effects in counteracting inflammation, modulation of inhibitory neurotransmitter and stimulation of spinal alpha7 receptor. The last mechanism, however, is unlikely since several reports suggested that central alpha7 receptor are not involved in thermal and mechanical nociceptive hypersensitivity in mice.

Interestingly, in situations poorly responsive to nonsteroidal anti-inflammatory drugs (NSAID), or when such agents are contra-indicated, PAMs can be successfully

exploited in the treatment of inflammatory pain conditions. [55] The effects of the NSAID diclofenac and PNU-120596 on pro-inflammatory cytokine levels induced by carrageenan administration were evaluated (**Figure 11**). Both agents attenuated paw swelling and reduced levels of the pro-inflammatory cytokine IL-6 within the hind paw edema, but only PNU-120596 significantly decreased the carrageenan-induced increase in levels of TNF-α. The antagonization of these inflammatory effects induced by MLA proved alpha7 mediation.
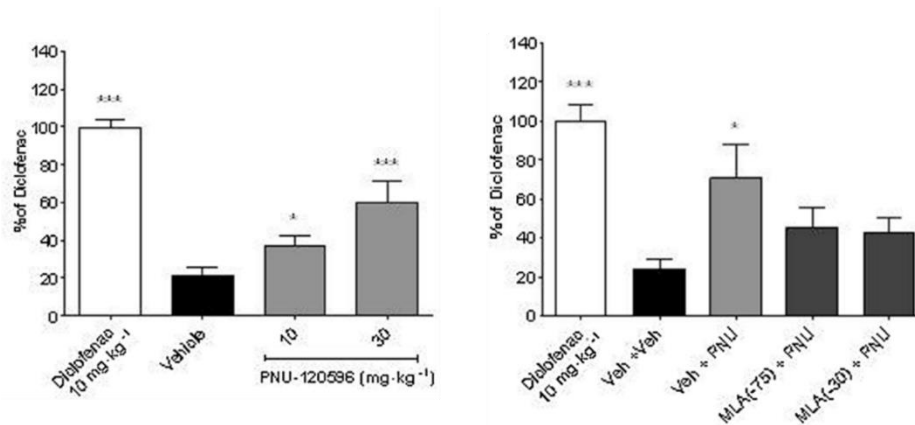
Figure 11. *Anti-hyperalgesic actions of PAM PNU-120596 mediated by activation of alpha7 nAChR. The rats were injected with either CFA (left panel) or carrageenan (right panel) into the plantar surface of the hind paw. They were then administered either drug or vehicle and the effects of PNU-120596 and vehicle on mechanical hyperalgesia were evaluated (left panel). The anti-hyperalgesic actions of PNU-120596 are mediated via activation of alpha7 nAChRs (right panel): MLA reverted the PNU-120596 effects. Diclofenac was included as a positive control and data expressed as a percentage of diclofenac mediated anti-hyperalgesia.* [55]

1.5 Alpha7 nAChR Silent agonists

The profile of an alpha7 silent agonist is revealed by electrophysiological experiments performed in presence of the compound of interest, the control acetylcholine and a type II PAM. The representative traces of the alpha7 receptor responses evoked by applications of a silent agonist, collected on human alpha7 nAChRs expressed in *Xenopus leavis* oocytes, are depicted in **Figure 12**. [30, 56] A compound acting as a silent ligand is characterized by lack of agonist activity (**Figure 12, panel A**): its application to the alpha7 receptor subtype results in no or very weak activation and channel opening, so that no ion channel fluxes are measured, proving that a silent agonist does not act like a conventional alpha7 agonist. Co-application of a silent agonist with the agonist ACh causes inhibition of the alpha7 receptor responsivity to acetylcholine and therefore diminished ion currents evoked by the agonist (**Figure 12, panel B**). This inhibition proves that the silent agonist binds the alpha7 receptor at the acetylcholine orthosteric binding site.
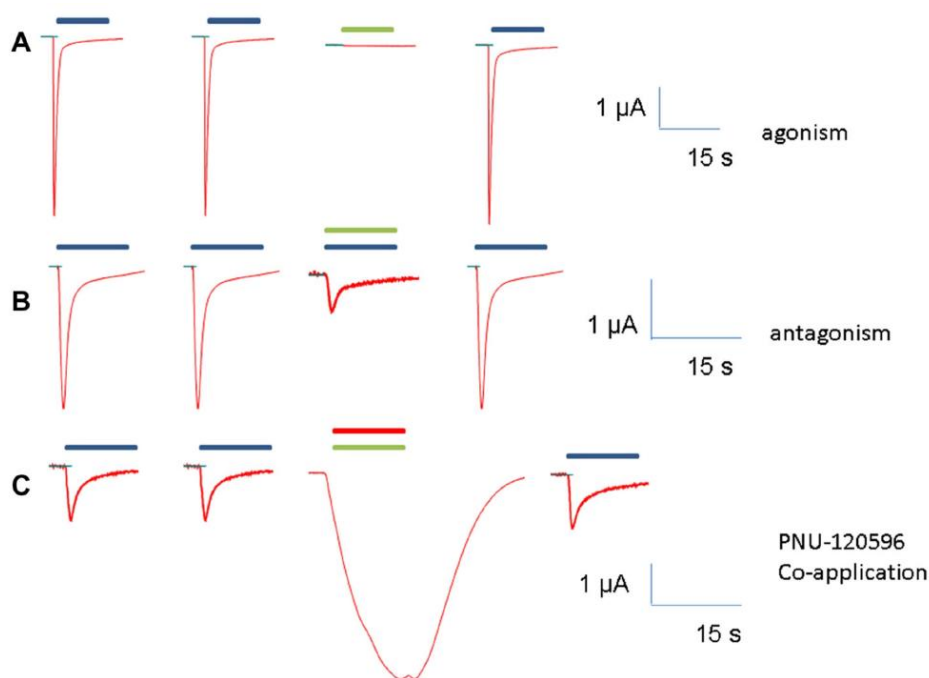
Figure 12. Representative traces of the alpha7 nAChR response to applications of a silent agonist. Blue bars represent duration of ACh applications, green bars the silent agonist application, and the red bar represents PNU-120596 application (adapted from Bioorganic & Medicinal Chemistry Letters 2013). [30]

A silent compound preferentially induces non-conducting alpha7 receptor states (D_s or D_i), thus appearing as an agonist when co-applied with a type II PAM, namely PNU-120596. Evoking a very large channel response in co-application with PNU-120596 (**Figure 12, panel C**), the silent agonist reveals to selectively promote the desensitized state D*s* of the receptor. By definition, the type II PAM does not possess alpha7 detectable activity by its own, but it is able to destabilize a form of desensitization unique to this receptor subtype,

thereby promoting protracted bursts of channel opening. [56] In the light of the described electrophysiological profile, a silent agonist is a ligand that binds to the orthosteric binding site of the receptor, acts as a desensitizer when applied alone, inducing conformational changes converting the alpha7 nAChR subtype from a resting state selectively into a desensitized state that can be probed by type II PAM (PNU-120596) and behaves as a channel activator in the presence of type II PAM. Alpha7 silent agonists. The binding of a silent agonist to the alpha7 receptor subtype does not promote the typical nAChRs ionotropic activation and the corresponding pharmacological profile associated is independent from ion channel currents, but rather more similar to a metabotropic signal transduction mechanism. Therefore, silent agonists represent molecular tools of great interest to study functions of the alpha7 nAChR that do not involve ion conducting states, and may constitute a new potential alternative for the development of alpha7 nAChR therapeutics. Despite being electrophysiologically revealed by co-application with a type II PAM, silent agonists do not require this co-application in order to exert their pharmacological activities in in vitro and in vivo tests. [56]

1.5.1 Pharmacophore model for alpha7 Silent agonism

Because of the intriguing profile displayed by silent agonists, a preliminar pharmacophore model for alpha7 silent agonism has been hypothesized and defined starting from the chemical structures of the known alpha7 silent agonists KC-1 and NS-6740 (**Figure 13**).

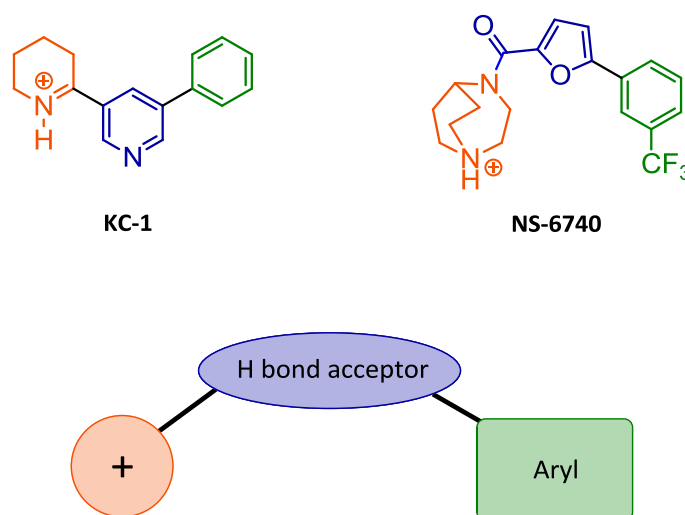*Figure 13. Structures of two alpha7 silent agonists and the putative pharmacophore model for alpha7 silent agonism (adapted from adapted from Bioorganic & Medicinal Chemistry Letters 2013).* [30]

Despite quite different molecular skeletons, some relevant structural features are commonly present in compounds KC-1 and NS-6740:
- a positively charged centre;
- a heterocyclic ring with hydrogen bonding capability;

- an aryl group, potentially suitable for a wide range of structural modifications.

All the elements of the pharmacophore model are in specific angular relationships between each other. The positively charge centre of the illustrated pharmacophore can be represented by either a permanently positively charged ion, i.e. a quaternary ammonium head, or by a protonable nitrogen. Therefore, tertiary amines, protonable at physiological pH, constitute a good alternative to permanently charged quaternary ammonium salts.

According to more recent evidences, the minimal pharmacophore for the alpha7 silent agonism corresponds to the tetraethylammonium (TEA) group, which selectively induces the D_s state of the receptor. [57] This finding originates from the minimum requirements for selective agonist activation of the human alpha7 nAChR subtype, i.e. diethyldimethylammonium (diEdiMA). Starting from it, progressive replacement of methyl groups with ethyl groups led first to triethylmethylammonium (triEMA), a partial agonist of the alpha7 receptor, and then to TEA, an alpha7 silent agonist (**Figure 14**).
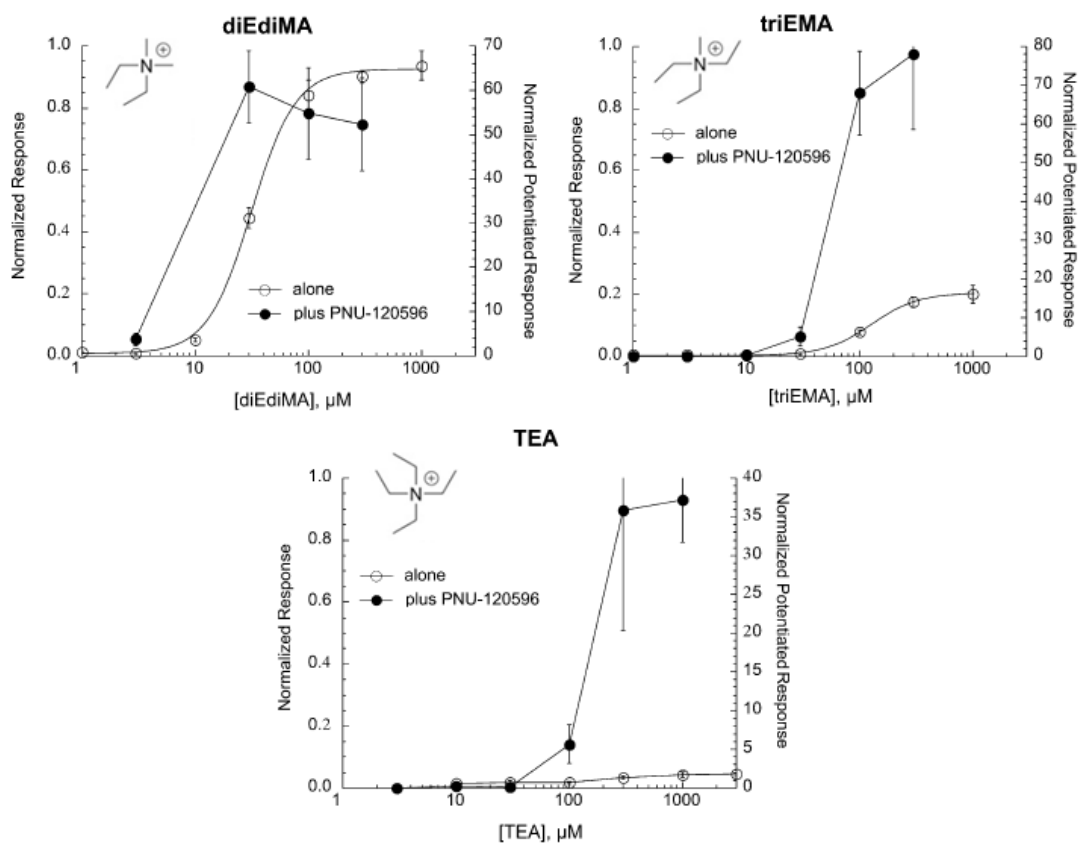
Figure 14. Structures and relative electrophysiological profiles of diEdiMA, triEMA and TEA. Concentration-response studies reported the orthosteric (drug applied alone) and allosterically potentiated (drug co-applied with 10 µM PNU-120596) activation of alpha7 nAChR (adapted from The Journal of Pharmacology and Experimental Therapeutics 2014). [57]

The observed pharmacological profiles suggest that additional ethyl group makes the positively charged nitrogen atom of the compound bulkier, more shielded and less effective for forming the cation-π bond interaction responsible for receptor activation. An optimal Connolly solvent-excluded volume for alpha7 silent agonism has been hypothesized as well

and, according to electrostatic effects, in order to possess the desired silent profile, the core pharmacophore elements should possess an estimated solvent-excluded volume between 130 and 180 Å^3.

1.5.2 Alpha7 Silent agonists in inflammatory processes

Recent evidences highlighted alpha7 signal transductions not dependent from ion channel activation and the linkage between this alpha7 metabotropic-like transmission and inflammation. Indeed, silent agonists by triggering metabotropic ways proved to be useful pharmacological tools in the treatment of inflammatory conditions. [29, 56]

The alpha7 silent agonist NS-6740 (**Figure 13**) preferentially induces non-conducting desensitized conformational states of the receptor and data collected on this compound revealed its therapeutic potential in treating chronic pain and inflammation diseases.

NS-6740, with an agonist activity less than 2% compared to ACh, was first characterized as a very weak agonist [29] and subsequently revealed to be a silent agonist when co-applied with the PAM PNU-120596, promoting the conformational changes associated with desensitization rather than activation. [56] In preliminary *in vitro* studies, [29] NS-6740 appeared to be more efficacious than classical alpha7 agonists in modulating the inflammatory function of microglia and suppressing LPS-stimulated secretion of TNF-α in rat cultured cells, with some effects at 25 μM and complete inhibition at 50 and 100 μM (**Figure 15**).

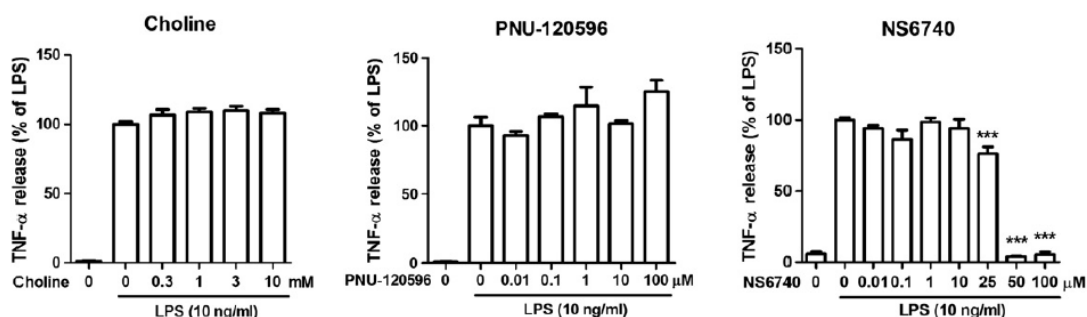***Figure 15.*** *Effects of the alpha7 agonist choline, PAM PNU-120596 and silent agonist NS-6740 on LPS-induced TNF-α release in rat microglia enriched cultures. Rat microglia enriched cultures were exposed to 0.3–10 μM choline, 0.01–100 μM PNU-120596 or 0.01–100 μM NS6740 for 30 min before adding 10 ng/ml LPS for another 4h after which the culture media were collected and submitted to an ELISA for TNF-α (adapted from Journal of Neuroimmunology 2012).* [29]

Since the same effects were not observed with alpha7 agonists (i.e. choline) or alpha7 PAMs (i.e. PNU-120596), the hypothesis of receptor non-conducting states involved in modulation of inflammation was further suggested. Although nicotine is effective in reducing pro-inflammatory cytokines release after LPS stimulation in cultured rodent microglia by alpha7 receptor mediation, these anti-inflammatory effects occur at high nicotine concentrations, known to promote desensitization of the alpha7 receptor and,

therefore, the neuroprotective and anti-inflammatory effects of nicotine are hypothesized to depend on this conformational state

In *in vivo* mouse models of chronic pain, including peripheral neuropathy, NS-6740 recently showed significant dose- and time-dependent antinoceptive activity in several pain models. [56] In the formalin test, NS-6740 reduced inflammation both in the early and the late phases (**Figure 16**) and pretreatment with MLA totally blocked the observed anti-nociceptive effects, thus proving the alpha7 mediation. In addition, NS-6740 decreased the edema associated with inflammation in the formalin test, as measured by the difference in the ipsilateral paw diameter before and after injection of the compound (**Figure 18**).
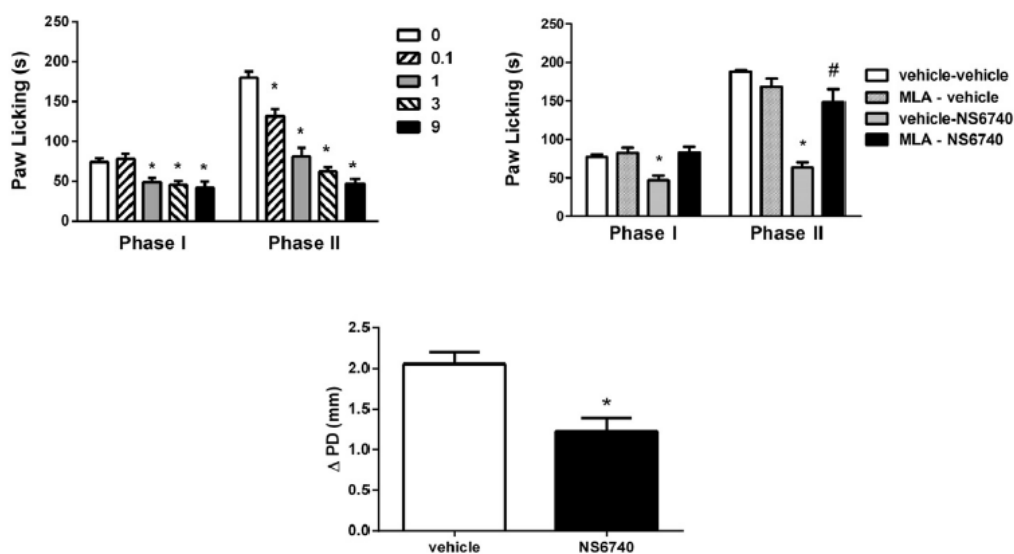

Figure 16. *The anti-nociceptive effects of NS-6740 in in vivo mouse models of chronic pain. The anti-nociceptive effects of NS-6740 after administration of various doses (0.1, 1, 3, and 9 mg/kg, i.p.) on formalin-induced pain behavior in the mouse. The cumulative pain response of time of licking was measured during the period of 0-5 min (first phase) and 20-40 min (second phase) (upper left panel). Blockage of NS-6740 antinociceptive effects in the formalin test by the alpha7 antagonist MLA (10 mg/kg, s.c.) given 15 min before an active dose of 3 mg/kg of NS6740 or vehicle. Fifteen min later, mice were injected with formalin and then observed for pain behaviors (upper right panel). Anti-edema effect of NS-6740 (9 mg/kg) in the formalin test as measured by the difference in the ipsilateral paw diameter before and after injection (ΔPD) (lower panel) (adapted from Neuropharmacology 2015).* [56]

Furthermore, NS-6740 significantly attenuated the chronic pain associated with chronic constrictive injury (CCI) and showed anti-allodynic effects in a dose- and time-dependent way (**Figure 17**). The anti-allodynic effects of NS-6740 were mediated by the alpha7 receptor since they were totally blocked by pretreatment with MLA (**Figure 19**).
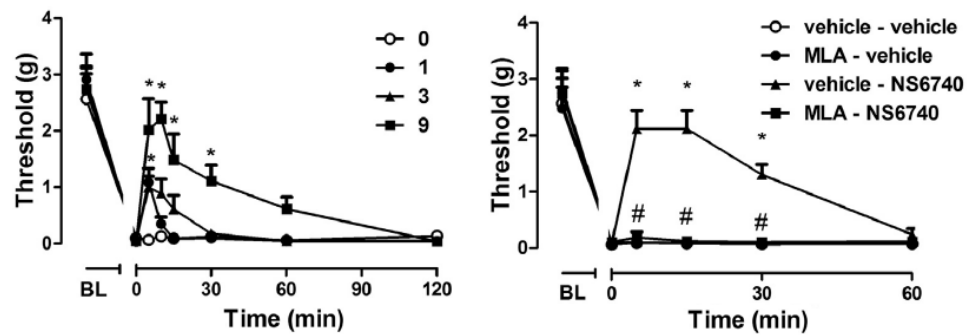
Figure 17. *The antiallodynic effects of NS-6740 in chronic constrictive injury (CCI) neuropathic model are dose-dependent (left panel) and mediated by the alpha7 nAChR, since blocked by mecamylamine (MLA) (right panel). NS-6740 (1, 3, and 9 mg/kg, i.p.) significantly attenuated CCI-induced mechanical allodynia in a dose-related manner (left panel). Blockade of the antiallodynic effect of NS-6740 in the CCI test by MLA (10 mg/kg, s.c.) given 15 min before an active dose of 9 mg/kg of NS-6740 or vehicle proved alpha7 mediation (adapted from Neuropharmacology 2015). [56]*

According to time and concentration dependence, the observed effects were consistent with an alpha7 desensitized state induced by NS-6740, supporting the thesis that analgesia can be achieve through non conducting state of alpha7 nAChR signal transduction.

All these evidences underlined the therapeutic potential of alpha7 selective silent agonists in mouse models of tonic and chronic pain and in the treatment of diseases characterized by strong inflammatory components. While alpha7 agonists better treat cognitive diseases, silent agonists show greater activity on pain and inflammation inhibition, suggesting that therapeutic agents like NS-6740 or other silent agonists favor peripheral pain and inflammatory disorders treatment over cognitive diseases. A possible explanation consists in the peculiar alpha7 receptors expressed by immune cells, which are not conductive in response to ACh and therefore may modulate unique intracellular signaling pathways.

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CHAPTER II

Aim of the thesis

The current PhD research project aimed at investigating the role of the alpha7 nAChRs in the cholinergic inflammatory cascade to elucidate the mechanistic complexity surrounding this research area through the design, synthesis and pharmacological evaluation of novel heterocyclic ligands targeting and selectively activating this subtype.

With the purpose of deepening the inflammatory processes mediated by the alpha7 nicotinic cholinergic pathway, this research focused on the allosteric modulation, less explored with respect to the orthosteric activation, and on the new emerging silent agonism mode of stimulating the alpha7 receptor. [26, 30] As reported in the introduction, both these alpha7 activators are involved in the modulation of inflammatory signals.

2.1 Alpha7 PAM derivatives

The first part of the present research project aimed at identifying and characterizing novel alpha7 PAMs. To this purpose, it was exploited the molecular hybridization approach which is an emerging structural modification tool to design new molecules with improved bioactivity when compared to the parent compounds. This strategy was applied to the alpha7 type I PAMs, 5-hydroxyindole (5-HI) and genistein, (**Figure 18**). 5-HI shows a quite low alpha7 PAM activity (EC_{50} 1.6 mM [26]) but it's characterized by a simple and flexible structure prone to molecular functionalization. On the other hand, the alpha7 PAM activity of genistein is fairly greater (EC_{50} 40 μM [26]). Both these two PAMs are characterized by biological activities other than the alpha7 allosteric modulation. 5-HI possesses potentiating effect on $5-HT_3$ serotonin receptors, due to its structural similarity to serotonin, [58, 59, 60] whereas genistein is endowed with tyrosine kinases inhibitory effect and estrogen-like properties. [61, 62] Therefore, the hybridization approach aimed at both enhancing the 5-HI PAM activity by means of genistein elements introduction and reducing the unwanted side biological properties of the parent compounds to achieve a more selective biological profile.
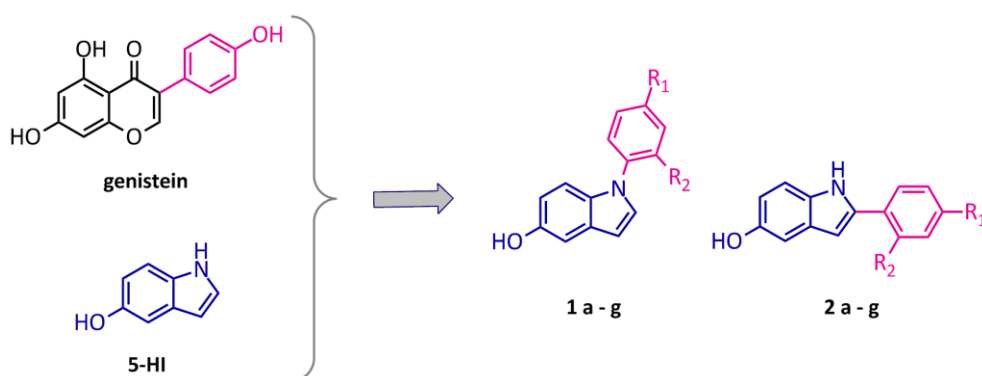

Figure 18. Hybridization approach to novel PAM derivatives.

By comparing the structures of the two model compounds, similarities between the molecular scaffolds were highlighted:

▪ the hetero aromatic nucleus: 3-phenyl-4*H*-chromen-4-one and indole moiety
▪ a hydroxyl group in position 7 and in position 5 of genistein and 5-HI, respectively
▪ a hydrogen bonding acceptor group: ketone carbonyl fuction and nitrogen atom.

Conversely, the most prominent difference is represented by the presence of a phenyl ring in position 3 of genistein which is not detected in 5-HI. According to these observations, two first sets of derivatives, **1a-g** and **2a-g**, (**Figure 19**) were planned to overcome the underlined disparity. Indeed, the heteroaromatic nucleus of 5-hydroxyindole was connected to a variable substituted phenyl ring by exploring two different positions of linking, the nitrogen or the adjacent carbon atom in position 2, thus obtaining the two parallel series of target compounds **1a-g** and **2a-g**,respectively.

	R_1	R_2
1a	OH	H
1b	OH	CH_3
1c	NH_2	H
1d	$NHCH_3$	H
1e	NH_2	Cl
1f	$NHCH_3$	Cl
1g	OH	Cl

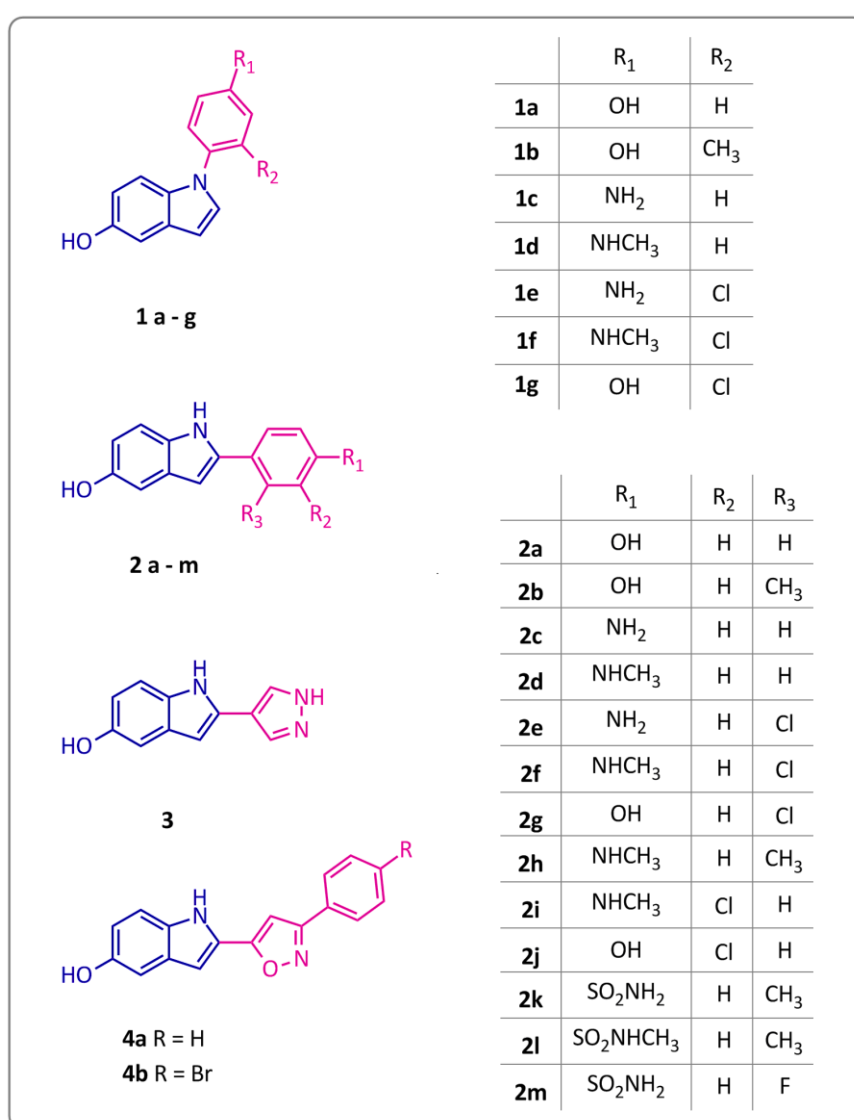|	R_1	R_2	R_3
2a	OH	H	H
2b	OH	H	CH_3
2c	NH_2	H	H
2d	$NHCH_3$	H	H
2e	NH_2	H	Cl
2f	$NHCH_3$	H	Cl
2g	OH	H	Cl
2h	$NHCH_3$	H	CH_3
2i	$NHCH_3$	Cl	H
2j	OH	Cl	H
2k	SO_2NH_2	H	CH_3
2l	SO_2NHCH_3	H	CH_3
2m	SO_2NH_2	H	F

1 a - g

2 a - m

3

4a R = H
4b R = Br

Figure 19. Structures of target compounds related to 5-HI as novel potential alpha7 PAMs.

In both series of the new derivatives, hydrophilic substituents were inserted in *para* position of the phenyl ring to evaluate potential polar interaction within the binding pocket of the receptor. Besides, the relevance of coplanarity between the heteroaromatic nucleus and the phenyl ring in the ligand-receptor interaction was assessed by introduction of a methyl group or a chloro atom in *ortho* position.

Data collected on preliminary electrophysiological experiments performed on these derivatives (**1a-g** and **2a-g**) evidenced the promising enhancement in PAM activity of compounds **2b**, **2f** and **2g**. Considering their structural features, new modifications were planned to further investigate the *C*-aryl substitution of 5-HI and novel derivatives **2h-m** (**Figure 19**) were synthetized. Derivative **2h** combines the *ortho*-methyl group of derivative **2b** and the *para*-methylamino group of derivative **2g**. In compounds **2i** and **2j** the chloro atom of **2f** and **2g** was shifted from *ortho* to *meta* position, while conserving the *para* substituent, in order to study the correlation between position and substituent nature on allosteric activity. To further explore *para*-polar substitutions and in analogy to the type II PAM TQS (**Figure 6**), sulfonamide and methylsulfonamide groups were introduced (2**k-m**). The 5-HI nucleus was then linked to 4-pyrrole in derivative **3** (**Figure 19**) to investigate the effect of heteroaromatic substitution, while preserving hydrogen bond and polar interaction capability. Finally, taking into account the type II PAM PNU-120596 (**Figure 6**), derivatives **4a-b** (**Figure 19**) were designed by introducing a 1,2-isoxazole ring on 5-HI, with the aim of studying also the effect of an enhanced hindrance on PAM profile.

To evaluate the alpha7 PAM activity with respect to a further structural similarity between the new 5-HI derivatives and genistein, a small set of compounds **5a-c** (**Figure 20**) were parallelly prepared by introducing a few exploited substituents on the phenyl ring of genistein. Compounds 5**a** and **5c** were already known for their estrogenic activity. [63]
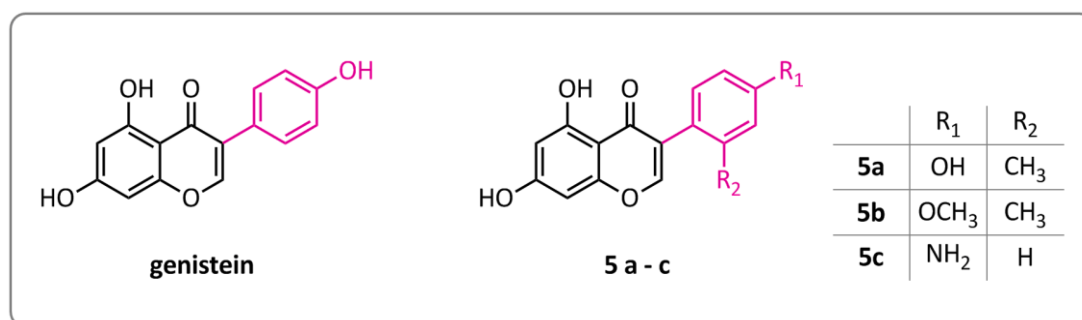

Figure 20. Structures of target compounds related to genistein as novel potential alpha7 PAMs

2.2 Alpha7 Silent agonist derivatives

In addition to positive allosteric modulators, the current research project was focused on the investigation of alpha7 nAChR signal transduction mediated by compounds characterized by the silent agonism profile. As previously mentioned, silent agonists represent an emerging mode of activation of the alpha7 receptor not involving ion-channel transduction and a preliminary pharmacophore model for alpha7 silent agonism has been recently defined (**Figure 13**). [30, 57] To further shed light on the pharmacophore characteristics and on the peculiar pharmacological profile of silent agonists in the modulation of inflammatory conditions, three different series of new potential silent derivatives were designed and synthesized.

2.2.1 diEPP derivatives

The compound *N*,*N*-diethyl-*N*'-phenylpiperazinium (diEPP) (**Figure 21**) was recently reported to present the alpha7 silent agonism profile [57] and it was therefore selected to be the model compound for the first series of new derivatives. The structure of diEPP well lends the compound to functionalization and thus investigation of potential further enhancement of the silent agonist activity.
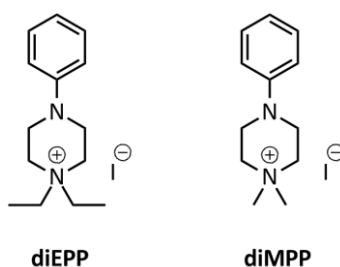

Figure 21. *Structures of the alpha7 silent agonist diEPP and the alpha7 agonist diMPP.*

The model compound diEPP arose from an initial comparison between the minimal pharmacophore for selective activation of the alpha7 receptor subtype, i.e. the diethyldimethylammonium (diEdiMA) cation, and the one for alpha7 silent agonism, i.e. the tetraethylammonium (TEA) cation. [57] The ganglionic agonist diMPP (*N*,*N*-dimethyl-*N*'-phenylpiperazinium) (**Figure 21**), resembles the diEdiMA cation accountable for selective alpha7 activation, but also proved to be a full agonist of this subtype. According to the TEA minimal pharmacophore for silent agonism, substitution of both the methyl groups of diMPP with ethyl groups was hypothesized to turn diMPP into an alpha7 silent agonist. The current hypothesis was confirmed by diEPP, the diethyleted equivalent of diMPP. Replacement of methyl groups with ethyl ones led to loss of the alpha3beta4 efficacy component present in the parent compound diMPP.

Detailed investigation of the diEPP profile showed that the compound was unable to evoke an agonist response after application at 30 μM (**Figure 22,** green dots), but co-application of diEPP with the PAM PNU-120596 led to an activation of the alpha7 receptor (**Figure 22**, pink dots), thus revealing the alpha7 silent agonism profile.
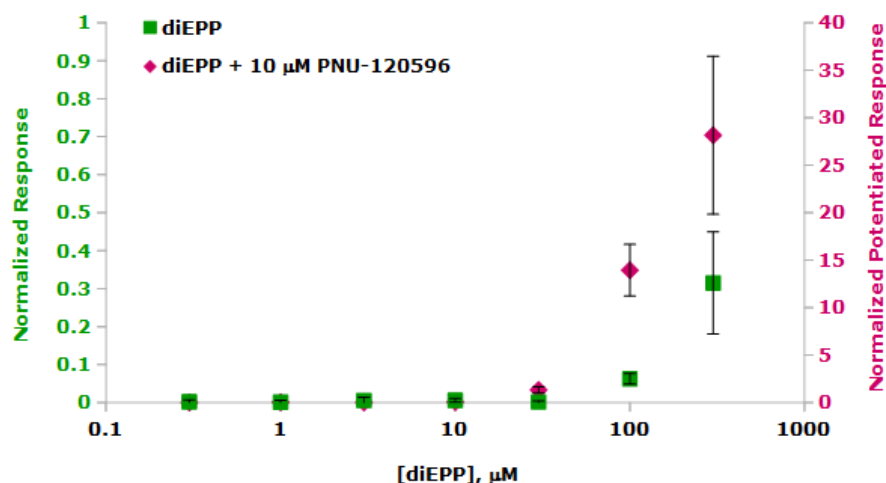
Figure 22. *diEPP electrophysiological data on human alpha7 nAChRs expressed in Xenopus oocytes. Normalized response refers to receptor response to application of test compound at the concentration specified. Normalized potentiated response refers to receptor response of co-application of test compound at the concentration specified and 10 μM PNU-120596. Data are reported as net charge mean ± SEM (standard error of mean) and relative to the control ACh.*

In order to investigate the ability of the phenyl ring of diEPP to establish additional interaction within the alpha7 binding pocket, the structure of the diEPP backbone was varied addressing different parts of the parent compound moiety (**Figure 23**). The first set of derivatives **6a-u** aimed at investigating the effects of different substituents on the phenyl ring of diEPP on the electrophysiological behaviour, with the goal to obtain compounds with an improved IC_{50}. In the second set of derivatives (**7** and **8**) the nature of the linkage between the two rings was modified to test the role of the *N*-aryl linkage for the silent agonist activity. Functionalization of the phenyl ring led to diEPP derivatives **6a-u** (**Figure 23**), characterized by variations in both functional groups identity and position to study their influences on the silent profile. Several different groups were introduced in the *ortho*, *meta* and *para* positions of the aromatic ring, aiming at extended structure-activity relationships between nature and/or positon of the substituent and the alpha7 silent agonism profile. The different substituents were selected focusing on their capability of establishing different electrostatic, bonding and spatial interactions within the binding pocket, potentially affecting the alpha7 silent agonism profile. Electron donor and electron acceptor, H-bond donor and acceptor, polar and non-polar, halogens and different bulky groups were inserted on the aromatic part of the parent compound diEPP. Methyl and methoxide groups were the first introduced, then the trifluoromethyl group, bioisoster of the methyl one, was investigated, followed by the halogen atoms. The choice of the trifluoromethyl group was guided by the known silent agonist NS-6740, which presents a CF_3 group on the *meta* position of the phenyl ring. Subsequently, cyano and the corresponding amide groups were taken into account. Finally, hydroxyl and naphthalene groups were introduced to investigate the influence of a more polar and a more bulky

group respectively. The *ortho* substitution was considered aiming at investigating the impact of the aromatic ring orientation in the space on the corresponding activity.
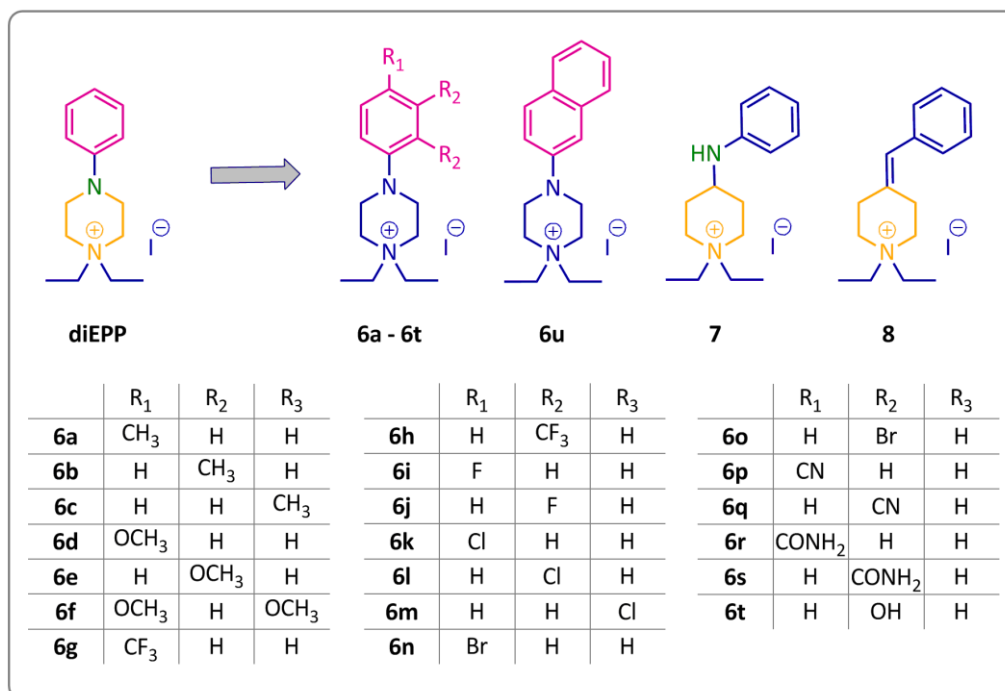

	R_1	R_2	R_3		R_1	R_2	R_3		R_1	R_2	R_3
6a	CH_3	H	H	6h	H	CF_3	H	6o	H	Br	H
6b	H	CH_3	H	6i	F	H	H	6p	CN	H	H
6c	H	H	CH_3	6j	H	F	H	6q	H	CN	H
6d	OCH_3	H	H	6k	Cl	H	H	6r	$CONH_2$	H	H
6e	H	OCH_3	H	6l	H	Cl	H	6s	H	$CONH_2$	H
6f	OCH_3	H	OCH_3	6m	H	H	Cl	6t	H	OH	H
6g	CF_3	H	H	6n	Br	H	H				

***Figure 23.** Structures of target compounds related to diEPP as novel potential alpha7 silent agonists.*

The second set of diEPP analogues (**Figure 23**) arose from the idea of testing different spatial orientation of the molecule and the variations addressed the nature of the linkage between the aliphatic and the aromatic rings. The piperazines ring of the parent compound was replaced by the corresponding piperidine one and two different linkages between the aromatic portion and the piperidine moiety were investigated for testing the importance of the *N*-aryl linkage in the reference compound . The two rings were indeed connected through an amino (**7**) or a methylene (**8**) bridge, which, conferring a different grade of flexibility and interaction capability to the molecule, might affect the interactions within the binding pocket.

2.2.2 Quinuclidine derivatives

Two series of compounds characterized by a quinuclidine spirocyclic-Δ^2-isoxazoline nucleus (**9a-h** and **10a-h**) and a 3-substituted-5-(quinuclidin-3-ylmethyl)-1,2,4-oxadiazole (**11a-m** and **12a-m**) were designed and synthesized. These derivatives were planned according to the structural and electrostatic features described in the preliminar pharmacophore model of a typical alpha7 silent agonist. [30] These characteristics make the silent agonism prone to well bear a wide range of structural modifications. The positively charged centre, in particular, may be represented by both a permanent positively charged ion, i.e. a quaternary ammonium cation, or by a protonatable nitrogen. Therefore, tertiary amines, which can be protonated at physiological pH, may be a suitable alternative to permanently charged quaternary ammonium salts.

2.2.2.1 Spirocyclic quinuclidinyl-Δ²-isoxazoline derivatives

In the recent past years the research group in which I developed the current PhD project has extensively studied the nicotinic cholinergic transmission through the design and synthesis of numerous ligands interacting with different nACh receptor subtypes. [64, 65] Within this research line, a library of quaternary ammonium compounds previously synthesized as alpha7 subtype-selective nicotinic ligands, but endowed with a high alpha7 binding affinity ($K_i > 3$ µM), has been selected to be analyzed as potential silent agonists. From the screening four compounds ICH-12, SG-3, SG-6 and MR-6 (**Figure 24**) revealed to possess the alpha7 silent agonism profile of interest.

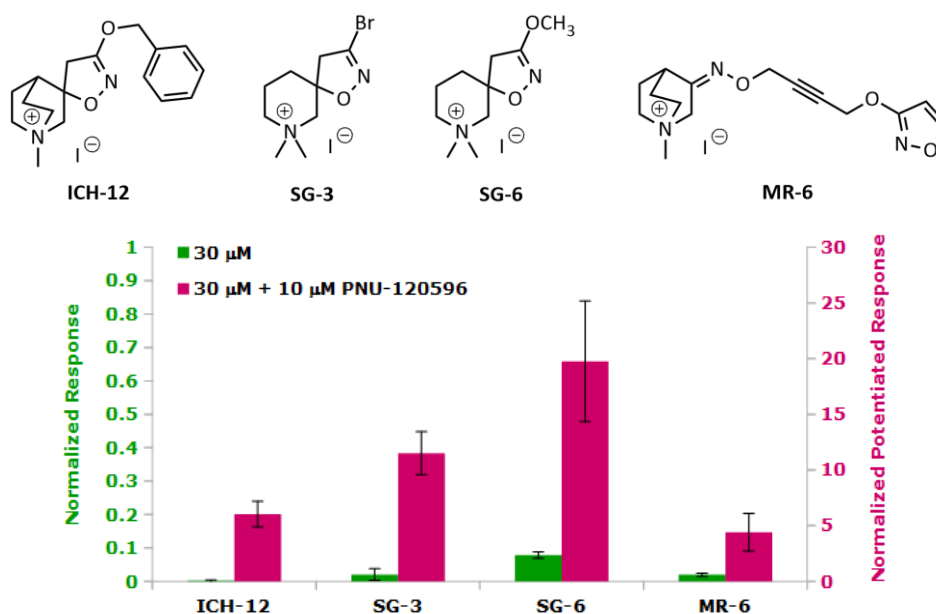***Figure 24.*** *Structures and corresponding electrophysiological data of ICH-12, SG-3, SG-6 and MR-6 collected on human alpha7 nAChRs expressed in Xenopus oocytes. Normalized response refers to receptor response to 30 µM application of test compound. Normalized potentiated response to receptor response with 30 µM test compound and 10 µM PNU-120596 co-application. Data are reported as net charge mean ± SEM (standard error of mean) and relative to the control ACh.*

Therefore, among them has been chosen the model compound for developing the new series of quinuclidine spirocyclic-Δ^2-isoxazoline derivatives. In particular, the emerged compounds ICH-12, SG-3, SG-6, MR-6 appeared to have structural characteristics conform to the general pharmacophoric requirements described for the silent agonism and resulted to have a good silent profile:

- they all lack of agonist activity: the agonist evoked response, normalized for the acetylcholine response, is less than 10% (green bars);
- co-application of the compound with the type II PAM PNU-120596 evoked great responses of the alpha7 receptor, thus revealing their ability to selectively induce a desensitized PNU-sensitive state of the alpha7 receptor, typical characteristic of silent agonism (pink bars).

Despite the highlighted common features, the four compounds differ in the ratio of silent agonist (pink bars) *versus* agonist activity (green bars). In particular, SG-6, showed the greatest sensibility to PNU-120596 co-application, but it also had the highest agonist activity. Conversely, ICH-12 possessed a good silent agonism, with a potentiated response 6-fold greater than the acetylcholine control and a non-detectable agonism. Consequently, because of the optimum ratio of silent agonist over agonist activity, ICH-12 resulted to be the best candidate to be further manipulated and it was selected as model compound for designing the new silent derivatives. To identify the best portion of the molecule to which address the modifications, the structure of ICH-12 was divided into three major fragments: the quinuclidine ring, the Δ^2-isoxazoline nucleus and the benzyl moiety. The quinuclidine ring, common to two out of four tested compounds, was hypothesized to be relevant for silent agonism and it was therefore preserved in the new designed derivatives. Similarly, the heterocyclic isoxazoline ring was maintained since prevalently present in the emerged compounds. The benzyl moiety of ICH-12 represents the most variable portion within the analysed set of compounds and therefore, the planned modifications were addressed to this fragment. In particular, preserving the spiro quinuclidinyl-Δ^2-isoxazoline core as key element, novel derivatives **9a-h** and **10a-h** (**Figure 25**) arose by introduction of different suitable substituents in the *meta* position of the phenyl ring analogously to the silent compound NS6740. The substituent groups introduced were selected according to their different nature and represent a reasonable set of diversification in terms of electrostatic properties, bonding interactions and bulky features, thus potentially able of establishing putative additional interaction within the alpha7 binding pocket. The new derivatives were designed either as protonatable tertiary amines or permanently charged quaternary methyl ammonium salts. The trifluoromethyl group is present in the structure of the known alpha7 silent agonists NS-6740 and it was therefore the first substituent introduced on the benzyl ring (**9a** and **10a**). To investigate the potential influence of halogen bond interactions over the silent agonist activity of the parent compound, derivatives **9b-e** and **10b-e** were designed by inserting the different halogen atoms in the *meta* position of the aromatic moiety. Furthermore, a methyl group (**9f** and **10f**) as bioisosteric replacement of the trifluoromethyl group and a methoxide group (**9g** and **10g**) as fragment able of accepting hydrogen-bond interactions were introduced. Finally, the naphthalene (**9h** and **10h**) moiety

was picked to evaluate whether the binding pocket of the receptor would bear a greater steric hindrance and still preserve the silent agonist activity.
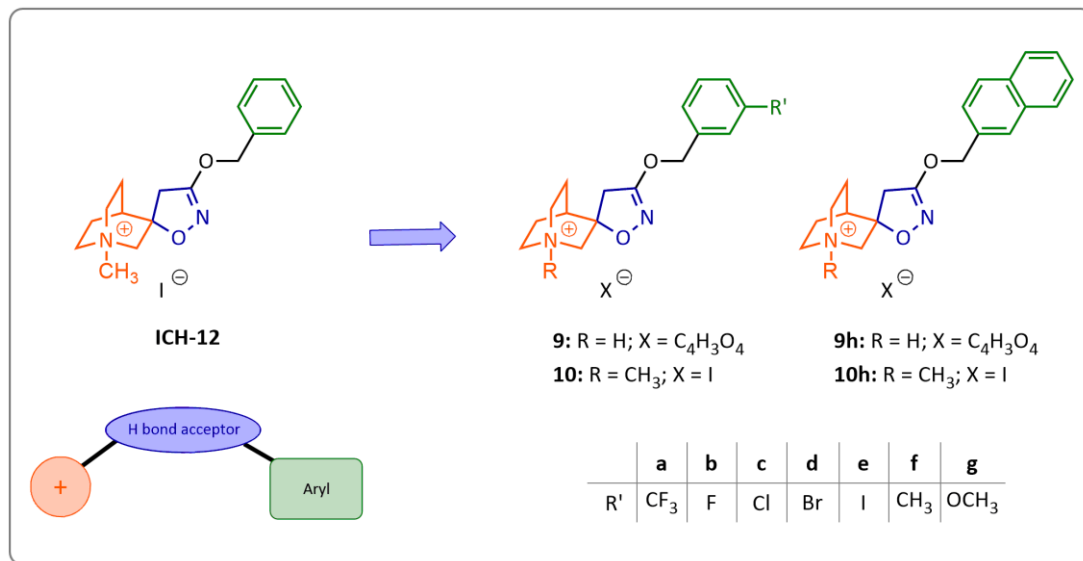

Figure 25. *Structures of target compounds related to ICH-12 as novel potential alpha7 silent agonists.*

2.2.2.2 Quinuclidine-1,2,4-oxadiazole derivatives

To further explore the pharmacophore requirements for alpha7 silent agonism, a second series of quinuclidine-related compounds (**11a-m** and **12a-m**, **Figure 26**) was designed. In comparison with the previous series, the new derivatives differs in the nature of the central portion of the scaffold, bearing a 1,2,4-oxadiazole ring instead of an isoxazoline one. Noteworthy, the 1,2,4-oxadiazole ring was chosen according to the structural and spatial determinants of the known silent agonist NS-6740. The distances between the basic core and the atom with hydrogen bond properties of NS-6740 are maintained in the new derivatives. With respect to the Δ^2-isoxazoline system, the additional nitrogen atom of the 1,2,4-oxadiazole nucleus should contribute to investigate the relevance of hydrogen bonding in the receptor interaction of this pharmacophoric region. The quinuclidine basic core was preserved as positively charged centre and was linked through a methylene bridge to the position 5 of the oxadiazole which is connected in position 3 to an aliphatic/aromatic substituent. In particular, suitably substituted phenyl rings and heteroaromatic cores (i.e. pyridine, furan and thiophene) were linked to the 1,2,4-oxadiazole nucleus. The phenyl ring linked to the oxadiazole moiety was substituted with trifluoromethyl, fluoro, chloro, bromine and iodine groups, respectively **11a-e** and **12a-e**, in comparison with the previous set of compounds. The corresponding oxadiazoles derivatives of ICH-12 was designed as well, therefore unsubstituted phenyl compounds (**11f** and **12f**) were planned and synthesized, and besides the methyl and methoxide derivatives (**11g-h** and **12g-h**) were introduced. To evaluate the influence of a different side aromatic centre, analogues containing heteroaromatic rings, like pyridine, furan and thiophene were

selected and introduced in position 3 of the oxadiazoles ring (**11j-m** and **12j-m**). The target compounds wer designed either as protonatable tertiary amines or quaternary methyl ammonium salts.
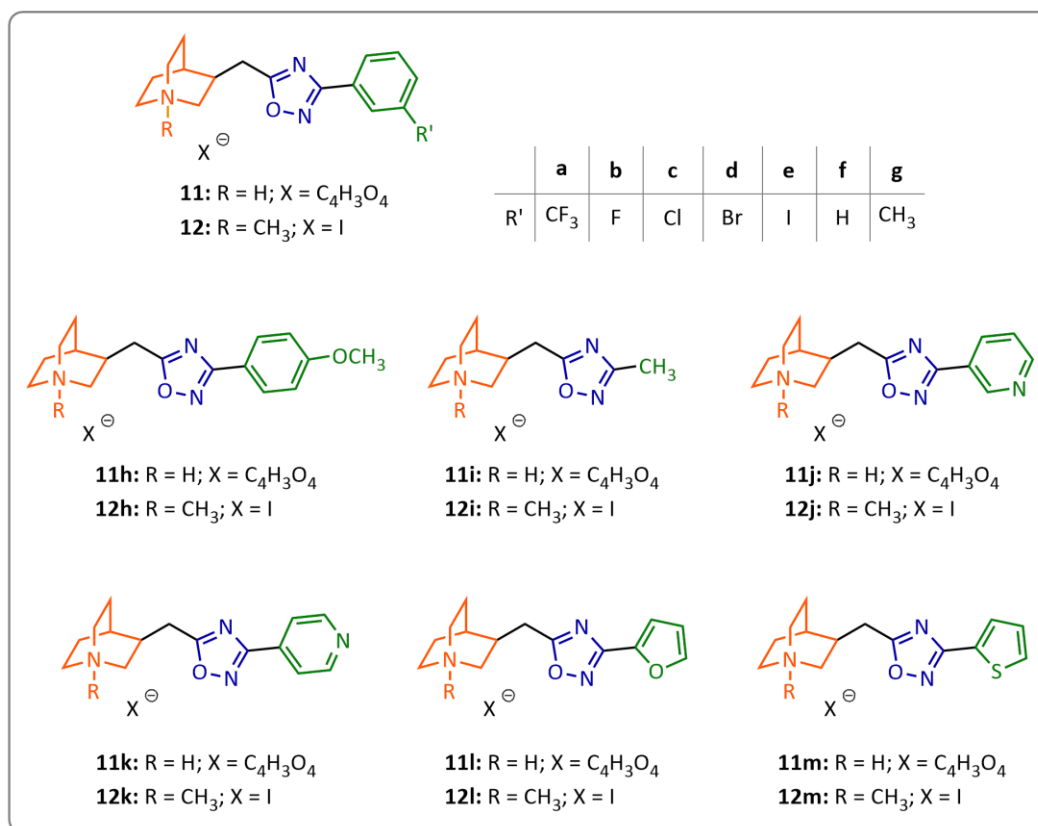

11: R = H; X = $C_4H_3O_4$
12: R = CH_3; X = I

	a	b	c	d	e	f	g
R'	CF_3	F	Cl	Br	I	H	CH_3

11h: R = H; X = $C_4H_3O_4$
12h: R = CH_3; X = I

11i: R = H; X = $C_4H_3O_4$
12i: R = CH_3; X = I

11j: R = H; X = $C_4H_3O_4$
12j: R = CH_3; X = I

11k: R = H; X = $C_4H_3O_4$
12k: R = CH_3; X = I

11l: R = H; X = $C_4H_3O_4$
12l: R = CH_3; X = I

11m: R = H; X = $C_4H_3O_4$
12m: R = CH_3; X = I

Figure 26. Structures of target compounds containing the 1,2,4-oxadiazole nucleus as novel potential alpha7 silent agonists.
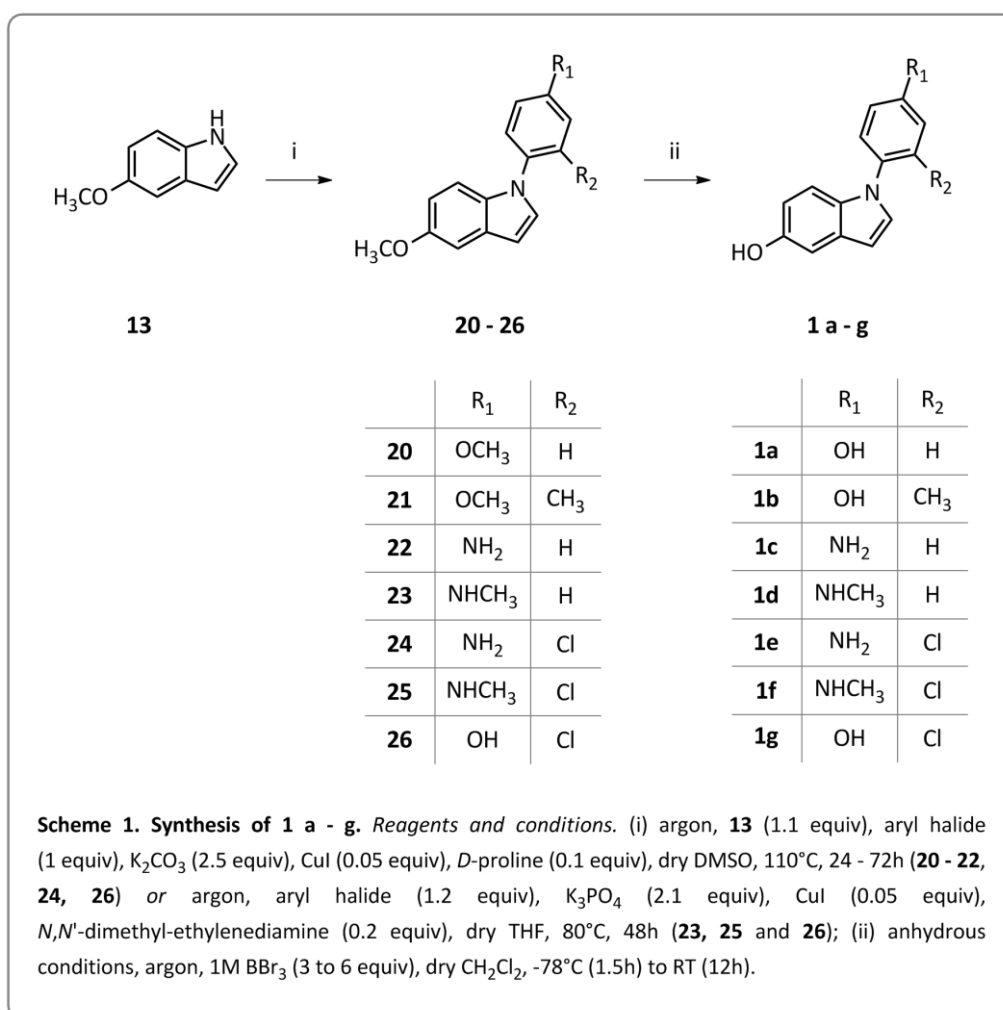
CHAPTER III

Chemistry

3.1 Synthetic approaches to the PAM derivatives
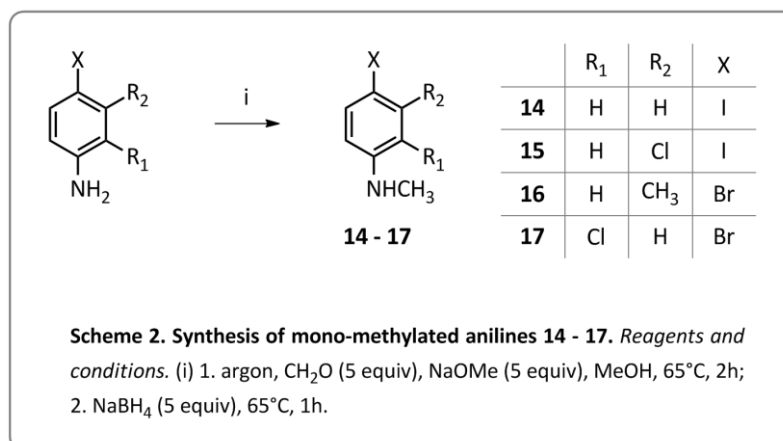
3.1.1 5-Hydroxyindole derivatives

3.1.1.1 Synthesis of compounds 1a-g

The novel *N*-aryl 5-hydroxyindole-related derivatives **1a-g** were achieved through the two steps general synthetic approach depicted in **Schemes 1**, following known literature procedures that have been optimized depending on the aryl group introduced. The key step for the synthesis was represented by a copper-catalyzed Ullmann-type aryl amination between commercially available 5-methoxyindole (**13**) and the appropriate aryl halide, a reaction largely exploited to obtain *N*-arylindoles, promoted and accelerated by the alpha-amino acid. **[66, 67, 68, 69]** Among the different amino acids, proline was the best choice because of its low reactivity toward coupling with aryl halides. **[67]** Therefore, the key reaction step to derivatives **1a-g** was performed by treating **13** with the conveniently substituted aryl halides in presence of the catalytic complex formed by copper iodide and *D*-proline. The reaction proceeded smoothly and afforded the desired coupled intermediates **20-22** and **24** in quite good yields (29-74%).

	R₁	R₂		R₁	R₂
20	OCH₃	H	**1a**	OH	H
21	OCH₃	CH₃	**1b**	OH	CH₃
22	NH₂	H	**1c**	NH₂	H
23	NHCH₃	H	**1d**	NHCH₃	H
24	NH₂	Cl	**1e**	NH₂	Cl
25	NHCH₃	Cl	**1f**	NHCH₃	Cl
26	OH	Cl	**1g**	OH	Cl

Scheme 1. Synthesis of 1 a - g. *Reagents and conditions.* (i) argon, **13** (1.1 equiv), aryl halide (1 equiv), K₂CO₃ (2.5 equiv), CuI (0.05 equiv), *D*-proline (0.1 equiv), dry DMSO, 110°C, 24 - 72h (**20 - 22**, **24**, **26**) *or* argon, aryl halide (1.2 equiv), K₃PO₄ (2.1 equiv), CuI (0.05 equiv), *N,N'*-dimethyl-ethylenediamine (0.2 equiv), dry THF, 80°C, 48h (**23**, **25** and **26**); (ii) anhydrous conditions, argon, 1M BBr₃ (3 to 6 equiv), dry CH₂Cl₂, -78°C (1.5h) to RT (12h).
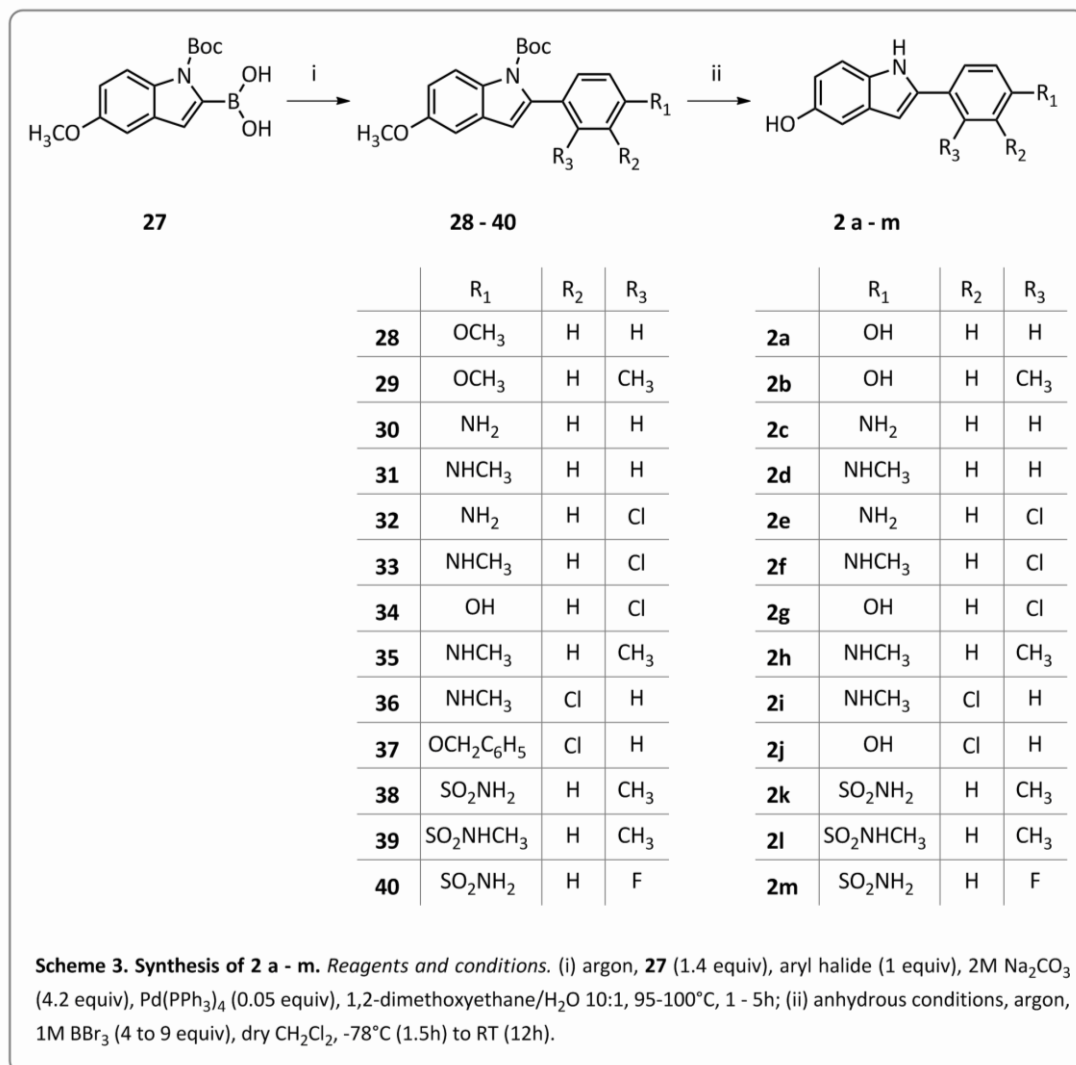
Alternatively, amine derivatives **23** and **25** were prepared by reacting 5-methoxyindole (**13**) and the appropriate aryl halides in presence of *N,N'*-dimethylethylenediamine, a ligand evenly able to promote the nitrogen-carbon bond formation as well as the alpha-amino acids. [70] Attempts to obtain intermediate **26**, applying both the synthetic routes illustrated, failed, also when protection of the hydroxyl group was introduced. Therefore, compound **1g** was not achieved. Cleavage of the methoxide protecting group in strong acidic conditions afforded the desired final compounds **1a-f**. [71] Compound **1f** quickly degraded to a violet oil even under argon atmosphere at -20°C and, consequently, it was not submitted to pharmacological assays.

When the coupling reaction involved a methylated aniline, the aryl halide of interest was obtained from the corresponding aromatic amine through a reductive amination in presence of formaldehyde and sodium borohydride (**Scheme 2**). [72] This protocol allowed to selectively achieve mono-methylation of the aniline to give **14-17**. Methyl anilines **14** and **15** were reacted with **13** to obtain derivatives **23** and **25**, whereas **16** and **17**, here described, were reacted with only *N*-Boc-5-methoxy-2-indolylboronic acid (**27**) to obtain derivatives **35** and **36** of the next series of 5-hydroxyindole derivatives **2a-m**.

	R_1	R_2	X
14	H	H	I
15	H	Cl	I
16	H	CH$_3$	Br
17	Cl	H	Br

Scheme 2. Synthesis of mono-methylated anilines 14 - 17. *Reagents and conditions.* (i) 1. argon, CH$_2$O (5 equiv), NaOMe (5 equiv), MeOH, 65°C, 2h; 2. NaBH$_4$ (5 equiv), 65°C, 1h.

3.1.1.2 Synthesis of compounds 2a-m and 3

As shown in **Scheme 3**, to obtain compounds **2a-m**, the synthetic key step is represented by a Suzuki cross-coupling reaction between the commercially available *N*-Boc-5-methoxy-2-indolylboronic acid (**27**) and the appropriate aryl halides, by using the palladium catalyst tetrakis(triphenylphosphine)palladium(0) in basic medium. [73]
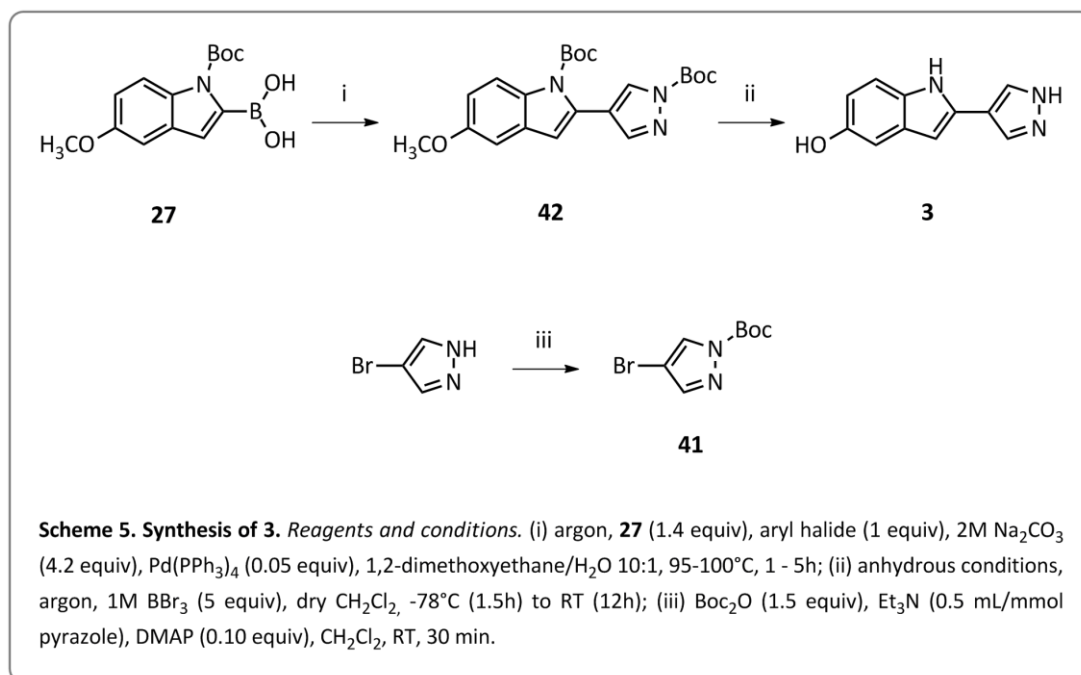

	R_1	R_2	R_3
28	OCH_3	H	H
29	OCH_3	H	CH_3
30	NH_2	H	H
31	$NHCH_3$	H	H
32	NH_2	H	Cl
33	$NHCH_3$	H	Cl
34	OH	H	Cl
35	$NHCH_3$	H	CH_3
36	$NHCH_3$	Cl	H
37	$OCH_2C_6H_5$	Cl	H
38	SO_2NH_2	H	CH_3
39	SO_2NHCH_3	H	CH_3
40	SO_2NH_2	H	F

	R_1	R_2	R_3
2a	OH	H	H
2b	OH	H	CH_3
2c	NH_2	H	H
2d	$NHCH_3$	H	H
2e	NH_2	H	Cl
2f	$NHCH_3$	H	Cl
2g	OH	H	Cl
2h	$NHCH_3$	H	CH_3
2i	$NHCH_3$	Cl	H
2j	OH	Cl	H
2k	SO_2NH_2	H	CH_3
2l	SO_2NHCH_3	H	CH_3
2m	SO_2NH_2	H	F

Scheme 3. Synthesis of 2 a - m. *Reagents and conditions.* (i) argon, **27** (1.4 equiv), aryl halide (1 equiv), 2M Na_2CO_3 (4.2 equiv), $Pd(PPh_3)_4$ (0.05 equiv), 1,2-dimethoxyethane/H_2O 10:1, 95-100°C, 1 - 5h; (ii) anhydrous conditions, argon, 1M BBr_3 (4 to 9 equiv), dry CH_2Cl_2, -78°C (1.5h) to RT (12h).

The reaction conditions illustrated allowed achieving the coupling intermediates **28-40** in good to excellent yields (39-100%). The methyl anilines **14-17** to obtain derivatives **31**, **33, 35** and **36** were synthesized as previously mentioned (**Scheme 2**). [72] Conversely, for intermediates **38** and **39**, the benzenesulfonamides **18** and **19** were synthesized in quantitative yields starting from the corresponding sulfonyl chloride and the appropriate amine, according to the procedure reported in **Scheme 4**. [74]

Scheme 4. Synthesis of sulfonamides 18 - 19.
Reagents and conditions. (i) amine, CH₃CN, 0°C to RT, 10 min.
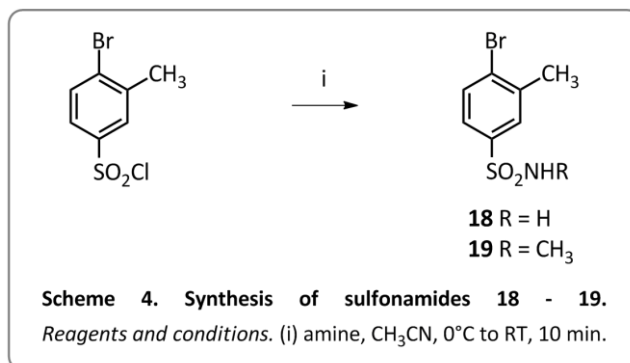
Methoxide and Boc groups were simultaneously removed by treatment of intermediates **28-40** with a solution of boron tribromide to provide the target compounds **2a–m**.
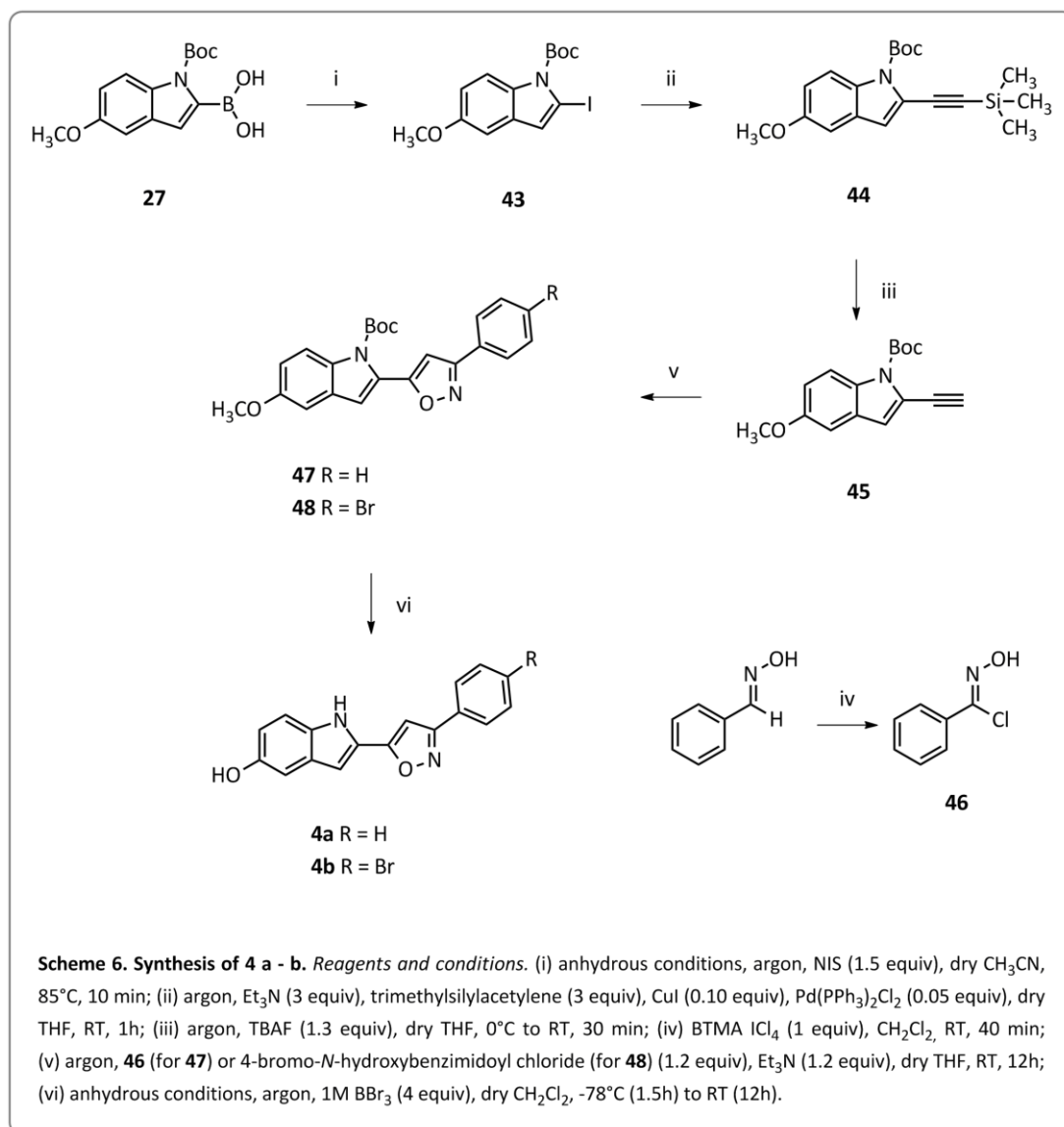
Finally, compound **3** was synthesized applying the Suzuki coupling to the Boc intermediate **41**, quantitately prepared from 4-bromopyrazole according to a common literature procedure, [75] followed by the usual deprotection (**Scheme 5**).

Scheme 5. Synthesis of 3. *Reagents and conditions.* (i) argon, **27** (1.4 equiv), aryl halide (1 equiv), 2M Na₂CO₃ (4.2 equiv), Pd(PPh₃)₄ (0.05 equiv), 1,2-dimethoxyethane/H₂O 10:1, 95-100°C, 1 - 5h; (ii) anhydrous conditions, argon, 1M BBr₃ (5 equiv), dry CH₂Cl₂, -78°C (1.5h) to RT (12h); (iii) Boc₂O (1.5 equiv), Et₃N (0.5 mL/mmol pyrazole), DMAP (0.10 equiv), CH₂Cl₂, RT, 30 min.

3.1.1.3 Synthesis of compounds 4a-b

The synthetic route to indole derivatives **4a** and **4b** is illustrated in **Scheme 6.** Despite the several procedures reported in literature, [76, 77] direct iodination of the carbon 2 of 5-methoxyindole was not successful. Therefore, the iodide intermediate **43** was obtained by treatment of the boronic acid **27** with *N*-iodosuccinimide (NIS) in acetonitrile. [78, 79] Subsequent Sonogashira coupling between **43** and trimethylsilylacetylene in presence of bis(triphenylphosphine)palladium(II) dichloride, copper iodide and

trimethylamine afforded the protected alkyne derivate **44** quantitatively. In order to obtain the free alkyne key intermediate **45**, the silyl protecting group of **44** was removed in strong acidic condition by treatment with tetrabutylammonium fluoride.

Scheme 6. Synthesis of 4 a - b. *Reagents and conditions.* (i) anhydrous conditions, argon, NIS (1.5 equiv), dry CH₃CN, 85°C, 10 min; (ii) argon, Et₃N (3 equiv), trimethylsilylacetylene (3 equiv), CuI (0.10 equiv), Pd(PPh₃)₂Cl₂ (0.05 equiv), dry THF, RT, 1h; (iii) argon, TBAF (1.3 equiv), dry THF, 0°C to RT, 30 min; (iv) BTMA ICl₄ (1 equiv), CH₂Cl₂, RT, 40 min; (v) argon, **46** (for **47**) or 4-bromo-*N*-hydroxybenzimidoyl chloride (for **48**) (1.2 equiv), Et₃N (1.2 equiv), dry THF, RT, 12h; (vi) anhydrous conditions, argon, 1M BBr₃ (4 equiv), dry CH₂Cl₂, -78°C (1.5h) to RT (12h).

The 1,3-dipolar cycloaddition between the dienophile alkyne **45** and the appropriate nitrile oxides*, in situ* by dehydrochlorination of the corresponding hydroximoyl chlorides, [80] led to the formation of the corresponding 3,5-disubstituted-1,2-isoxazole **47** and **48**. Hydroximoyl chlorides are the stable precursor of the 1,3-dipole nitrile oxide and are readily prepared by treating the corresponding aldehyde oxime with benzyl(trimethyl)ammonium tetrachloroiodate (BTMA ICl₄). [65] Finally, the methoxide and Boc groups, were cleaved by reacting intermediates **47** and **48** with boron tribromide, affording the desired compounds **4a** and **4b**.

3.1.2 Genistein derivatives 5a-c

The synthetic approaches to the novel genistein-related derivatives **5a-c**, adapted from known literature procedures, are depicted in **Schemes 7** and **8**. Attempts to obtain an enamino ketone by reacting *N,N*-dimethylformamide dimethyl acetal directly with **49** were not successful, presumably because dimethylformamide dimethyl acetal is capable of reacting with phenols. [81, 82] Therefore, protection of two hydroxyl substituents in the triol moiety was considered to be crucial. **Scheme 7** illustrates the general optimized synthetic approach applied to achieve the desired key intermediate 3-iodochromone **52** through a three steps synthetic procedure. Commercially available trihydroxyacetophenone **49** was first protected by treatment with 2-methoxyethoxymethyl chloride (MEMCl) and *N,N*-diisopropylethylamine (DIPEA) to give the desired protected intermediate **50**, which was subsequently and quantitatively converted into the corresponding enamino ketone **51** by reaction with dimethylformamide dimethyl acetal (DMF/DMA). The reaction was explored heating either with the support of the microwaves or in routine conditions (oil bath), and no significant differences were observed. In presence of iodine, **51** underwent tandem cyclization and iodination to provide **52**. [81]

Scheme 7. Synthesis of 52. *Reagents and conditions.* (i) anhydrous conditions, argon; 1. DIPEA (2.83 equiv), dry CH_2Cl_2, 0°C, 30'; 2. MEMCl (2.36 equiv), 0°C, 1h; (ii) DMF/DMA (1 equiv), MW, 85°C, 1h; (iii) pyridine (1.2 equiv), iodine (0.9 equiv), $CHCl_3$, RT, 12h.
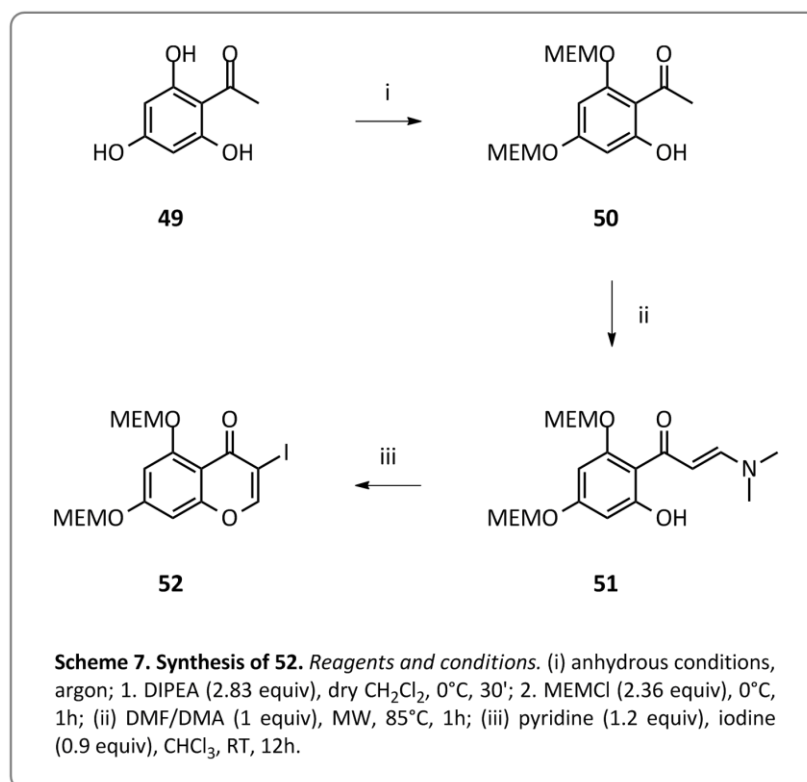
In addition to the desired product, the reaction with iodine caused the formation of a phenyl ring iodinated by-product. The amount of by-product was limited, even if not completely avoided, by reducing the amount of iodine added from an equimolar to 0.9:1 ratio with intermediate **51** and its portionwise addition to the reaction mixture. The 3-iodochromone **52** is a key intermediate in the synthesis of the isoflavones because of the

excellent electrophilic properties of iodine and its strategic position next to the double bond, which favor palladium catalyzed Suzuki reaction to insert the final ring of the isoflavones (**Scheme 8**). [83] Compound **52** was reacted with the appropriate phenyl boronic acid in basic medium using palladium on charcoal as catalyst to give the corresponding protected intermediates **53-55**. If not commercially available, boronic acid intermediates were obtained reacting the corresponding aryl halides with triisopropyl borate. [84]

	R_1	R_2
53	OH	H
54	OCH_3	CH_3
55	NH_2	H

	R_1	R_2
genistein	OH	H
5a	OH	CH_3
5b	OCH_3	CH_3
5c	NH_2	H

Scheme 8. Synthesis of genistein and derivatives 5 a - c. *Reagents and conditions.* (i) anhydrous conditions, argon, phenylboronic acid (2 equiv), Na_2CO_3 (3 equiv), Pd/C (10% w/w), DME/H_2O 1:1, 80-90°C, 1 - 3h; (ii) 12N HCl, CHCl$_3$/MeOH 1:1, 65°C, 1h (**genistein** and **5b**) or anhydrous conditions, argon, 1M BBr$_3$ (7 equiv), dry CH_2Cl_2, -78°C (1h) to RT (2h) (**5a**) or 4M HCl in 1,4-dioxane, RT, 2h (**5c**).

Cleavage of the ether MEM protecting groups of intermediates **53-55** to afford the target compounds **5a-c** was performed in strong acidic conditions, in presence of hydrochloric acid or boron tribromide. Refluxing **53** with concentrate hydrochloric acid provided the model compound genistein which was synthesized to obtain comparable pharmacological data. Depending on the different reaction conditions, deprotection of the intermediate **54** afforded derivatives **5a** or **5b**: treatment with boron tribromide led to cleavage of both the MEM and the methoxide groups thus affording **5a**, while**,** by reaction with concentrate hydrochloric acid, cleavage of only MEM groups was achieved leading to **5b**. Finally, the aminoalchol **5c**, together with salification of the aniline, was obtained from intermediate **55** by using hydrochloric acid.

3.2 Synthetic approaches to the Silent agonist derivatives

3.2.1 diEPP derivatives

3.2.1.1 Synthesis of compounds 6a-u

The experimental work on diEPP derivatives described in this thesis has been carried out in Gainesville (FL, USA) under the supervision of Dr. Nicole A. Horenstein during my one year stay at the Department of Chemistry, University of Florida as a visiting researcher.

diEPP derivative **6a-q** and **6t-u** were synthesized applying the two steps synthetic protocol depicted in **Scheme 9**.

		R_1	R_2	R_3			R_1	R_2	R_3
57	6a	CH_3	H	H	66	6j	H	F	H
58	6b	H	CH_3	H	67	6k	Cl	H	H
59	6c	H	H	CH_3	68	6l	H	Cl	H
60	6d	OCH_3	H	H	69	6m	H	H	Cl
61	6e	H	OCH_3	H	70	6n	Br	H	H
62	6f	OCH_3	H	OCH_3	71	6o	H	Br	H
63	6g	CF_3	H	H	72	6p	CN	H	H
64	6h	H	CF_3	H	73	6q	H	CN	H
65	6i	F	H	H	74	6t	H	OH	H

Scheme 9. Synthesis of 6 a - u. *Reagents and conditions.* (i) anhydrous conditions, argon, 1-ethylpiperazine (1.5 equiv), aryl halide (1 equiv), K_2CO_3 (halide = I) or K_3PO_4 (halide = Br) (2 equiv), CuI (0.10 equiv), *L*-proline (0.20 equiv), dry DMSO, 90-100°C, 14h to 112h and 30 min; (ii) EtI (7 equiv), dry THF, 80-90°C, 17h to 68h and 30 min.

The key reaction involved the appropriate substituted aryl halides and 1-ethylpiperazine, which were reacted in a copper-catalyzed Ullman-type reaction to obtain formation of a carbon-nitrogen bond in presence of the catalytic complex formed by copper iodide and *L*-proline, accordingly to a procedure previously mentioned, to provide the coupled intermediates **57-75**. Depending on the different reactivity of the halides towards the coupling reaction, potassium carbonate or potassium phosphate tribasic was used as base. Indeed, aryl bromides, being less reactive, need stronger basic condition to efficiently undergo the desired coupling. [66, 67, 68, 69] The coupling reaction was inhibited by sterically hindered aryl halides, like ortho-substituents (**59** and **69**), and this inhibition resulted to be greater compared to *N*-aryl-5-hydroxyindoles. Aryl iodides usually provided better coupling yields (52-83%) than bromides (20-64%). The coupled piperazines **57-75** were converted into the corresponding quaternary ammonium salts **6a-q** and **6t-u** by reaction with iodoethane. The carboxamide derivatives **6r** and **6s** were obtained by hydration of the corresponding nitriles **6p** and **6s** in presence of acetaldoxime and tetrakis(triphenylphosphine)palladium(0) (**Scheme 10**). [85]

	R_1	R_2
6p	CN	H
6q	H	CN

	R_1	R_2
6r	$CONH_2$	H
6s	H	$CONH_2$

Scheme 10. Synthesis of 6r and 6s. *Reagents and conditions.* (i) anhydrous conditions, argon, acetaldoxime (2 equiv), Pd(PPh₃)₄ (0.05 equiv), EtOH, reflux, 63 - 87h.
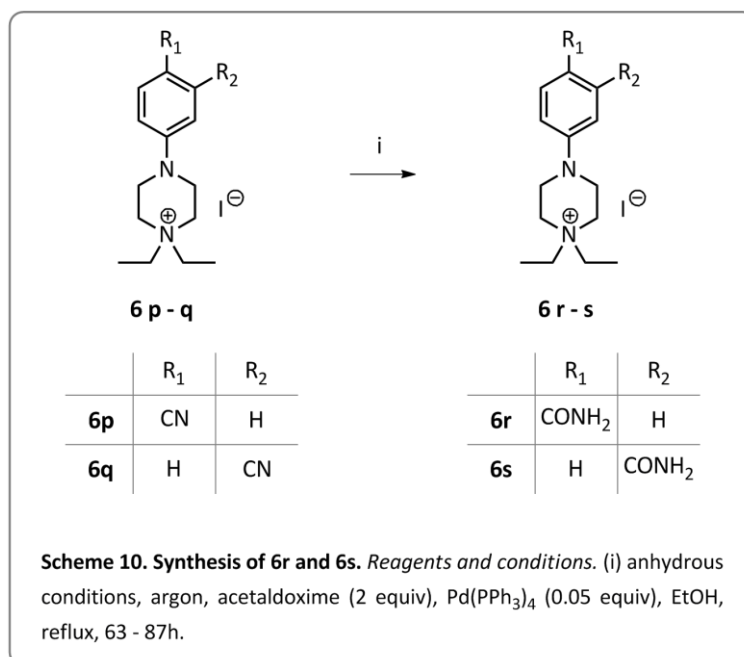
3.2.1.2 Synthesis of compound 7

To achieve the phenylamino diEPP analogue **7** (**Scheme 11**), commercially available 1-ethyl-4-piperidone and aniline underwent a one-pot reductive amination in presence of sodium cyanoborohydride, at pH 6-7, to give intermediate **76**. [86] The pH discriminates the ratio of reduction between imine and carbonyl compounds induced by sodium cyanoborohydride: at pH of 3-4 carbonyl compounds are readily reduced to alcohols, while at 6-7 reductive amination selectively happens. [87] Protection of the aromatic amino group of **76** was obtained with trifluoroacetic anhydride (TFAA) and triethylamine, [88]

affording intermediate **77**, which was subsequently reacted with iodoethane to provide the quaternary ammonium salt **78**. Finally, cleavage of the trifluoroacetyl group refluxing **78** with potassium carbonate in a mixture of methanol-water [88, 89] led the desired final compound **7**.

Scheme 11. Synthesis of 7. *Reagents and conditions.* (i) anhydrous conditions, argon, 1-ethyl-4-piperidone (1.2 equiv), aniline (1 equiv), NaBH$_3$CN (1.2 equiv), AcOH to pH 6-7, dry MeOH, RT to 65°C, 7d; (ii) anhydrous conditions, argon, TFAA (3 equiv), Et$_3$N (4 equiv), dry CH$_2$Cl$_2$, 0°C, 5h; (iii) EtI (7 equiv), copper metal, dry THF, 90°C, 22h; (iv) K$_2$CO$_3$ (12 equiv), MeOH/H$_2$O 7:1, 65°C, 1h.
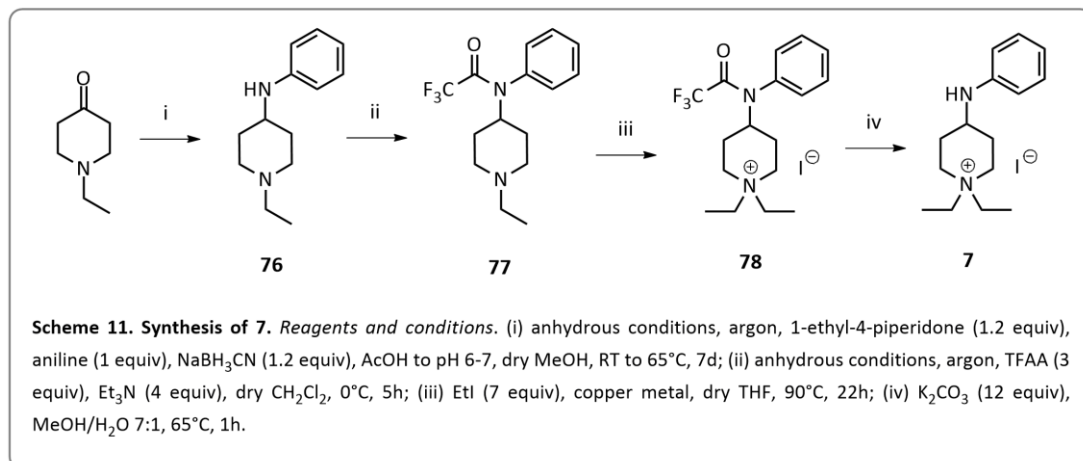
3.2.1.3 Synthesis of compound 8

The diEPP derivative **8** was synthesized according to the synthetic procedure reported in **Scheme 12**, with the key step being represented by the Wittig-Horner reaction between 1-ethyl-4-piperidone and diethyl benzylphosphonate in presence of sodium hydride and [15-crown-5] to give the alkene intermediate **79**. [90] The crown ether was found to be essential to accelerate the reaction rate by complexing the cation from sodium hydride. The olefinated product **79** was then converted into the corresponding quaternary ammonium salt by treatment with iodoethane, affording the final product **8**.

Scheme 12. Synthesis of 8. *Reagents and conditions.* (i) 1-ethyl-4-piperidone (1 equiv), diethyl benzylphosphonate (1.5 equiv), NaH (1.5 equiv), 15-Crown-5 (0.2 equiv), dry THF, 0°C to RT, 4d; (ii) EtI (7 equiv), copper metal, EtOH, 90°C, 51h.
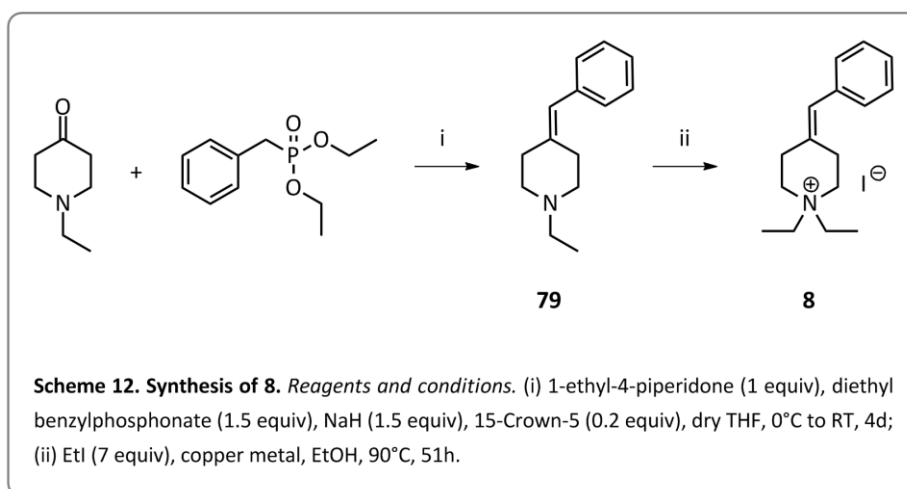
3.2.2 Quinuclidine derivatives

3.2.2.1 Synthesis of compounds 9a-h and 10a-h

The key intermediate to derivatives **9a-h** and **10a-h** is represented by bromo-Δ^2–isoxazoline **83**, synthesized exploiting a 1,3-dipolar cycloaddition-based approach by reacting the dipolaphile **81** and the 1,3-dipole nitrile oxide, *in situ* generated from its stable precursor 1,1-dibromoformaldoxime (**82**) (**Scheme 13**). [64]

Scheme 13. Synthesis of 83. *Reagents and conditions.* (i) 1. anhydrous conditions, argon, $CH_3P^+(C_6H_5)_3$ Br- (1.5 equiv), $(CH_3)_3COK$ (1.4 equiv), dry Et_2O, 0°C to 35°C, 45 min; 2. 3-quinuclidinone (1 equiv), dry Et_2O, 0°C to RT, 12h; (ii) argon, 1M BH_3 (1 equiv), dry THF, 0°C to RT, 1.5h; (iii) **82** (3 equiv), K_2CO_3 (5 equiv), EtOAc, RT, 24h; (iv) 1. glyoxylic acid (1 equiv), NH_2OH HCl (1 equiv), H_2O, RT, 20h; 2. $NaHCO_3$ (2.07 equiv), Br_2 (1.4 equiv), CH_2Cl_2, 0°C to RT, 3h.

Quinuclidin-3-one underwent a Wittig-Horner reaction with the appropriate ylide in a basic medium to afford quantitatively the alkene derivative **80**. Without further purification, 3-methylenequinuclidine **80** was subsequently reacted with borane in tetrahydrofuran complex to obtain the corresponding boranyl protected intermediate **81**. The boron complex **81** was converted into the spirocyclic derivative **83** by virtue of a 1,3-dipolar cycloaddition reaction with **82**, synthesized from glyoxylic acid, in presence of potassium carbonate. [64] The key intermediate **83** underwent, in basic medium, efficient nucleophilic displacement of the bromine atom by suitably meta-substituted benzyl alcohols to provide the corresponding isoxazolines **84-90** and the free base derivative **94** (**Scheme 14**).

Scheme 14. Synthesis of 91 - 98. *Reagents and conditions.* (i) anhydrous conditions, argon, 1. 3-R-benzyl alcohol (1.5 equiv), NaH 60% (1.65 equiv), dry THF, 0°C to RT, 30 min ; 2. **83** (1 equiv), dry THF, 0°C to RT, 2 h; (ii) argon, CF$_3$COOH (5 equiv), acetone, 0°C to RT, 12 h.
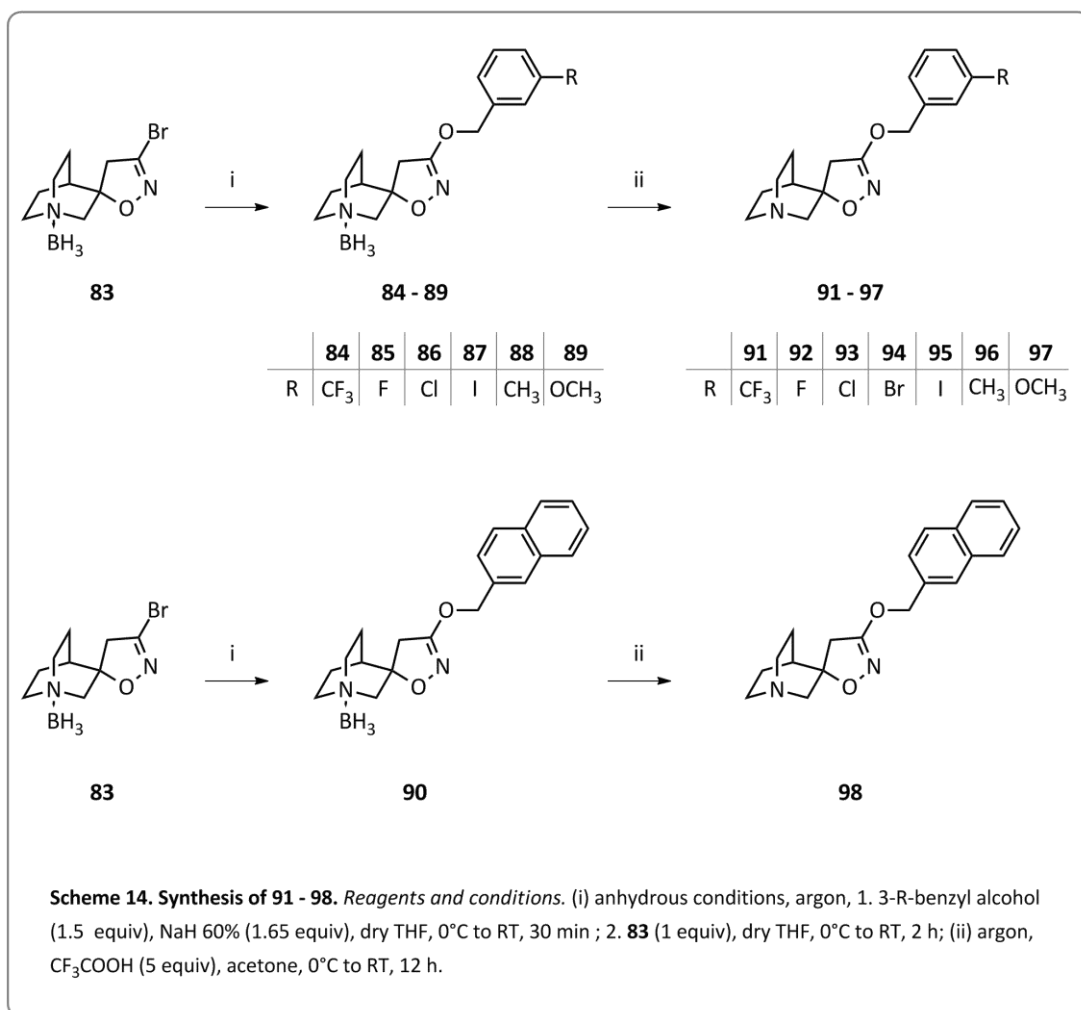
The nucleophilic substitution reaction between **83** and the appropriate alcohol fairly proceeded in presence of sodium hydride. The synthesis of **94** was performed with potassium carbonate in acetonitrile and directly afforded the tertiary amine base since the cleavage of the boranyl group occurred simultaneously. Treatment of intermediates **84-90** with trifluoroacetic acid in acetone readily afforded the corresponding quinuclidine free bases **91-93** and **95-98** in quantitative yields. [64] Tertiary amines **91-98** were treated with fumaric acid to provide the related crystalline salts **9a-h** or, alternatively, with methyl iodide to give the corresponding methyl quaternary ammonium salts **10a-h** (**Scheme 15**). [64]

	91	92	93	94	95	96	97
R	CF$_3$	F	Cl	Br	I	CH$_3$	OCH$_3$

	a	b	c	d	e	f	g
R	CF$_3$	F	Cl	Br	I	CH$_3$	OCH$_3$

Scheme 15. Synthesis of 9 a - h and 10 a - h. *Reagents and conditions.* (i) fumaric acid (1 equiv), MeOH, 2h, RT; (ii) CH$_3$I (8 equiv), MeOH, 12h, RT.
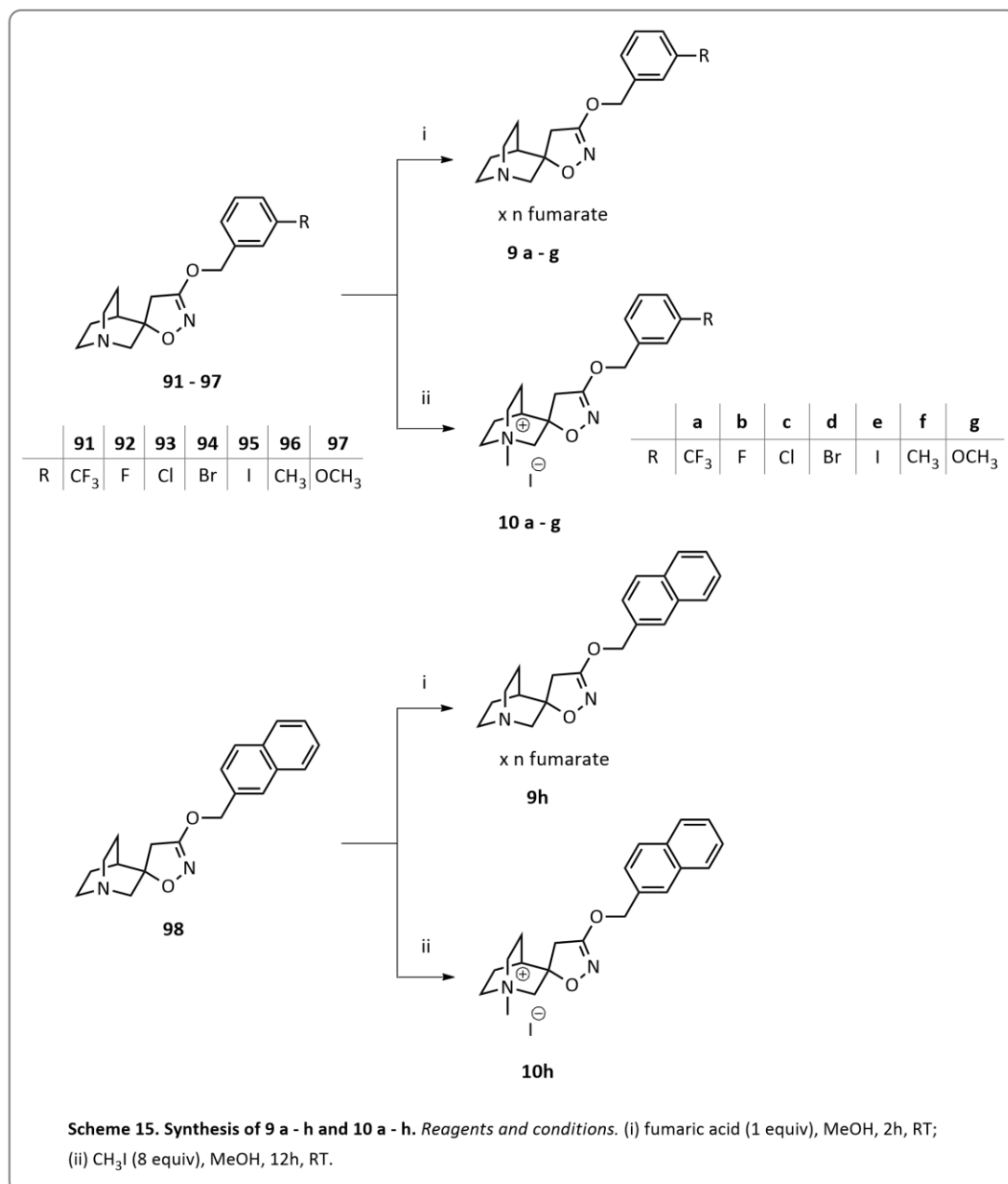
3.2.2.2 Synthesis of compounds 11a-m and 12a-g

Derivatives **11a-m** and **12a-m** were synthesized exploiting the procedure depicted in **Schemes 16-20**. First, quinuclidin-3-one was converted into methyl quinuclidinylidene acetate **99** by means of a Wittig-Horner reaction with trimethyl phosphonoacetate and sodium hydride (**Scheme 16**). [91] The Wittig olefination provided the desired alkene in a mixture of the two isomers (*E*) and (*Z*), which were not separated and directly treated with hydrogen in presence of palladium on charcoal catalyst to achieve reduction of the olefinic double bond and obtain intermediate **100**. [92]

Scheme 16. Synthesis of 101. *Reagents and conditions.* (i) 1. anhydrous conditions, argon, $(CH_3O)_2P(O)CH_2CO_2CH_3$ (1.2 equiv), NaH 60% (1.2 equiv), dry THF, 0°C to RT, 1h; 2. quinuclidin-3-one (1 equiv), RT, 12h; (ii) argon, H_2, Pd/C 10%, MeOH, RT, 1h; (iii) argon, 1M BH_3 (1 equiv), dry THF, 0°C to RT, 1.5h.
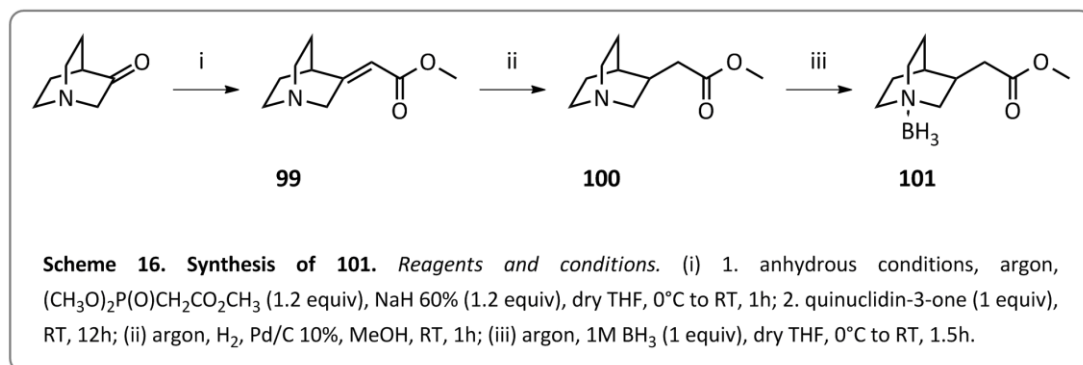
Similarly to the previous series approach, protection of the basic center of the quinuclidine ring with borane complex provided the *N*-boranyl methyl ester **101**. When the protection was performed on intermediate **99**, the subsequent reduction hardly occurred, while cleavage of the boranyl protecting group happened. The key step to the final compounds **11a-m** and **12a-m** is represented by a cycloaddition reaction between methyl ester **101** and variously substituted amidoximes **102-114** (**Figure 27**). At first, methyl ester **100** was directly reacted with the appropriate amidoxime to gain the corresponding 1,2,4-oxadiazole derivative, but poor reaction yields and tricky purification steps occurred.

The appropriate amidoximes **102-114** were either purchased or readily and quantitatively synthesized from the corresponding nitrile precursors according to a general standard literature procedure, by refluxing them with hydroxylamine (**Scheme 17**). [91]

Scheme 17. Synthesis of amidoxime. *Reagents and conditions:* (i) NH_2OH (4 equiv), EtOH, 90°C, 1h.
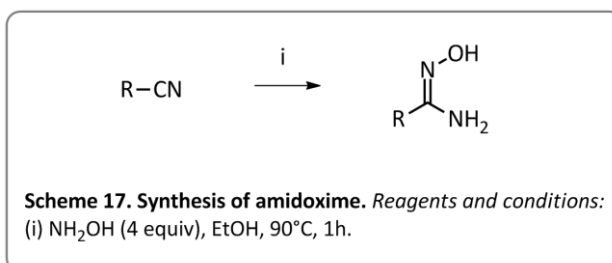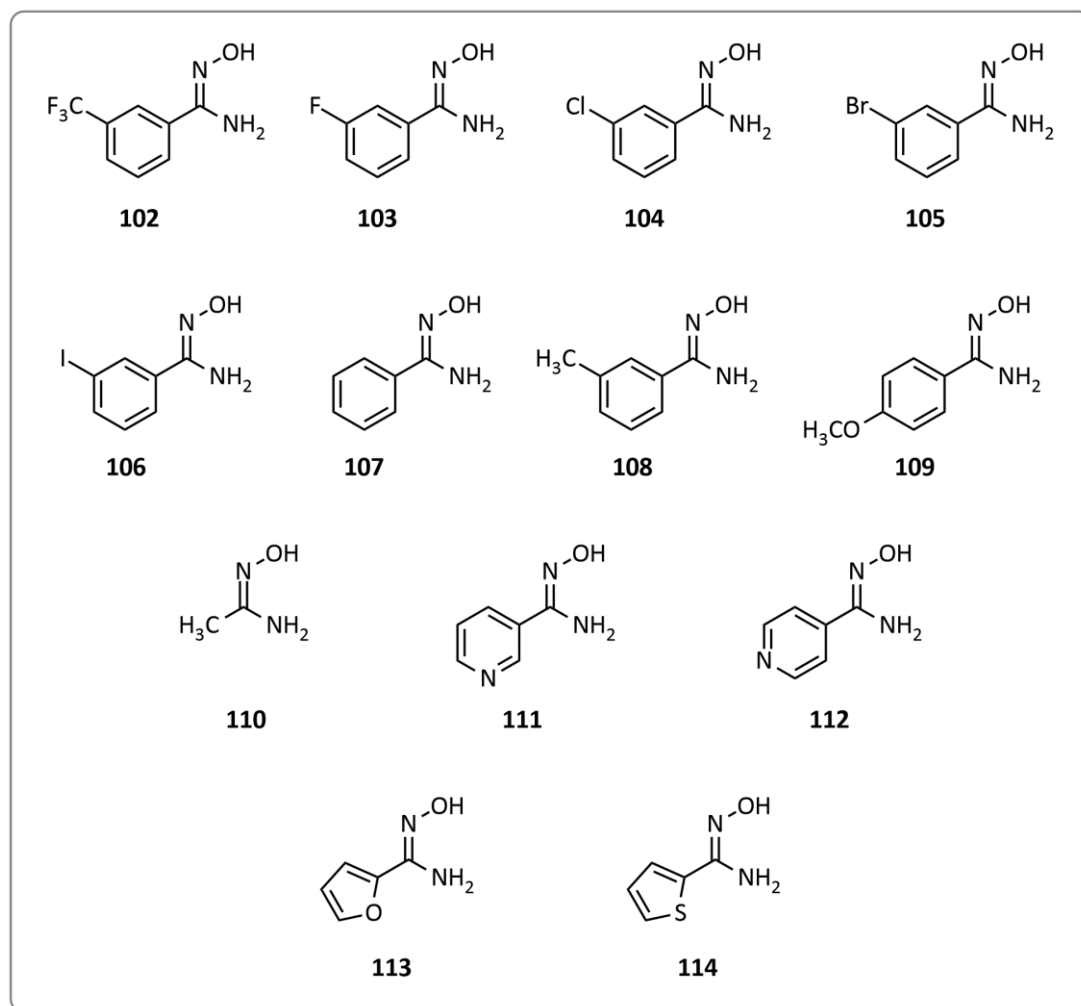
Figure 27. *Amidoximes used in the synthesis of 1,2,4-oxadiazoles derivatives.*

Oxadiazole intermediates **115-126** were synthesized, according to the general protocol depicted in **Scheme 18**, by reacting the free quinuclidine base ester **100** or the *N*-boranyl methyl ester **101** with the appropriate amidoxime in presence of an excess of a base, either sodium hydride or cesium carbonate. While successfully affording the oxadiazole compound in most cases (**115**, **123-126**), sodium hydride protocol provided intermediates **116-121** only in traces (less than 10% yield) and **122** poorly. Therefore, for compounds **116-122** the use of cesium carbonate was successfully explored and the coupling reaction of amidoximes **103-109** with the methyl ester **101** proceeded smoothly, affording the desired 1,2,4-oxadiazole intermediates **116-122** in high yields. Besides providing the desired derivatives in greater quantities, cesium carbonate allowed the reaction to occur in non-anhydrous conditions and in presence of air atmosphere.
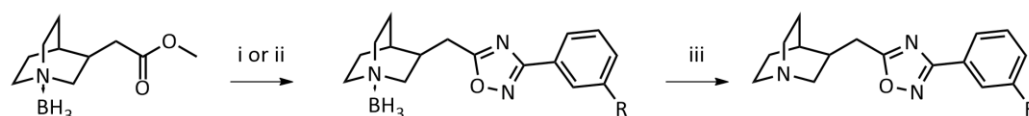
R	CF$_3$	F	Cl	Br	I	H	CH$_3$
	115	116	117	118	119	120	121
	127	128	129	130	131	132	133
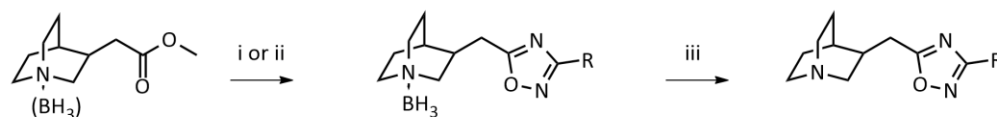
R	4-methoxyphenyl	CH$_3$	pyridin-3-yl	pyridin-4-yl
	122		123	124
	134	135	136	137
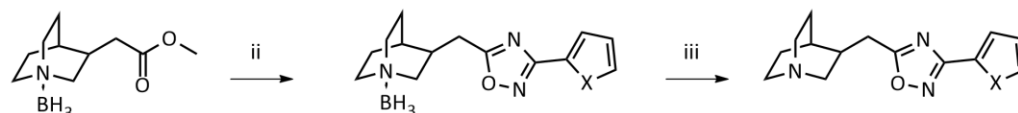
X	O	S
	125	126
	138	139

Scheme 18. Synthesis of 127 - 139. *Reagents and conditions.* (i) 1. **103 - 109** (3 equiv), Cs$_2$CO$_3$ (3 equiv), THF, 50°C, 20 min; 2. **101** (1 equiv), 80°C, 12h; (ii) 1. anhydrous conditions, argon, **102** or **110 - 112** or **125 - 126** (3 equiv), molecular sieves, dry THF, RT, 30 min; 2. NaH 60% (3 equiv), 0°C to RT, 20 min; 3. **101** or **100** (for **110**) (1 equiv), 80°C, 2h; (iv) argon, CF$_3$COOH (5 equiv), acetone, 0°C to RT, 12h.

Treatment of intermediates **115-126** with trifluoroacetic acid in acetone afforded the quinuclidinyl free bases **127-139** quantitatively, which were then reacted either with fumaric acid or methyl iodide in methanol to give, respectively, the desired final fumaric salts **11a-m** (**Scheme 19**) or the quaternary methyl ammonium derivatives **12a-g** (**Scheme 20**). The planned methyl derivative **12h-m** have not been achieved yet.

127 - 133

	127	**128**	**129**	**130**	**131**	**132**	**133**
R	CF$_3$	F	Cl	Br	I	H	CH$_3$

11 a - g

11	**a**	**b**	**c**	**d**	**e**	**f**	**g**
R	CF$_3$	F	Cl	Br	I	H	CH$_3$

134 - 137

	134	**135**	**136**	**137**
R	4-methoxy-phenyl	CH$_3$	pyridin-3-yl	pyridin-4-yl

11 h - k

11	**h**	**i**	**j**	**k**
R	4-methoxy-phenyl	CH$_3$	pyridin-3-yl	pyridin-4-yl

138 - 139

	138	**139**
X	O	S

11 l - m

11	**l**	**m**
X	O	S

Scheme 19. Synthesis of 11 a - m. *Reagents and conditions.* (i) fumaric acid (1 equiv), MeOH, 2h, RT.

127 - 133

	127	**128**	**129**	**130**	**131**	**132**	**133**
R	CF$_3$	F	Cl	Br	I	H	CH$_3$

12 a - g

12	**a**	**b**	**c**	**d**	**e**	**f**	**g**
R	CF$_3$	F	Cl	Br	I	H	CH$_3$

Scheme 20. Synthesis of 12 a - g. *Reagents and conditions.* (i) CH$_3$I (8 equiv), MeOH, 12h, RT.

CHAPTER IV

Results and discussion

4.1 General pharmacological considerations

The pharmacological assays performed on the novel derivatives synthesized during this experimental PhD project were carried out in the research group coordinated by Prof. Roger L. Papke at the Department of Pharmacology and Therapeutics, College of Medicine, University of Florida, Gainesville. Here are reported the pharmacological results available only for some target compounds, because many derivatives are still under investigation.

The alpha 7 activity profile of the tested compounds was evaluated in electrophysiological assays using human alpha7 nAChRs expressed in *Xenopus* oocytes and two electrode voltage clamping.

As previously mentioned in the introduction chapter, the relationship between the peak current and the net charge evoked by agonist application to the alpha7 receptor depends on the effective agonist concentration applied. This phenomenon is ascribable to the concentration-dependent desensitization of this receptor subtype induced by high levels of agonist bind site occupancy and permits to estimate the EC_{50} (half maximal effective concentration) and I_{max} (maximal inhibition) values for a tested drug from the single-concentration responses evoked by application of the compound. [93] For each compound, the ratio of net charge to peak current allows estimation of the functional concentration, which correspond to the EC_{50} for that compound. The efficacy of a compound can be estimated from the comparison between the net charge response of the compound to the expected value of net charge response obtained by a full agonist applied at the estimated functional concentration. In the investigation of the agonist activity of a novel compound, the experiments were carried out using acetylcholine at a concentration of 60 μM as full agonist; this specific concentration corresponds to the EC_{80} for the net charge response of ACh. Therefore, peak currents and net charges measured for a specific compound and normalized for the ACh control offer an idea of the relationship between the compound concentration tested (i.e. 30 μM) and its EC_{80}. The concentration of the tested compound which leads to a ratio of the normalized measures equaled one would correspond to the same effective concentration as 60 μM ACh. The potency of a compound tested at a specific concentration can be estimated by the relative value of the net charge response and varies independently from the compound efficacy. Specifically, compounds with a high peak current to net charge ratio, but small net charge are likely to be less efficacious than less potent. The explanation for the present consideration derive from the alpha7 receptor synchronization which causes extremely sharp peak responses with minimal net charge area, [25] resulting in an over-estimation of the response when evaluated considering the peak current.

Depending on the specific aim of the investigation, the electrophysiological assays performed followed different protocols and the main features are here summarized.

ALPHA7 AGONIST ACTIVITY. Experiments to test the agonist activity of a compound consist in two initial controls, the test and a control at the end. All the controls involve application of ACh at a concentration of 60 µM, while the test consists in application of 30 µM of the compound. The average response of the two initial controls is taken as reference value to normalize the compound response. The concentration of 30 µM proved to the best value for testing the compound since it is high enough to provide a detectable response and low enough to avoid possible complications, like channel blocking. Finally, the control at the end of the test aims at determining the desensitization or rundown of the alpha7 receptor. For the generation of a concentration-response curve (CRC), ACh controls and experimental compounds applications alternated. The responses evoked at the alpha7 nAChR were quantified as peak current and net charge and analyzed.

POSITIVE ALLOSTERIC MODULATION OF THE ALPHA7 nAChR. Experiments to test the allosteric modulation induced by the tested compound consist in the evaluation of first the agonist profile followed by the proper allosteric modulation. The test to assess the agonist activity is performed in the same conditions depicted in the previous subchapter, applying 30 µM of tested compound. In the investigation of the allosteric component, the test aims at determining the rate of potentiation in the receptor response evoked by the putative allosteric modulator co-application. The test consists of two initial controls, the test and a control at the end. All the controls involve application of ACh at a concentration of 60 µM, while the test consists in a co-application of 60 µM of ACh with usually 30 µM of tested compound, or even higher compound concentrations. The average response of the two initial controls is taken as reference value to normalize the compound response. Finally, the control at the end of the test aims at determining the desensitization or rundown of the alpha7 receptor. For the generation of a concentration-response curve (CRC), ACh controls and experimental compound co-applications alternated. The responses evoked at the alpha7 nAChR were quantified as peak current and net charge and analyzed.

SILENT AGONISM OF THE ALPHA7 nAChR. In order to evaluate the silent agonism profile of the novel derivatives, the partial agonism activity and the potentiated response in presence of the type II PAM PNU-120596 were quantified and compared. The induction of PAM-sensitive desensitization, typical of a silent agonist, was detected evaluating the net charge response evoked by co-application of the tested compound PNU-120596. In details, experiments consist in two initial controls, the test and a control at the end. All the controls involve application of ACh at a concentration of 60 µM, while the test consists in a co-application of 30 µM of the compound with 10 µM of PNU-120596. The average response of the two initial controls is taken as reference value to normalize the compound response. Finally, the control at the end of the test aims at determining the desensitization or rundown of the alpha7 receptor. The corresponding values were reported relative to the control response evoked by 60 µM ACh alone. The responses evoked at the alpha7 nAChR were quantified as peak current and net charge and analyzed.

4.2 Pharmacological evaluation of 5-hydroxyindole derivatives

The alpha7 nAChR profile of the target 5HI *N*-aryl derivatives **1a-e** was defined according to the experimental procedures described in the general section and the results are reported in **Table 1**.

Compound	Agonist response		Agonist potentiated response	
	Peak current	Net Charge	Peak current	Net Charge
5-HI	0.002 ± 0.000	0.000 ± 0.005	1.092 ± 0.178	1.252 ± 0.112
1a	0.006 ± 0.001	0.009 ± 0.007	1.337 ± 0.195	1.200 ± 0.031
1b	0.018 ± 0.011	0.009 ± 0.004	1.064 ± 0.177	1.258 ± 0.086
1c	0.002 ± 0.000	0.000 ± 0.003	1.094 ± 0.232	1.089 ± 0.076
1d	0.009 ± 0.003	0.005 ± 0.002	1.612 ± 0.204	1.242 ± 0.087
1e	0.009 ± 0.003	0.004 ± 0.005	0.977 ± 0.079	0.958 ± 0.029

*Table 1. Electrophysiological data of derivatives **1a-e** on human alpha7 nAChRs expressed in Xenopus oocytes. Data are reported as mean ± SEM (standard error of mean) and relative to the control ACh. Agonist response refers to receptor response to 30 μM application of test compound. Agonist potentiated response refers to receptor response with 60 μM ACh (agonist) and 30 μM test compound co-application.*

The set of data collected on human alpha7 nAChRs expressed by Xenopus oocytes suggests that linking a variously substituted aromatic ring on the nitrogen atom of 5-hydroxyindole (5-HI) does not lead to improvement of the parent compound PAM activity. Indeed, despite not showing agonist activity at the receptor subtype of interest, the tested compounds were not able to further improve the 5-HI agonist potentiated response. However, derivatives **1a**, **1b** and **1d** displayed a PAM activity comparable to the one of the reference compound 5-HI, suggesting that the PAM binding pocket of the alpha7 receptor well tolerate bulky groups on the indole moiety.

The activity profile of the novel 5HI C-aryl derivatives **2a-g** was investigated according to the experimental procedures described in the general section and the results are reported in **Table 2** and **Table 3**.

Differently from the *N*-aryl series, electrophysiological assays data performed on 5-hydoxyindole derivatives **2a-g** showed interesting results. In particular, **2b** (4-hydroxy-2-methylphenyl), **2f** (2-chloro-4-(methylamino)phenyl) and **2g** (2-chloro-4-hydroxyphenyl) derivatives showed enhanced PAM activity compared to the parent compound, thus suggesting that the modifications in C-2 position of the indole system are favourable. Polar substituents in the *para* position of the aryl ring are well-tolerated, but not sufficient to induce PAM activity improvement. Indeed, all derivatives **2a-g** are characterized by the presence of polar groups in *para*, but only the few of them with an additional substitution in the *ortho* position display enhanced PAM activity (**2b**, **2f**, and **2g**). Noteworthy, *para*

substitution *per se* as well as primary aniline lead to suppression of the allosteric component (see data on compounds **2c-e**). From these experimental evidences it is possible to hypothesize that di-substituted phenyl rings linked to position 2 of 5-HI lead to potentiation of the PAM activity of the parent compound. In particular, the best results were obtained with hydroxyl or methylamino groups in *para* and methyl or chloro groups in *ortho* of the aromatic ring, leading to compound **2b** which shows a potentiated activity (net charge) (2.386±0.159) double of the parent compound 5-HI (1.252±0.112) and comparable to the one of genistein (2.733± 0.162).

Compound	Agonist response		Agonist potentiated response	
	Peak current	Net Charge	Peak current	Net Charge
5-HI	0.002 ± 0.000	0.000 ± 0.005	1.092 ± 0.178	1.252 ± 0.112
2a	0.001 ± 0.000	0.002 ± 0.000	0.984 ± 0.035	1.189 ± 0.028
2b	0.009 ± 0.003	0.000 ± 0.002	1.627 ± 0.164	2.386 ± 0.159
2c	0.003 ± 0.002	0.003 ± 0.001	0.994 ± 0.055	1.017 ± 0.018
2d	0.001 ± 0.000	0.000 ± 0.000	1.110 ± 0.062	1.043 ± 0.063
2e	0.002 ± 0.000	0.008 ± 0.004	1.215 ± 0.061	1.057 ± 0.011
2f	0.001 ± 0.000	0.003 ± 0.002	1.457 ± 0.094	1.534 ± 0.069
2g	0.002 ± 0.000	0.003 ± 0.001	2.031 ± 0.225	1.712 ± 0.117

*Table 2. Electrophysiological data of derivatives **2a-g** on human alpha7 nAChRs expressed in Xenopus oocytes. Data are reported as mean ± SEM (standard error of mean) and relative to the control ACh. Agonist response refers to receptor response to 30 μM application of test compound. Agonist potentiated response refers to receptor response with 60 μM ACh (agonist) and 30 μM test compound co-application.*

The most promising compounds were tested at different concentrations (**Table 3**) and the corresponding CRCs were generated (**Figure 28**). According to the data collected, all the three derivatives showed better PAM activity compared to the reference compound at any of the concentrations tested. Compound **2g** is more potent and about twice as efficacious as 5-HI, while derivatives **2b** and **2f** are far more efficacious than the parent compound, but not much more potent.

	Agonist potentiated response							
	5-HI		2b		2f		2g	
	Peak current	Net Charge	Peak current	Net Charge	Peak current	Net Charge	Peak current	Net Charge
3 µM	0.935 ± 0.038	1.014 ± 0.035	0.985 ± 0.071	1.045 ± 0.032	1.532 ± 0.160	1.116 ± 0.031	1.278 ± 0.102	1.091 ± 0.013
10 µM	1.059 ± 0.167	1.018 ± 0.038	1.060 ± 0.052	1.148 ± 0.030	1.131 ± 0.102	1.218 ± 0.018	1.883 ± 0.398	1.611 ± 0.290
30 µM	1.259 ± 0.231	1.084 ± 0.012	1.690 ± 0.284	1.580 ± 0.063	2.041 ± 0.187	1.749 ± 0.058	1.882 ± 0.097	1.828 ± 0.087
100 µM	1.177 ± 0.064	1.153 ± 0.053	2.579 ± 0.256	2.780 ± 0.278	2.660 ± 0.393	3.454 ± 0.175	2.690 ± 0.318	2.662 ± 0.159
300 µM	1.802 ± 0.328	1.526 ± 0.148	3.636 ± 0.202	4.569 ± 0.699	5.545 ± 1.215	6.308 ± 0.269	3.324 ± 0.510	2.960 ± 0.115
1000 µM	1.879 ± 0.199	2.356 ± 0.236	3.893 ± 0.569	6.061 ± 0.761	5.702 ± 2.088	7.368 ± 0.380	2.527 ± 0.146	3.436 ± 0.085

Table 3. *CRCs Electrophysiological data of derivatives **2b, 2f and 2g** on human alpha7 nAChRs expressed in Xenopus oocytes. Data are reported as mean ± SEM (standard error of mean) and relative to the control ACh. Agonist potentiated response refers to receptor response with 60 µM ACh (agonist) and the reported concentration of the test compound co-application.*
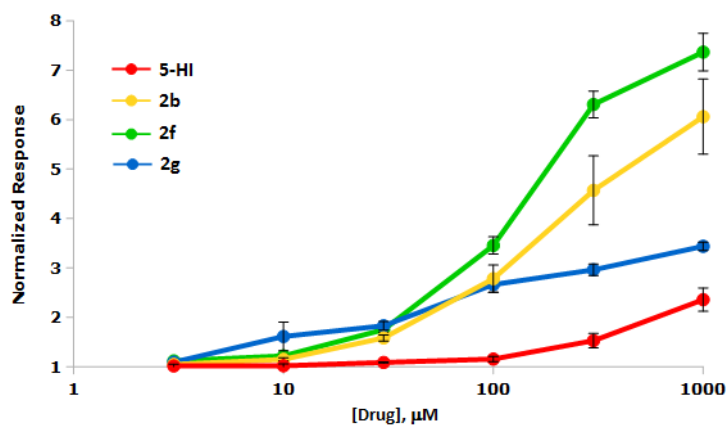
Figure 28. *Net-charge responses collected in CRCs experiments performed in oocytes expressing human α7 receptors for derivatives **2b, 2f** and **2g** compared to 5-HI.*

CRCs data further confirmed the improvement in positive allosteric modulation achieved with the new derivatives **2b**, **2f** and **2g**, both in potency and in efficacy and therefore the relevance of substitutions in *ortho* and *para* positions of the phenyl ring. Starting from them, new modifications were addressed to these highlighted positions by introducing different polar groups in *para* and methyl or halogens in *ortho*, thus obtaining compounds **2h-m**, as previously mentioned in the aim section. These new derivatives, together with **3** and **4a-b**, are currently under investigation to evaluate their PAM activity.

4.3 Pharmacological evaluation of genistein derivatives

The potential positive allosteric modulatory profile of the novel genistein-related derivatives **5a-c** was investigated according to the general protocols illustrated in the general pharmacological consideration section and the results are summarized in **Table 4**. The parent compound genistein was included in the series for comparison.

The novel derivatives were first assessed to determine the corresponding agonist activity. Ideally, the agonist activity of a compound planned to be a PAM should be very low, if not equal to zero. In the case of the present compounds, all the new genistein-related derivatives did not show agonism towards the alpha7 nAChR subtype, therefore becoming candidates for PAM activity investigation.

Compound	Agonist response		Agonist potentiated response	
	Peak current	Net Charge	Peak current	Net Charge
genistein	0.010 ± 0.001	0.004 ± 0.004	2.237 ± 0.323	2.733 ± 0.162
5a	0.008 ± 0.001	0.004 ± 0.004	1.746 ± 0.436	1.811 ± 0.116
5b	0.014 ± 0.002	0.000 ± 0.000	0.946 ± 0.107	1.017 ± 0.031
5c	0.014 ± 0.005	0.002 ± 0.002	0.926 ± 0.107	0.949 ± 0.045

Table 4. *Electrophysiological data of derivatives **5a-c** on human alpha7 nAChRs expressed in Xenopus oocytes. Data are reported as mean ± SEM (standard error of mean) and relative to the control ACh. Agonist response refers to receptor response to 30 μM application of test compound. Agonist potentiated response refers to receptor response with 60 μM ACh (agonist) and 30 μM test compound co-application.*

As reported in **Table 4**, co-application of the tested compound (30 μM) with the control represented by the agonist acetylcholine (60 μM) did not lead to any improvement in the PAM activity compared to the parent compound genistein, which remains the best PAM of the series. Indeed, derivatives **5a**-**c**, when co-applied with ACh, evoked an alpha7 potentiated response lower than the one evoked by genistein. In the least two cases (**5b** and **5c**) no potentiation occurred, since for these two compounds, the net charges measured after co-application with ACh equal the responses evoked by ACh alone. Only compound **5a** was able to induce potentiation in the agonist evoked response, showing a potentiated response close to the one of the parent compound (0.66-fold of the genistein potentiation). Indeed, substitutions with either methoxide or amino groups lead to suppression of the PAM activity of the compound.

4.4 Pharmacological evaluation of diEPP derivatives

The activity profile of diEPP derivatives **6a-u**, **7** and **8** synthesized was evaluated according to the experimental procedures highlighted in the general chapter and the results are summarized in **Tables 5**. The parent compound diEPP was included in the series for comparison. [57]

Compound	Agonist response		Potentiated response	
	Peak current	Net Charge	Peak current	Net Charge
diEPP	0.002 ± 0.001	0.002 ± 0.003	0.347 ± 0.083	1.333 ± 0.334
6a	0.002 ± 0.001	0.011 ± 0.003	2.112 ± 1.191	3.605 ± 2.021
6b	0.005 ± 0.002	0.001 ± 0.000	0.073 ± 0.020	0.228 ± 0.111
6c	0.148 ± 0.013	0.319 ± 0.066	12.220 ± 2.327	16.357 ± 2.448
6d	0.019 ± 0.003	0.063 ± 0.014	6.445 ± 1.971	11.610 ± 0.294
6e	0.010 ± 0.010	0.011 ± 0.011	0.409 ± 0.140	0.592 ± 0.234
6f	0.010 ± 0.006	0.014 ± 0.009	0.635 ± 0.117	1.136 ± 0.072
6g	0.029 ± 0.002	0.032 ± 0.003	27.896 ± 2.555	61.772 ± 7.736
6h	0.002 ± 0.000	0.000 ± 0.002	1.106 ± 0.684	3.061 ± 2.079
6i	0.005 ± 0.001	0.010 ± 0.002	7.121 ± 1.382	22.937 ± 5.771
6j	0.010 ± 0.002	0.061 ± 0.089	0.033 ± 0.005	0.163 ± 0.110
6k	0.168 ± 0.025	0.234 ± 0.048	12.834 ± 4.596	18.252 ± 6.190
6l	0.015 ± 0.002	0.020 ± 0.003	4.694 ± 1.609	4.227 ± 0.991
6m	0.231 ± 0.033	0.367 ± 0.054	26.608 ± 7.557	36.624 ± 6.081
6n	0.310 ± 0.052	0.270 ± 0.017	6.115 ± 0.952	12.347 ± 3.453
6o	0.018 ± 0.005	0.040 ± 0.006	11.853 ± 4.151	9.982 ± 1.744
6p	0.102 ± 0.011	0.234 ± 0.024	47.538 ± 15.479	67.989 ± 25.437
6q	0.041 ± 0.018	0.022 ± 0.024	0.640 ± 0.173	1.493 ± 0.668
6r	0.023 ± 0.004	0.065 ± 0.009	13.996 ± 4.168	43.947 ± 8.505
6s	0.010 ± 0.002	0.056 ± 0.022	10.001 ± 1.890	50.716 ± 8.493
6t	0.191 ± 0.013	0.283 ± 0.013	10.413 ± 2.625	31.444 ± 5.086
6u	0.102 ± 0.022	0.143 ± 0.018	9.709 ± 1.320	15.892 ± 2.256
7	0.003 ± 0.001	0.002 ± 0.004	1.494 ± 0.654	5.611 ± 2.138
8	0.042 ± 0.018	0.071 ± 0.027	5.352 ± 1.044	9.892 ± 3.057

Table 5. *Electrophysiological data of diEPP derivatives **6a-u, 7** and **8** on human alpha7 nAChRs expressed in Xenopus oocytes. Data are reported as mean ± SEM (standard error of mean) and relative to the control ACh. Agonist response refers to receptor response to 30 μM application of test compound. Potentiated response refers to receptor response with 30 μM test compound and 10 μM PNU-120596 co-application.*

According to these results, the electrophysiological profile of the novel diEPP derivatives **6a-u** is greatly influenced by the nature and sensitive to the position of the aryl substituent. Indeed, the tested compounds exhibited a broad range of activities at the alpha7 receptor subtype, including partial agonism and various degrees of silent agonism, while some compounds were effectively inactive, displaying no agonism and very weak silent agonism.

Partial agonists

Among the tested compounds, some derivatives emerged as partial agonists of the alpha7 nAChR. Compounds **6c** (*ortho*-methyl), **6k** (*para*-chloro), **6m** (*ortho*-chloro), **6n** (*para*-bromo), **6p** (*para*-cyano), **6t** (*meta*-hydroxy) and **6u** (2-naphthalene) were identified as alpha7 partial agonists according to their ability to evoke a net charge response, when applied alone, greater than the threshold value of a tenth of the ACh control (**Table 6**).

Compound	Agonist response	
	Peak current	Net Charge
diEPP	0.002 ± 0.001	0.002 ± 0.003
6c	0.148 ± 0.013	0.319 ± 0.066
6k	0.168 ± 0.025	0.234 ± 0.048
6m	0.231 ± 0.033	0.367 ± 0.054
6n	0.310 ± 0.052	0.270 ± 0.017
6p	0.102 ± 0.011	0.234 ± 0.024
6t	0.191 ± 0.013	0.283 ± 0.013
6u	0.102 ± 0.022	0.143 ± 0.018

Table 6. *Electrophysiological data of diEPP derivatives, emerged as partial agonists, on human alpha7 nAChRs expressed in Xenopus oocytes. Data are reported as mean ± SEM (standard error of mean) and relative to the control ACh. Agonist response refers to receptor response to 30 μM application of test compound.*

According to the considerations previously introduced about the alpha7 receptor pharmacology, at a specific functional concentration, a partial agonist would have net charges responses lower than that predicted for a full agonist. As depicted in **Table 3**, compounds **6c**, **6k**, **6m**, **6n**, **6p**, **6t** and **6u** have a ratio peak current to net charge lower than one, thus confirming their partial agonism profile. Among this set of compounds, the **6k** (*para*-chloro) and **6u** (2-naphthalene) diEPP derivatives presented the greatest peak current to net charge ratios, therefore they are the most potent partial agonists of the series. Being a partial agonist, the *meta*-hydroxyl compound **6t**, suggested a unique hydrogen bond interaction of the phenolic group capable of inducing a conducting state of the alpha7 receptor. Based on the observations concerning the estimation of potency and efficacy from peak currents and net charges evoked at the alpha7 receptor, compound **6c** (*ortho*-methyl) and compound **6m** (*ortho*-chloro) are the most efficacious diEPP derivatives of the series, suggesting that *ortho*-substituents of the diEPP moiety promoted alpha7 partial

agonism. Indeed, despite not having the best estimated potency, they show the highest net charge responses. Insertion of a naphthalene group (**6u**) on the diEPP structure was meant to investigate the endurance of the alpha7 binding pocket towards groups with a greater bulkiness. The partial agonism activity of the compound suggests larger groups promote conductive states of the receptor.

Inactive compounds

Within the diEPP set of compounds synthesized, four derivatives showed no significant activity at the selected nicotinic receptor subtype. Indeed, compounds **6b** (*meta*-methyl), **6e** (*meta*-methoxide), **6f** (*ortho, para*-methoxide), **6j** (*meta*-fluoro) and **6q** (*meta*-cyano) lacked of both the partial agonist and the silent agonist activities (**Table 7**).

Compound	Agonist response		Potentiated response	
	Peak current	Net Charge	Peak current	Net Charge
diEPP	0.002 ± 0.001	0.002 ± 0.003	0.347 ± 0.083	1.333 ± 0.334
6b	0.005 ± 0.002	0.001 ± 0.000	0.073 ± 0.020	0.228 ± 0.111
6e	0.010 ± 0.010	0.011 ± 0.011	0.409 ± 0.140	0.592 ± 0.234
6f	0.010 ± 0.006	0.014 ± 0.009	0.635 ± 0.117	1.136 ± 0.072
6j	0.010 ± 0.002	0.061 ± 0.089	0.033 ± 0.005	0.163 ± 0.110
6q	0.041 ± 0.018	0.022 ± 0.024	0.640 ± 0.173	1.493 ± 0.668

Table 7. *Electrophysiological data of inactive diEPP derivatives on human alpha7 nAChRs expressed in Xenopus oocytes. Data are reported as mean ± SEM (standard error of mean) and relative to the control ACh. Agonist response refers to receptor response to 30 μM application of test compound. Potentiated response refers to receptor response with 30 μM test compound and 10 μM PNU-120596 co-application.*

Silent agonists

Within the diEPP series, several substitutions on the aromatic ring of the parent compound led to compounds exhibiting the desired alpha7 silent agonism profile, with little or no partial agonism activity, and a potentiated response evoked by PNU-120596 greater than the parent compound diEPP (**Table 8**). Compounds **6a** (*para*-methyl), **6d** (*para*-methoxide), **6g** (*para*-trifluoromethyl), **6h** (*meta*-trifluoromethyl), **6i** (*para*-fluoro), **6l** (*meta*-chloro), **6o** (*meta*-bromo), **6r** (*para*-carboxamide) and **6s** (*meta*-carboxamide) emerged as alpha7 silent agonists. The two diEPP derivatives **7** and **8**, characterized by a piperidine instead of a piperazines ring, showed enhanced alpha7 silent agonism compared to the parent compound, thus suggesting that the nitrogen atom of the *N*-aryl bond does not play an essential role in silent agonism within the diEPP framework. [57]

	Agonist response		Potentiated response	
Compound	Peak current	Net Charge	Peak current	Net Charge
diEPP	0.002 ± 0.001	0.002 ± 0.003	0.347 ± 0.083	1.333 ± 0.334
6a	0.002 ± 0.001	0.011 ± 0.003	2.112 ± 1.191	3.605 ± 2.021
6d	0.019 ± 0.003	0.063 ± 0.014	6.445 ± 1.971	11.610 ± 0.294
6g	0.029 ± 0.002	0.032 ± 0.003	27.896 ± 2.555	61.772 ± 7.736
6h	0.002 ± 0.000	0.000 ± 0.002	1.106 ± 0.684	3.061 ± 2.079
6i	0.005 ± 0.001	0.010 ± 0.002	7.121 ± 1.382	22.937 ± 5.771
6l	0.015 ± 0.002	0.020 ± 0.003	4.694 ± 1.609	4.227 ± 0.991
6o	0.018 ± 0.005	0.040 ± 0.006	11.853 ± 4.151	9.982 ± 1.744
6r	0.023 ± 0.004	0.065 ± 0.009	13.996 ± 4.168	43.947 ± 8.505
6s	0.010 ± 0.002	0.056 ± 0.022	10.001 ± 1.890	50.716 ± 8.493
7	0.003 ± 0.001	0.002 ± 0.004	1.494 ± 0.654	5.611 ± 2.138
8	0.042 ± 0.018	0.071 ± 0.027	5.352 ± 1.044	9.892 ± 3.057

Table 8. Electrophysiological data of diEPP derivatives, emerged as silent agonists, on human alpha7 nAChRs expressed in Xenopus oocytes. Data are reported as mean ± SEM (standard error of mean) and relative to the control ACh. Agonist response refers to receptor response to 30 μM application of test compound. Potentiated response refers to receptor response with 30 μM test compound and 10 μM PNU-120596 co-application. Highlighted the best silent agonists of the series.

The results collected on the alpha7 receptor suggested that the substituents introduced on the aromatic ring of the parent compound diEPP influenced the silent agonism activity by means of different effects. Compared to the reference compound diEPP, enhancement of the silent agonism may arise from either decrement of the agonism, increase of the potentiated PNU-120596 response induced by greater induction and stabilization of the desensitized state, or both effects.

By modifications of the diEPP moiety several derivatives with enhanced silent agonism were achieved and, in particular, the best results were obtained with substitutions addressed to the *meta* and the *para* positions of the aromatic ring involving small-to-medium size groups. In particular, compounds **6g** (*para*-trifluoromethyl), **6i** (*para*-fluoro), **6r** (*para*-carboxamide) and **6s** (*meta*-carboxamide) showed the best silent agonism profile of the series (**Figure 29**). Despite being quite different in the possible interactions with the receptor binding site, in the diEPP series the trifluoromethyl group, fluorine atoms and carboxamides greatly enhanced the alpha7 silent agonist activity of the parent compound. To attribute the observed profile to a specific common effect, the properties of these functional groups were examined:

- polarity: it was the first property considered due to the presence of fluorine in **6g** and **6i**, and oxygen or amino groups in **6r** and **6s**. However, polarity by itself is not sufficient to fully explain enhanced silent agonism, since not all polar groups induced this behavior, i.e. the hydroxyl group of derivative **6t**;

- lipophilicity: in previously reported cases, fluorine and the trifluoromethyl groups proved to enhance the lipophilicity of a compound. [94] If enhanced lipophilicity was the key to silent agonism improvement, substituents like methyl would increase the silent agonist activity compared to the parent compound, but, within the tested series, both *meta-* (**6b**) and *ortho-*methyl (**6c**) derivatives failed to effectively promote the desired profile, further supporting the hypothesis of polar interactions involved in the silent activity;

- hydrogen bonding: it takes part to protein-ligand binding and while carboxamide groups are known to be good hydrogen bond acceptors, fluorine does not really participate to them. Therefore, if hydrogen bonds were the reasons for the observed profile, carboxamides would show greater improvement than the other groups, and this is not the case.
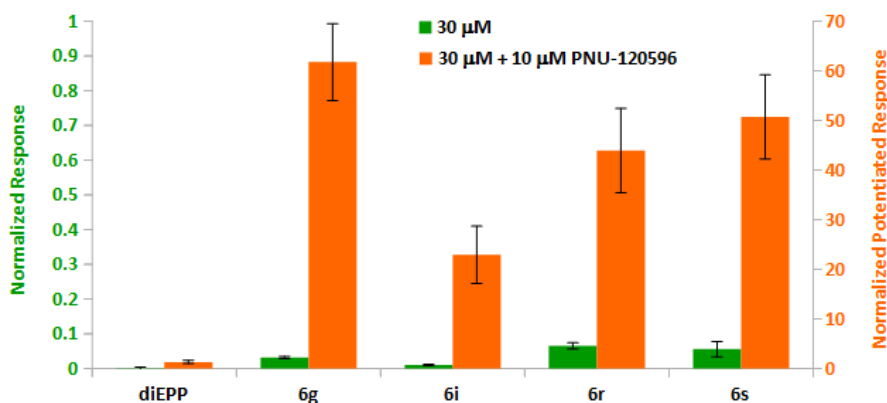
Figure 29. Electrophysiological data of the best silent agonists of the diEPP series. Normalized response refers to receptor response to 30 μM application of test compound. Normalized potentiated response to receptor response with 30 μM test compound and 10 μM PNU-120596 co-application. Data are reported as net charge mean ± SEM (standard error of mean) and relative to the control ACh.

The enhanced silent agonism achieved with trifluoromethyl, fluoro and carboxamide groups arose from a combination of multifactorial effects, which bear in common the ability to selectively stabilize the desensitized state of the alpha7 receptor sensitive to PNU-120596 conferred by point-to-point interactions between the tested compounds and the binding pocket of the receptor.

Introduction of a halogen group in the *meta* position of the diEPP aromatic moiety produced intriguing results and the potentiated response with PNU co-application increased from fluoro to chloro to bromo diEPP derivatives, therefore halogen bonding interactions were accounted for silent agonism. The halogen bond consists in a noncovalent interaction occurring between a covalently bonded halogen atom and a negatively charged atom in another molecule. [95, 96] Polarizability and electronegativity of the halogen involved affect the strength of the resulting bond: the greater halogen polarizability and the lower the electronegativity of the halogen give rise to the stronger halogen bonds, and the

potency of the halogen bond interaction increases in the order fluorine-chlorine-bromine-iodine. [97, 98] Fluorine is the least polarizable and the most electronegative halogen, and therefore the least prone to form halogen bonds. Considering the *meta*-substituted diEPP derivatives, the potentiated responses evoked by PAM co-application increased from fluorine (**6j**), to hydrogen (diEPP parent compound), to chlorine (**6l**), to bromine (**6o**). The observed trend is consistent with the halogen-bond interaction hypothesis between the compound and an appropriate electron-donor residue within the receptor binding pocket. According to this hypothesis, electron-rich or electron-donating substituents in the *meta* position would show low silent agonism profile, and diEPP derivatives data support this statement. Indeed, *meta*-methoxide (**6e**) and *meta*-cyano (**6q**) diEPP derivatives showed PAM-dependent responses respectively half and equivalent to the one evoked by the parent compound.

Conversely to the *meta* position, chlorine and bromine substitutions in the *ortho* or the *para* positions did not result in compounds characterized by the silent agonism profile, but rather in partial agonists. The *para*-fluoro (**6i**) and the *para*-trifluoromethyl (**6g**) diEPP derivatives, however, represent two of the best silent agonists of the series, thus indicating a unique behavior of fluorine groups, difficult to explain in absence of high resolution structure. [94] However, the most likely hypothesis to explain the activity showed by fluorine diEPP relies on the fluorine bond.

In some cases, when the quaternary ammonium diEPP derivative appeared as a silent agonist, the corresponding tertiary amine was investigated. The activities of the selected ethyl phenyl piperazines **63** (*para*-trifluoromethyl), **65** (*para*-fluoro), **68** (*meta*-chloro) and **71** (*meta*-bromo) are reported in **Table 9**. These compounds were mostly inactive at the alpha7 receptor, with no partial agonism and weak responses evoked by PAM co-application. Within these tertiary amine compounds, compound **71** showed a silent agonist activity 5-fold greater than the parent compound and close to the corresponding quaternary derivative. Consistent with these results, the permanent positive charge is not mandatory for silent agonist activity, when suitable structural features are present independently from the core charged nitrogen. The present statement becomes particularly relevant in the development of silent agonists required to cross the blood-brain barrier to explicate their therapeutic functions. However, in order to target the peripheral immune system to exert anti-inflammatory activities, quaternary ammonium salts of the series emerged as better candidates according to their superior silent agonism profile.

Compound	Agonist response		Potentiated response	
	Peak current	Net Charge	Peak current	Net Charge
diEPP	0.002 ± 0.001	0.002 ± 0.003	0.347 ± 0.083	1.333 ± 0.334
6h	0.002 ± 0.000	0.000 ± 0.002	1.106 ± 0.684	3.061 ± 2.079
63	0.005 ± 0.001	0.000 ± 0.004	0.396 ± 0.230	1.387 ± 0.723
6i	0.005 ± 0.001	0.010 ± 0.002	7.121 ± 1.382	22.937 ± 5.771
65	0.006 ± 0.001	0.000 ± 0.003	0.014 ± 0.002	0.035 ± 0.004
6l	0.015 ± 0.002	0.020 ± 0.003	4.694 ± 1.609	4.227 ± 0.991
68	0.009 ± 0.002	0.009 ± 0.008	0.074 ± 0.028	0.148 ± 0.067
6o	0.018 ± 0.005	0.040 ± 0.006	11.853 ± 4.151	9.982 ± 1.744
71	0.007 ± 0.001	0.000 ± 0.003	2.166 ± 0.709	6.930 ± 2.425

Table 9. Comparison between tertiary amine and quaternary ammonium salts of some silent agonists of the diEPP series. . Electrophysiological data of diEPP derivatives, emerged as silent agonists, on human alpha7 nAChRs expressed in Xenopus oocytes. Data are reported as mean ± SEM (standard error of mean) and relative to the control ACh. Agonist response refers to receptor response to 30 μM application of test compound. Potentiated response refers to receptor response with 30 μM test compound and 10 μM PNU-120596 co-application.

Aiming at investigating the alpha7 silent agonism profile with structural modifications of the diEPP moiety, compounds **7** and **8** were synthesized to test the influence of the nitrogen atom in the piperazine ring attached to the phenyl group on the silent agonism. In both cases, the tested compounds showed enhanced PNU-120596 potentiation compared to the parent compound, with **7** been superior for the better ratio of desensitization to residual partial agonism compared to **8**. Indeed, compound **7** evoked a partial agonism response not detectable within experimental error. The more flexibility conferred by the amino bridge to compound **7** with respect to the methylene bridge of compound **8** may be ascribable for the observed difference.

CHAPTER V

Experimental section

5.1 Materials and methods

^{1}H and ^{13}C NMR spectra were recorded on a Varian Mercury-300 (300 and 75 MHz, respectively) or Varian Inova-500 (500 and 126 MHz) instruments. Chemical shifts (δ scale) are reported in parts-per-million (*ppm*) and coupling constants (*J*) in hertz (*Hz*). For each compound analyzed, the specific deuterated solvent (CDCl$_3$, CD$_3$OD, (CD$_3$)$_2$CO, or (CD$_3$)$_2$SO) used is reported. Chemical shifts (δ scale) are reported in parts per million (ppm) relative to the peak of the internal standard TMS (δ = 0.00 ppm) for CDCl$_3$, CD$_3$OD, (CD$_3$)$_2$CO or relative to the central peak of the solvent (δ = 2.50 ppm for (CD$_3$)$_2$SO) in ^{1}H NMR and relative to the central peak of the solvent (δ = 77.16 ppm for CDCl$_3$, 49.00 for CD$_3$OD, 39.52 for (CD$_3$)$_2$SO (DMSO-*d*6), and 29.84 for (CD$_3$)$_2$CO) in ^{13}C NMR. Processing of the spectra was performed with MestReNova 8.1.1.

Mass spectra (ESI-MS) were registered with Varian 320 LC-MS/MS instrument, on a Hewlett-Packard 5988A spectrometer or on an Agilent 6220 ESI TOF (Santa Clara, CA) mass spectrometer equipped with electrospray and DART sources operated in positive ion mode. Data are reported as mass/charge ratio (*M/Z*).

Melting points were obtained on a Büchi Mod. B 540 instrument or on an MFB-595010M Gallenkamp apparatus equipped with a digital thermometer, and not corrected.

Reactions were monitored by TLC analyses performed on commercial silica gel 60 F254 aluminium sheets/glass plates (Merck or EMD Millipore) or neutral alumina TLC plates (Fluka). Unless otherwise noted, the TLC analyses were performed on silica gel sheets/glass plates. Spots were observed with UV-lamp (λ = 365 nm) and/or evidenced with different TLC stains: a solution of KMnO$_4$ in 0.1 N NaOH, a 5% solution of phosphomolybdic acid in ethanol and, for amines and quaternary ammonium salts, Dragendorff reagent (a solution of KBiI$_4$ in CH$_3$COOH and H$_2$O).

Column chromatographies were performed on silica gel Sigma-Aldrich (230-400 mesh) or neutral alumina as stationary phase; eluents have been specified time to time.

All reagents were of reagent quality or were purified before use. Organic solvents for the reactions were of analytical grade or were purified by standard procedures. Several reactions were carried out in anhydrous condition (flame-dried glassware) and under inert (argon or nitrogen) atmosphere; those conditions are implied whenever in the synthetic procedure dry solvents are listed.

5.2 Synthesis of PAM derivatives

5.2.1 5-Hydroxyindole derivatives

5.2.1.1 General procedure for *N*-methylation of anilines (14-17)

The appropriate aniline (1 equiv) was dissolved in MeOH under argon atmosphere. Formaldehyde 37% (5 equiv) and sodium methoxide (5 equiv) were subsequently added. The reaction mixture was heated to reflux (65°C) for 2h and then cooled to 0°C with an ice bath. Sodium borohydride (5 equiv) was then slowly added. The reaction mixture was heated to reflux again for 1h and monitored by TLC in cyclohexane/EtOAc 4:1. Upon completion, the mixture was cooled to room temperature, concentrated under reduced pressure, slowly diluted with water and then extracted with EtOAc (x 3). The organic layers were combined, dried over Na_2SO_4, filtered and concentrated *in vacuo* to give the desired methylated compound quantitatively.

5.2.1.2 General procedure for the synthesis of sulfonamides (18-19)

To a solution of the appropriate sulfonyl chloride in acetonitrile (0.10 mL/mmol sulfonyl chloride), cooled at 0°C, the appropriate amine was added dropwise. The resulting mixture was stirred at room temperature till complete consumption of the starting materials (which usually occurred within 10 min), then diluted with deionized water and extracted with EtOAc (x 3). The combined organic phases were dried over Na_2SO_4, filtered and concentrated under reduced pressure to provide the pure desired compound in quantitative yields.

4-iodo-*N*-methylaniline (14)

The title compound was prepared according to general procedure **5.2.1.1** by reacting 4-iodoaniline (865 mg, 3.95 mmol, 1 equiv), formaldehyde 37% (1.47 mL, 19.75 mmol, 5 equiv) and sodium borohydride (747 mg, 19.75 mmol, 5 equiv). Evaporation of the solvent afforded the desired compound as a yellow oil (909 mg, yield 99%).
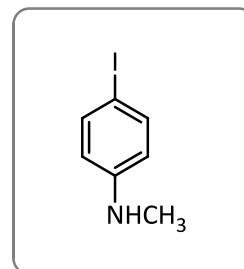
14: yellow oil; TLC (PMA): R_f = 0.55 (cyclohexane/EtOAc 4:1).
^{1}H NMR (CDCl$_3$, 300 MHz) δ 7.45 (d, *J* = 8.3 Hz, 2H), 6.40 (d, *J* = 8.3 Hz, 2H), 3.72 (br s, 1H), 2.80 (s, 3H).
^{13}C NMR (CDCl$_3$, 75 MHz) δ 148.7, 137.6, 114.6, 77.6, 30.5.

3-chloro-4-iodo-*N*-methylaniline (15)

The title compound was prepared according to general procedure **5.2.1.1** by reacting 3-chloro-4-iodoaniline (1 g, 3.95 mmol, 1 equiv), formaldehyde 37% (1.47 mL, 19.75 mmol, 5 equiv) and sodium borohydride (747 mg, 19.75 mmol, 5 equiv). Evaporation of the solvent afforded the desired compound as a yellow oil (1.01 g, yield 96%).
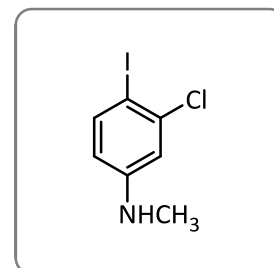
15: yellow oil; TLC (PMA): R_f = 0.51 (cyclohexane/EtOAc 4:1).
^{1}H NMR (CDCl$_3$, 300 MHz) δ 7.53 (d, *J* = 8.7 Hz, 1H), 6.73 (d, *J* = 2.7 Hz, 1H), 6.26 (dd, *J* = 8.7, 2.7 Hz, 1H), 4.16 (br s, 1H), 2.81 (s, 3H).
^{13}C NMR (CDCl$_3$, 75 MHz) δ 150.3, 139.9, 138.7, 113.1, 112.7, 80.5, 30.5.

4-bromo-*N*,3-dimethylaniline (16)

The title compound was prepared according to general procedure **5.2.1.1** by reacting 4-bromo-3-methylaniline (300 mg, 1.61 mmol, 1 equiv), formaldehyde 37% (600 μL, 8.05 mmol, 5 equiv) and sodium borohydride (305 mg, 8.05 mmol, 5 equiv). Evaporation of the solvent afforded the desired compound as a yellow oil (296 mg, yield 92%).
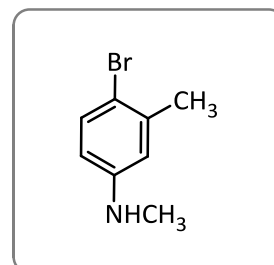
16: yellow oil; TLC (PMA): R_f = 0.42 (cyclohexane/EtOAc 9:1).
^{1}H NMR (CDCl$_3$, 300 MHz) δ 7.31 (d, *J* = 8.6 Hz, 1H), 6.49 (d, *J* = 2.8 Hz, 1H), 6.33 (dd, *J* = 8.6, 2.9 Hz, 1H), 3.65 (br s, 1H), 2.81 (s, 3H), 2.35 (s, 3H).
^{13}C NMR (CDCl$_3$, 75 MHz) δ 148.7, 138.1, 132.6, 114.7, 111.6, 111.5, 30.7, 23.1.

4-bromo-2-chloro-*N*-methylaniline (17)

The title compound was prepared according to general procedure **5.2.1.1** by reacting 4-bromo-2-chloroaniline (300 mg, 1.45 mmol, 1 equiv), formaldehyde 37% (540 μL, 7.25 mmol, 5 equiv) and sodium borohydride (274 mg, 7.25 mmol, 5 equiv). Evaporation of the solvent afforded the desired compound as a yellow oil (313 mg, yield 98%).
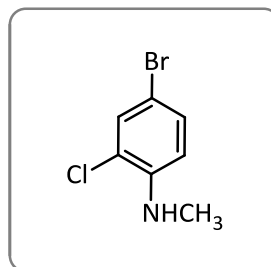
17: yellow oil; TLC (PMA): R_f = 0.51 (cyclohexane/EtOAc 4:1).
^{1}H NMR (CDCl$_3$, 300 MHz) δ 7.22 (d, *J* = 1.7 Hz, 1H), 7.09 (dd, *J* = 8.7, 1.6 Hz, 1H), 6.32 (d, *J* = 8.7 Hz, 1H), 4.21 (br s, 1H), 2.70 (s, 3H).
^{13}C NMR (CDCl$_3$, 75 MHz) δ 144.1, 131.0, 130.5, 119.5, 111.6, 107.2, 30.2.

4-bromo-3-methylbenzenesulfonamide (18)

The title compound was prepared according to general procedure **5.2.1.2** by reacting 4-bromo-3-methylbenzene-1-sulfonyl chloride (200 mg, 0.75 mmol, 1 equiv) and aqueous ammonia (340 μL) in acetonitrile (80 μL). Evaporation of the solvent afforded the desired compound as a yellow oil (184 mg, yield 98%).
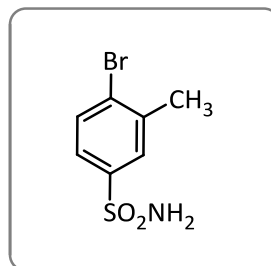
18: yellow oil; TLC (PMA): R_f = 0.42 (cyclohexane/EtOAc 9:1).
^{1}H NMR (CDCl$_3$, 300 MHz) δ 7.72 (d, *J* = 2.2 Hz, 1H), 7.61 (d, *J* = 8.3 Hz, 1H), 7.52 (dd, *J* = 8.4, 2.1 Hz, 1H), 4.86 (br s, 2H), 2.40 (s, 3H).
^{13}C NMR (CD$_3$OD, 75 MHz) δ 144.4, 140.3, 134.0, 129.8, 129.2, 126.1, 23.0.

4-bromo-*N*,3-dimethylbenzenesulfonamide (19)

The title compound was prepared according to general procedure **5.2.1.1** by reacting 4-bromo-3-methylbenzene-1-sulfonyl chloride (200 mg, 0.75 mmol, 1 equiv) and methylamine solution 40% in water (350 μL) in acetonitrile (80 μL). Evaporation of the solvent afforded the desired compound as a yellow oil (190 mg, yield 96%).
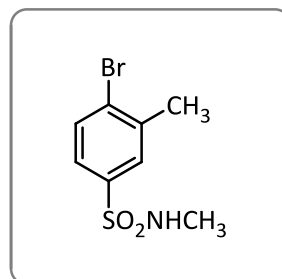
19: yellow oil; TLC (PMA): R_f = 0.42 (cyclohexane/EtOAc 9:1).
^{1}H NMR (CDCl$_3$, 300 MHz) δ 7.66 (d, *J* = 2.1 Hz, 1H), 7.60 (d, *J* = 8.3 Hz, 1H), 7.46 (dd, *J* = 8.4, 2.3 Hz, 1H), 4.97 (br s, 1H), 2.57 (s, 3H), 2.39 (s, 3H).
^{13}C NMR (CDCl$_3$, 75 MHz) δ 139.6, 138.0, 133.2, 130.1, 129.2, 126.0, 29.3, 23.1.

5.2.1.3 Experimental procedures for the synthesis of 1a-f

5.2.1.3.1 General procedure for *N*-arylation of 5-methoxyindole (20-26)

5.2.1.3.1.1: The coupling reaction was carried out under inert argon atmosphere. 5-methoxyindole (**13**) (1.1 equiv), K_2CO_3 (2.5 equiv), CuI (0.05 equiv) and *D*-proline (0.1 equiv) were dissolved in DMSO (2 mL/mmol arylbenzene). The mixture was gently heated to 110°C (± 5°C) under an inert atmosphere for 20 min, then the appropriate aryl halide (1 equiv) was added dropwise. The reaction was stirred at 110°C till complete consumption of the starting materials. Upon completion, the cooled solution was first filtered through a Celite pad, then partitioned between EtOAc and deionized water, and the aqueous layer extracted with EtOAc (x 3). The combined organic phases were washed with brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude material was subsequently purified by silica gel column chromatography to give the pure product.

5.2.2.3.1.2: The coupling reaction was carried out under inert argon atmosphere. 5-methoxyindole (**13**) (1 equiv, 1M), K_3PO_4 (2.1 equiv), CuI (0.05 equiv), *N,N'*-dimethylethylenediamine (0.2 equiv) and the appropriate aryl halide (1.2 equiv) were dissolved in dry THF. The mixture was gently heated to 80°C (± 5°C) under an inert atmosphere till complete consumption of the starting materials. Upon completion (48h), the cooled solution was diluted with EtOAc, filtered through a plug of silica gel and eluted with EtOAc. The filtrate was concentrated under reduced pressure and then the crude material was purified by silica gel column chromatography to provide the pure product.

5.2.1.3.2 General procedure for demethylation (1a-f)

The reaction was carried out in anhydrous conditions and under inert argon atmosphere. To a stirred solution of the appropriate methoxy derivative (1 equiv) in dry CH_2Cl_2 (5.5 mL/mmol methoxy derivative), cooled at -78°C, 1M BBr_3 in hexanes (3 equiv/methoxy group) was added dropwise. The reaction mixture was stirred at -78°C for 1.5h and then at RT for 12h. Upon completion, the reaction was cooled at 0°C, quenched by the addition of 5% aqueous solution of Na_2CO_3 and extracted with CH_2Cl_2/*i*-PrOH 9:1 (x 3). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The crude material was purified by silica gel column chromatography eluting in CH_2Cl_2/MeOH to afford the pure product.

5-methoxy-1-(4-methoxyphenyl)-1*H*-indole (20)

The title compound was prepared according to general procedure **5.2.1.3.1.1** by reacting 5-methoxyindole (**13**) (800 mg, 5.44 mmol, 1.1 equiv) and 4-iodoanisole (1.16 g, 4.95 mmol, 1 equiv). Upon completion (TLC in cyclohexane/EtOAc 9:1), standard workup was applied and the crude material was purified by silica gel column chromatography eluting in cyclohexane/EtOAc 95:5 to give the pure product as a yellow oil (925 mg, yield 74%).
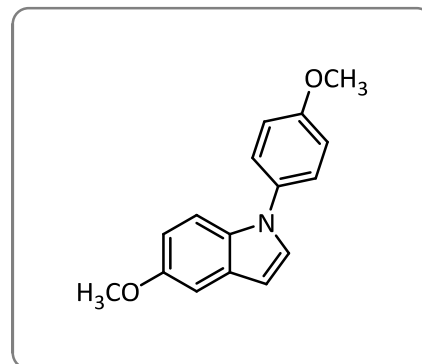
20: yellow oil; TLC (KMnO$_4$; PMA): R$_f$ = 0.33 (cyclohexane/EtOAc 9:1).
^{1}H NMR (CDCl$_3$, 300 MHz) δ 7.31 (d, *J* = 8.9 Hz, 2H), 7.27 (d, *J* = 9.0 Hz, 1H), 7.18 (d, *J* = 3.2 Hz, 1H), 7.06 (d, *J* = 2.5 Hz, 1H), 6.95 (d, *J* = 8.9 Hz, 2H), 6.79 (dd, *J* = 8.9, 2.5 Hz, 1H), 6.50 (d, *J* = 3.2 Hz, 1H), 3.80 (s, 6H).
^{13}C NMR (CDCl$_3$, 75 MHz) δ 158.1, 154.5, 133.0, 131.6, 129.5, 128.7, 125.6, 114.7, 112.4, 111.2, 102.6, 55.8, 55.5.

1-(4-hydroxyphenyl)-1*H*-indol-5-ol (1a)

The title compound was prepared according to general procedure **5.2.1.3.2** by reacting **20** (925 mg, 3.65 mmol, 1 equiv) and 1M BBr$_3$ in hexanes (22 mL, 22 mmol, 6 equiv) in dry CH$_2$Cl$_2$. Upon completion (TLC in CH$_2$Cl$_2$/MeOH 95:5), standard workup was applied and the crude material was purified by silica gel column chromatography eluting in CH$_2$Cl$_2$/MeOH 98:2 to give the pure product as a white solid (286 mg, yield 35%).
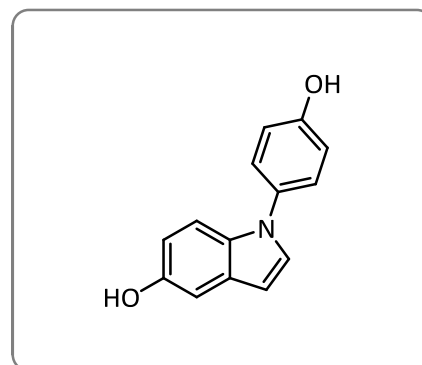
1a: white solid; TLC (KMnO$_4$; PMA): R$_f$ = 0.40 (CH$_2$Cl$_2$/MeOH 95:5); m.p.: 141.9°C; MS (ESI) m/z for C$_{14}$H$_{12}$NO$_2^+$ [M+H]$^+$ calcd. 226.1, found 226.0.
^{1}H NMR (CD$_3$OD, 300 MHz) δ 7.08 (d, *J* = 8.7 Hz, 3H), 7.04 (d, *J* = 3.1 Hz, 1H), 6.90 (d, *J* = 1.9 Hz, 1H), 6.79 (d, *J* = 8.7 Hz, 2H), 6.61 (dd, *J* = 8.8, 2.1 Hz, 1H), 6.29 (d, *J* = 2.7 Hz, 1H).
^{13}C NMR (CD$_3$OD, 75 MHz) δ 157.0, 151.8, 133.3, 132.7, 131.2, 129.7, 126.6, 117.0, 112.8, 111.7, 105.9, 102.7.

5-methoxy-1-(4-methoxy-2-methylphenyl)-1*H*-indole (21)

The title compound was prepared according to general procedure **5.2.1.3.1.1** by reacting 5-methoxyindole (**13**) (1 g, 6.79 mmol, 1.1 equiv) and 4-bromo-3-methylanisole (1.24 mg, 6.17 mmol, 1 equiv). Upon completion (TLC in cyclohexane/EtOAc 95:5), standard workup was applied and the crude material was purified by silica gel column chromatography eluting in cyclohexane/EtOAc 98:2 to give the pure product as a brown oil (483 mg, yield 29%).
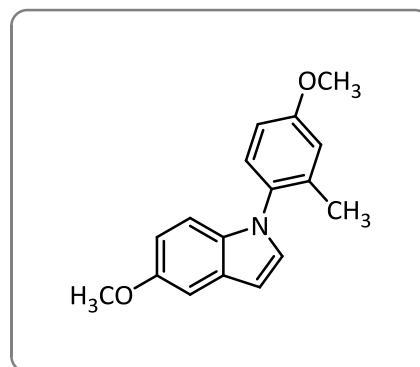
21: brown oil; TLC (KMnO$_4$; PMA): R$_f$ = 0.39 (cyclohexane/EtOAc 95:5).
^{1}H NMR (CDCl$_3$, 300 MHz) δ 7.09 (d, *J* = 8.5 Hz, 1H), 7.03 (d, *J* = 2.3 Hz, 1H), 6.98 (d, *J* = 3.1 Hz, 1H), 6.80 (d, *J* = 8.9 Hz, 1H), 6.76 (d, *J* = 2.8 Hz, 1H), 6.74 − 6.67 (m, 2H), 6.45 (dd, *J* = 3.1, 0.5 Hz, 1H), 3.74 (s, 3H), 3.72 (s, 3H), 1.90 (s, 3H).
^{13}C NMR (CDCl$_3$, 75 MHz) δ 159.2, 154.4, 137.4, 132.8, 131.4, 129.6, 129.2, 128.6, 116.2, 112.3, 111.9, 111.3, 102.5, 101.9, 55.9, 55.5, 17.9.

1-(4-hydroxy-2-methylphenyl)-1*H*-indol-5-ol (1b)

The title compound was prepared according to general procedure **5.2.1.3.2** by reacting **21** (100 mg, 0.37 mmol, 1 equiv) and 1M BBr$_3$ in hexanes (2.25 mL, 2.25 mmol, 6 equiv) in dry CH$_2$Cl$_2$. Upon completion (TLC in CH$_2$Cl$_2$/MeOH 98:2), standard workup was applied and the crude material was purified by silica gel column chromatography eluting in CH$_2$Cl$_2$/MeOH 98:2 to give the pure product as a white solid (61 mg, yield 69%).
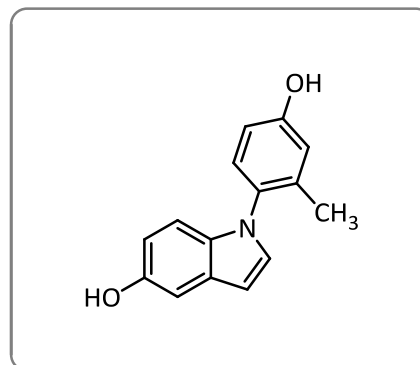
1b: white solid; TLC (KMnO$_4$; PMA): R$_f$ = 0.30 (CH$_2$Cl$_2$/AcOEt 93:7); m.p.: 58.1-61.4°C; MS (ESI) m/z for C$_{15}$H$_{14}$NO$_2^+$ [M+H]$^+$ calcd. 240.1, found 240.0.
^{1}H NMR (CD$_3$OD, 300 MHz) δ 6.93 (d, *J* = 3.2 Hz, 1H), 6.90 (dd, *J* = 4.4, 1.3 Hz, 1H), 6.88 (d, *J* = 2.2 Hz, 1H), 6.68 (d, *J* = 2.8 Hz, 1H), 6.65 − 6.57 (m, 2H), 6.55 (dd, *J* = 8.8, 2.3 Hz, 1H), 6.29 (d, *J* = 3.1 Hz, 1H), 1.77 (s, 3H).
^{13}C NMR (CD$_3$OD, 75 MHz) δ 158.3, 151.7, 138.4, 133.9, 131.5, 130.6, 130.3, 130.1, 118.2, 114.4, 112.7, 111.7, 105.7, 102.0, 17.6.

4-(5-methoxy-1H-indol-1-yl)aniline (22)

The title compound was prepared according to general method **5.2.1.3.1.1** by reacting 5-methoxyindole (**13**) (1.30 g, 8.83 mmol, 1.1 equiv) and 4-iodoaniline (1.76 g, 8.03 mmol, 1 equiv). Upon completion (TLC in cyclohexane/EtOAc 4:1), standard workup was applied and the crude material was purified by silica gel column chromatography eluting in cyclohexane/EtOAc 85:15 to give the pure product as a brown oil (1.04 g, yield 54%).
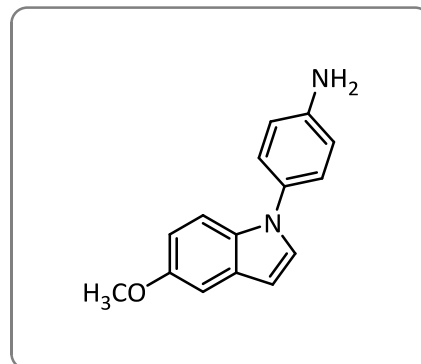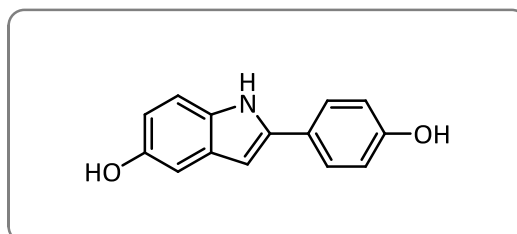
22: brown oil; TLC (KMnO$_4$; PMA): R$_f$ = 0.25 (cyclohexane/EtOAc 4:1).
^{1}H NMR (CDCl$_3$, 300 MHz) δ 7.20 (d, J = 8.9 Hz, 1H), 7.11 − 7.03 (m, 3H), 7.01 (d, J = 1.7 Hz, 1H), 6.78 − 6.69 (m, 1H), 6.56 (dd, J = 8.5, 1.4 Hz, 2H), 6.44 − 6.39 (m, 1H), 3.72 (s, 3H), 3.54 (br s, 2H).
^{13}C NMR (CDCl$_3$, 75 MHz) δ 154.3, 145.3, 131.8, 130.9, 129.3, 128.9, 125.7, 115.6, 112.2, 111.3, 102.6, 102.1, 55.9.

1-(4-aminophenyl)-1*H*-indol-5-ol (1c)

The title compound was prepared according to general procedure **5.2.1.3.2** by reacting **22** (1.04 g, 4.36 mmol, 1 equiv) and 1M BBr$_3$ in hexanes (13.1 mL, 13.08 mmol, 3 equiv) in dry CH$_2$Cl$_2$. Upon completion (TLC in CH$_2$Cl$_2$/MeOH 95:5), standard workup was applied and the crude material was purified by silica gel column chromatography eluting in CH$_2$Cl$_2$/MeOH 98:2 to give the pure product as a white solid (476 mg, yield 49%).
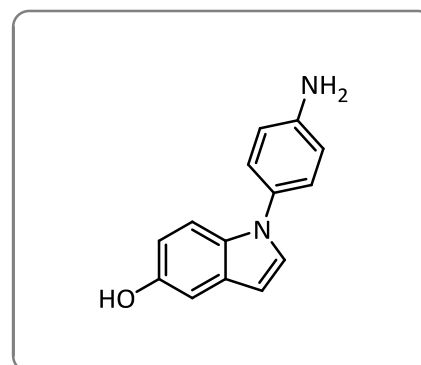
1c: white solid; TLC (KMnO$_4$; PMA): R$_f$ = 0.31 (CH$_2$Cl$_2$/MeOH 98); m.p.: 134.0-135.7°C; MS (ESI) m/z for C$_{14}$H$_{13}$N$_2$O$^+$ [M+H]$^+$ calcd. 225.1, found 225.0.
^{1}H NMR ((CD$_3$)$_2$SO, 300 MHz) δ 8.78 (br s, 1H), 7.33 (d, J = 3.1 Hz, 1H), 7.17 (d, J = 8.8 Hz, 1H), 7.13 (d, J = 8.6 Hz, 2H), 6.90 (d, J = 1.8 Hz, 1H), 6.69 (d, J = 8.7 Hz, 2H), 6.64 (dd, J = 8.9, 2.3 Hz, 1H), 6.38 (dd, J = 3.1, 0.6 Hz, 1H), 5.24 (br s, 2H).
^{13}C NMR ((CD$_3$)$_2$SO, 75 MHz) δ 151.1, 147.4, 130.4, 129.3, 128.8, 128.1, 124.9, 114.3, 111.8, 110.6, 104.4, 101.1.

4-(5-methoxy-1H-indol-1-yl)-*N*-methylaniline (23)

The title compound was prepared according to general procedure **5.2.1.3.1.2** by reacting 5-methoxyindole (**13**) (400 mg, 2.72 mmol, 1 equiv) and 4-iodo-*N*-methylaniline (**14**) (760 mg, 3.26 mmol, 1.2 equiv). Upon completion (TLC in cyclohexane/CH_2Cl_2 1:1), standard workup was applied and the crude material was purified by silica gel column chromatography eluting in cyclohexane/CH_2Cl_2 3:2 to afford the desired product as a yellow oil (244 mg, yield 36%).
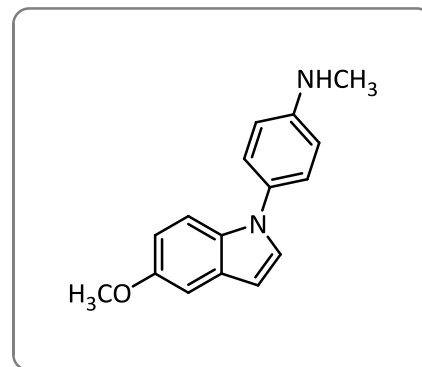
23: yellow oil; TLC ($KMnO_4$; PMA): R_f = 0.28 (cyclohexane/EtOAc 9:1).
^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.25 (d, *J* = 8.9 Hz, 1H), 7.18 (d, *J* = 8.8 Hz, 2H), 7.14 (d, *J* = 3.4 Hz, 1H), 7.04 (d, *J* = 2.4 Hz, 1H), 6.76 (dd, *J* = 8.9, 2.4 Hz, 1H), 6.60 (d, *J* = 8.8 Hz, 2H), 6.46 (dd, *J* = 3.1, 0.8 Hz, 1H), 3.78 (s, 3H), 2.79 (s, 3H).
^{13}C NMR ($CDCl_3$, 75 MHz) δ 154.4, 148.1, 132.0, 130.0, 129.3, 129.0, 126.0, 112.9, 112.3, 111.4, 102.6, 102.0, 56.0, 31.0.

1-(4-(methylamino)phenyl)-1*H*-indol-5-ol (1d)

The title compound was prepared according to general procedure **5.2.1.3.2** by reacting **23** (244 mg, 0.97 mmol, 1 equiv) and 1M BBr_3 in hexanes (2.9 mL, 2.90 mmol, 3 equiv) in dry CH_2Cl_2. Upon completion (TLC in CH_2Cl_2/MeOH 95:5), standard workup was applied and the crude material was purified by silica gel column chromatography eluting in CH_2Cl_2/MeOH 98:2 to give the pure product as an off-white solid (70 mg, yield 30%).
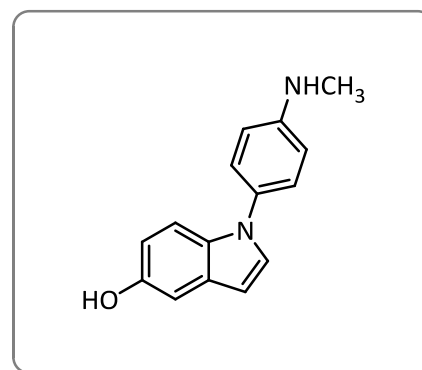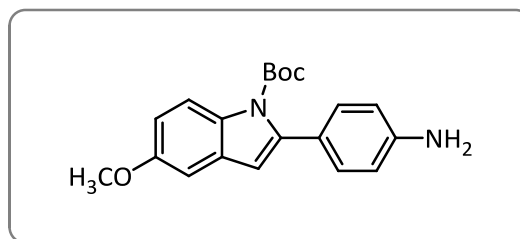
1d: off-white solid; TLC ($KMnO_4$; PMA): R_f = 0.34 (CH_2Cl_2/MeOH 98:2); m.p.: 61.5°C; MS (ESI) m/z for $C_{15}H_{15}N_2O^+$ [M+H]$^+$ calcd. 239.1, found 239.0.
^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.22 – 7.14 (m, 3H), 7.13 (d, *J* = 3.1 Hz, 1H), 6.97 (d, *J* = 2.4 Hz, 1H), 6.67 – 6.58 (m, 3H), 6.39 (d, *J* = 3.1 Hz, 1H), 4.23 (br s, 2H), 2.78 (s, 3H).
^{13}C NMR ($CDCl_3$, 75 MHz) δ 149.7, 147.9, 132.1, 130.2, 129.6, 129.4, 126.0, 113.2, 111.8, 111.3, 105.3, 101.7, 31.1.

3-chloro-4-(5-methoxy-1H-indol-1-yl)aniline (24)

The title compound was prepared according to general method **5.2.1.3.1.1** by reacting 5-methoxyindole (**13**) (800 mg, 5.44 mmol, 1.1 equiv) and 3-chloro-4-iodoaniline (1.26 g, 4.95 mmol, 1 equiv). Upon completion (TLC in cyclohexane/EtOAc 4:1), standard workup was applied and the crude material was purified by silica gel column chromatography eluting in cyclohexane/EtOAc 85:15 to give the pure product as a brown oil (424 mg, yield 36%).
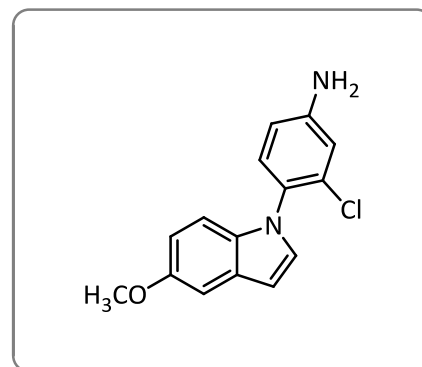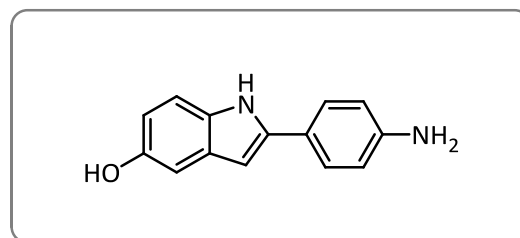

24: brown oil; TLC (KMnO$_4$; PMA): R$_f$ = 0.23 (cyclohexane/EtOAc 4:1).
^{1}H NMR (CDCl$_3$, 300 MHz) δ 7.08 – 7.01 (m, 3H), 6.89 (dd, J = 8.9, 0.5 Hz, 1H), 6.74 (dd, J = 8.9, 2.4 Hz, 1H), 6.70 (d, J = 2.5 Hz, 1H), 6.52 – 6.44 (m, 2H), 3.76 (s, 3H), 3.61 (br s, 2H).
^{13}C NMR (CDCl$_3$, 75 MHz) δ 154.4, 147.3, 132.8, 132.7, 130.2, 129.9, 128.7, 127.4, 115.9, 113.8, 112.3, 111.4, 102.7, 102.2, 56.0.

1-(4-amino-2-chlorophenyl)-1*H*-indol-5-ol (1e)

The title compound was prepared according to general procedure **5.2.1.3.2** by reacting 3-chloro-4-(5-methoxy-1*H*-indol-1-yl)aniline (**24**) (424 mg, 1.55 mmol, 1 equiv) and 1M BBr$_3$ in hexanes (4.65 mL, 4.65 mmol, 3 equiv) in dry CH$_2$Cl$_2$. Upon completion (TLC in CH$_2$Cl$_2$/MeOH 95:5), standard workup was applied and the crude material was purified by silica gel column chromatography eluting in CH$_2$Cl$_2$/MeOH 99:1 to give the pure product as a yellowish solid (76 mg, yield 19%).
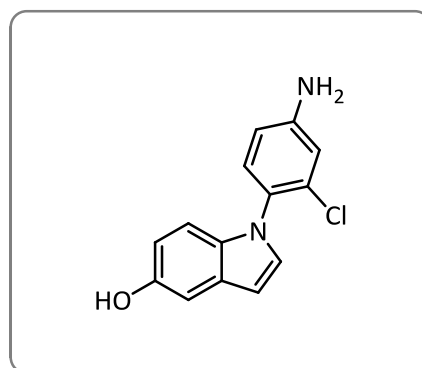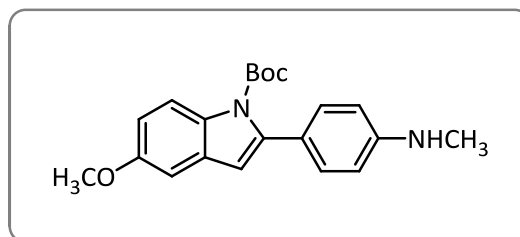

1e: yellowish solid; TLC (KMnO$_4$; PMA): R$_f$ = 0.49 (CH$_2$Cl$_2$/MeOH 95:5); m.p.: 133.1°C; MS (ESI) m/z for C$_{14}$H$_{12}$ClN$_2$O$^+$ [M+H]$^+$ calcd. 259.1, found 259.0.
^{1}H NMR (CD$_3$OD, 300 MHz) δ 7.12 – 7.06 (m, 2H), 6.96 (dd, J = 2.3, 0.5 Hz, 1H), 6.87 (d, J = 2.5 Hz, 1H), 6.81 (dd, J = 8.7, 0.7 Hz, 1H), 6.73 – 6.63 (m, 2H), 6.41 (dd, J = 3.1, 0.8 Hz, 1H), 3.76 – 3.63 (m, 0.3H, DIPE 13% residual), 1.12 (d, J = 6.1 Hz, 1.5H, DIPE 13% residual).
^{13}C NMR (CD$_3$OD, 75 MHz) δ 151.9, 150.5, 133.9, 133.5, 131.0, 130.9, 130.4, 127.3, 116.3, 114.7, 112.7, 111.8, 105.7, 102.3.

3-chloro-4-(5-methoxy-1*H*-indol-1-yl)-*N*-methylaniline (25)

The title compound was prepared according to general procedure **5.2.1.3.1.2** by reacting 5-methoxyindole (**13**) (124 mg, 0.84 mmol, 1 equiv) and 3-chloro-4-iodo-*N*-methylaniline (**15**) (271 mg, 1.01 mmol, 1.2 equiv). Upon completion (TLC in cyclohexane/CH$_2$Cl$_2$ 1:1), standard workup was applied and the crude material was purified by silica gel column chromatography eluting in cyclohexane/CH$_2$Cl$_2$ 3:2 to afford the desired product as a yellow oil (137 mg, yield 57%).
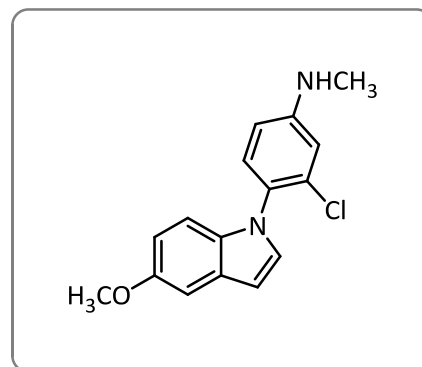
25: yellow oil; TLC (KMnO$_4$; PMA): R$_f$ = 0.21 (cyclohexane/CH$_2$Cl$_2$ 1:1).
^{1}H NMR (CDCl$_3$, 300 MHz) δ 7.07 (d, *J* = 9.2 Hz, 1H), 7.04 (d, *J* = 1.1 Hz, 1H), 6.90 (d, *J* = 8.9 Hz, 1H), 6.74 (dd, *J* = 8.9, 2.3 Hz, 1H), 6.61 (d, *J* = 2.6 Hz, 1H), 6.47 (dd, *J* = 3.1, 0.8 Hz, 1H), 6.42 (dd, *J* = 8.6, 2.5 Hz, 1H), 3.76 (s, *J* = 4.0 Hz, 3H), 2.73 (s, 3H).
^{13}C NMR (CDCl$_3$, 75 MHz) δ 154.4, 149.8, 133.0, 132.8, 130.0, 130.0, 128.6, 126.0, 112.7, 112.2, 111.5, 111.3, 102.6, 102.0, 56.0, 30.6.

1-(2-chloro-4-(methylamino)phenyl)-1*H*-indol-5-ol (1f)

The title compound was prepared according to general procedure **5.2.1.3.2** by reacting **25** (137 mg, 0.48 mmol, 1 equiv) and 1M BBr$_3$ in hexanes (1.44 mL, 1.44 mmol, 3 equiv) in dry CH$_2$Cl$_2$. Upon completion (TLC in CH$_2$Cl$_2$/MeOH 98:2), standard workup was applied and the crude material was purified by silica gel column chromatography eluting in CH$_2$Cl$_2$/MeOH 99:1 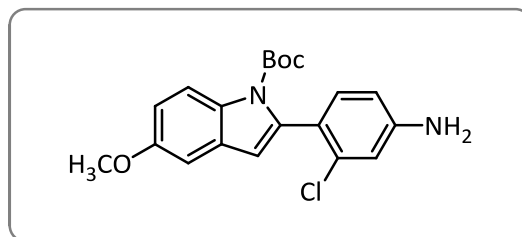to give the pure product as a white solid (26 mg, yield 20%),which resulted to be unstable and quickly degraded to a violet oil.
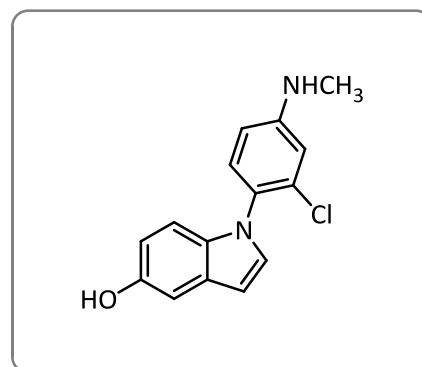
1f: white solid (previous degradation); TLC (KMnO$_4$; PMA): R$_f$ = 0.36 (CH$_2$Cl$_2$/MeOH 98:2).
^{1}H NMR (CD$_3$OD, 300 MHz) δ 7.02 (d, *J* = 8.6 Hz, 1H), 6.98 (d, *J* = 3.2 Hz, 1H), 6.86 (d, *J* = 2.3 Hz, 1H), 6.71 (d, *J* = 8.7 Hz, 1H), 6.64 (d, *J* = 2.6 Hz, 1H), 6.56 (dd, *J* = 8.8, 2.3 Hz, 1H), 6.50 (dd, *J* = 8.6, 2.6 Hz, 1H), 6.31 (d, *J* = 3.2 Hz, 1H), 2.70 (s, 3H).
^{13}C NMR (CD$_3$OD, 75 MHz) δ 152.2, 151.9, 134.0, 133.8, 131.0, 130.4, 126.4, 113.2, 112.6, 112.1, 111.8, 105.7, 102.3, 30.4.

5.2.1.4 Experimental procedures for the synthesis of 2a-m, 3 and 4a-b

5.2.2.4.1 General procedure for the Suzuki-Miyaura cross-coupling (28-40 and 42)

The coupling reaction was carried out under inert argon atmosphere. To a stirred and degassed solution of *N*-Boc-5-methoxy-2-indolylboronic acid (**27**) (1.4 equiv), appropriate aryl halide (1 equiv) and Pd(PPh$_3$)$_4$ (0.05 equiv) in 1,2-dimethoxyethane (18 mL/mmol indole) was added a degassed aqueous solution of 2M Na$_2$CO$_3$ (4.2 equiv). The resulting mixture was stirred at reflux (95-100°C) till complete consumption of the starting material. Upon completion (TLC cyclohexane/EtOAc), deionized water (13 mL/mmol indole) was added to the cooled solution, the organic layer was separated and the water phase was extracted with EtOAc (x 3). The combined organic phases were dried over Na$_2$SO$_4$, filtered and concentrated under reduced pressure. The crude material was purified by silica gel column chromatography to give the cross-coupling pure product.

5.2.1.4.2 General procedure for demethylation and Boc cleavage (2a-m and 3)

The reaction was carried out in anhydrous conditions and under inert argon atmosphere. To a stirred solution of the appropriate methoxy intermediate (1 equiv) in dry CH$_2$Cl$_2$ (5.5 mL/mmol methoxy intermediate), cooled at -78°C, 1M BBr$_3$ in hexanes (3 equiv/methoxy group, 1 equiv/Boc group) was added dropwise. The reaction mixture was stirred at -78°C for 1.5h and then at RT for 12h. Upon completion, the reaction was cooled at 0°C, quenched by the addition of 5% aqueous solution of Na$_2$CO$_3$ and extracted with CH$_2$Cl$_2$ (x 3). The combined organic layers were washed with brine, dried over Na$_2$SO$_4$, filtered and evaporated under reduced pressure. The crude material was subsequently purified by silica gel column chromatography to give the pure product.

tert-butyl 5-methoxy-2-(4-methoxyphenyl)-1*H*-indole-1-carboxylate (28)

The title compound was prepared according to general procedure **5.2.1.4.1** by reacting *N*-Boc-5-methoxy-2-indolylboronic acid (**27**) (400 mg, 1.37 mmol, 1.4 equiv) with 4-iodoanisole (230 mg, 0.98 mmol, 1 equiv). The mixture was heated at 95°C for 2.5h. Upon completion (TLC cyclohexane/EtOAc 9:1), after standard workup, the crude material was purified by silica gel column chromatography eluting in cyclohexane/EtOAc 97:3 to provide the pure product as a pale yellow oil (277 mg, yield 80%).
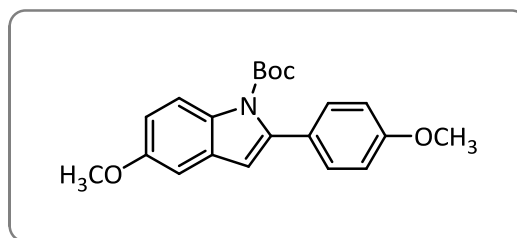
28: pale yellow oil; TLC (KMnO$_4$; PMA): R$_f$ = 0.33 (cyclohexane/EtOAc 9:1).
^{1}H NMR (CDCl$_3$, 300 MHz) δ 7.99 (d, *J* = 9.0 Hz, 1H), 7.24 (d, *J* = 8.8 Hz, 2H), 6.91 (d, *J* = 2.5 Hz, 1H), 6.84 (dd, *J* = 9.0, 2.4 Hz, 3H), 6.34 (s, 1H), 3.76 (s, 3H), 3.75 (s, 3H), 1.27 (s, 9H).
^{13}C NMR (CDCl$_3$, 75 MHz) δ 159.3, 156.1, 150.4, 141.2, 132.1, 130.2, 130.0, 127.5, 116.2, 113.3, 112.8, 109.6, 102.9, 83.3, 55.8, 55.5, 27.8.

2-(4-hydroxyphenyl)-1*H*-indol-5-ol (2a)

The title compound was prepared according to general procedure **5.2.1.4.1** by reacting **28** (260 mg, 0.74 mmol, 1 equiv) with 1M BBr$_3$ (6.66 mL, 6.66 mmol) in dry CH$_2$Cl$_2$. Upon completion (TLC in CH$_2$Cl$_2$/MeOH 95:5), standard workup was applied and the crude material was purified by silica gel column chromatography eluting in CH$_2$Cl$_2$/MeOH 95:5 to give the pure product as a white solid (94 mg, yield 57%).
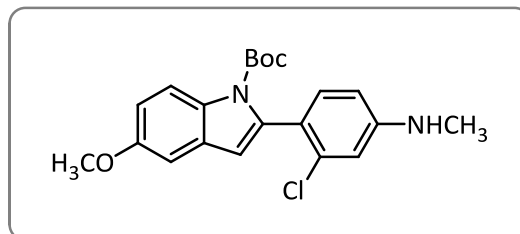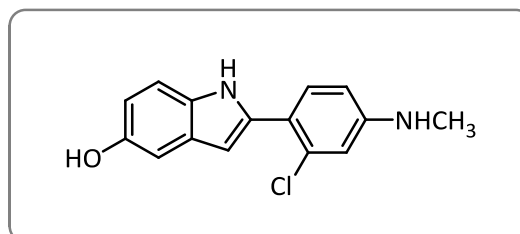

1a: white solid; TLC (KMnO$_4$; PMA): R$_f$ = 0.25 (CH$_2$Cl$_2$/MeOH 95:5); m.p.: 244°C (dec.); MS (ESI) *m/z* per C$_{14}$H$_{12}$NO$_2^+$ [M+H$^+$]$^+$ calcd. 226.1, found 226.1.
^{1}H NMR (CD$_3$OD, 300 MHz) δ 7.48 (d, *J* = 8.6 Hz, 1H), 7.08 (d, *J* = 8.6 Hz, 1H), 6.79 (d, *J* = 2.3 Hz, 1H), 6.74 (d, *J* = 8.7 Hz, 1H), 6.53 (dd, *J* = 8.6, 2.4 Hz, 1H), 6.38 (s, 1H), 3.25 (s, 0.16H, 5% MeOH residual)).
^{13}C NMR (CD$_3$OD, 75 MHz) δ 158.0, 151.5, 140.5, 133.6, 131.5, 127.4, 126.0, 116.6, 112.2, 111.9, 105.0, 97.5.

tert-butyl 5-methoxy-2-(4-methoxy-2-methylphenyl)-1*H*-indole-1-carboxylate (29)

The title compound was prepared according to general procedure **5.2.1.4.2** by reacting *N*-Boc-5-methoxy-2-indolylboronic acid (**27**) (400 mg, 1.37 mmol, 1.4 equiv) with 4-bromo-3-methylanisole (140 μL, 197 mg, 0.98 mmol, 1 equiv). The mixture was

heated at 95°C for 3h. Upon completion (TLC cyclohexane/EtOAc 9:1), after standard workup, the crude material was purified by silica gel column chromatography eluting in cyclohexane/EtOAc 98:2 to provide the pure product as a pale yellow oil (284 mg, yield 79%).
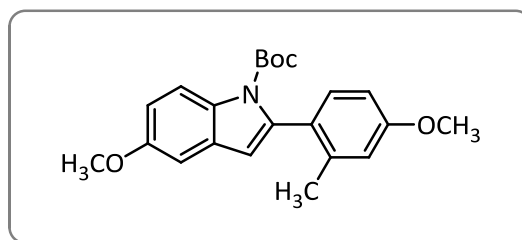
29: pale yellow oil; TLC (KMnO$_4$; PMA): R$_f$ = 0.41 (cyclohexane/EtOAc 9:1).
^{1}H NMR (CDCl$_3$, 300 MHz) δ 8.06 (d, *J* = 9.0 Hz, 1H), 7.08 (d, *J* = 8.2 Hz, 1H), 6.92 (d, *J* = 2.4 Hz, 1H), 6.85 (dd, *J* = 9.0, 2.5 Hz, 1H), 6.73 – 6.62 (m, 2H), 6.26 (s, 1H), 3.76 (s, 3H), 3.73 (s, 3H), 2.07 (s, 3H), 1.18 (s, 9H).
^{13}C NMR (CDCl$_3$, 75 MHz) δ 159.6, 156.0, 150.2, 140.0, 138.8, 131.5, 130.7, 130.1, 127.9, 116.4, 115.2, 112.7, 110.3, 109.6, 102.8, 82.8, 55.8, 55.4, 27.7, 20.4.

2-(4-hydroxy-2-methylphenyl)-1*H*-indol-5-ol (2b)

The title compound was prepared according to general procedure **5.2.1.4.2** by reacting *tert*-butyl 5-methoxy-2-(4-methoxy-2-methylphenyl)-1*H*-indole-1-carboxylate (**29**) (260 mg, 0.71 mmol, 1 equiv) with 1M BBr$_3$ (6.39 mL, 6.39 mmol, 9 equiv) in dry

CH$_2$Cl$_2$. Upon completion (TLC in CH$_2$Cl$_2$/MeOH 95:5), standard workup was applied and the crude material was purified by silica gel column chromatography eluting in CH$_2$Cl$_2$/MeOH 95:5 to give the pure product as a light yellow solid (93 mg, yield 55%).
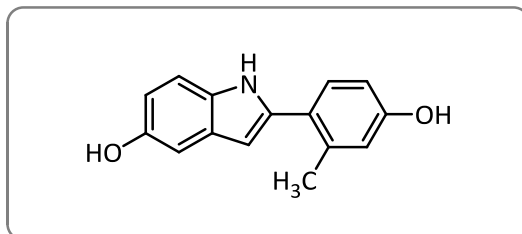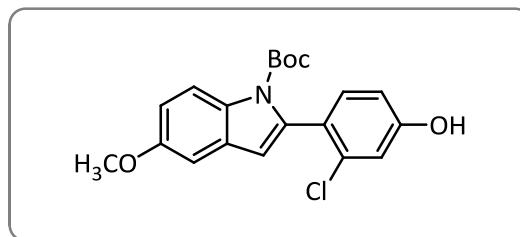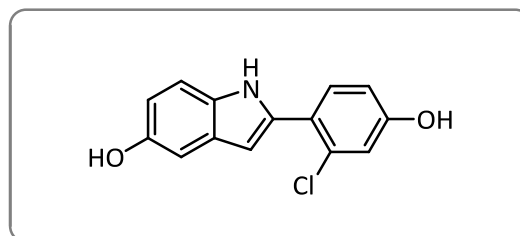
2b: light yellow solid; TLC (KMnO$_4$; PMA): R$_f$ = 0.30 (CH$_2$Cl$_2$/MeOH 95:5); m.p.: 199.5°C; MS (ESI) *m/z* per C$_{15}$H$_{14}$NO$_2^+$ [M+H$^+$]$^+$ calcd. 240.1, found 239.9.
^{1}H NMR (CD$_3$OD, 300 MHz) δ 7.19 (d, *J* = 8.3 Hz, 1H), 7.08 (d, *J* = 8.6 Hz, 1H), 6.83 (d, *J* = 2.3 Hz, 1H), 6.65 – 6.52 (m, 3H), 6.15 (s, 1H), 5.31 (s, 0.08H, 3% MeOH residual), 2.28 (s, 3H).
^{13}C NMR (CD$_3$OD, 75 MHz) δ 157.8, 151.2, 140.1, 138.6, 132.9, 131.4, 131.0, 126.2, 118.3, 113.8, 112.1, 111.8, 104.9, 101.5, 21.4.

tert-butyl 2-(4-aminophenyl)-5-methoxy-1*H*-indole-1-carboxylate (30)

The title compound was prepared according to general procedure **5.2.1.4.1** by reacting *N*-Boc-5-methoxy-2-indolylboronic acid (**27**) (1 g, 3.44 mmol, 1.4 equiv) with 4-iodoaniline (538 mg, 2.45 mmol, 1 equiv). The mixture was heated at 95°C for 2h. Upon completion (TLC cyclohexane/EtOAc 9:1), after standard workup, the crude material was purified by silica gel column chromatography eluting in cyclohexane/EtOAc 9:1 to 4:1 to provide the pure product as a brown oil (334 mg, yield 40%).

30: brown oil; TLC (KMnO$_4$; PMA): R$_f$ = 0.17 (cyclohexane/EtOAc 4:1).
^{1}H NMR (CDCl$_3$, 300 MHz) δ 7.97 (d, *J* = 9.0 Hz, 1H), 7.12 (d, *J* = 8.2 Hz, 2H), 6.91 (d, *J* = 2.6 Hz, 1H), 6.83 (dd, *J* = 9.0, 2.6 Hz, 1H), 6.63 (d, *J* = 8.3 Hz, 2H), 6.32 (s, 1H), 3.77 (s, 3H), 1.29 (s,9H).
^{13}C NMR (CDCl$_3$, 75 MHz) δ 156.0, 150.5, 145.9, 141.8, 132.1, 130.3, 129.9, 125.4, 116.1, 114.6, 112.5, 109.1, 102.9, 83.2, 55.8, 27.9.

2-(4-aminophenyl)-1*H*-indol-5-ol (2c)

The title compound was prepared according to general procedure **5.2.1.4.2** by reacting *tert*-butyl 2-(4-aminophenyl)-5-methoxy-1*H*-indole-1-carboxylate (**30**) (300 mg, 0.89 mmol, 1 equiv) with 1M BBr$_3$ (3.56 mL, 3.56 mmol, 4 equiv) in dry CH$_2$Cl$_2$. Upon completion (TLC in CH$_2$Cl$_2$/MeOH 98:2), standard workup was applied and the crude material was purified by silica gel column chromatography eluting in CH$_2$Cl$_2$/MeOH 98:2 to give the pure product as a light-grey solid (80 mg, yield 40%).
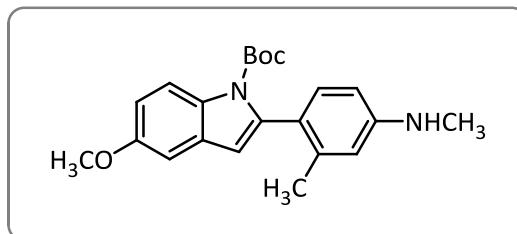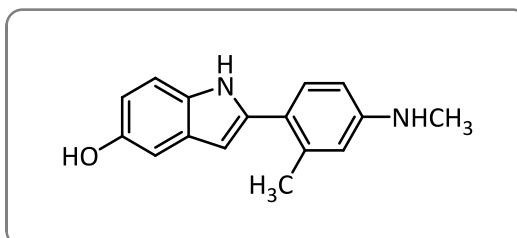
2c: light grey solid; TLC (KMnO$_4$; PMA): R$_f$ = 0.19 (CH$_2$Cl$_2$/MeOH 98:2); m.p.: 257.3°C (dec.);
MS (ESI) *m/z* per C$_{14}$H$_{13}$N$_2$O$^+$ [M+H$^+$]$^+$ calcd. 225.1, found 225.0.
^{1}H NMR ((CD$_3$)$_2$SO, 300 MHz) δ 10.83 (s, 1H), 8.52 (s, 1H), 7.46 (d, *J* = 8.5 Hz, 2H), 7.09 (d, *J* = 8.5 Hz, 1H), 6.75 (d, *J* = 2.0 Hz, 1H), 6.61 (d, *J* = 8.5 Hz, 2H), 6.51 (dd, *J* = 8.5, 2.3 Hz, 1H), 6.39 (s, 1H), 5.23 (s, 2H).
^{13}C NMR ((CD$_3$)$_2$SO, 75 MHz) δ 150.6, 148.2, 139.5, 131.2, 129.8, 125.9, 120.2, 113.9, 111.0, 110.3, 103.3, 94.9.

tert-butyl 5-methoxy-2-(4-(methylamino)phenyl)-1*H*-indole-1-carboxylate (31)

The title compound was prepared according to general procedure **5.2.1.4.1** by reacting *N*-Boc-5-methoxy-2-indolylboronic acid (**27**) (1.45 g, 4.97 mmol, 1.4 equiv) with 4-iodo-*N*-methylaniline (**14**) (827 mg, 3.55 mmol, 1 equiv). The mixture was heated at 95°C for 2h. Upon completion (TLC cyclohexane/EtOAc 9:1), after standard workup, the crude material was purified by silica gel column chromatography eluting in cyclohexane/EtOAc 9:1 to 85:15 to provide the pure product as a brown oil (486 mg, yield 39%).

31: brown oil; TLC (KMnO$_4$; PMA): R$_f$ = 0.22 (cyclohexane/EtOAc 9:1).
^{1}H NMR (CDCl$_3$, 300 MHz) δ 7.97 (d, *J* = 9.0 Hz, 1H), 7.22 − 7.14 (m, 3H), 6.91 (d, *J* = 2.5 Hz, 1H), 6.83 (dd, *J* = 9.0, 2.6 Hz, 1H), 6.66 (d, *J* = 8.6 Hz, 2H), 6.33 (s, *J* = 0.6 Hz, 1H), 3.78 (s, *J* = 7.5 Hz, 4H), 2.82 (s, 3H), 1.31 (s, *J* = 9.8 Hz, 9H).
^{13}C NMR (CDCl$_3$, 75 MHz) δ 156.1, 150.5, 146.8, 141.7, 132.2, 130.3, 130.0, 126.0, 116.1, 113.5, 112.6, 109.3, 102.9, 83.3, 55.8, 29.8, 27.9.

2-(4-(methylamino)phenyl)-1*H*-indol-5-ol (2d)

The title compound was prepared according to general procedure **5.2.1.4.2** by reacting *tert*-butyl 5-methoxy-2-(4-(methyl-amino)phenyl)-1H-indole-1-carboxylate (**31**) (480 mg, 1.36 mmol, 1 equiv) with 1M BBr$_3$ (5.44 mL, 5.44 mmol, 4 equiv) in dry CH$_2$Cl$_2$. Upon completion (TLC in CH$_2$Cl$_2$/MeOH 98:2), standard workup was applied and the crude material was purified by silica gel column chromatography eluting in CH$_2$Cl$_2$/MeOH 98:2 to give the pure product as a light grey solid (105 mg, yield 32%).
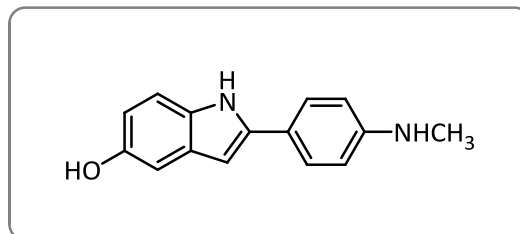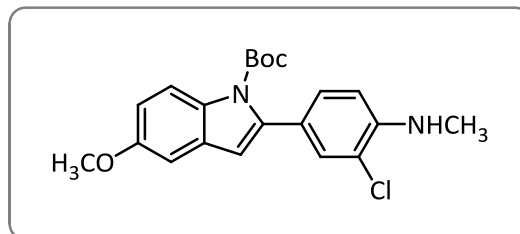
2d: light grey solid; TLC (KMnO$_4$; PMA): R$_f$ = 0.30 (CH$_2$Cl$_2$/MeOH 98:2); m.p.: 246.2°C (dec.);
MS (ESI) *m/z* per C$_{15}$H$_{15}$N$_2$O$^+$ [M+H$^+$]$^+$ calcd. 239.1, found 239.0.
^{1}H NMR ((CD$_3$)$_2$SO, 300 MHz) δ 10.87 (s, 1H), 8.53 (s, 1H), 7.54 (d, *J* = 8.5 Hz, 2H), 7.09 (d, *J* = 8.6 Hz, 1H), 6.74 (d, *J* = 2.3 Hz, 1H), 6.58 (d, *J* = 8.7 Hz, 2H), 6.50 (dd, *J* = 8.6, 2.3 Hz, 1H), 6.40 (s, 1H), 5.83 (q, *J* = 4.8 Hz, 1H), 2.71 (d, *J* = 5.0 Hz, 3H).
^{13}C NMR ((CD$_3$)$_2$SO, 75 MHz) δ 150.6, 149.3, 139.4, 131.2, 129.8, 125.9, 120.0, 111.7, 111.0, 110.4, 103.3, 95.0, 29.6.

tert-butyl 2-(4-amino-2-chlorophenyl)-5-methoxy-1*H*-indole-1-carboxylate (32)

The title compound was prepared according to general procedure **5.2.1.4.1** by reacting *N*-Boc-5-methoxy-2-indolylboronic acid (**27**) (1 g, 3.44 mmol, 1.4 equiv) with 3-chloro-4-iodoaniline (624 mg, 2.45 mmol, 1 equiv). The mixture was heated at 95°C for 5h. Upon completion (TLC cyclohexane/EtOAc 9:1), after standard workup, the crude material was purified by silica gel column chromatography eluting in cyclohexane/EtOAc 9:1 to 85:15 to provide the pure product as a light brown oil (899 mg, yield 98%).
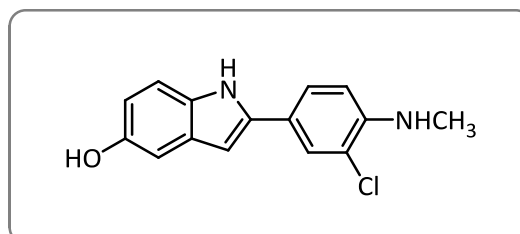
32: light brown oil; TLC (KMnO$_4$; PMA): R$_f$ = 0.19 (cyclohexane/EtOAc 9:1).
^{1}H NMR (CDCl$_3$, 300 MHz) δ 8.06 (d, *J* = 9.0 Hz, 1H), 7.02 (d, *J* = 8.2 Hz, 1H), 6.91 (d, *J* = 2.4 Hz, 1H), 6.84 (dd, *J* = 9.0, 2.6 Hz, 1H), 6.62 (d, *J* = 2.2 Hz, 1H), 6.46 (dd, *J* = 8.2, 2.3 Hz, 1H), 6.30 (s, 1H), 3.74 (s, 3H), 1.25 (s, 9H).
^{13}C NMR (CDCl$_3$, 75 MHz) δ 155.8, 150.1, 147.6, 138.1, 134.9, 131.7, 131.6, 129.8, 124.3, 116.3, 115.1, 113.0, 112.8, 110.0, 103.0, 82.9, 55.7, 27.7.

2-(4-amino-2-chlorophenyl)-1*H*-indol-5-ol (2e)

The title compound was prepared according to general procedure **5.2.1.4.2** by reacting *tert*-butyl 2-(4-amino-2-chloro phenyl)-1*H*-indole-5-methoxy-1*H*-indole-1-carboxylate (**32**) (899 mg, 2.41 mmol, 1 equiv) with 1M BBr$_3$ (9.64 mL, 9.64 mmol, 4 equiv) in dry CH$_2$Cl$_2$. Upon completion (TLC in CH$_2$Cl$_2$/MeOH 95:5), standard workup was applied and the crude material was purified by silica gel column chromatography eluting in CH$_2$Cl$_2$/MeOH 98:2 to give the pure product as a yellowish solid (486 mg, 78%).
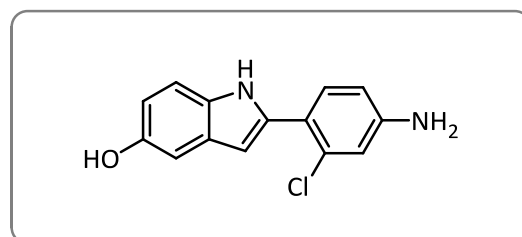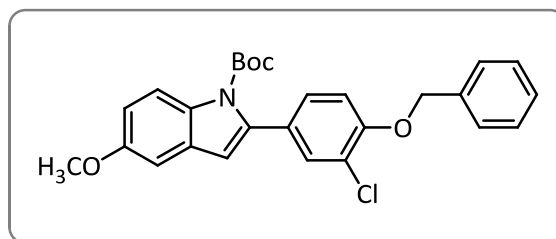
2e: yellowish solid; TLC (KMnO$_4$; PMA): R$_f$ = 0.36 (CH$_2$Cl$_2$/MeOH 95:5); m.p.: 174.6°C; MS (ESI) *m/z* per C$_{14}$H$_{12}$ClN$_2$O$^+$ [M+H$^+$]$^+$ calcd. 259.1, found 259.0
^{1}H NMR (CD$_3$OD, 300 MHz) δ 7.35 (d, *J* = 8.4 Hz, 1H), 7.19 (d, *J* = 8.6 Hz, 1H), 6.90 (d, *J* = 2.2 Hz, 1H), 6.81 (d, *J* = 2.3 Hz, 1H), 6.67 (dd, *J* = 4.0, 2.3 Hz, 1H), 6.64 (dd, *J* = 4.2, 2.3 Hz, 1H), 6.50 (s, 1H), 3.48 (q, *J* = 7.0 Hz, 0.19H, 6% Et2O residual), 1.17 (t, *J* = 7.0 Hz, 0.33H, 6% Et2O residual).
^{13}C NMR (CD$_3$OD, 75 MHz) δ 151.3, 149.6, 137.8, 133.4, 132.8, 132.0, 130.6, 130.6, 122.1, 117.0, 114.8, 112.3, 112.2, 104.9, 101.9.

tert-butyl 2-(2-chloro-4-(methylamino)phenyl)-5-methoxy-1*H*-indole-1-carboxylate (33)

The title compound was prepared according to general procedure **5.2.1.4.1** by reacting *N*-Boc-5-methoxy-2-indolylboronic acid (**27**) (344 mg, 1.18 mmol, 1.4 equiv) with 3-chloro-4-iodo-*N*-methylaniline (**15**) (225 mg, 0.84 mmol, 1 equiv). The mixture was heated at 95°C for 5h. Upon completion (TLC cyclohexane/EtOAc 9:1), after standard workup, the crude material was purified by silica gel column chromatography eluting in cyclohexane/EtOAc 9:1 to provide the pure product as a light brown oil (185 mg, yield 56%).

33: light brown oil; TLC (KMnO$_4$; PMA): R$_f$ = 0.26 (cyclohexane/EtOAc 9:1).
^{1}H NMR (CDCl$_3$, 300 MHz) δ 8.06 (d, *J* = 9.0 Hz, 1H), 7.07 (d, *J* = 8.3 Hz, 1H), 6.93 (d, *J* = 2.4 Hz, 1H), 6.85 (dd, *J* = 9.0, 2.6 Hz, 1H), 6.56 (d, *J* = 2.3 Hz, 1H), 6.41 (dd, *J* = 8.3, 2.4 Hz, 1H), 6.31 (s, 1H), 3.76 (s3H), 2.74 (s, 3H), 1.27 (s, 9H).
^{13}C NMR (CDCl$_3$, 75 MHz) δ 155.9, 150.2, 138.4, 135.1, 131.6, 129.9, 122.9, 116.4, 113.0, 112.2, 110.4, 110.0, 102.9, 82.8, 55.8, 30.7, 27.8.

2-(2-chloro-4-(methylamino)phenyl)-1*H*-indol-5-ol (2f)

The title compound was prepared according to general procedure **5.2.1.4.2** by reacting *tert*-butyl 2-(2-chloro-4-(methyl-amino)phenyl)-5-methoxy-1*H*-indole-1-carboxylate (**33**) (185 mg, 0.48 mmol, 1 equiv) with 1M BBr$_3$ (1.92 mL, 1.92 mmol, 4 equiv) in dry CH$_2$Cl$_2$. Upon completion (TLC in CH$_2$Cl$_2$/MeOH 98:2), standard workup was applied and the crude material was purified by silica gel column chromatography eluting in CH$_2$Cl$_2$ – MeOH 99:1 to give the pure product as a light yellow solid (70 mg, 54%).

2f: light yellow solid; TLC (KMnO$_4$; PMA): R$_f$ = 0.36 (CH$_2$Cl$_2$/MeOH 98:2); m.p.: 148.8°C; MS (ESI) *m/z* per C$_{15}$H$_{14}$ClN$_2$O$^+$ [M+H$^+$]$^+$ calcd. 273.1, found 273.0.
^{1}H NMR (CD$_3$OD, 300 MHz) δ 7.29 (d, *J* = 8.5 Hz, 1H), 7.09 (d, *J* = 8.6 Hz, 1H), 6.80 (d, *J* = 2.4 Hz, 1H), 6.58 (d, *J* = 2.4 Hz, 1H), 6.54 (dd, *J* = 8.6, 2.4 Hz, 1H), 6.50 (dd, *J* = 8.6, 2.4 Hz, 1H), 6.40 (s, 1H), 2.69 (s, 3H).
^{13}C NMR (CD$_3$OD, 75 MHz) δ 151.7, 151.4, 138.1, 133.8, 132.9, 132.0, 130.8, 121.0, 113.9, 113.8, 112.3, 112.2, 112.1, 104.9, 101.7, 101.7, 30.3.

tert-butyl 2-(2-chloro-4-hydroxyphenyl)-5-methoxy-1*H*-indole-1-carboxylate (34)

The title compound was prepared according to general procedure **5.2.1.4.1** by reacting *N*-Boc-5-methoxy-2-indolylboronic acid (**27**) (400 mg, 1.37 mmol, 1.4 equiv) with 4-bromo-3-chlorophenol (204 mg, 0.98 mmol, 1 equiv). The mixture was heated at 95°C for 5h. Upon completion (TLC cyclohexane/EtOAc 9:1), after standard workup, the crude material was purified by silica gel column chromatography eluting in cyclohexane/EtOAc 9:1 to provide the pure product as a brown oil (190 mg, yield 52%).


34: brown oil; TLC (KMnO$_4$; PMA): R$_f$ = 0.28 (cyclohexane/EtOAc 85:15).
^{1}H NMR (CDCl$_3$, 300 MHz) δ 8.02 (d, *J* = 9.0 Hz, 1H), 7.12 (d, *J* = 8.3 Hz, 1H), 6.94 (d, *J* = 2.5 Hz, 1H), 6.91 – 6.83 (m, 1H), 6.81 (d, *J* = 2.5 Hz, 1H), 6.65 (dd, *J* = 8.3, 2.5 Hz, 1H), 6.47 (br s, 1H), 6.33 (s, 1H), 3.77 (s, 3H), 1.29 (s, 9H).
^{13}C NMR (CDCl$_3$, 75 MHz) δ 156.8, 155.9, 150.3, 137.7, 134.9, 131.8, 131.5, 129.9, 126.6, 116.4, 113.7, 113.3, 110.6, 103.3, 83.7, 55.9, 27.8.

2-(2-chloro-4-hydroxyphenyl)-1*H*-indol-5-ol (2g)

The title compound was prepared according to general procedure **5.2.1.4.2** by reacting *tert*-butyl 2-(2-chloro-4-hydroxy-phenyl)-5-methoxy-1*H*-indole-1-carboxylate (**34**) (190 mg, 0.51 mmol, 1 equiv) with 1M BBr$_3$ (3.06 mL, 3.06 mmol) in dry CH$_2$Cl$_2$. Upon completion (TLC in CH$_2$Cl$_2$/MeOH 95:5), standard workup was applied and the crude material was purified by silica gel column chromatography eluting in CH$_2$Cl$_2$/MeOH 95:5 to give the pure product as a white solid (91 mg, yield 69%).
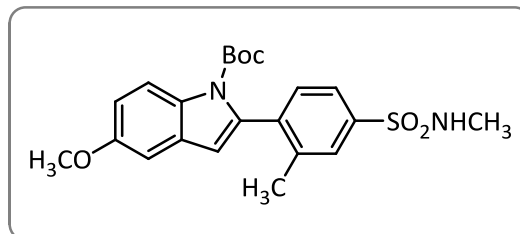

2g: white solid; TLC (KMnO$_4$; PMA): R$_f$ = 0.30 (CH$_2$Cl$_2$/MeOH 95:5); m.p.: 198.4°C; MS (ESI) *m/z* per C$_{14}$H$_{11}$ClNO$_2$$^+$ [M+H$^+$]$^+$ calcd. 260.1, found 260.0.
^{1}H NMR (CD$_3$OD, 300 MHz) δ 7.43 (d, *J* = 8.5 Hz, 1H), 7.22 (d, *J* = 8.7 Hz, 1H), 7.00 – 6.93 (m, 2H), 6.80 (dd, *J* = 8.5, 2.5 Hz, 1H), 6.71 (dd, *J* = 8.6, 2.4 Hz, 1H), 6.56 (s, *J* = 0.7 Hz, 1H), 5.37 (s, 0.07H, 4% CH$_2$Cl$_2$ residual).
^{13}C NMR (CD$_3$OD, 75 MHz) δ 158.6, 151.3, 137.2, 133.5, 132.9, 132.4, 130.5, 124.7, 118.0, 115.5, 112.5, 112.4, 105.1, 102.4.

tert-butyl 5-methoxy-2-(2-methyl-4-(methylamino)phenyl)-1*H*-indole-1-carboxylate (35)

The title compound was prepared according to general procedure **5.2.1.4.1** by reacting *N*-Boc-5-methoxy-2-indolylboronic acid (**27**) (306 mg, 1.05 mmol, 1.4 equiv) with 4-bromo-*N*,3-dimethylaniline (**16**) (150 mg, 0.75 mmol, 1 equiv). The mixture was heated at 95°C for 3h. Upon completion (TLC cyclohexane/EtOAc 9:1), after standard workup, the crude material was purified by silica gel column chromatography eluting in cyclohexane/EtOAc 9:1 to provide the pure product as a yellow oil (212 mg, yield 77%).
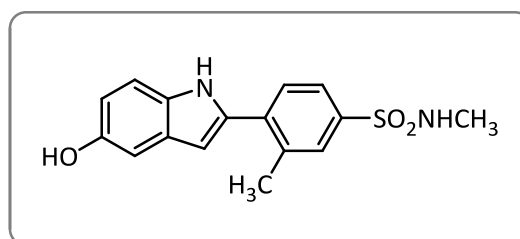
35: yellow oil; TLC (KMnO$_4$; PMA): R$_f$ = 0.45 (cyclohexane/EtOAc 4:1).
^{1}H NMR (CDCl$_3$, 300 MHz) δ 8.04 (d, *J* = 9.0 Hz, 1H), 6.95 (d, *J* = 7.8 Hz, 1H), 6.90 (d, *J* = 2.5 Hz, 1H), 6.81 (dd, *J* = 9.0, 2.6 Hz, 1H), 6.38 – 6.28 (m, 2H), 6.23 (s, 1H), 3.73 (s, 3H), 2.71 (s, 3H), 2.00 (s, 3H), 1.19 (s, 9H).
^{13}C NMR (CDCl$_3$, 75 MHz) δ 155.9, 150.3, 149.4, 141.0, 138.0, 131.5, 130.6, 130.2, 124.2, 116.3, 113.4, 112.4, 109.3, 109.1, 102.8, 82.5, 55.7, 30.8, 27.7, 20.3.

2-(2-methyl-4-(methylamino)phenyl)-1*H*-indol-5-ol (2h)

The title compound was prepared according to general procedure **5.2.1.4.2** by reacting *tert*-butyl 5-methoxy-2-(2-methyl-4-(methylamino)phenyl)-1*H*-indole-1-carboxylate (**35**) (212 mg, 0.58 mmol, 1 equiv) with 1M BBr$_3$ (2.32 mL, 2.32 mmol, 4 equiv) in dry CH$_2$Cl$_2$. Upon completion (TLC in CH$_2$Cl$_2$/MeOH 95:5), standard workup was applied and the crude material was purified by silica gel column chromatography eluting in CH$_2$Cl$_2$/MeOH 95:5 to give the pure product as a yellowish solid (134 mg, yield 91%).

2h: yellowish solid; TLC (KMnO$_4$; PMA): R$_f$ = 0.47 (CH$_2$Cl$_2$/MeOH 95:5); m.p.: 90.5°C (dec.); MS (ESI) *m/z* per C$_{16}$H$_{17}$N$_2$O$^+$ [M+H$^+$]$^+$ calcd. 253.1, found 253.2.
^{1}H NMR (CD$_3$OD, 300 MHz) δ 7.27 (d, *J* = 8.1 Hz, 1H), 7.16 (d, *J* = 8.6 Hz, 1H), 6.89 (d, *J* = 2.2 Hz, 1H), 6.62 (dd, *J* = 8.6, 2.4 Hz, 1H), 6.57 – 6.47 (m, 2H), 6.22 (s, 1H), 3.75 – 3.64 (m, 0.2H, DIPE residual), 2.78 (s, 3H), 2.39 (s, 3H), 1.11 (d, *J* = 6.1 Hz, 1.4H, 12% DIPE residual).
^{13}C NMR (CD$_3$OD, 75 MHz) δ 151.2, 150.6, 140.7, 137.8, 132.8, 131.0, 130.8, 123.3, 115.8, 112.0, 111.5, 111.2, 104.8, 30.7, 21.6.

tert-butyl 2-(3-chloro-4-(methylamino)phenyl)-5-methoxy-1*H*-indole-1-carboxylate (36)

The title compound was prepared according to general procedure **5.2.1.4.1** by reacting *N*-Boc-5-methoxy-2-indolylboronic acid (**27**) (516 mg, 1.77 mmol, 1.4 equiv) with 4-bromo-2-chloro-*N*-methylaniline (**17**) (279 mg, 1.26 mmol, 1 equiv). The mixture was heated at 95°C for 1h. Upon completion (TLC cyclohexane/EtOAc 95:5), after standard workup, the crude material was purified by silica gel column chromatography eluting in cyclohexane/EtOAc 95:5 to provide the pure product as a yellow oil (394 mg, yield 81%).
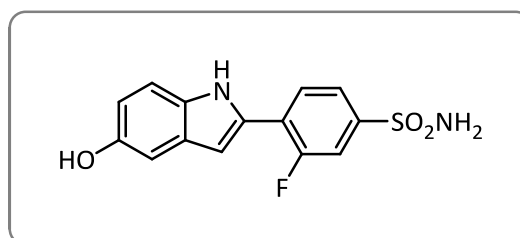
36: yellow oil; TLC (KMnO$_4$; PMA): R$_f$ = 0.41 (cyclohexane/EtOAc 95:5).
^{1}H NMR (CD$_3$OD, 300 MHz) δ 8.00 (d, *J* = 9.1 Hz, 1H), 7.18 (d, *J* = 2.0 Hz, 1H), 7.10 (dd, *J* = 8.3, 2.0 Hz, 1H), 6.94 (d, *J* = 2.5 Hz, 1H), 6.83 (dd, *J* = 9.0, 2.6 Hz, 1H), 6.59 (d, *J* = 8.4 Hz, 1H), 6.32 (s, 1H), 2.84 (s, 3H), 1.28 (s, 9H).
^{13}C NMR (CD$_3$OD, 75 MHz) δ 157.4, 151.6, 146.3, 141.8, 133.4, 131.5, 130.4, 129.4, 124.5, 119.0, 116.7, 113.6, 110.7, 110.1, 103.8, 84.2, 56.0, 30.4, 27.9.

2-(3-chloro-4-(methylamino)phenyl)-1*H*-indol-5-ol (2i)

The title compound was prepared according to general procedure **5.2.1.4.2** by reacting *tert*-butyl 2-(3-chloro-4-(methylamino)phenyl)-5-methoxy-1*H*-indole-1-carboxylate (**36**) (394 mg, 1.02 mmol, 1 equiv) with 1M BBr$_3$ (4.08 mL, 4.08 mmol, 4 equiv) in dry CH$_2$Cl$_2$. Upon completion (TLC in CH$_2$Cl$_2$/MeOH 95:5), standard workup was applied and the crude material was purified by silica gel column chromatography eluting in CH$_2$Cl$_2$/MeOH 98:2 to give the pure product as a white solid (236 mg, yield 85%).
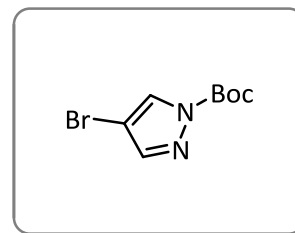
2i: white solid; TLC (KMnO$_4$; PMA): R$_f$ = 0.54 (CH$_2$Cl$_2$/MeOH 95:5); m.p.: 200.7°C (dec.);
MS (ESI) *m/z* per C$_{15}$H$_{14}$ClN$_2$O$^+$ [M+H$^+$]$^+$ calcd. 273.1, found 273.0.
^{1}H NMR (CD$_3$OD, 300 MHz) δ 7.63 (d, J = 2.1 Hz, 1H), 7.50 (dd, J = 8.5, 2.1 Hz, 1H), 7.17 (d, J = 8.6 Hz, 1H), 6.90 (d, J = 2.3 Hz, 1H), 6.68 – 6.60 (m, 2H), 6.44 (s, 1H), 2.83 (s, 3H).
^{13}C NMR (CD$_3$OD, 75 MHz) δ 151.5, 145.9, 139.8, 133.6, 131.5, 126.6, 125.9, 123.4, 120.2, 112.2, 111.9, 111.9, 105.0, 97.4, 30.4.

tert-butyl 2-(3-chloro-4-phenoxyphenyl)-5-methoxy-1*H*-indole-1-carboxylate (37)

The title compound was prepared according to general procedure **5.2.1.4.1** by reacting *N*-Boc-5-methoxy-2-indolylboronic acid (**27**) (200 mg, 0.69 mmol, 1.4 equiv) with 1-benzyloxy-4-bromo-2-chlorobenzene (146 mg, 0.49 mmol, 1 equiv). The mixture was heated at 95°C for 5h. Upon completion (TLC cyclohexane/EtOAc 95:5), after standard workup, the crude material was purified by silica gel column chromatography eluting in cyclohexane/EtOAc 95:5, and then further purified by another column chromatography in cyclohexane/CH$_2$Cl$_2$ 3:7 to provide the pure product as a yellow oil (202 mg, yield 89%).

37: yellow oil; TLC (KMnO$_4$; PMA): R$_f$ = 0.41 (cyclohexane/EtOAc 95:5).
^{1}H NMR (CDCl$_3$, 300 MHz) δ 8.01 (d, *J* = 9.1 Hz, 1H), 7.45 – 7.38 (m, 2H), 7.38 – 7.22 (m, 3H), 7.20 – 7.18 (m, 1H), 7.18 – 7.12 (m, 1H), 6.92 (d, *J* = 2.5 Hz, 2H), 6.91 – 6.83 (m, 2H), 6.37 (s, 1H), 5.15 (s, 2H), 3.79 (s, 3H), 1.26 (s, 9H).
^{13}C NMR (CDCl$_3$, 75 MHz) δ 156.2, 153.7, 150.1, 139.5, 136.5, 132.2, 130.9, 129.9, 128.7, 128.7, 128.1, 128.0, 127.2, 122.6, 116.3, 113.4, 113.2, 110.1, 103.0, 83.6, 70.9, 55.8, 27.8.

2-(3-chloro-4-hydroxyphenyl)-1*H*-indol-5-ol (2j)

The title compound was prepared according to general procedure **5.2.1.4.2** by reacting *tert*-butyl 2-(3-chloro-4-phenoxy-phenyl)-5-methoxy-1*H*-indole-1-carboxylate (**37**) (202 mg, 0.44 mmol, 1 equiv) with 1M BBr$_3$ (2.20 mL, 2.20 mmol, 5 equiv) in dry CH$_2$Cl$_2$. Upon completion (TLC in CH$_2$Cl$_2$/MeOH 95:5), standard workup was applied and the crude material was purified by silica gel column chromatography eluting in CH$_2$Cl$_2$/MeOH 97:3 to give the pure product as a white solid (65 mg, yield 78%).

2j: white solid; TLC (KMnO$_4$; PMA): R$_f$ = 0.60 (cyclohexane/CH$_2$Cl$_2$ 3:7); m.p.: 210.0°C (dec.); MS (ESI) *m/z* per C$_{14}$H$_{11}$ClNO$_2$$^+$ [M+H$^+$]$^+$ calcd. 260.1, found 259.6.
^{1}H NMR (CD$_3$OD, 300 MHz) δ 7.70 (t, *J* = 2.5 Hz, 1H), 7.51 (dt, *J* = 8.5, 2.6 Hz, 1H), 7.22 – 7.15 (m, 1H), 6.95 (dd, *J* = 8.5, 2.8 Hz, 1H), 6.89 (t, *J* = 2.4 Hz, 1H), 6.69 – 6.61 (m, 1H), 6.54 – 6.50 (m, 1H), 3.34 (s, 0.07H, 2% MeOH residual).
^{13}C NMR (CD$_3$OD, 75 MHz) δ 153.6, 151.6, 139.0, 133.7, 131.3, 127.4, 125.8, 122.1, 117.9, 112.5, 112.3, 105.1, 98.3.
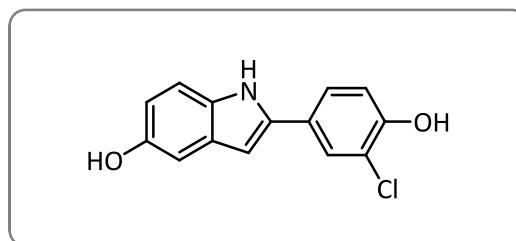
tert-butyl 5-methoxy-2-(2-methyl-4-sulfamoylphenyl)-1*H*-indole-1-carboxylate (38)

The title compound was prepared according to general procedure **5.2.1.4.1** by reacting *N*-Boc-5-methoxy-2-indolylboronic acid (**27**) (244 mg, 0.84 mmol, 1.4 equiv) with 4-bromo-3-methylbenzenesulfonamide (**18**) (150 mg, 0.60 mmol, 1 equiv). The
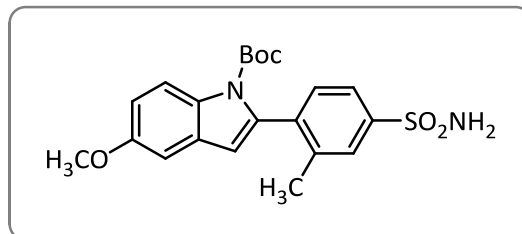
mixture was heated at 95°C for 3h. Upon completion (TLC cyclohexane/EtOAc 7:3), after standard workup, the crude material was purified by silica gel column chromatography eluting in cyclohexane/EtOAc 7:3 to provide the pure product as a yellow oil (200 mg, yield 80%).

38: yellow oil; TLC (KMnO$_4$; PMA): R$_f$ = 0.28 (cyclohexane/EtOAc 7:3).
^{1}H NMR (CDCl$_3$, 300 MHz) δ 8.02 (d, *J* = 9.0 Hz, 1H), 7.78 − 7.64 (m, 2H), 7.28 (d, *J* = 8.0 Hz, 1H), 6.90 (d, *J* = 2.4 Hz, 1H), 6.86 (dd, *J* = 9.0, 2.5 Hz, 1H), 6.28 (s, 1H), 5.37 (br s, 2H), 3.74 (s, 3H), 2.13 (s, 3H), 1.17 (s, 9H).
^{13}C NMR (CDCl$_3$, 75 MHz) δ 156.1, 149.7, 141.7, 139.9, 138.9, 137.9, 131.5, 130.2, 129.9, 127.1, 123.3, 116.5, 113.5, 110.2, 103.2, 83.8, 55.8, 27.6, 20.0.

4-(5-hydroxy-1*H*-indol-2-yl)-3-methylbenzenesulfonamide (2k)

The title compound was prepared according to general procedure **5.2.1.4.2** by reacting *tert*-butyl 5-methoxy-2-(2-methyl-4-sulfamoylphenyl)-1*H*-indole-1-carboxylate (**38**) (145 mg, 0.35 mmol, 1 equiv) with 1M BBr$_3$ (1.40 mL, 1.40 mmol, 4 equiv) in dry
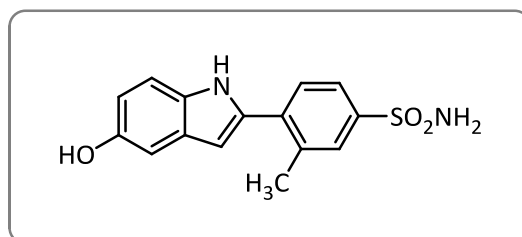
CH$_2$Cl$_2$. Upon completion (TLC in CH$_2$Cl$_2$/MeOH 95:5), standard workup was applied and the crude material was purified by silica gel column chromatography eluting in CH$_2$Cl$_2$/MeOH 95:5 to give the pure product as a white solid (76 mg, yield 72%).
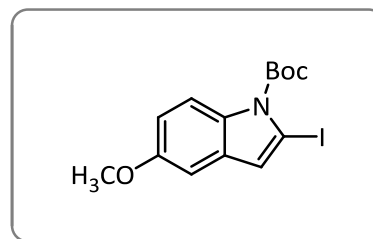
2k: white solid; TLC (KMnO$_4$; PMA): R$_f$ = 0.47 (CH$_2$Cl$_2$/MeOH 95:5); m.p.: 257.9°C; MS (ESI) *m/z* per C$_{15}$H$_{15}$N$_2$O$_3$S$^+$ [M+H$^+$]$^+$ calcd. 303.1, found 302.9.
^{1}H NMR (CD$_3$OD, 300 MHz) δ 7.84 (d, *J* = 0.7 Hz, 1H), 7.78 (dd, *J* = 8.2, 2.0 Hz, 1H), 7.66 (d, *J* = 8.2 Hz, 1H), 7.24 (d, *J* = 8.7 Hz, 1H), 6.96 (d, *J* = 2.3 Hz, 1H), 6.72 (dd, *J* = 8.7, 2.4 Hz, 1H), 6.51 (s, *J* = 0.7 Hz, 1H), 3.35 (s, 0.67H, 22% MeOH residual), 2.59 (s, 3H).
^{13}C NMR (CD$_3$OD, 75 MHz) δ 151.7, 143.1, 138.2, 138.0, 137.8, 133.5, 130.7, 130.2, 129.4, 124.6, 113.4, 112.6, 105.3, 103.9, 21.6.

tert-butyl 5-methoxy-2-(2-methyl-4-(*N*-methylsulfamoyl)phenyl)-1*H*-indole-1-carboxylate (39)

The title compound was prepared according to general procedure **5.2.1.4.1** by reacting *N*-Boc-5-methoxy-2-indolylboronic acid (**27**) (162 mg, 0.56 mmol, 1.4 equiv) with 4-bromo-3-methylbenzenesulfonamide (**19**) (105 mg, 0.40 mmol, 1 equiv). The mixture was heated at 95°C for 3h. Upon completion (TLC cyclohexane/EtOAc 7:3), after standard workup, the crude material was purified by silica gel column chromatography eluting in cyclohexane/EtOAc 7:3 to provide the pure product as a yellow oil (167 mg, yield 98%).
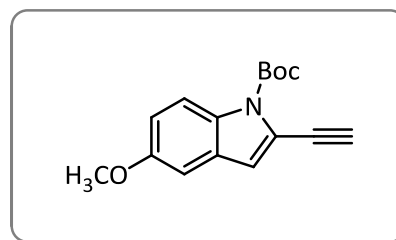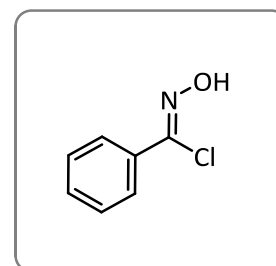
39: yellow oil; TLC (KMnO$_4$; PMA): R$_f$ = 0.38 (cyclohexane/EtOAc 7:3).
^{1}H NMR (CDCl$_3$, 300 MHz) δ 8.08 (d, *J* = 9.1 Hz, 1H), 7.72 − 7.60 (m, 2H), 7.33 (d, *J* = 7.9 Hz, 1H), 6.95 (d, *J* = 2.5 Hz, 1H), 6.89 (dd, *J* = 9.0, 2.5 Hz, 1H), 6.32 (s, 1H), 4.83 (q, *J* = 5.3 Hz, 1H), 3.78 (s, 3H), 2.62 (d, *J* = 5.4 Hz, 3H), 2.17 (s, 3H), 1.16 (s, 9H).
^{13}C NMR (CDCl$_3$, 75 MHz) δ 156.3, 149.7, 140.2, 139.0, 138.4, 137.9, 131.6, 130.3, 129.9, 128.0, 124.3, 116.5, 113.6, 110.2, 103.2, 83.5, 55.8, 29.4, 27.6, 20.1.

4-(5-hydroxy-1*H*-indol-2-yl)-*N*,3-dimethylbenzenesulfonamide (2l)

The title compound was prepared according to general procedure **5.2.1.4.2** by reacting *tert*-butyl 5-methoxy-2-(2-methyl-4-(*N*-methylsulfamoyl)phenyl)-1*H*-indole-1-carboxylate (**39**) (167 mg, 0.39 mmol, 1 equiv) with 1M BBr$_3$ (1.56 mL, 1.56 mmol, 4 equiv) in dry CH$_2$Cl$_2$. Upon completion (TLC in CH$_2$Cl$_2$/MeOH 95:5), standard workup was applied and the crude material was purified by silica gel column chromatography eluting in CH$_2$Cl$_2$/MeOH 95:5 to give the pure product as a yellowish solid (115 mg, yield 93%).

2l: yellowish solid; TLC (KMnO$_4$; PMA): R$_f$ = 0.38 (CH$_2$Cl$_2$/MeOH 95:5); m.p.: 160.0°C (dec.); MS (ESI) *m/z* per C$_{16}$H$_{17}$N$_2$O$_3$S$^+$ [M+H$^+$]$^+$ calcd. 317.1, found 317.1.
^{1}H NMR (CD$_3$OD, 300 MHz) δ 7.79 − 7.67 (m, 3H), 7.25 (d, *J* = 8.7 Hz, 1H), 6.96 (d, *J* = 2.0 Hz, 1H), 6.73 (dd, *J* = 8.7, 2.4 Hz, 1H), 6.53 (s, 1H), 3.35 (s, 0.10H, 3% MeOH residual), 2.59 (s, 3H), 2.56 (s, 3H).
^{13}C NMR (CD$_3$OD, 75 MHz) δ 151.8, 138.7, 138.7, 138.2, 137.6, 133.5, 130.7, 130.4, 125.7, 113.5, 112.6, 105.3, 104.0, 29.3, 21.6.

tert-butyl 2-(2-fluoro-4-sulfamoylphenyl)-5-methoxy-1*H*-indole-1-carboxylate (40)

The title compound was prepared according to general procedure **5.2.1.4.1** by reacting *N*-Boc-5-methoxy-2-indolylboronic acid (**27**) (100 mg, 0.34 mmol, 1.4 equiv) with 4-bromo-3-fluorobenzenesulfonamide (62 mg, 0.25 mmol, 1 equiv). The mixture was heated at 95°C for 5h. Upon completion (TLC cyclohexane/EtOAc 7:3), after standard workup, the crude material was purified by silica gel column chromatography eluting in cyclohexane/EtOAc 3:2 to provide the pure product as a yellow oil (106 mg, yield 100%).
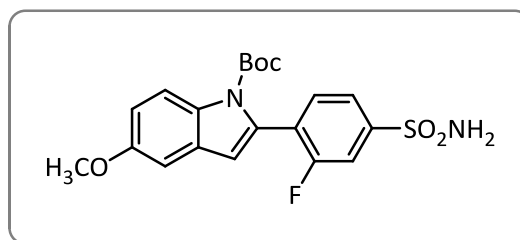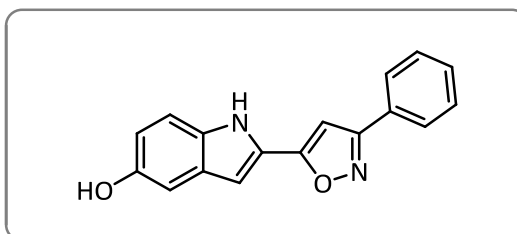
40: yellow oil; TLC (KMnO$_4$; PMA): R$_f$ = 0.30 (cyclohexane/EtOAc 7:3).
^{1}H NMR (CD$_3$OD, 300 MHz) δ 8.07 (d, J = 9.1 Hz, 1H), 7.79 (dd, J = 8.0, 1.8 Hz, 1H), 7.68 (dd, J = 8.2, 1.4 Hz, 1H), 7.64 (dd, J = 6.4, 5.6 Hz, 1H), 7.11 (d, J = 2.5 Hz, 1H), 6.97 (dd, J = 9.1, 2.6 Hz, 1H), 6.67 (s, 1H), 3.84 (s, 3H), 1.35 (s, 9H).
^{13}C NMR (CDCl$_3$, 75 MHz) δ 159.6 (d, J = 253.0 Hz), 156.2, 149.8, 143.1 (d, J = 7.0 Hz), 132.9, 132.2, 131.1 (d, J = 3.3 Hz), 129.6, 128.3 (d, J = 5.4 Hz), 122.1 (d, J = 3.7 Hz), 116.5, 114.4, 113.7 (d, J = 25.4 Hz), 112.1, 103.3, 84.3, 55.9, 27.8.

3-fluoro-4-(5-hydroxy-1*H*-indol-2-yl)benzenesulfonamide (2m)

The title compound was prepared according to general procedure **5.2.1.4.2** by reacting *tert*-butyl 2-(2-fluoro-4-sulfamoylphenyl)-5-methoxy-1*H*-indole-1-carboxylate (**40**) (106 mg, 0.25 mmol, 1 equiv) with boron tribromide 1M (1.0 mL, 1.0 mmol, 4 equiv) in dry CH$_2$Cl$_2$. Upon completion (TLC in CH$_2$Cl$_2$/MeOH 95:5), standard workup was applied and the crude material was purified by silica gel column chromatography eluting in CH$_2$Cl$_2$/MeOH 97:3 to give the pure product as a yellowish solid (65 mg, yield 78%).

2m: yellowish solid; TLC (KMnO$_4$; PMA): R$_f$ = 0.55 (CH$_2$Cl$_2$/MeOH 9:1); m.p.: 240.7°C (dec.); MS (ESI) *m/z* per C$_{14}$H$_{12}$FN$_2$O$_3$S$^+$ [M+H$^+$]$^+$ calcd. 307.1, found 306.9.
^{1}H NMR (CD$_3$OD, 300 MHz) δ 7.95 (t, J = 7.9 Hz, 1H), 7.79 − 7.66 (m, 2H), 7.28 (dd, J = 8.8, 0.5 Hz, 1H), 6.99 − 6.87 (m, 2H), 6.76 (dd, J = 8.7, 2.4 Hz, 1H), 3.35 (s, 0.24H, 8% MeOH residual).
^{13}C NMR (CD$_3$OD, 75 MHz) δ 159.9 (d, J = 252.4 Hz), 152.1, 144.3 (d, J = 7.1 Hz), 134.0, 132.0 (d, J = 2.9 Hz), 130.7 (d, J = 1.2 Hz), 128.9 (d, J = 3.9 Hz), 125.8 (d, J = 12.2 Hz), 123.4 (d, J = 3.4 Hz), 115.4 (d, J = 26.2 Hz), 114.6, 112.9, 105.4, 104.8 (d, J = 8.9 Hz).

tert-butyl 4-bromo-1H-pyrazole-1-carboxylate (41)

4-bromo-1*H*-pyrazole (0.98 g, 6.7 mmol, 1 equiv) was dissolved in CH_2Cl_2 (18 mL), Boc_2O (2.19 g, 10.0 mmol, 1.5 equiv), Et_3N (3.5 mL) and DMAP (65 mg, 0.67 mmol, 0.10 equiv) were added and the resulting mixture was stirred at RT for 30 min. Upon completion (TLC cyclohexane/EtOAc 9:1), the solvent was evaporated and the residue was extracted with EtOAc (x 3). The crude material was then purified by silica gel column chromatography eluting in cyclohexane/EtOAc 9:1 to provide the pure product as a yellow oil (1.49 g, yield 90%).

41: yellow oil; TLC (KMnO$_4$; PMA): R$_f$ = 0.32 (cyclohexane/EtOAc 9:1).
^{1}H NMR (CDCl$_3$, 300 MHz) δ 8.26 (s, 1H), 7.82 (s, 1H), 1.81 (s, 9H).
^{13}C NMR (CDCl$_3$, 75 MHz) δ 144.5, 130.5, 97.4, 86.4, 28.0.

tert-butyl 2-(1-(*tert*-butoxycarbonyl)-1*H*-pyrazol-4-yl)-5-methoxy-1*H*-indole-1-carboxylate (42)

The title compound was prepared according to general procedure **5.2.1.4.1** by reacting *N*-Boc-5-methoxy-2-indolylboronic acid (**27**) (300 mg, 1.03 mmol, 1.4 equiv) with *tert*-butyl 4-bromo-1*H*-pyrazole-1-carboxylate (**41**) (182 mg, 0.74 mmol, 1 equiv). The mixture was heated at 95°C for 5h. Upon completion (TLC cyclohexane/EtOAc 9:1), after standard workup, the crude material was purified by silica gel column chromatography eluting in cyclohexane/EtOAc 4:1 to provide the pure product as a yellow oil (174 mg, yield 57%).
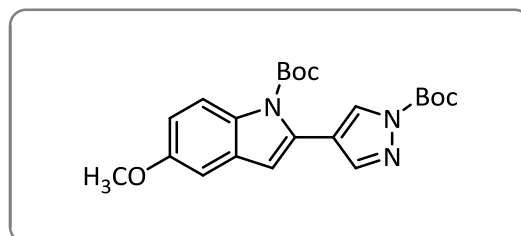
42: yellow oil; TLC (KMnO$_4$; PMA): R$_f$ = 0.55 (cyclohexane/EtOAc 7:3).
^{1}H NMR (CD$_3$OD, 300 MHz) δ 8.24 (s, 1H), 7.95 (d, *J* = 9.1 Hz, 1H), 7.83 (s, 1H), 6.93 (d, *J* = 2.5 Hz, 1H), 6.84 (dd, *J* = 9.1, 2.6 Hz, 1H), 6.50 (s, 1H), 3.76 (s, 3H), 1.63 (s, 9H), 1.47 (s, 9H).
^{13}C NMR (CD$_3$OD, 75 MHz) δ 157.5, 151.2, 148.6, 145.6, 133.1, 131.4, 131.1, 130.7, 119.5, 117.3, 114.4, 111.9, 103.9, 87.1, 85.2, 56.0, 28.2, 28.1.

2-(1H-pyrazol-4-yl)-1*H*-indol-5-ol (3)

The title compound was prepared according to general procedure **5.2.1.4.2** by reacting *tert*-butyl 2-(1-(tert-butoxycarbo-nyl)-1H-pyrazol-4-yl)-5-methoxy-1*H*-indole-1-carboxylate (**42**) (174 mg, 0.42 mmol, 1 equiv) with 1M BBr$_3$ (2.10 mL, 2.10 mmol, 5 equiv) in dry CH$_2$Cl$_2$. Upon completion (TLC in CH$_2$Cl$_2$/MeOH 95:5), standard workup was applied and the crude material was purified by silica gel column chromatography eluting in CH$_2$Cl$_2$/MeOH 9:1 to give the pure product as a white solid (65 mg, yield 78%).
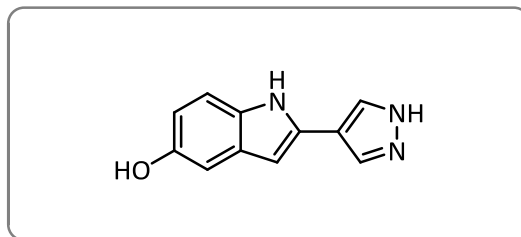
3: white solid; TLC (KMnO$_4$; PMA): R$_f$ = 0.38 (CH$_2$Cl$_2$/MeOH 9:1); m.p.: 240.1°C (dec.); MS (ESI) *m/z* per C$_{11}$H$_{10}$N$_3$O$^+$ [M+H$^+$]$^+$ calcd. 200.1, found 200.2.
^{1}H NMR (CD$_3$OD, 300 MHz) δ 7.93 (s, 2H), 7.15 (d, *J* = 8.6 Hz, 1H), 6.87 (d, *J* = 2.3 Hz, 1H), 6.62 (dd, *J* = 8.6, 2.4 Hz, 1H), 6.40 (s, 1H).
^{13}C NMR (CD$_3$OD, 75 MHz) δ 151.6, 133.1, 131.4, 116.8, 112.0, 111.8, 104.9, 97.9.

tert-butyl 2-iodo-5-methoxy-1*H*-indole-1-carboxylate (43)

In a flame dried flask, fluxed with argon, *N*-Boc-5-methoxy-2-indolylboronic acid (**27**) (1 g, 3.44 mmol, 1 equiv) was dissolved in dry acetonitrile (17 mL). *N*-iodosuccinimide (1.16 g, 5.16 mmol, 1.5 equiv) was added and the resulting mixture was heated at reflux (85°C) till complete consumption of the starting material, protected from light and air (TLC in cyclohexane/EtOAc 7:3). Upon completion (10 min), the solvent was removed under reduced pressure, the residue was dissolved in EtOAc. The organic phase was washed with deionized water (x 1), with aqueous Na_2SO_3 (1M, x 1), aqueous $NaHCO_3$ (1M, x 1) and deionized water (x 1). The organic phase was then dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude was purified by two silica gel column chromatographies (cyclohexane/EtOAc 95:5 to 9:1 and then in cyclohexane/CH_2Cl_2 7:3 to 3:2) to provide the pure product as a pale yellow oil (786 mg, yield 61%).

43: pale yellow oil; TLC ($KMnO_4$; PMA): R_f = 0.40 (cyclohexane/EtOAc 97:3).
^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.97 (s, *J* = 9.1 Hz, 1H), 6.91 – 6.86 (m, 2H), 6.84 (dd, *J* = 9.1, 2.3 Hz, 1H), 3.83 (s, 3H), 1.71 (s, 9H).
^{13}C NMR ($CDCl_3$, 75 MHz) δ 155.7, 149.1, 132.3, 131.8, 121.7, 116.2, 113.1, 101.6, 85.0, 75.1, 55.6, 28.3.

tert-butyl 5-methoxy-2-((trimethylsilyl)ethynyl)-1*H*-indole-1-carboxylate (44)

In inert argon atmosphere, to a stirred solution of **43** (786 mg, 2.11 mmol, 1 equiv) in dry THF (10 mL) were added Et_3N (881 μL, 639 mg, 6.32 mmol, 3 equiv) and then trimethylsilylacetylene (893 μL, 621 mg, 6.32 mmol, 3 equiv). The mixture was degassed for 10 min fluxing argon. Then, CuI (40 mg, 0.21 mmol, 0.10 equiv) and $Pd(PPh_3)_2Cl_2$ (74 mg, 0.11 mmol, 0.05 equiv) were added and the resulting final mixture was stirred at RT till complete consumption of the starting material (TLC in cyclohexane/EtOAc 97:3). Upon completion (1h), the reaction mixture was filtered over a Celite pad, washing with EtOAc. The filtrate was extracted with EtOAc and washed with water. The combined organic phases were then dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude was purified by silica gel column chromatography eluting in cyclohexane/EtOAc 97:3 to provide the desired product as a yellow oil (684 mg, yield 94%).
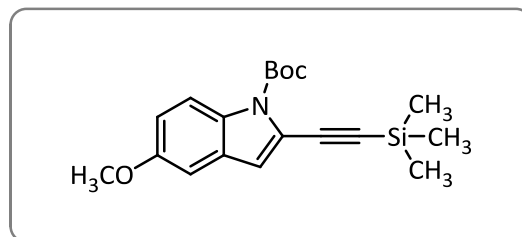
44: yellow oil; TLC ($KMnO_4$; PMA): R_f = 0.43 (cyclohexane/EtOAc 97:3).
^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.09 (d, *J* = 9.1 Hz, 1H), 6.97 (dd, *J* = 9.1, 2.5 Hz, 1H), 6.91 (d, *J* = 2.4 Hz, 1H), 6.88 (s, 1H), 3.81 (s, 3H), 1.70 (s, 9H), 0.30 (s, 9H).
^{13}C NMR ($CDCl_3$, 75 MHz) δ 156.2, 149.4, 131.0, 129.2, 120.7, 117.5, 116.4, 115.0, 102.4, 100.7, 97.2, 84.3, 55.5, 28.3, -0.1.

tert-butyl 2-ethynyl-5-methoxy-1*H*-indole-1-carboxylate (45)

To a stirred solution of *tert*-butyl 5-methoxy-2-((trimethylsilyl)ethynyl)-1H-indole-1-carboxylate (**44**) (684 mg, 1.99 mmol, 1 equiv) in dry THF (20 mL), under argon atmosphere and cooled to 0°C, tetrabutylammonium fluoride (677 mg, 2.59 mmol, 1.3 equiv) was added. The resulting mixture was stirred at RT and monitored by TLC (cyclohexane/EtOAc 97:3). Upon completion (30 min), the mixture was extracted with EtOAc (x 3). The combined organic phases were then dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude was purified by silica gel column chromatography eluting in cyclohexane/EtOAc 95:5 to provide the desired product as a yellow oil (502 mg, yield 93%).

45: yellow oil; TLC ($KMnO_4$; PMA): R_f = 0.30 (cyclohexane/EtOAc 97:3).
^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.01 (d, *J* = 8.8 Hz, 1H), 7.00 – 6.92 (m, 2H), 6.89 (s, 1H), 3.83 (s, 3H), 1.68 (s, 9H).
^{13}C NMR ($CDCl_3$, 75 MHz) δ 156.3, 149.5, 130.9, 129.2, 120.1, 117.5, 116.6, 115.2, 102.7, 84.8, 83.5, 76.5, 55.8, 28.3.

N-hydroxybenzimidoyl chloride (46)

Benzaldehyde oxime (190 mg, 1.57 mmol, 1 equiv) was dissolved in CH_2Cl_2 and benzyl(trimethyl)ammonium tetrachloroiodate (657 mg, 1.57 mmol, 1 equiv) was added. The greenish suspension dissolved within 10 min after vigorous stirring at RT to result in a yellow solution. After stirring for additional 30 min (TLC in cyclohexane/EtOAc 9:1), the mixture was diluted with Et_2O and the resulting precipitate was filtered off. The filtrate was evaporated under reduced pressure to provide the pure compound as a yellow oil (244 mg, yield 100%).

46: yellow oil; TLC ($KMnO_4$; PMA): R_f = 0.30 (cyclohexane/EtOAc 97:3).
^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.60 (br s, 1H), 7.86 – 7.78 (m, 2H), 7.51 – 7.38 (m, 3H).

tert-butyl 5-methoxy-2-(3-phenylisoxazol-5-yl)-1*H*-indole-1-carboxylate (47)

To a stirred solution of *tert*-butyl 2-ethynyl-5-methoxy-1*H*-indole-1-carboxylate (**45**) (100 mg, 0.37 mmol, 1 equiv) in dry THF (10 mL), under argon atmosphere, Et₃N (62 μL, 45 mg, 0.44 mmol, 1.2 equiv) and *N*-hydroxybenzimidoyl chloride (**46**) (69 mg, 0.44 mmol, 1.2 equiv) were added. The resulting mixture was stirred at RT and monitored by TLC (cyclohexane/EtOAc 9:1). Upon completion (12h), the mixture was diluted with EtOAc and washed with saturated aqueous solution of NaHCO₃ (x 2) and brine (x 1). The organic phase was then dried over Na₂SO₄, filtered and concentrated under reduced pressure. The crude was purified by silica gel column chromatography eluting in cyclohexane/EtOAc 9:1 to provide the desired product as a yellow oil (139 mg, yield 97%).
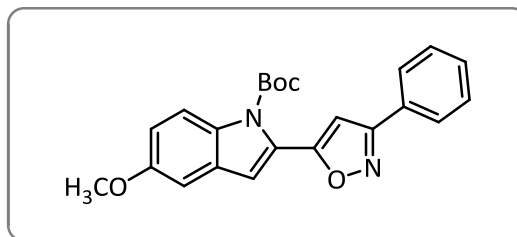
47: yellow oil; TLC (KMnO₄; PMA): R_f = 0.30 (cyclohexane/EtOAc 97:3).
^{1}H NMR (CDCl₃, 300 MHz) δ 8.07 (d, *J* = 8.8 Hz, 1H), 7.83 – 7.76 (m, 2H), 7.45 – 7.37 (m, 3H), 7.00 – 6.93 (m, 2H), 6.82 (s, 1H), 6.71 (s, 1H), 3.80 (s, 3H), 1.43 (s, 9H).
^{13}C NMR (CDCl₃, 75 MHz) δ 164.6, 162.5, 156.4, 149.4, 132.5, 130.2, 129.1, 129.1, 126.9, 126.3, 116.6, 115.5, 113.4, 103.3, 102.2, 84.6, 55.8, 27.9.

2-(3-phenylisoxazol-5-yl)-1*H*-indol-5-ol (4a)

The title compound was prepared according to general procedure **5.2.1.4.2** by reacting *tert*-butyl 5-methoxy-2-(3-phenyl-isoxazol-5-yl)-1*H*-indole-1-carboxylate (**47**) (139 mg, 0.36 mmol, 1 equiv) with 1M BBr₃ (1.44 mL, 1.44 mmol, 4 equiv) in dry CH₂Cl₂. Upon completion (TLC in CH₂Cl₂/MeOH 98:2), standard workup was applied and the crude material was purified by silica gel column chromatography eluting in CH₂Cl₂/MeOH 98:2 to give the pure product as a white solid (73 mg, yield 73%).

4a: white solid; TLC (KMnO₄; PMA): R_f = 0.47 (CH₂Cl₂/MeOH 95:5); m.p.: 257.9°C; MS (ESI) *m/z* per $C_{17}H_{13}N_2O_2^+$ [M+H⁺]⁺ calcd. 277.1, found 276.9.
^{1}H NMR (CD₃OD, 300 MHz) δ 7.92 – 7.85 (m, 2H), 7.55 – 7.47 (m, 3H), 7.28 (d, *J* = 8.8 Hz, 1H), 7.05 (s, 1H), 7.00 – 6.97 (m, 1H), 6.90 (d, *J* = 0.5 Hz, 1H), 6.81 (dd, *J* = 8.8, 2.4 Hz, 1H).
^{13}C NMR (CD₃OD, 75 MHz) δ 166.2, 164.3, 152.4, 134.1, 131.3, 130.3, 130.2, 130.1, 127.8, 126.9, 115.4, 113.2, 105.6, 103.2, 97.9.

tert-butyl 2-(3-(4-bromophenyl)isoxazol-5-yl)-5-methoxy-1*H*-indole-1-carboxylate (48)

To a stirred solution of *tert*-butyl 2-ethynyl-5-methoxy-1*H*-indole-1-carboxylate (**45**) (100 mg, 0.37 mmol, 1 equiv) in dry THF (10 mL), under argon atmosphere, Et$_3$N (62 μL, 45 mg, 0.44 mmol, 1.2 equiv) and 4-bromo-*N*-hydroxybenzimidoyl chloride (104 mg, 0.44 mmol, 1.2 equiv) were added. The resulting mixture was stirred at RT and monitored by TLC (cyclohexane/EtOAc 9:1). Upon completion (12h), the mixture was diluted with EtOAc and washed with saturated aqueous solution of NaHCO$_3$ (x 2) and brine (x 1). The organic phase was then dried over Na$_2$SO$_4$, filtered and concentrated under reduced pressure. The crude was purified by silica gel column chromatography eluting in cyclohexane/EtOAc 9:1 to provide the desired product as a yellow oil (105 mg, yield 60%).
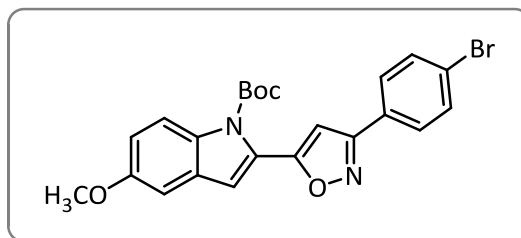
48: yellow oil; TLC (KMnO$_4$; PMA): R$_f$ = 0.29 (cyclohexane/EtOAc 97:3).
^{1}H NMR (CDCl$_3$, 300 MHz) δ 8.08 – 8.02 (m, 1H), 7.67 (d, *J* = 8.6 Hz, 2H), 7.55 (d, *J* = 8.6 Hz, 2H), 7.19 (s, 1H), 7.00 – 6.93 (m, 2H), 6.83 (s, 1H), 6.70 (s, 1H), 3.80 (s, 3H), 1.44 (s, 9H).
^{13}C NMR (CDCl$_3$, 75 MHz) δ 165.0, 161.6, 156.4, 149.4, 132.6, 132.4, 129.1, 128.5, 128.1, 126.2, 124.5, 116.7, 115.7, 113.6, 103.4, 102.0, 84.7, 55.9, 28.0.

2-(3-(4-bromophenyl)isoxazol-5-yl)-1*H*-indol-5-ol (4b)

The title compound was prepared according to general procedure **5.2.1.4.2** by reacting *tert*-butyl 2-(3-(4-bromo-phenyl)isoxazol-5-yl)-5-methoxy-1*H*-indole-1-carboxylate (**48**) (105 mg, 0.22 mmol, 1 equiv) with 1M BBr$_3$ (0.88 mL, 0.88 mmol, 4 equiv) in dry CH$_2$Cl$_2$. Upon completion (TLC in CH$_2$Cl$_2$/MeOH 97:3), standard workup was applied and the crude material was purified by silica gel column chromatography eluting in CH$_2$Cl$_2$/MeOH 97:3 to give the pure product as a white solid (73 mg, yield 73%).
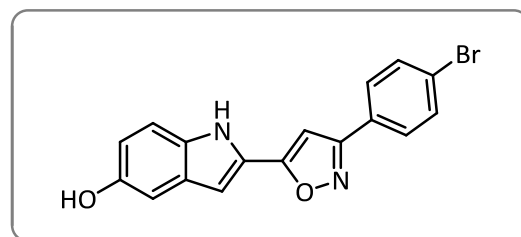
4b: white solid; TLC (KMnO$_4$; PMA): R$_f$ = 0.47 (CH$_2$Cl$_2$/MeOH 95:5); m.p.: 257.9°C; MS (ESI) m/z per C$_{17}$H$_{12}$BrN$_2$O$_2^+$ [M+H$^+$]$^+$ calcd. 355.1, found 355.0.
^{1}H NMR (CD$_3$OD, 300 MHz) δ 7.81 (d, *J* = 8.6 Hz, 2H), 7.67 (d, *J* = 8.6 Hz, 2H), 7.28 (d, *J* = 8.8 Hz, 1H), 7.04 (s, 1H), 6.98 (d, *J* = 2.0 Hz, 1H), 6.89 (d, *J* = 0.7 Hz, 1H), 6.81 (dd, *J* = 8.8, 2.3 Hz, 1H).
^{13}C NMR (CD$_3$OD, 75 MHz) δ 166.5, 163.3, 152.5, 134.1, 133.3, 130.3, 129.6, 129.4, 126.7, 125.4, 115.5, 113.2, 105.6, 103.3, 97.7.

5.2.2 Genistein derivatives 5a-c

5.2.2.1 Synthesis of the key intermediate 52

1-(2-hydroxy-4,6-bis((2-methoxyethoxy)methoxy)phenyl)ethanone (50)

In a flame dried flask and under argon atmosphere, 2',4',6'-trihydroxyacetophenone mono-hydrate (**49**) (7.0 g, 37.60 mmol, 1 equiv) was suspended in dry CH_2Cl_2 (210 mL), cooled at 0°C and stirred for 5 min to obtain an homogeneous pale-yellow suspension. DIPEA (18.53 mL, 106.41 mmol, 2.83 equiv) was added dropwise and the mixture was stirred at 0°C for 30 min. Then, at 0°C, MEM-chloride (10.13 mL, 88.74 mmol, 2.36 equiv) was added dropwise. The final bright yellow solution was stirred at 0°C for 1h and the reaction propagation was monitored by TLC (cyclohexane/EtOAc 1:1). Upon completion, the reaction was quenched with distilled water (100 mL), the organic layer was separated and then the aqueous layer was extracted with CH_2Cl_2 (x 3). The combined organic layers were dried over Na_2SO_4, filtered and the solvent was evaporated under reduced pressure. The crude product was purified by silica gel column chromatography (cyclohexane/EtOAc 1:1) to yield **50** as a pale-yellow oil (8.03 g, yield 62%).
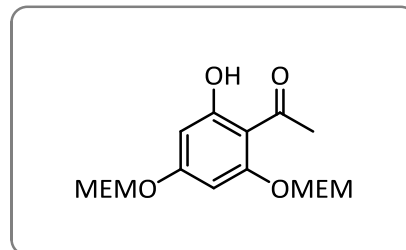
50: pale-yellow oil; TLC (KMnO₄; PMA): R_f = 0.30 (cyclohexane/EtOAc 1:1).
^1H NMR (CDCl₃, 300 MHz) δ 6.26 (s, 2H), 5.33 (s, 2H), 5.25 (s, 2H), 3.84 – 3.77 (m, 4H), 3.58 – 3.53 (m, 4H), 3.38 (s, 3H), 3.37 (s, 3H), 2.63 (s, 3H).
^{13}C NMR (CDCl₃, 75 MHz) δ 203.3, 166.9, 163.5, 160.4, 107.0, 97.3, 94.2, 93.6, 93.1, 71.8, 71.6, 68.6, 68.3, 58.2, 33.2, 33.2.

3-(dimethylamino)-1-(2-hydroxy-4,6-bis((2-methoxyethoxy)methoxy)phenyl)prop-2-en-1-one (51)

50 (6.0 g, 17.42 mmol, 1 equiv) was dissolved in DMF (15 mL) and DMF/DMA (2.08 g, 17.42 mmol, 1 equiv) was added. The stirred solution was heated at 80°C for 6h. The reaction was monitored by TLC in cyclohexane/EtOAc 1:1. After cooling to RT, the solvent was evaporated under reduced pressure to **51** as a reddish oil (6.96 g, yield 100%).
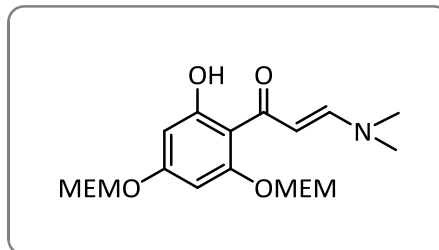
51: reddish oil; TLC (PMA; Dragendorff): R_f = 0.17 (cyclohexane/EtOAc 1:1).
^{1}H NMR (CDCl$_3$, 300 MHz) δ 7.81 (d, J = 12.3 Hz, 1H), 6.17 (d, J = 12.3 Hz, 1H), 6.15 (s, 1H), 6.10 (s, 1H), 5.21 (s, 2H), 5.14 (s, 2H), 3.76 − 3.68 (m, 4H), 3.49 − 3.44 (m, 4H), 3.27 (s, 6H), 3.05 (br s, 3H), 2.82 (br s, 3H).
^{13}C NMR (CDCl$_3$, 75 MHz) δ 189.6, 166.6, 161.0, 158.7, 154.5, 106.7, 97.7, 96.6, 94.3, 94.0, 92.8, 71.4, 68.3, 67.8, 58.8, 45.1, 37.1.

3-iodo-5,7-bis((2-methoxyethoxy)methoxy)-4*H*-chromen-4-one (52)

To a vigorous stirred solution of **51** (4.43 g, 11.09 mmol, 1 equiv) in CHCl$_3$ (148 mL) was first added pyridine (1.08 mL, 13.31 mmol, 1.2 equiv) and then iodine portionwise (2.53 g, 9.98 mmol, 0.9 equiv) over 1h. The reaction mixture was stirred for 12h at RT and monitored by TLC in cyclohexane/EtOAc 1:1. Upon completion, saturated aqueous solution of Na$_2$S$_2$O$_3$ was added and the mixture was stirred at RT for 10 min. The mixture was then diluted with deionized water and extracted with CH$_2$Cl$_2$, adding CuSO$_4$ to complex pyridine. The combined organic layers were dried over Na$_2$SO$_4$, filtered and the solvents evaporated *in vacuo*. The crude product was purified by silica gel column chromatography (cyclohexane/EtOAc 1:1) to yield **52** as a yellow oil (1.92 g, yield 40%).
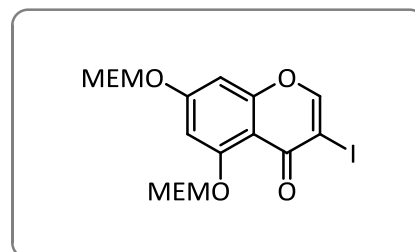
52: yellow oil. TLC (PMA; Dragendorff): 0.21 (cyclohexane/EtOAc 1:1).
^{1}H NMR (CDCl$_3$, 300 MHz) δ 8.03 (s, 1H), 6.70 (d, J = 2.0 Hz, 1H), 6.67 (d, J = 2.0 Hz, 1H), 5.31 (s, 2H), 5.24 (s, 2H), 3.87 − 3.81 (m, 2H), 3.78 − 3.72 (m, 2H), 3.53 − 3.45 (m, 4H), 3.29 (s, 3H), 3.29 (s, 3H).
^{13}C NMR (CDCl$_3$, 75 MHz) δ 171.3, 161.5, 159.2, 158.0, 155.7, 108.7, 102.1, 96.8, 94.3, 93.3, 89.3, 71.4, 68.3, 59.0, 58.9, 30.2, 29.7.

5.2.2.2 General procedure for the Suzuki-Miyaura cross-coupling (53-55)

A solution of **52** (1 equiv), appropriate phenylboronic acid (2 equiv), Na_2CO_3 (3 equiv), Pd/C 10% (10% w/w mol equiv) in 1,2-dimethoxyethane/water 1:1 (4 mL/mmol starting material) was heated to reflux (80-90°C) in a microwave reactor or in an oil bath until complete consumption of the starting material. The reaction was monitored by TLC in cyclohexane/EtOAc. Upon completion, the cooled mixture was filtered through a Celite pad, diluted with deionized water and then extracted with CH_2Cl_2 (x 3). The combined organic layers were dried over Na_2SO_4, filtered and evaporated under reduced pressure. The crude product was purified by silica gel column chromatography, eluting in cyclohexane/EtOAc to yield the desired pure product.

5.2.2.3 General procedure for the cleavage of MEM protecting groups (genistein and 5a-c)

A mixture of the 3-aryl-5,7-bis((2-methoxyethoxy)methoxy)-4*H*-chromen-4-one, $CHCl_3$ (2.76 mL/mmol starting material), MeOH (2.76 mL/mmol starting material) and 12N HCl (1 mL/0.55 mmol starting material) was refluxed for 1h. The reaction was quenched with deionized water and the mixture was extracted with CH_2Cl_2 (x 3). The combined organic layers were washed with deionized water, dried over Na_2SO_4, filtered and the solvents evaporated under reduced pressure. The crude product was purified by silica gel column chromatography, eluting in CH_2Cl_2/MeOH to yield the desired pure product.

3-(4-hydroxyphenyl)-5,7-bis((2-methoxyethoxy)methoxy)-4*H*-chromen-4-one (53)

The title compound was prepared according to the general procedure 5.**2.2.2** by reacting **52** (250 mg, 0.52 mmol, 1 equiv) with 4-hydroxyphenylboronic acid (144 mg, 1.04 mmol, 2 equiv). The mixture was heated in an oil bath at 80°C for 2h, monitored by TLC in 100% EtOAc. Upon completion, the cooled mixture was filtered through a Celite pad, diluted with deionized water and then extracted with CH_2Cl_2 (x 3). The combined organic layers were dried over Na_2SO_4, filtered and the solvents evaporated under reduced pressure. The crude product was purified by silica gel column chromatography, eluting in 100% EtOAc to yield the desired product **53** (143 mg, yield 61%).
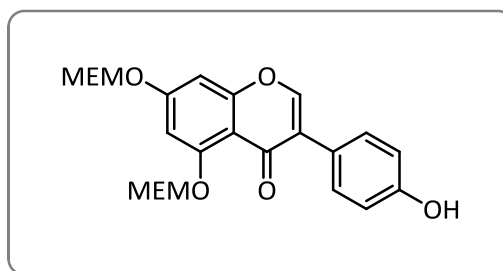
53: yellow oil; TLC ($KMnO_4$; PMA): R_f = 0.36 (EtOAc).
^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.86 (br s, 1H), 7.70 (s, 1H), 7.26 − 7.18 (m, 2H), 6.80 (d, *J* = 8.6 Hz, 2H), 6.69 (dd, *J* = 7.9, 2.3 Hz, 2H), 5.31 (s, 2H), 5.25 (s, 2H), 3.85 − 3.73 (m, 4H), 3.54 − 3.43 (m, 4H), 3.31 (s, 3H), 3.27 (s, 3H).
^{13}C NMR ($CDCl_3$, 75 MHz) δ 176.0, 161.3, 159.4, 158.6, 157.0, 150.7, 130.4, 126.1, 122.8, 115.6, 110.8, 101.3, 96.7, 93.9, 93.3, 71.5, 71.4, 68.2, 68.1, 59.0, 58.8.

5,7-dihydroxy-3-(4-hydroxyphenyl)-4*H*-chromen-4-one (genistein)

The title compound was prepared according to general procedure **5.2.2.3** by reacting **53** (143 mg, 0.32 mmol, 1 equiv) with 12 N HCl (200 μL) in $CHCl_3$ (2 mL) and MeOH (2 mL). The mixture was refluxed for 1h. Upon completion, the reaction was quenched with deionized water, and the mixture was extracted with CH_2Cl_2 (x 3). The combined organic layers were washed with H_2O, dried over Na_2SO_4, filtered and the solvents evaporated *in vacuo*. The crude product was purified by silica gel column chromatography (CH_2Cl_2/MeOH 95:5) to yield the desired product as a pale yellow oil, which was then re-crystalized from EtOH (56 mg, yield 65%).
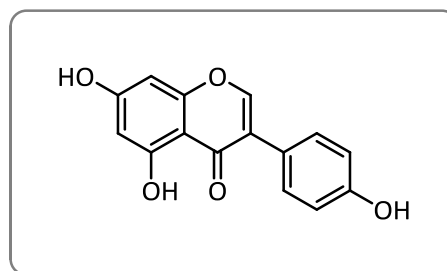
genistein: white solid; TLC ($KMnO_4$; PMA): R_f = 0.46 (CH_2Cl_2/MeOH 9:1); m.p.: 307.4-309.8°C (dec.); MS (ESI) m/z for $C_{15}H_{11}O_5^+$ [M+H]$^+$ calcd. 271.1, found 271.0.
^{1}H NMR ((CD_3)$_2$CO, 300 MHz) δ 8.93 (br s, 1H), 8.01 (s, 1H), 7.31 (d, *J* = 8.7 Hz, 2H), 6.77 (d, *J* = 8.7 Hz, 2H), 6.27 (d, *J* = 2.2 Hz, 1H), 6.14 (d, *J* = 2.2 Hz, 1H), 2.97 (br s, 2H).
^{13}C NMR ((CD_3)$_2$CO, 75 MHz) δ 181.6, 165.0, 163.9, 159.0, 158.4, 154.3, 131.2, 124.0, 123.0, 116.0, 106.2, 99.8, 94.5.

4-methoxy-2-methylphenylboronic acid (56)

To a stirred solution of 4-bromo-3-methylanisole (883 µL, 6.25 mmol, 1 equiv) in dry THF (6 mL), cooled at -78°C, 2.5M *n*-BuLi in hexanes (6 mL, 15 mmol, 2.4 equiv) was added dropwise. After 1h, triisopropyl borate (3.5 mL, 15 mmol, 2.4 equiv) was added and the reaction was warmed to RT. The solution was stirred at RT for 3h till complete consumption of the starting material (TLC in cyclohexane/EtOAc 1:1). Upon completion, saturated aqueous solution of NH_4Cl was added and the mixture was extracted with CH_2Cl_2 (x 3). The combined organic layers were dried over Na_2SO_4, filtered and evaporated under reduced pressure. The crude material was subsequently purified by silica gel column chromatography eluting in cyclohexane/EtOAc 1:1 to afford the pure product as a white solid (1.02 g, yield 98%).
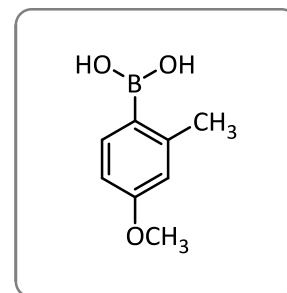
56: white solid; TLC ($KMnO_4$; PMA): R_f = 0.32 (cyclohexane/EtOAc 1:1); m.p.: 176.6-184.6°C.
^{1}H NMR ($CDCl_3$, 300 MHz,): δ 8.16 (d, *J* = 8.3 Hz, 1H), 6.83 (dd, *J* = 2.5, 8.3 Hz, 1H), 6.79 (d, *J* = 2.5 Hz, 1H), 3.86 (s, 3H), 2.79 (s, 3H).
^{13}C NMR ($CDCl_3$, 75 MHz): δ 162.7, 148.8, 139.5, 116.4, 110.6, 55.2, 23.5.

3-(4-methoxy-2-methylphenyl)-5,7-bis((2-methoxyethoxy)methoxy)-4*H*-chromen-4-one (54)

The title compound was prepared according to general procedure **5.2.2.2** by reacting **52** (150 mg, 0.31 mmol, 1 equiv) with 4-methoxy-2-methylphenylboronic acid (**56**) (103 mg, 0.62 mmol, 2 equiv). The mixture was heated in a microwave reactor at 80°C for 1h (TLC cyclohexane/EtOAc 1:1). Upon completion, the cooled mixture was filtered through a Celite pad, diluted with deionized water and then extracted with CH_2Cl_2 (x 3). The combined organic layers were dried over Na_2SO_4, filtered and the solvents evaporated *in vacuo*. The crude product was purified by silica gel column chromatography eluting in 100% EtOAc to yield the desired product as a dark yellow oil (122 mg, yield 83%).
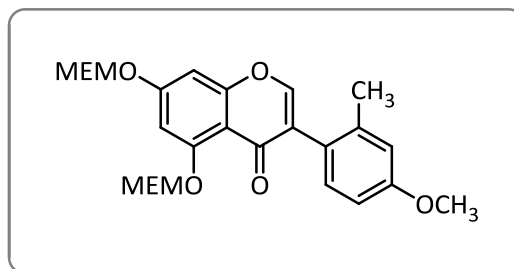
54: dark yellow oil; TLC ($KMnO_4$; PMA): R_f = 0.50 (EtOAc).
^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.65 (s, 1H), 7.06 (d, *J* = 8.3 Hz, 1H), 6.80 – 6.72 (m, 4H), 5.36 (s, 2H), 5.33 (s, 2H), 3.91 – 3.88 (m, 2H), 3.86 – 3.82 (m, 2H), 3.80 (s, 3H), 3.59 – 3.53 (m, 4H), 3.38 (s, 3H), 3.35 (s, 3H), 2.21 (s, 3H).
^{13}C NMR ($CDCl_3$, 75 MHz) δ 175.3, 161.2, 159.7, 159.7, 158.5, 151.4, 139.6, 131.8, 126.9, 124.2, 115.8, 111.5, 111.0, 102.6, 97.4, 94.8, 93.4, 71.6, 68.3, 59.2, 59.0, 55.3, 20.5.

5,7-dihydroxy-3-(4-hydroxy-2-methylphenyl)-4*H*-chromen-4-one (5a)

In anhydrous conditions and under argon atmosphere, to a stirred solution of **54** (122 mg, 0.26 mmol, 1 equiv) in dry CH_2Cl_2 (6 mL), cooled at -78°C, 1M BBr_3 in hexanes (1.82 mL, 1.82 mmol, 7 equiv) was slowly added. The reaction mixture was stirred at -78°C for 1h and then at RT until completion. After 2h (TLC in CH_2Cl_2/MeOH 95:5), the reaction was quenched by additioning 5% aqueous solution of Na_2CO_3 and extracted with CH_2Cl_2/*i*-PrOH 9:1 (x 3). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered and evaporated *in vacuo*. The crude material wassubsequently purified by silica gel column chromatography eluting in CH_2Cl_2/MeOH 97:3 and then re-crystalized from EtOH to provide the pure compound (45 mg, yield 62%).
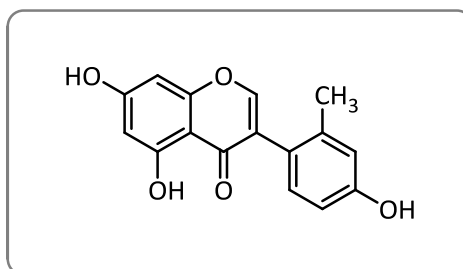
5a: pale yellow solid; TLC (KMnO$_4$; PMA): R_f = 0.34 (CH_2Cl_2/MeOH 95:5); m.p.: 283.3-284.6°C (dec.); MS (ESI) m/z for $C_{16}H_{13}O_5^+$ [M+H]$^+$ calcd. 285.1, found 285.1.
^{1}H NMR ((CD$_3$)$_2$CO, 300 MHz) δ 9.67 (br s, 1H), 8.43 (br s, 1H), 7.98 (s, 1H), 7.04 (d, *J* = 8.2 Hz, 1H), 6.77 (d, *J* = 2.3 Hz, 1H), 6.70 (dd, *J* = 8.2, 2.6 Hz, 1H), 6.42 (d, *J* = 2.2 Hz, 1H), 6.29 (d, *J* = 2.1 Hz, 1H), 3.23 (br s, 1H), 2.17 (s, 3H).
^{13}C NMR ((CD$_3$)$_2$CO, 75 MHz) δ 181.6, 165.0, 163.8, 159.2, 158.5, 155.4, 140.3, 132.7, 125.1, 122.9, 117.6, 113.3, 106.0, 99.8, 94.5, 20.3.

5,7-dihydroxy-3-(4-methoxy-2-methylphenyl)-4*H*-chromen-4-one (5b)

The title compound was prepared according to general procedure **5.2.2.3** by reacting **54** (53 mg, 0.11 mmol, 1 equiv) with 12N HCl (60 µL) in CHCl$_3$ (330 µL) and MeOH (330 µL). The mixture was refluxed for 1h. Upon completion (TLC in 100% EtOAc), the reaction was quenched with deionized water and the mixture was extracted with CH_2Cl_2 (x 3). The combined organic layers were washed with H_2O, dried over Na_2SO_4, filtered and the solvents evaporated *in vacuo*. The crude product was purified by silica gel column chromatography (CH_2Cl_2/MeOH 95:5) to yield the desired product as a yellow oil, then re-crystalized from EtOH to provide a yellowish solid (25 mg, yield 75%).
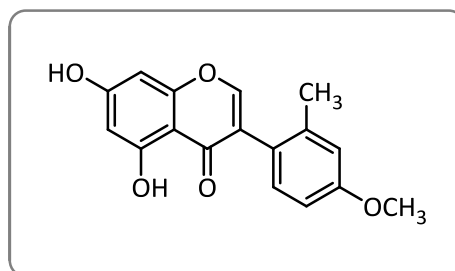
5b: yellowish solid; TLC (KMnO$_4$; PMA): R_f = 0.47 (CH_2Cl_2/MeOH 9:1); m.p.: 223.8-225.3°C (dec.); MS (ESI) m/z for $C_{17}H_{15}O_5^+$ [M+H]$^+$ calcd. 299.1, found 299.1.
^{1}H NMR ((CD$_3$)$_2$CO, 300 MHz) δ 9.72 (br s, 1H), 8.01 (s, 1H), 7.14 (d, *J* = 8.3 Hz, 1H), 6.86 (d, *J* = 2.5 Hz, 1H), 6.79 (dd, *J* = 8.3, 2.6 Hz, 1H), 6.43 (d, *J* = 2.1 Hz, 1H), 6.29 (d, *J* = 2.1 Hz, 1H), 3.81 (s, 3H), 3.02 (br s, 1H), 2.22 (s, 3H).
^{13}C NMR ((CD$_3$)$_2$CO, 75 MHz) δ 181.5, 165.0, 163.8, 160.9, 159.3, 155.5, 140.41, 132.7, 125.0, 124.1, 116.3, 111.7, 99.9, 94.6, 55.5, 20.4.

3-(4-aminophenyl)-5,7-bis((2-methoxyethoxy)methoxy)-4*H*-chromen-4-one (55)

The title compound was prepared according to general procedure **5.2.2.2** by reacting **52** (1.0 g, 2.08 mmol, 1 equiv) with 4-aminophenylboronic acid pinacol ester (912 mg, 4.16 mmol, 2 equiv). The mixture was heated in oil bath at 90°C for 3h and monitored by TLC in 100% EtOAc. Upon completion, the cooled mixture was filtered through a Celite pad, diluted with deionized water and then extracted with CH_2Cl_2 (x 3). The combined organic layers were dried over Na_2SO_4, filtered and the solvents evaporated *in vacuo*. The crude product was purified by silica gel column chromatography (EtOAc) to give the desired product as a yellow oil (600 mg, yield 65%).
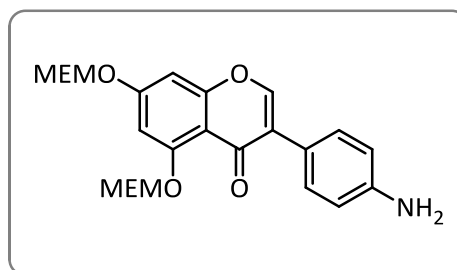
55: yellow oil; TLC ($KMnO_4$; PMA): R_f = 0.35 (EtOAc).
^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.69 (s, *J* = 0.6 Hz, 1H), 7.25 (d, *J* = 8.1 Hz, 2H), 6.71 − 6.67 (m, 2H), 6.64 (d, *J* = 8.2 Hz, 2H), 5.32 (s, 2H), 5.25 (s, 2H), 3.88 − 3.81 (m, 2H), 3.81 − 3.73 (m, 2H), 3.54 − 3.44 (m, 4H), 3.31 (s, 3H), 3.29 (s, 3H).
^{13}C NMR ($CDCl_3$, 75 MHz) δ 175.5, 161.0, 159.4, 158.5, 150.1, 146.5, 130.2, 126.2, 121.8, 114.9, 111.3, 102.0, 97.1, 94.4, 93.3, 71.5, 68.2, 59.1, 59.0.

3-(4-aminophenyl)-5,7-dihydroxy-4H-chromen-4-one hydrochloride (5c)

The title compound was prepared by reacting **55** (133 mg, 0.30 mmol, 1 equiv) with 4M HCl in 1,4-dioxane (6 mL). The mixture was stirred at room temperature for 2h. Upon completion (TLC in 100% EtOAc), the solvent was removed under reduced pressure and the crude was then re-crystalized from EtOH, affording the desired product as a brownish solid (80 mg, yield 87%).
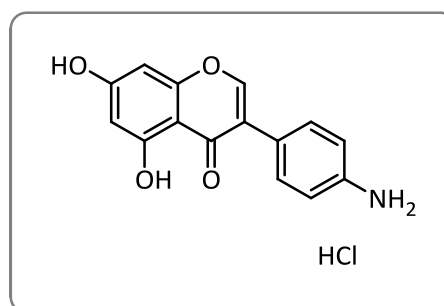
5c: brownish solid; TLC ($KMnO_4$; PMA): R_f = 0.46 (cyclohexane/EtOAC 1:1); m.p.: 272.6-275.0°C; MS (ESI) m/z for $C_{15}H_{12}NO_4^+$ [M]$^+$ calcd. 270.1, found 270.0.
^{1}H NMR (($CD_3)_2$SO, 300 MHz) δ 8.23 (s, 1H), 7.76 (d, *J* = 8.4 Hz, 2H), 7.47 (d, *J* = 8.4 Hz, 2H), 6.38 (d, *J* = 2.1 Hz, 1H), 6.25 (d, *J* = 2.1 Hz, 1H).
^{13}C NMR (($CD_3)_2$SO, 75 MHz) δ 181.9, 165.0, 164.0, 159.0, 153.7, 149.5, 130.6, 124.5, 119.9, 114.8, 106.2, 99.7, 94.4.

5.3 Silent agonists derivatives

5.3.1 Experimental procedures for the synthesis of 6a-u, 7 and 8

5.3.1.1 General procedure for the coupling between 1-ethylpiperazine and the appropriate aryl halide (57-75)

The coupling reaction is carried out in anhydrous conditions and under argon atmosphere. In a flame-dried and argon fluxed flask, K_2CO_3 (2 equiv, for aryl iodide) or K_3PO_4 (2 equiv, for aryl bromide), CuI (0.1 equiv), and *L*-proline (0.2 equiv) were suspended in dry DMSO (1.6 mL/mmol 1-ethylpiperazine). Then, the appropriate aryl halide (1 equiv) and 1-ethylpiperazine (1.5 equiv) were subsequently added. The resulting mixture was heated at 90-100°C until completion (TLC in hexanes/EtOAc 9:1). Upon completion, to the cooled mixture was added deionized water, the organic layer was separated, and the aqueous layer was extracted with EtOAc (x 3). The combined organic layers were washed once with saturated brine, dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified on a silica gel column chromatography eluted with a mixture of EtOAc/MeOH to provide the corresponding pure aniline. When coupling aryl di-bromides, 1-ethylpiperazine and the aryl bromide were used in a 1:1 stoichiometric ratio.

5.3.1.2 General procedure for ethylation (6a-q and 6t-u)

In a sealed vial, the coupled compound **57-75** (1 equiv) was dissolved in dry THF and some copper was added as stabilizer. EtI (7 equiv) was added and the resulting mixture was heated at 90°C until complete consumption of the starting material (TLC in CH_2Cl_2/MeOH 9:1). Upon completion, the mixture was cooled to RT and the solvent removed under reduced pressure. The crude was then purified by re-crystallization from a proper solvent, directly or after a chromatography (silica column, elution in CH_2Cl_2/MeOH 9:1)..

5.3.1.3 General procedure for hydration of nitrile to amide (6r-s)

In anhydrous conditions and under argon atmosphere, in a sealed vial, the appropriate benzonitrile derivative (2 equiv), acetaldoxime (4 equiv) and $Pd(PPh_3)_4$ (0.1 equiv) were put in a sealed vial and dissolved in EtOH. The resulting mixture was refluxed until complete consumption of the starting material and monitored by TLC on neutral alumina CH_2Cl_2/MeOH 95:5 or 85:15. Upon completion, the reaction was cooled to RT, filtered through a Celite pad and washed with hot EtOH. The solvent was evaporated under reduced pressure and the residue was purified with neutral alumina column chromatography, eluting in CH_2Cl_2/MeOH 95:5 to 85:15. The collected fractions were then re-crystallized from MeOH or EtOH to afford the pure compound.

1-ethyl-4-*p*-tolylpiperazine (57)

The title compound was prepared according to the general procedure described in **5.3.1.1**, by reacting 4-iodotoluene (114 mg, 0.53 mmol, 1 equiv) and 1-ethylpiperazine (100 µL, 0.79 mmol, 1.5 equiv). After standard workup, **57** was obtained as an orange oil (70 mg, yield 65 %).

57: orange oil; TLC (KMnO$_4$): R$_f$ = 0.51 (CH$_2$Cl$_2$/MeOH 9:1).
^{1}H NMR (CDCl$_3$, 300 MHz) δ 6.99 (d, *J* = 8.2 Hz, 2H), 6.77 (d, *J* = 8.3 Hz, 2H), 3.14 − 3.06 (m, 4H), 2.59 − 2.49 (m, 4H), 2.40 (q, *J* = 7.2 Hz, 2H), 2.18 (s, 3H), 1.05 (t, *J* = 7.2 Hz, 3H).
^{13}C NMR (CDCl$_3$, 75 MHz) δ 149.3, 129. 7, 129.2, 116.5, 53.0, 52.4, 49.7, 20.5, 12.0.
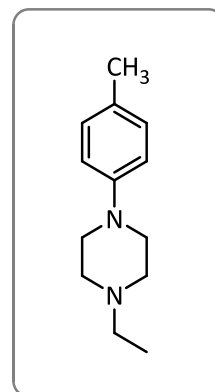
1,1-diethyl-4-*p*-tolylpiperazin-1-ium iodide (6a)

According to the general procedure **5.3.1.2**, EtI (141 µL, 1.75 mmol, 7 equiv) was added to a solution of 1-ethyl-4-*p*-tolylpiperazine **(57)** (50 mg, 0.25 mmol, 1 equiv) in dry THF. Evaporation of the solvent under reduced pressure afforded the crude quaternary salt quantitatively that was re-crystalized from *i*-PrOH to give the pure final compound as a yellow solid (60 mg, yield 68%).

6a: yellow solid; TLC (KMnO$_4$; Dragendorff): R$_f$ = 0.37 (CH$_2$Cl$_2$/MeOH 4:1); m.p.: 147.4-148.8°C; HMRS [M]$^+$ (C$_{15}$H$_{25}$N$_2$$^+$) Calcd 233.2012, Found 233.2017.
^{1}H NMR ((CD$_3$)$_2$SO, 300 MHz) δ 7.09 (d, *J* = 8.3 Hz, 2H), 6.91 (d, *J* = 8.4 Hz, 2H), 3.58 − 3.50 (m, 4H), 3.51 − 3.37 (m, 8H), 2.22 (s, 3H), 1.21 (t, *J* = 7.2 Hz, 6H).
^{13}C NMR ((CD$_3$)$_2$SO, 75 MHz) δ 147.2, 129.5, 128.8, 115.8, 56.5, 51.9, 42.0, 20.1, 6.7.

## 1-ethyl-4-*m*-tolylpiperazine (58)

The title compound was prepared according to the general procedure described in **5.3.1.1**, by reacting 3-bromotoluene (320 µL, 450 mg, 2.63 mmol, 1 equiv) and 1-ethylpiperazine (500 µL, 3.94 mmol, 1.5 equiv). After standard workup, **58** was obtained as a yellow oil (106 mg, yield 20%).

58: yellow oil; TLC (KMnO$_4$): R$_f$ = 0.51 (CH$_2$Cl$_2$/MeOH 9:1).
^{1}H NMR (CDCl$_3$, 300 MHz) δ 7.15 (tt, *J* = 8.1, 1.1 Hz, 1H), 6.78 − 6.74 (m, 2H), 6.74 − 6.66 (m, 1H), 3.26 − 3.18 (m, 4H), 2.66 − 2.59 (m, 4H), 2.49 (q, *J* = 7.2 Hz, 2H), 2.32 (s, 3H), 1.13 (t, *J* = 7.2 Hz, 3H).
^{13}C NMR (CDCl$_3$, 75 MHz) δ 151.5, 138.9, 129.0, 120.7, 117.0, 113.3, 53.0, 52.4, 49.2, 21.9, 12.0.

1,1-diethyl-4-*m*-tolylpiperazin-1-ium iodide (6b)

According to the general procedure **5.3.1.2**, EtI (276 µL, 3.43 mmol, 7 equiv) was added to a solution of 1-ethyl-4-*m*-tolylpiperazine **(58)** (101 mg, 0.49 mmol, 1 equiv) in dry THF. Evaporation of the solvent under reduced pressure afforded the crude quaternary salt quantitatively that was first purified by silica gel column chromatography, eluting in CH$_2$Cl$_2$/MeOH 9:1, and then re-crystalized from THF and EtOH to give the pure final compound as yellow crystals (77 mg, yield 43%).

6b: yellow crystals; TLC (KMnO$_4$; Dragendorff): R$_f$ = 0.37 (CH$_2$Cl$_2$/MeOH 4:1); m.p.: 144.0-145.0°C; HMRS [M]$^+$ (C$_{15}$H$_{25}$N$_2^+$) Calcd 233.2012 Found 233.2023.
^{1}H NMR (CD$_3$OD, 500 MHz) δ 7.16 (t, *J* = 7.9 Hz, 1H), 6.88 − 6.85 (m, 1H), 6.82 (dd, 1H), 6.78 − 6.74 (m, 1H), 3.64 − 3.59 (m, 4H), 3.56 − 3.48 (m, 8H), 2.31 (s, 3H), 1.40 − 1.33 (m, 6H).
^{13}C NMR (CD$_3$OD, 126 MHz) δ 150.9, 140.2, 130.1, 123.0, 118.3, 114.7, 58.8, 54.2, 44.1, 21.7, 7.4.

1-ethyl-4-*o*-tolylpiperazine (59)

The title compound was prepared according to the general procedure described in **5.3.1.1**, by reacting 2-iodotoluene (1.34 mL, 2.29 g, 10.50 mmol, 1 equiv) and 1-ethylpiperazine (2.0 mL, 15.75 mmol, 1.5 equiv). After standard workup, **59** was obtained as a reddish oil (386 mg, yield 18%).

59: reddish oil; TLC (KMnO$_4$): R$_f$ = 0.51 (CH$_2$Cl$_2$/MeOH 9:1).

^{1}H NMR (CDCl$_3$, 300 MHz) δ 7.20 − 7.12 (m, 2H), 7.06 − 7.01 (m, 1H), 7.00 − 6.94 (m, 1H), 3.00 − 2.94 (m, 4H), 2.69 − 2.57 (m, 4H), 2.51 (q, *J* = 7.3 Hz, 2H), 2.30 (s, 3H), 1.14 (t, *J* = 7.3 Hz, 3H).

^{13}C NMR (CDCl$_3$, 75 MHz) δ 151.6, 132.6, 131.1, 126.6, 123.2, 119.1, 53.4, 52.5, 51.7, 18.0, 12.1.

1,1-diethyl-4-o-tolylpiperazin-1-ium iodide (6c)

According to the general procedure **5.3.1.2**, EtI (1.06 mL, 13.16 mmol, 7 equiv) was added to a solution of 1-ethyl-4-*o*-tolylpiperazine **(59)** (385 mg, 1.88 mmol, 1 equiv) in dry THF. Evaporation of the solvent under reduced pressure afforded the crude quaternary salt quantitatively that was re-crystalized from THF/ethanol/MeOH to give the pure final compound as bright white crystals (301 mg, yield 45%).

6c: bright white crystals; TLC (KMnO$_4$; Dragendorff): R$_f$ = 0.37 (CH$_2$Cl$_2$/MeOH 4:1); m.p.: 158.5-160.2°C; HMRS [M]$^+$ (C$_{15}$H$_{25}$N$_2$$^+$) Calcd 233.2012 Found 233.2023.
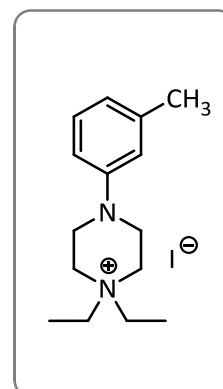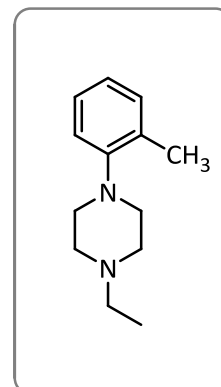
^{1}H NMR (CD$_3$OD, 500 MHz) δ 7.26 − 7.16 (m, 3H), 7.07 − 7.01 (m, 1H), 3.67 − 3.62 (m, 4H), 3.60 (q, *J* = 7.4 Hz, 4H), 3.29 − 3.23 (m, 4H), 2.33 (s, 3H), 1.38 (t, *J* = 7.1 Hz, 6H).

^{13}C NMR (CD$_3$OD, 126 MHz) δ 150.6, 134.2, 132.3, 127.9, 125.8, 120.9, 59.6, 46.4, 17.9, 7.4.
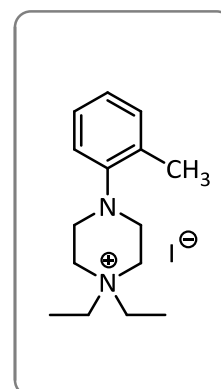
1-ethyl-4-(4-methoxyphenyl)piperazine (60)

The title compound was prepared according to the general procedure described in **5.3.1.1**, by reacting 4-iodoanisole (616 mg, 2.63 mmol, 1 equiv) and 1-ethylpiperazine (500 µL, 3.94 mmol, 1.5 equiv). After standard workup, **60** was obtained as a pale yellow solid (302 mg, yield 52%).

60: pale yellow solid; TLC (KMnO$_4$):
R$_f$ = 0.44 (CH$_2$Cl$_2$/MeOH 9:1); m.p.:56.6-57.8 °C.
^{1}H NMR (CDCl$_3$, 300 MHz) δ 6.84 (d, J = 9.1 Hz, 2H), 6.77 (d, J = 9.2 Hz, 2H), 3.70 (s, 3H), 3.09 − 3.02 (m, 4H), 2.60 − 2.52 (m, 4H), 2.41 (q, J = 7.3 Hz, 2H), 1.06 (t, J = 7.2 Hz, 3H).
^{13}C NMR (CDCl$_3$, 75 MHz) δ 153.7, 145.7, 118.1, 114.4, 55.5, 52.9, 52.3, 50.6, 12.0.
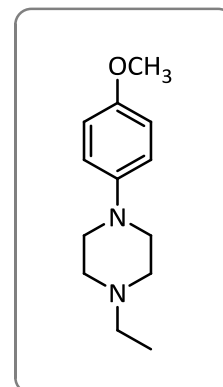
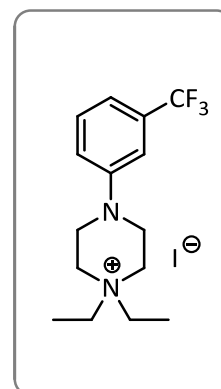### 1,1-diethyl-4-(4-methoxyphenyl)piperazin-1-ium iodide (6d)

According to the general procedure **5.3.1.2**, EtI (692 µL, 8.61 mmol, 7 equiv) was added to a solution of 1-ethyl-4-(4-methoxyphenyl)piperazine **(60)** (270 mg, 1.23 mmol, 1 equiv) in dry THF. Evaporation of the solvent under reduced pressure afforded the crude quaternary salt quantitatively that was re-crystalized from THF and EtOH to give the pure final compound as bright yellowish crystals (334 mg, yield 72%).

6d: bright yellowish crystal; TLC (KMnO$_4$; Dragendorff): R$_f$ = 0.35 (CH$_2$Cl$_2$/MeOH 4:1); m.p.: 150.5-153.1°C; HMRS [M]$^+$ (C$_{15}$H$_{25}$N$_2$O$^+$) Calcd 249.1961 Found 249.1972.
^{1}H NMR (CD$_3$OD, 500 MHz) δ 7.01 (d, J = 9.1 Hz, 2H), 6.88 (d, J = 9.1 Hz, 2H), 3.75 (s, 3H), 3.65 − 3.59 (m, 4H), 3.54 (q, J = 7.3 Hz, 4H), 3.45 − 3.39 (m, 4H), 1.36 (t, J = 7.3 Hz, 6H).
^{13}C NMR (CD$_3$OD, 126 MHz) δ 156.4, 145.0, 119.9, 115.6, 59.0, 56.0, 54.3, 45.4, 7.4.
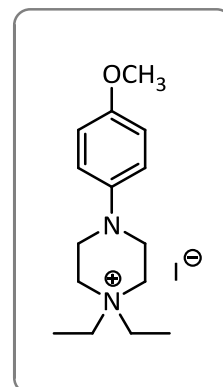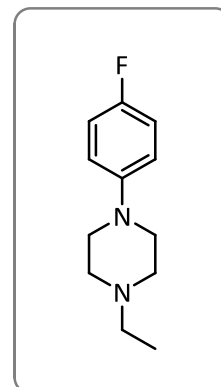
1-ethyl-4-(3-methoxyphenyl)piperazine (61)

The title compound was prepared according to the general procedure described in **5.3.1.1**, by reacting 3-iodoanisole (1.25 mL, 2.46 g, 10.50 mmol, 1 equiv) and 1-ethylpiperazine (2.0 mL, 15.75 mmol, 1.5 equiv). After standard workup, **61** was obtained as a red oil (1.62 g, yield 70%).
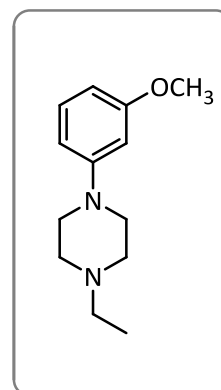
61: red oil; TLC (KMnO$_4$): R$_f$ = 0.50 (CH$_2$Cl$_2$/MeOH 9:1).
^{1}H NMR (CDCl$_3$, 300 MHz) δ 7.08 (t, J = 8.2 Hz, 1H), 6.46 (ddd, J = 8.3, 2.3, 1.0 Hz, 1H), 6.39 (t, J = 2.3 Hz, 1H), 6.33 (ddd, J = 8.2, 2.3, 0.9 Hz, 1H), 3.70 (s, 3H), 3.17 − 3.10 (m, 4H), 2.55 − 2.49 (m, 4H), 2.39 (q, J = 7.2 Hz, 2H), 1.05 (t, J = 7.2 Hz, 3H).
^{13}C NMR (CDCl$_3$, 75 MHz) δ 160.7, 152.8, 129.8, 108.9, 104.4, 102.5, 55.2, 52.9, 52.4, 49.1, 12.1.

### 1,1-diethyl-4-(3-methoxyphenyl)piperazin-1-ium iodide (6e)

According to the general procedure **5.3.1.2**, EtI (1.28 mL, 15.96 mmol, 7 equiv) was added to a solution of 1-ethyl-4-(3-methoxyphenyl)piperazine **(61)** (503 mg, 2.28 mmol, 1 equiv) in dry THF. Evaporation of the solvent under reduced pressure afforded the crude quaternary salt quantitatively that was re-crystalized from THF and EtOH to give the pure final compound as white crystals (649 mg, yield 76%).
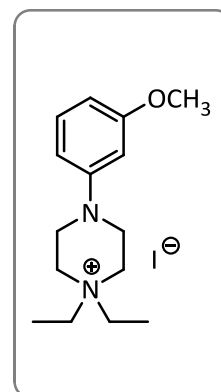
6e: white crystals; TLC (KMnO$_4$; Dragendorff): R$_f$ = 0.35 (CH$_2$Cl$_2$/MeOH 4:1); m.p.: 146.0-147.0°C; HMRS [M]$^+$ (C$_{15}$H$_{25}$N$_2$O$^+$) Calcd 249.1961, Found 249.1966.
^{1}H NMR (CD$_3$OD, 500 MHz) δ 7.19 (t, J = 8.2 Hz, 1H), 6.62 (dd, J = 8.3, 2.1 Hz, 1H), 6.57 (t, J = 2.4 Hz, 1H), 6.51 (dd, J = 8.2, 2.3 Hz, 1H), 3.77 (s, 3H), 3.64 − 3.60 (m, 4H), 3.57 − 3.48 (m, 8H), 1.36 (t, J = 7.4 Hz, 6H).
^{13}C NMR (CD$_3$OD, 126 MHz) δ 162.2, 152.2, 131.1, 110.1, 107.3, 104.0, 58.7, 55.8, 54.3, 44.0, 7.4.

1-(2,4-dimethoxyphenyl)-4-ethylpiperazine (62)

The title compound was prepared according to the general procedure described in **5.3.1.1**, by reacting 1-bromo-2,4-dimethoxybenzene (2.28 g, 1.51 mL, 10.5 mmol, 1 equiv) and 1-ethylpiperazine (2.0 mL, 15.75 mmol, 1.5 equiv). After standard workup, **62** was obtained as a brown oil (839 mg, yield 32%).

62: brown oil; TLC (KMnO$_4$): R$_f$ = 0.36 (CH$_2$Cl$_2$/MeOH 9:1).
^{1}H NMR (CDCl$_3$, 300 MHz) δ 6.88 (d, J = 8.5 Hz, 1H), 6.48 (d, J = 2.6 Hz, 1H), 6.43 (ddd, J = 8.6, 2.7, 0.8 Hz, 1H), 3.84 (s, 3H), 3.78 (s, 3H), 3.14 – 2.97 (m, 4H), 2.75 – 2.57 (m, 4H), 2.49 (q, J = 7.2 Hz, 2H), 1.13 (t, J = 7.2 Hz, 3H).
^{13}C NMR (CDCl$_3$, 75 MHz) δ 156.2, 153.5, 135.4, 118.6, 103.4, 100.0, 55.6, 55.5, 53.3, 52.5, 51.3, 12.1.
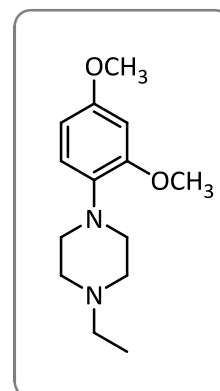
4-(2,4-dimethoxyphenyl)-1,1-diethylpiperazin-1-ium iodide (6f)

According to the general procedure **5.3.1.2**, EtI (1.13 mL, 14.07 mmol, 7 equiv) was added to a solution of 1-(2,4-dimethoxyphenyl)-4-ethylpiperazine **(62)** (502 mg, 2.01 mmol, 1 equiv) in dry THF. Evaporation of the solvent under reduced pressure afforded the crude quaternary salt quantitatively that was re-crystalized from THF and EtOH to give the pure final compound as a white solid (236 mg, yield 29%).

6f: white solid; TLC (KMnO$_4$; Dragendorff):R$_f$ = 0.34 (CH$_2$Cl$_2$/MeOH 4:1); m.p.: 187.0-188.8°C; HMRS [M]$^+$ (C$_{16}$H$_{27}$N$_2$O$_2$$^+$) Calcd 279.2067 Found 279.2078.
^{1}H NMR (CD$_3$OD, 300 MHz) δ 7.01 (d, J = 8.6 Hz, 1H), 6.57 (d, J = 2.7 Hz, 1H), 6.49 (dd, J = 8.7, 2.7 Hz, 1H), 3.84 (s, 3H), 3.76 (s, 3H), 3.64 – 3.49 (m, 8H), 3.36 – 3.31 (m, 4H), 1.36 (t, J = 7.3 Hz, 6H).
^{13}C NMR (CD$_3$OD, 75 MHz) δ 158.8, 155.2, 133.8, 121.5, 105.3, 100.9, 59.3, 56.2, 56.0, 54.5, 45.6, 7.5.

1-ethyl-4-(4-(trifluoromethyl)phenyl)piperazine (63)

The title compound was prepared according to the general procedure described in **5.3.1.1**, by reacting 1-iodo-4-(trifluoromethyl)benzene (2.86 g, 1.54 mL, 10.50 mmol, 1 equiv) and 1-ethylpiperazine (2.0 mL, 15.75 mmol, 1.5 equiv). After standard workup, **63** was obtained as a white solid (2.20 g, yield 81%).

63: white solid; TLC (KMnO$_4$): R$_f$ = 0.51 (CH$_2$Cl$_2$/MeOH 9:1); m.p.: 56.3-56.5°C; HMRS [M+H]$^+$ (C$_{13}$H$_{18}$F$_3$N$_2$$^+$) Calcd 259.1417, Found 259.1426.
^{1}H NMR (CDCl$_3$, 300 MHz) δ 7.48 (d, J = 8.6 Hz, 2H), 6.92 (d, J = 8.6 Hz, 2H), 3.36 − 3.26 (m, 4H), 2.64 − 2.56 (m, 4H), 2.48 (q, J = 7.2 Hz, 2H), 1.14 (t, J = 7.2 Hz, 3H).
^{13}C NMR (CDCl$_3$, 126 MHz) δ 153.5, 126.4, 125.0 (q, J = 276 Hz) 120.4 (q, J = 37 Hz), 114.5, 52.7, 52.5, 48.1, 12.1.
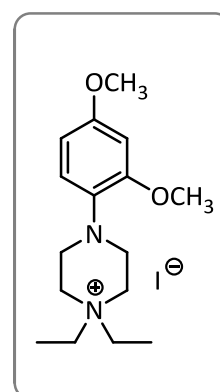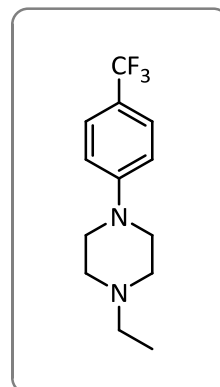
1,1-diethyl-4-(4-(trifluoromethyl)phenyl)piperazin-1-ium iodide (6g)

According to the general procedure **5.3.1.2**, EtI (1.55 mL, 19.32 mmol, 7 equiv) was added to a solution of 1-ethyl-4-(4-(trifluoromethyl)phenyl)piperazine **(63)** (714 mg, 2.76 mmol, 1 equiv) in dry THF. Evaporation of the solvent under reduced pressure afforded the crude quaternary salt quantitatively that was purified by silica gel column chromatography, eluting in CH$_2$Cl$_2$/MeOH 95:5 to 9:1 affording a yellow solid (546 mg, yield 48%). Then, it was re-crystalized from MeOH to give the pure final compound as white crystals (70 mg, yield 6%). ^{1}H NMR spectrum comparison before and after re-crystallization revealed the crude product was more than 95% pure.
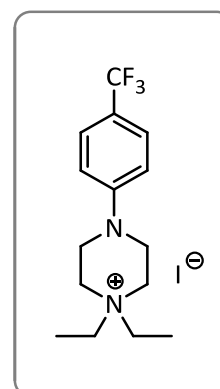
6g: white crystals; TLC (KMnO$_4$; Dragendorff): R$_f$ = 0.48 in CH$_2$Cl$_2$/MeOH 75:25); m.p.: 175.2-175.5°C; HMRS [M]$^+$ (C$_{15}$H$_{22}$F$_3$N$_2$$^+$) Calcd 287.1730 Found 287.1723.
^{1}H NMR ((CD$_3$)$_2$SO, 300 MHz) δ 7.59 (d, J = 8.4 Hz, 2H), 7.16 (d, J = 8.6 Hz, 2H), 3.74 − 3.61 (m, 4H), 3.61 − 3.54 (m, 4H), 3.50 (q, J = 7.2 Hz, 4H), 1.23 (t, J = 7.2 Hz, 6H).
^{13}C NMR ((CD$_3$)$_2$SO, 75 MHz) δ 151.9, 124.8 (q, J = 271 Hz), 126.2 (q, J = 4 Hz), 119.0 (q, J = 32 Hz), 114.6, 56.1, 51.9, 40.5, 6.8.

1-ethyl-4-(3-(trifluoromethyl)phenyl)piperazine (64)

The title compound was prepared according to the general procedure described in **5.3.1.1**, by reacting 3-bromobenzotrifluoride (2.36 g, 1.47 mL, 10.50 mmol, 1 equiv) and 1-ethylpiperazine (2.0 mL, 15.75 mmol, 1.5 equiv). After standard workup, **64** was obtained as a yellow oil (1.73 g, yield 64%).

64: yellow oil; TLC (KMnO$_4$): R$_f$ = 0.54 (CH$_2$Cl$_2$/MeOH 9:1); HMRS [M+H]$^+$ (C$_{13}$H$_{18}$F$_3$N$_2$$^+$) Calcd 259.1417, Found 259.1423.
^{1}H NMR (CDCl$_3$, 300 MHz) δ 7.39 – 7.29 (m, 1H), 7.14 – 7.10 (m, 1H), 7.09 – 7.03 (m, 2H), 3.31 – 3.21 (m, 4H), 2.66 – 2.57 (m, 4H), 2.48 (q, J = 7.2 Hz, 2H), 1.13 (t, J = 7.2 Hz, 3H).
^{13}C NMR (CDCl$_3$, 126 MHz) δ 151.5, 131.5 (q, J = 31 Hz) 129.6, 124.4, (q, J = 271 Hz) 118.6, 115.7, 112.1, 52.7, 52.4, 48.7, 12.1.
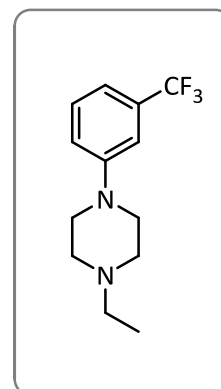
1,1-diethyl-4-(3-(trifluoromethyl)phenyl)piperazin-1-ium iodide (6h)

According to the general procedure **5.3.1.2**, EtI (1.53 mL, 18.97 mmol, 7 equiv) was added to a solution of 1-ethyl-4-(3-(trifluoromethyl)phenyl)piperazine **(64)** (701 mg, 2.71 mmol, 1 equiv) in dry THF. Evaporation of the solvent under reduced pressure afforded the crude quaternary salt quantitatively that was re-crystalized from MeOH to give the pure final compound as yellowish crystals (235 mg, yield 21%).

6h: yellowish crystals; TLC (KMnO$_4$): R$_f$ = 0.48 (CH$_2$Cl$_2$/MeOH 75:25); m.p.: 207-208°C; HMRS [M]$^+$ (C$_{15}$H$_{22}$F$_3$N$_2$$^+$) Calcd 287.1730 Found 287.1738.
^{1}H NMR (CD$_3$OD, 300 MHz) δ 7.52 – 7.43 (m, 1H), 7.32 – 7.26 (m, 2H), 7.23 – 7.16 (m, 1H), 3.69 – 3.60 (m, 8H), 3.56 (q, J = 7.3 Hz, 4H), 3.34 (s, methanol), 1.45 – 1.30 (m, 6H).
^{13}C NMR (CD$_3$OD, 75 MHz) 151.3, 132.5 (q, J = 32 Hz), 131.2, 125.7 (q, J = 273 Hz), 120.7, 118.0, 113.5, 58.5, 54.3, 43.4, 7.5.
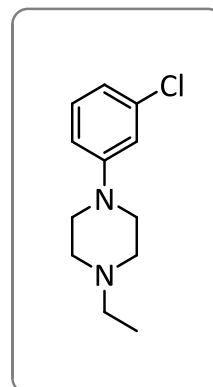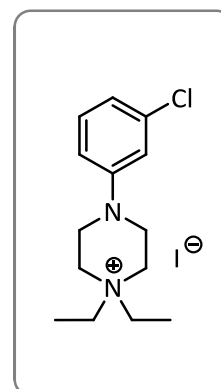
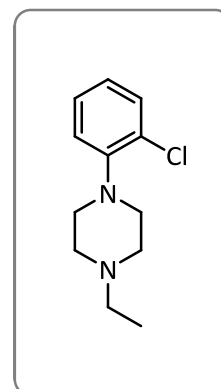## 1-ethyl-4-(4-fluorophenyl)piperazine (65)

The title compound was prepared according to the general procedure described in **5.3.1.1**, by reacting 4-fluoroiodobenzene (2.33 g, 1.21 mL, 10.50 mmol, 1 equiv) and 1-ethylpiperazine (2.0 mL, 15.75 mmol, 1.5 equiv). After standard workup, **65** was obtained as yellowish solid (1.77 g, yield 81%).

65: yellowish solid; TLC (KMnO$_4$): R$_f$ = 0.46 (CH$_2$Cl$_2$/MeOH 9:1); m.p.: 30.0-30.5°C.

^{1}H NMR (CDCl$_3$, 300 MHz) δ 7.02 – 6.92 (m, 2H), 6.92 – 6.84 (m, 2H), 3.20 – 3.10 (m, 4H), 2.66 – 2.57 (m, 4H), 2.48 (q, *J* = 7.3 Hz, 2H), 1.13 (t, *J* = 7.2 Hz, 3H).

^{13}C NMR (CDCl$_3$, 75 MHz) δ 157.2 (d, *J* = 239 Hz), 148.1, 117.8 (d, *J* = 7.7 Hz), 115.5 (d, *J* = 22.2 Hz), 52.9, 52.4, 50.2, 12.1.
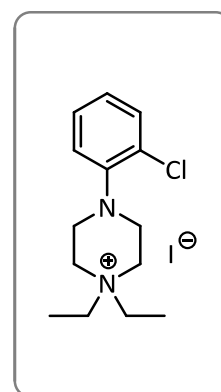
1,1-diethyl-4-(4-fluorophenyl)piperazin-1-ium iodide (6i)

According to the general procedure **5.3.1.2**, EtI (1.90 mL, 23.66 mmol, 7 equiv) was added to a solution of 1-ethyl-4-(4-fluorophenyl)piperazine **(65)** (704 mg, 3.38 mmol, 1 equiv) in dry THF. Evaporation of the solvent under reduced pressure afforded the crude quaternary salt quantitatively that was re-crystalized from THF and EtOH to give the pure final compound as white crystals (478 mg, yield 39%).

6i: white crystals; TLC (KMnO$_4$; Dragendorff): R$_f$ = 0.31 (CH$_2$Cl$_2$/MeOH 4:1); m.p.: 194.0-194.3°C; HMRS [M]$^+$ (C$_{14}$H$_{22}$FN$_2$$^+$) Calcd 237.1762 Found 237.1767.

^{1}H NMR (CD$_3$OD, 300 MHz) δ 7.08 – 7.04 (m, 2H), 7.04 – 7.01 (m, 2H), 3.66 – 3.59 (m, 4H), 3.58 – 3.50 (m, 4H), 3.50 – 3.43 (m, 4H), 1.43 – 1.30 (m, 6H).

^{13}C NMR (CD$_3$OD, 75 MHz) δ 159.2 (d, *J* = 240 Hz), 147.6, 119.7 (d, *J* = 8 Hz), 116.6 (d, *J* = 23 Hz), 58.8, 54.3, 44.8, 7.5.
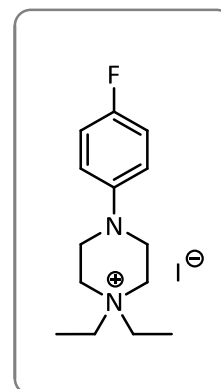
1-ethyl-4-(3-fluorophenyl)piperazine (66)

The title compound was prepared according to the general procedure described in **5.3.1.1**, by reacting 3-fluoroiodobenzene (2.33 g, 1.23 mL, 10.50 mmol, 1 equiv) and 1-ethylpiperazine (2.0 mL, 15.75 mmol, 1.5 equiv). After standard workup, **66** was obtained as a yellow oil (697 mg, yield 75%).
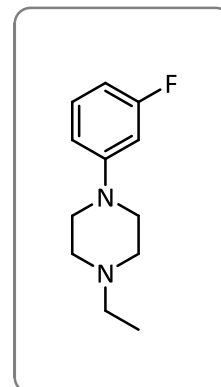
66: yellow oil; TLC (KMnO$_4$): R$_f$ = 0.49 (CH$_2$Cl$_2$/MeOH 9:1).
^{1}H NMR (CDCl$_3$, 300 MHz) δ 7.23 − 7.12 (m, 1H), 6.71 − 6.64 (m, 1H), 6.63 − 6.55 (m, 1H), 6.55 − 6.47 (m, 1H), 3.27 − 3.17 (m, 4H), 2.64 − 2.54 (m, 4H), 2.47 (q, J = 7.2 Hz, 2H), 1.13 (t, J = 7.2 Hz, 3H).
^{13}C NMR (CDCl$_3$, 75 MHz) δ 164.0 (d, J = 243 Hz), 153.1 (d, J = 10 Hz), 130.2 (d, J = 10 Hz), 111.1 (d, J = 2 Hz), 105.8 (d, J = 22 Hz), 102.7 (d, J = 25 Hz), 52.8, 52.5, 48.7, 12.1.

1,1-diethyl-4-(3-fluorophenyl)piperazin-1-ium iodide (6j)

According to the general procedure **5.3.1.2**, EtI (2.17 mL, 26.95 mmol, 7 equiv) was added to a solution of 1-ethyl-4-(3-fluorophenyl)piperazine **(66)** (801 mg, 3.85 mmol, 1 equiv) in dry THF. Evaporation of the solvent under reduced pressure afforded the crude quaternary salt quantitatively that was purified by silica gel column chromatography, eluting in CH$_2$Cl$_2$/MeOH 9:1. Despite several attempts in different solvents, crystals were not obtained. The pure final compound was afforded as an off-white solid (901 mg, yield 64%).
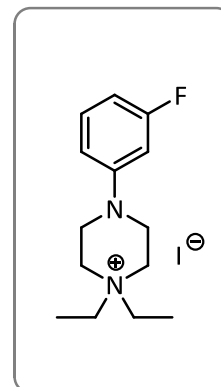
6j: off-white solid; TLC (KMnO$_4$; Dragendorff): R$_f$ = 0.33 in CH$_2$Cl$_2$/MeOH 4:1); m.p.: 125.0-127.0 °C; HMRS [M]$^+$ (C$_{14}$H$_{22}$FN$_2^+$) Calcd 237.1762 Found 237.1760.
^{1}H NMR ((CD$_3$)$_2$SO, 300 MHz) δ 7.35 − 7.23 (m, 1H), 6.92 − 6.80 (m, 2H), 6.71 − 6.61 (m, 1H), 3.62 − 3.51 (m, 8H), 3.48 (q, J = 7.2 Hz, 4H), 1.22 (t, J = 7.2 Hz, 6H).
^{13}C NMR ((CD$_3$)$_2$SO, 75 MHz) δ 163.1 (d, J = 241 Hz), 151.1 (d, J = 10 Hz), 130.5 (d, J = 10 Hz), 111.0 (d, J = 2 Hz), 105.7 (d, J = 21 Hz), 102.1 (d, J = 25 Hz), 56.1, 51.9, 41.1, 6.8.
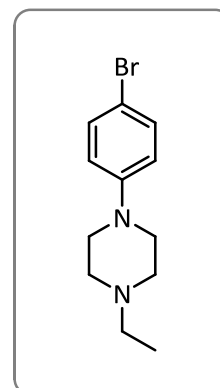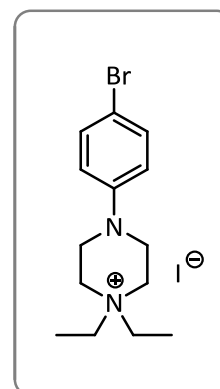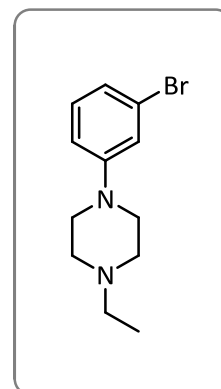
1-(4-chlorophenyl)-4-ethylpiperazine (67)

The title compound was prepared according to the general procedure described in **5.3.1.1**, by reacting 4-chlorobromobenzene (504 mg, 2.63 mmol, 1 equiv) and 1-ethylpiperazine (500 µL, 3.94 mmol, 1.5 equiv). After standard workup, **67** was obtained as a pale orange oil (146 mg, yield 25%).

67: pale orange oil; TLC (KMnO$_4$): R$_f$ = 0.55 (CH$_2$Cl$_2$/MeOH 9:1); HMRS [M+H]$^+$ (C$_{12}$H$_{18}$ClN$_2^+$) Calcd 225.1153, Found 225.1155.
^{1}H NMR (CDCl$_3$, 300 MHz) δ 7.13 (d, *J* = 9.1 Hz, 2H), 6.77 (d, *J* = 9.0 Hz, 2H), 3.15 − 3.08 (m, 4H), 2.57 − 2.51 (m, 4H), 2.41 (q, *J* = 7.2 Hz, 2H), 1.06 (t, *J* = 7.2 Hz, 3H).
^{13}C NMR (CDCl$_3$, 75 MHz) δ 150.1, 129.1, 124.6, 117.3, 52.8, 52.5, 49.3, 12.1.
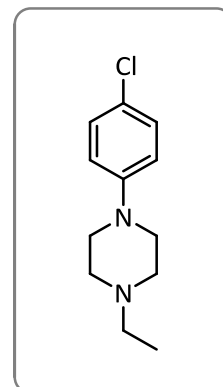
## 4-(4-chlorophenyl)-1,1-diethylpiperazin-1-ium iodide (6k)

According to the general procedure **5.3.1.2**, EtI (366 µL, 4.55 mmol, 7 equiv) was added to a solution of 1-(4-chlorophenyl)-4-ethylpiperazine **(67)** (146 mg, 0.65 mmol, 1 equiv) in dry THF. Evaporation of the solvent under reduced pressure afforded the crude quaternary salt quantitatively that was re-crystalized from THF and EtOH to give the pure final compound a yellowish solid (126 mg, yield 51%).

6k: yellowish solid; TLC (KMnO$_4$; Dragendorff): R$_f$ = 0.33 (CH$_2$Cl$_2$/MeOH 4:1); m.p.: 151.0-152.1°C; HMRS [M]$^+$ (C$_{14}$H$_{22}$ClN$_2^+$) Calcd 253.1466, Found 253.1476 [M], 255.1449 [M+2].
^{1}H NMR (CD$_3$OD, 500 MHz) δ 7.27 (d, *J* = 9.1 Hz, 2H), 7.01 (d, *J* = 9.0 Hz, 2H), 3.65 − 3.60 (m, 4H), 3.57 − 3.50 (m, 8H), 1.36 (t, *J* = 7.2 Hz, 6H).
^{13}C NMR (CD$_3$OD, 126 MHz) δ 149.6, 130.1, 127.0, 118.9, 58.6, 54.3, 43.9, 7.4.
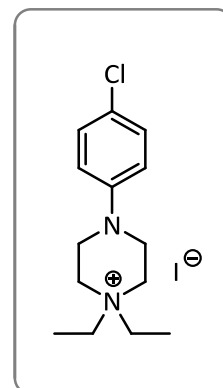
## 1-(3-chlorophenyl)-4-ethylpiperazine (68)

The title compound was prepared according to the general procedure described in **5.3.1.1**, by reacting 1-chloro-3-iodobenzene (1.3 mL, 2.50 g, 10.50 mmol, 1 equiv) and 1-ethylpiperazine (2.0 mL, 15.75 mmol, 1.5 equiv). After standard workup, **68** was obtained as an orange oil (1.96 g, yield 83%).

68: orange oil; TLC (KMnO$_4$): R$_f$ = 0.53 (CH$_2$Cl$_2$/MeOH 9:1).
HMRS [M+H]$^+$ (C$_{12}$H$_{18}$ClN$_2$$^+$) Calcd 225.1153, Found 225.1149
^{1}H NMR (CDCl$_3$, 300 MHz) δ 7.15 (t, J = 8.1 Hz, 1H), 6.88 (t, J = 2.2 Hz, 1H), 6.82 – 6.75 (m, 2H), 3.25 – 3.19 (m, 4H), 2.62 – 2.56 (m, 4H), 2.47 (q, J = 7.2 Hz, 2H), 1.13 (t, J = 7.2 Hz, 3H).
^{13}C NMR (CDCl$_3$, 75 MHz) δ 152.4, 135.0, 130.0, 119.2, 115.7, 113.8, 52.7, 52.4, 48.7, 12.1.

4-(3-chlorophenyl)-1,1-diethylpiperazin-1-ium iodide (6l)

According to the general procedure **5.3.1.2**, EtI (1.25 mL, 15.61 mmol, 7 equiv) was added to a solution of 1-(3-chlorophenyl)-4-ethylpiperazine **(68)** (501 mg, 2.23 mmol, 1 equiv) in dry THF. Evaporation of the solvent under reduced pressure afforded the crude quaternary salt quantitatively that was re-crystalized from THF and EtOH to give the pure final compound as white crystals (412 mg, yield 48%).

6l: white crystals; TLC (KMnO$_4$; Dragendorff): R$_f$ = 0.33 (CH$_2$Cl$_2$/MeOH 4:1); m.p.: 155.0-156.3°C; HMRS [M]$^+$ (C$_{14}$H$_{22}$ClN$_2$$^+$) Calcd 253.1466, Found 253.1475 [M], 255.1443 [M+2].
^{1}H NMR (CD$_3$OD, 300 MHz) δ 7.26 (t, J = 8.2 Hz, 1H), 7.05 (t, J = 2.2 Hz, 1H), 6.96 (ddd, J = 8.4, 2.5, 0.9 Hz, 1H), 6.90 (ddd, J = 7.9, 1.9, 0.8 Hz, 1H), 3.66 – 3.60 (m, 4H), 3.60 – 3.48 (m, 8H), 1.37 (t, J = 7.2 Hz, 6H).
^{13}C NMR (CD$_3$OD, 126 MHz) δ 152.1, 136.1, 131.5, 121.6, 117.2, 115.6, 58.5, 54.3, 43.5, 7.4.

## 1-(2-chlorophenyl)-4-ethylpiperazine (69)

The title compound was prepared according to the general procedure described in **5.3.1.1**, by reacting 1-chloro-2-iodobenzene (2.50 g, 10.50 mmol, 1 equiv) and 1-ethylpiperazine (2.0 mL, 15.75 mmol, 1.5 equiv). After standard workup, **69** was obtained as a brown oil (110 mg, yield 5%).

69: brown oil; TLC (KMnO$_4$): R$_f$ = 0.53 (CH$_2$Cl$_2$/MeOH 9:1).
^{1}H NMR (CDCl$_3$, 300 MHz) δ 7.35 (dd, J = 7.9, 1.5 Hz, 1H), 7.22 (td, J = 7.3, 1.5 Hz, 1H), 7.06 (dd, J = 8.0, 1.6 Hz, 1H), 6.97 (td, J = 7.6, 1.5 Hz, 1H), 3.18 – 3.06 (m, 4H), 2.75 – 2.62 (m, 4H), 2.53 (q, J = 7.2 Hz, 2H), 1.14 (t, J = 7.2 Hz, 3H).
^{13}C NMR (CDCl$_3$, 75 MHz) δ 149.3, 130.7, 128.9, 127.7, 123.8, 120.5, 53.0, 52.4, 51.1, 12.0.

4-(2-chlorophenyl)-1,1-diethylpiperazin-1-ium iodide (6m)

According to the general procedure **5.3.1.2**, EtI (276 μL, 3.43 mmol, 7 equiv) was added to a solution of 1-(2-chlorophenyl)-4-ethylpiperazine **(69)** (110 mg, 0.49 mmol, 1 equiv) in dry THF. Evaporation of the solvent under reduced pressure afforded the crude quaternary salt quantitatively that was first purified by silica gel column chromatography, eluting in CH$_2$Cl$_2$/MeOH 95:5, and then re-crystalized from THF and EtOH to give the pure final compound as white crystals (40 mg, yield 21%).

6m: white crystals; TLC (KMnO$_4$; Dragendorff): R$_f$ = 0.33 (CH$_2$Cl$_2$/MeOH 4:1); m.p.: 154.1-156.8°C; HMRS [M]$^+$ (C$_{14}$H$_{22}$ClN$_2$$^+$) Calcd 253.1472, Found 253.1483 [M], 255.1437 [M+2].
^{1}H NMR (CD$_3$OD, 300 MHz) δ 7.47 – 7.39 (m, 1H), 7.37 – 7.28 (m, 2H), 7.17 – 7.06 (m, 1H), 3.73 – 3.54 (m, 8H), 3.51 – 3.38 (m, 4H), 1.38 (t, J = 7.2 Hz, 6H).
^{13}C NMR (CD$_3$OD, 75 MHz) δ 148.4, 131.7, 130.0, 129.2, 126.5, 122.4, 59.3, 54.5, 45.7, 7.4.

1-(4-bromophenyl)-4-ethylpiperazine (70)

The title compound was prepared according to the general procedure described in **5.3.1.1**, by reacting 1,4-dibromobenzene (2.48 g, 10.50 mmol, 1 equiv) and 1-ethylpiperazine (1.33 mL, 10.50 mmol, 1.5 equiv). After standard workup, **70** was obtained as a off-white solid (913 mg, yield 32%).

70: off-white solid; TLC (KMnO$_4$): R$_f$ = 0.49 (CH$_2$Cl$_2$/MeOH 9:1); m.p.: 85.0-85.5°C.

^{1}H NMR (CDCl$_3$, 300 MHz) δ 7.33 (d, J = 9.0 Hz, 2H), 6.79 (d, J = 9.0 Hz, 2H), 3.24 − 3.13 (m, 4H), 2.65 − 2.55 (m, 4H), 2.47 (q, J = 7.2 Hz, 2H), 1.13 (t, J = 7.2 Hz, 3H).

^{13}C NMR (CDCl$_3$, 75 MHz) δ 150.4, 131.9, 117.6, 111.8, 52.8, 52.4, 49.1, 12.1.

4-(4-bromophenyl)-1,1-diethylpiperazin-1-ium iodide (6n)

According to the general procedure **5.3.1.2**, EtI (1.08 mL, 13.44 mmol, 7 equiv) was added to a solution of 1-(4-bromophenyl)-4-ethylpiperazine **(70)** (517 mg, 1.92 mmol, 1 equiv) in dry THF. Evaporation of the solvent under reduced pressure afforded the crude quaternary salt quantitatively that was first purified by silica gel column chromatography, eluting in CH$_2$Cl$_2$/MeOH 9:1 and then re-crystalized from THF and EtOH to give the pure final compound as white crystals (220 mg, yield 27%).

6n: white crystals; TLC (KMnO$_4$; Dragendorff): R$_f$ = 0.37 (CH$_2$Cl$_2$/MeOH 75:25); m.p.: 182.5-183.6°C; HMRS [M]$^+$ (C$_{14}$H$_{22}$BrN$_2$$^+$) Calcd 297.0961 Found 297.0965 [M], 299.0945 [M+2].

^{1}H NMR (CD$_3$OD, 300 MHz) δ 7.40 (d, J = 9.0 Hz, 2H), 6.97 (d, J = 9.1 Hz, 2H), 3.67 − 3.48 (m, 12H), 1.42 − 1.32 (m, 6H).

^{13}C NMR (CD$_3$OD, 75 MHz) δ 150.0, 133.1, 119.2, 114.1, 58.5, 54.2, 43.7, 7.3.

1-(3-bromophenyl)-4-ethylpiperazine (71)

The title compound was prepared according to the general procedure described in **5.3.1.1**, by reacting 1,3-dibromobenzene (3.72 g, 1.90 mL, 15.75 mmol, 1 equiv) and 1-ethylpiperazine (2.0 mL, 15.75 mmol, 1.5 equiv). After standard workup, **71** was obtained as a reddish oil (1.60 g, yield 38%).

71: reddish oil; TLC (KMnO$_4$): R$_f$ = 0.49 in CH$_2$Cl$_2$/MeOH 9:1; HMRS [M+H]$^+$ (C$_{12}$H$_{18}$BrN$_2$$^+$) Calcd 269.0648, Found 269.0641.
^{1}H NMR (CDCl$_3$, 300 MHz) δ 7.09 (t, J = 8.1 Hz, 1H), 7.05 – 7.01 (m, 1H), 6.97 – 6.91 (m, 1H), 6.86 – 6.79 (m, 1H), 3.26 – 3.16 (m, 4H), 2.63 – 2.53 (m, 4H), 2.46 (q, J = 7.2 Hz, 2H), 1.12 (t, J = 7.2 Hz, 3H).
^{13}C NMR (CDCl$_3$, 75 MHz) δ 152.5, 130.3, 123.3, 122.1, 118.6, 114.3, 52.7, 52.4, 48.7, 12.1.

4-(3-bromophenyl)-1,1-diethylpiperazin-1-ium iodide (6o)

According to the general procedure **5.3.1.2**, EtI (1.60 mL, 19.95 mmol, 7 equiv) was added to a solution of 1-(3-bromophenyl)-4-ethylpiperazine **(71)** (768 mg, 2.85 mmol, 1 equiv) in dry THF. Evaporation of the solvent under reduced pressure afforded the crude quaternary salt quantitatively that was re-crystalized from THF and EtOH to give the pure final compound as brownish/off-white crystals (403 mg, yield 33%).

6o: brownish/off-white crystals; TLC (KMnO$_4$; Dragendorff): R$_f$ = 0.37 (CH$_2$Cl$_2$/MeOH 75:25); m.p.: 198.8-199.5°C; HMRS [M]$^+$ (C$_{14}$H$_{22}$BrN$_2$$^+$) Calcd 297.0966 Found 297.0963 [M], 299.0944 [M+2].
^{1}H NMR (CD$_3$OD, 300 MHz) δ 7.23 – 7.16 (m, 2H), 7.08 – 6.98 (m, 2H), 3.67 – 3.50 (m, 12H), 1.37 (t, J = 7.2 Hz, 6H).
^{13}C NMR (CD$_3$OD, 75 MHz) δ 152.3, 131.8, 124.6, 124.1, 120.1, 116.1, 58.5, 54.2, 43.5, 7.5.
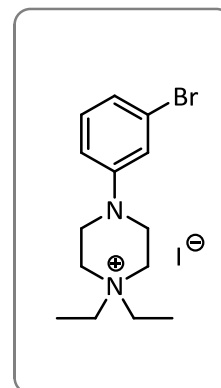
4-(4-ethylpiperazin-1-yl)benzonitrile (72)

The title compound was prepared according to the general procedure described in **5.3.1.1**, by reacting 4-bromobenzonitrile (191 mg, 1.05 mmol, 1 equiv) and 1-ethylpiperazine (200 µL, 1.57 mmol, 1.5 equiv) After standard workup, **72** was obtained as an orange oil (142 mg, yield 63%).

72: orange oil; TLC (KMnO$_4$): R$_f$ = 0.48 (CH$_2$Cl$_2$/MeOH 9:1); HMRS [M+H]$^+$ (C$_{13}$H$_{18}$N$_3^+$) Calcd 216.1495, Found 216.1499.
^{1}H NMR (CDCl$_3$, 300 MHz) δ 7.41 (d, J = 8.8 Hz, 1H), 6.79 (d, J = 9.3 Hz, 1H), 3.31 – 3.25 (m, 4H), 2.55 – 2.49 (m, 4H), 2.41 (q, J = 7.2 Hz, 2H), 1.06 (t, J = 7.2 Hz, 3H).
^{13}C NMR (CDCl$_3$, 75 MHz) δ 153.4, 133.5, 120.2, 114.2, 100.2, 52.4, 52.4, 47.2, 12.0.
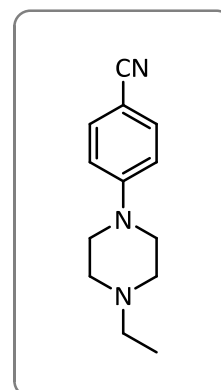
4-(4-cyanophenyl)-1,1-diethylpiperazin-1-ium iodide (6p)

According to the general procedure **5.3.1.2**, EtI (371 µL, 4.62 mmol, 7 equiv) was added to a solution of 4-(4-ethylpiperazin-1-yl)benzonitrile **(72)** (142 mg, 0.66 mmol, 1 equiv) in dry THF. Evaporation of the solvent under reduced pressure afforded the crude quaternary salt quantitatively that was first purified by silica gel column chromatography, eluting in CH$_2$Cl$_2$/MeOH 9:1, and then re-crystalized from THF and EtOH to give the pure final compound as light yellow crystals (35 mg, yield 14%).

6p: light yellow crystals; TLC (KMnO$_4$; Dragendorff): R$_f$ = 0.31 (CH$_2$Cl$_2$/MeOH 4:1); m.p.: 197.4-198.2°C; HMRS [M]$^+$ (C$_{15}$H$_{22}$N$_3^+$) Calcd 244.1808, Found 244.1813.
^{1}H NMR (CD$_3$OD, 300 MHz) δ 7.61 (d, J = 9.1 Hz, 2H), 7.12 (d, J = 9.1 Hz, 2H), 3.80 – 3.68 (m, 4H), 3.68 – 3.62 (m, 4H), 3.56 (q, J = 7.2 Hz, 4H), 1.37 (t, J = 7.2 Hz, 6H).
^{13}C NMR (CD$_3$OD, 75 MHz) δ 153.6, 134.6, 120.6, 116.2, 102.5, 58.2, 54.3, 42.1, 7.6.
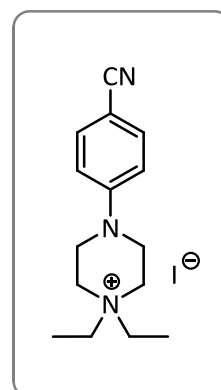
3-(4-ethylpiperazin-1-yl)benzonitrile (73)

The title compound was prepared according to the general procedure described in **5.3.1.1**, by reacting 3-bromobenzonitrile (1.92 g, 10.50 mmol, 1 equiv) and 1-ethylpiperazine (2.0 mL, 15.75 mmol, 1.5 equiv). After standard workup, **73** was obtained as a pale orange oil (1.36 g, yield 60%).

73: pale orange oil; TLC (KMnO$_4$): R$_f$ = 0.48 (CH$_2$Cl$_2$/MeOH 9:1).
^{1}H NMR (CDCl$_3$, 500 MHz) δ 7.35 – 7.28 (m, 1H), 7.14 – 7.07 (m, 3H), 3.29 – 3.22 (m, 4H), 2.65 – 2.58 (m, 4H), 2.48 (q, *J* = 7.2 Hz, 2H), 1.14 (t, *J* = 7.2 Hz, 3H).
^{13}C NMR (CDCl$_3$, 126 MHz) δ 151.5, 130.0, 122.5, 119.9, 119.5, 118.5, 113.2, 52.6, 52.4, 48.5, 12.1.
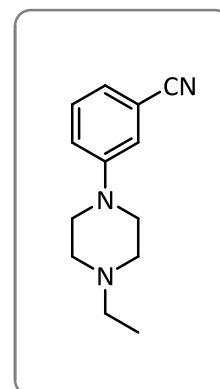
4-(3-cyanophenyl)-1,1-diethylpiperazin-1-ium iodide (6q)

According to the general procedure **5.3.1.2**, EtI (1.45 mL, 18.06 mmol, 7 equiv) was added to a solution of 3-(4-ethylpiperazin-1-yl)benzonitrile **(73)** (555 mg, 2.58 mmol, 1 equiv) in dry THF. Evaporation of the solvent under reduced pressure afforded the crude quaternary salt quantitatively that was first purified by silica gel column chromatography, eluting in CH$_2$Cl$_2$/MeOH 9:1 to 4:1, and then re-crystalized from THF/ethanol/MeOH to give the pure final compound as colorless crystals (140 mg, yield 15%).

6q: colorless crystals; TLC (KMnO$_4$; Dragendorff): R$_f$ = 0.31 (CH$_2$Cl$_2$/MeOH 4:1); m.p.: 210-212°C; HMRS [M]$^+$ (C$_{15}$H$_{25}$N$_2^+$) Calcd 244.1808 Found 244.1812.
^{1}H NMR (CD$_3$OD, 300 MHz) δ 7.50 – 7.40 (m, 1H), 7.40 – 7.32 (m, 2H), 7.22 (dt, *J* = 7.4, 1.3 Hz, 1H), 3.72 – 3.62 (m, 8H), 3.58 (q, *J* = 7.3 Hz, 4H), 1.38 (t, *J* = 7.3 Hz, 6H).
^{13}C NMR (CD$_3$OD, 75 MHz) δ 151.3, 131.5, 124.9, 121.7, 119.9, 114.2, 58.4, 54.2, 43.1, 7.4.
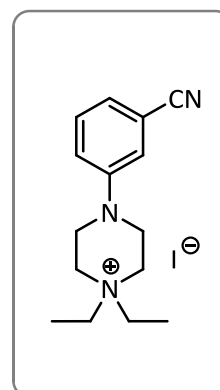
4-(4-carbamoylphenyl)-1,1-diethylpiperazin-1-ium iodide (6r)

The title compound was prepared according to the general procedure **5.3.1.3**, by reacting 4-(4-cyanophenyl)-1,1-diethylpiperazin-1-ium iodide **6p** (230 mg, 0.62 mmol, 2 equiv), acetaldoxime (73 mg, 1.24 mmol, 4 equiv) and Pd(PPh$_3$)$_4$ (35 mg, 0.03 mmol, 0.1 equiv) in EtOH. After standard workup, the crude was first purified by silica neutral alumina column chromatography, eluting in CH$_2$Cl$_2$/MeOH 95:5 to 85:15 and then re-crystalized from EtOH to give the pure final compound as a white solid (18 mg, yield 7%).
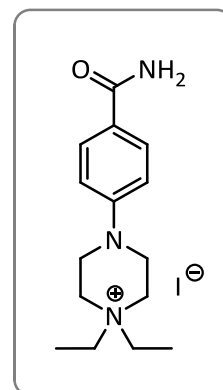
6r: white solids; TLC (alumina-KMnO$_4$; Dragendorff): R$_f$ = 0.42 (CH$_2$Cl$_2$/MeOH 4:1); m.p.: 238-239°C; HMRS [M]$^+$ (C$_{15}$H$_{24}$N$_3$O$^+$) Calcd 262.1914 Found 262.1923.

^{1}H NMR (CD$_3$OD, 300 MHz) δ 7.84 (d, *J* = 8.8 Hz, 2H), 7.07 (d, *J* = 8.9 Hz, 2H), 3.73 – 3.60 (m, 8H), 3.55 (q, *J* = 7.3 Hz, 4H), 1.37 (t, *J* = 7.3 Hz, 6H).

^{13}C NMR (CD$_3$OD, 75 MHz) δ 171.9, 153.4, 130.3, 125.8, 115.7, 58.3, 54.1, 42.6, 7.3.

4-(3-carbamoylphenyl)-1,1-diethylpiperazin-1-ium iodide (6s)

The title compound was prepared according to the general procedure **5.3.1.3.** by reacting 4-(3-cyanophenyl)-1,1-diethylpiperazin-1-ium iodide **6q** (273 mg, 0.74 mmol, 2 equiv), acetaldoxime (87 mg, 1.48 mmol, 4 equiv) and Pd(PPh$_3$)$_4$ (46 mg, 0.04 mmol, 0.1 equiv) in EtOH. After standard workup, the crude was first purified by silica neutral alumina column chromatography, eluting in CH$_2$Cl$_2$/MeOH 95:5 to 85:15 and then re-crystalized from MeOH to give the pure final compound as yellow crystals (77 mg, yield 27%).
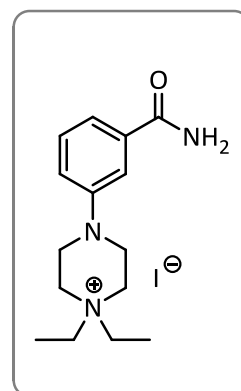
6s: yellow crystals; TLC (alumina-KMnO$_4$; Dragendorff): R$_f$ = 0.42 (CH$_2$Cl$_2$/MeOH 4:1); m.p.: 212-213°C; HMRS [M]$^+$ (C$_{15}$H$_{24}$N$_3$O$^+$) Calcd 262.1914 Found 262.1905.

^{1}H NMR (CD$_3$OD, 300 MHz) δ 7.54 – 7.51 (m, 1H), 7.45 – 7.35 (m, 2H), 7.26 – 7.21 (m, 1H), 3.68 – 3.59 (m, 8H), 3.56 (q, *J* = 7.3 Hz, 4H), 3.34 (s, MeOH 3% residual), 1.37 (t, *J* = 7.3 Hz, 6H).

^{13}C NMR (CD$_3$OD, 75 MHz) δ 172.3, 151.0, 135.9, 130.5, 120.9, 120.8, 116.4, 58.6, 54.2, 43.7, 7.4.

3-(4-ethylpiperazin-1-yl)phenol (74)

The title compound was prepared according to the general procedure described in **5.3.1.1**, by reacting 3-iodophenol (2.31 g, 10.50 mmol, 1 equiv) and 1-ethylpiperazine (2.0 mL, 15.75 mmol, 1.5 equiv). After standard workup, **74** was obtained as a brown solid (703 mg, yield 32%).

74: brown solid; TLC (KMnO$_4$): R$_f$ = 0.38 (CH$_2$Cl$_2$/MeOH 85:15); m.p.: 150.3-151.6°C; HMRS [M+H]$^+$ (C$_{12}$H$_{19}$N$_2$O$^+$) Calcd 207.1492, Found 207.1496.

^{1}H NMR (CD$_3$OD, 300 MHz) δ 7.03 (t, J = 8.1 Hz, 1H), 6.45 (ddd, J = 8.2, 2.4, 0.9 Hz, 1H), 6.40 (t, J = 2.3 Hz, 1H), 6.30 (ddd, J = 8.0, 2.3, 0.9 Hz, 1H), 5.48 (s, CH$_2$Cl$_2$), 3.20 – 3.11 (m, 4H), 2.68 – 2.57 (m, 4H), 2.49 (q, J = 7.2 Hz, 2H), 1.14 (t, J = 7.2 Hz, 3H).

^{13}C NMR (CD$_3$OD, 75 MHz) δ 159.2, 154.0, 130.8, 109.0, 108.3, 104.5, 53.7, 53.3, 50.0, 11.8.
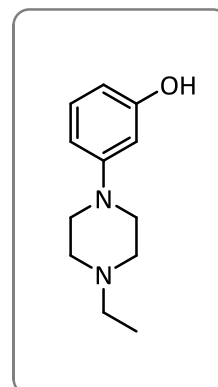
1,1-diethyl-4-(3-hydroxyphenyl)piperazin-1-ium iodide (6t)

According to the general procedure **5.3.1.2**, EtI (1.33 mL, 16.59 mmol, 7 equiv) was added to a solution of 3-(4-ethylpiperazin-1-yl)phenol **(74)** (489 mg, 2.37 mmol, 1 equiv) in dry THF. Evaporation of the solvent under reduced pressure afforded the crude quaternary salt quantitatively that was first purified by silica gel column chromatography, eluting in CH$_2$Cl$_2$/MeOH 9:1 to 85:15, and then re-crystalized from EtOH to give the pure final compound as off-white solid (186 mg, yield 22%).

6t: off-white solid; TLC (KMnO$_4$; Dragendorff): R$_f$ = 0.38 (CH$_2$Cl$_2$/MeOH 75:25); m.p.: 200-203°C; HMRS [M]$^+$ (C$_{14}$H$_{23}$N$_2$O$^+$) Calcd 235.1805 Found 235.1806.

^{1}H NMR ((CD$_3$)$_2$SO, 300 MHz) δ 9.26 (s, 1H), 7.04 (t, J = 8.1 Hz, 1H), 6.48 – 6.41 (m, 1H), 6.38 – 6.34 (m, 1H), 6.33 – 6.27 (m, 1H), 3.59 – 3.37 (m, 12H), 1.21 (t, J = 7.1 Hz, 6H).

^{13}C NMR ((CD$_3$)$_2$SO, 75 MHz) δ 158.1, 150.7, 129.7, 107.1, 106.6, 102.6, 56.4, 51.9, 41.5, 6.8.
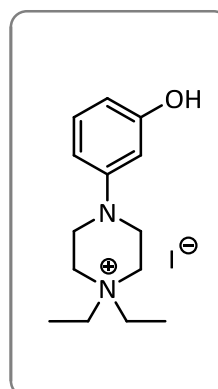
1-ethyl-4-(naphthalen-2-yl)piperazine (75)

The title compound was prepared according to the general procedure described in **5.3.1.1**, by reacting 2-bromonaphthalene (2.17 g, 10.5 mmol, 1 equiv) and 1-ethylpiperazine (2.0 mL, 15.75 mmol, 1.5 equiv). After standard workup, **75** was obtained as a brownish solid (1.02 g, yield 40%).
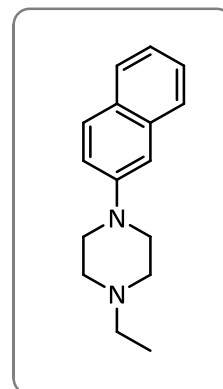
75: brownish solid; TLC (KMnO$_4$): R$_f$ = 0.38 (CH$_2$Cl$_2$/MeOH 9:1); m.p.: 69.1-70.0°C.
^{1}H NMR (CDCl$_3$, 300 MHz) δ 7.76 – 7.65 (m, 3H), 7.39 (ddd, J = 8.1, 6.8, 1.4 Hz, 1H), 7.33 – 7.24 (m, 2H), 7.12 (d, J = 2.5 Hz, 1H), 3.39 – 3.27 (m, 4H), 2.74 – 2.62 (m, 4H), 2.60 (s, DMSO), 2.51 (q, J = 7.2 Hz, 2H), 1.15 (t, J = 7.2 Hz, 3H).
^{13}C NMR (CDCl$_3$, 75 MHz) δ 149.3, 134.7, 128.8, 128.6, 127.5, 126.8, 126.3, 123.4, 119.4, 110.3, 53.0, 52.5, 49.6, 41.1 ((CH$_3$)$_2$SO) residual), 12.2.

1,1-diethyl-4-(naphthalen-2-yl)piperazin-1-ium iodide (6u)

According to the general procedure **5.3.1.2**, EtI (1.21 mL, 15.05 mmol, 7 equiv) was added to a solution of 1-ethyl-4-(naphthalen-2-yl)piperazine **(75)** (516 mg, 2.15 mmol, 1 equiv) in dry THF. Evaporation of the solvent under reduced pressure afforded the crude quaternary salt quantitatively that was first purified by silica gel column chromatography, eluting in CH$_2$Cl$_2$/MeOH 9:1 to 4:1, and then re-crystalized from THF and EtOH to give the pure final compound as a yellowish solid (430 mg, yield 51%).
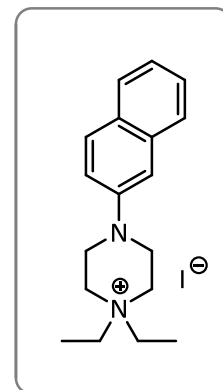
6u: yellowish solid; TLC (KMnO$_4$; Dragendorff): R$_f$ = 0.38 (CH$_2$Cl$_2$/MeOH 75:25); m.p.: 218-219°C; HMRS [M]$^+$ (C$_{18}$H$_{25}$N$_2$$^+$) Calcd 269.2018 Found 269.2013.
^{1}H NMR ((CD$_3$)$_2$SO, 300 MHz) δ 7.86 – 7.81 (m, 1H), 7.81 – 7.73 (m, 2H), 7.48 – 7.39 (m, 2H), 7.35 – 7.26 (m, 2H), 3.68 – 3.57 (m, 8H), 3.52 (q, J = 7.1 Hz, 4H), 1.25 (t, J = 7.2 Hz, 6H).
^{13}C NMR ((CD$_3$)$_2$SO, 75 MHz) δ 147.1, 134.0, 128.6, 128.1, 127.3, 126.6, 126.4, 123.5, 118.5, 109.8, 56.4, 52.0, 41.8, 6.8.

1-ethyl-*N*-phenylpiperidin-4-amine (76)

The reductive amination with NaBH$_3$CN was carried out in anhydrous conditions and under argon atmosphere. Aniline (1.41 mL, 15.47 mmol, 1 equiv) was dissolved in dry MeOH, freshly distilled from Na. To this solution, 1-ethyl-4-piperidone (2.5 mL, 18.56 mmol, 1.2 equiv) and NaBH$_3$CN (1.17 g, 18.56 mmol, 1.2 equiv) were subsequently added. The pH of the solution was adjusted to 6-7 with glacial acetic acid and then the resulting was stirred at RT (TLC in CH$_2$Cl$_2$/MeOH 95/5). Complete consumption of the starting material was not achieved, even after stirring for seven days and heating at reflux. The reaction was then quenched with saturated aqueous solution of NaHCO$_3$ and methanol was removed under reduced pressure. The residue was partitioned between EtOAc and water, and extracted with EtOAc (x 3). The combined organic layers were dried over MgSO$_4$, filtered and concentrated *in vacuo*. The residue was purified on silica gel column chromatography, eluting in CH$_2$Cl$_2$/MeOH 93:7 to provide the desired product as an off-white solid (1.88 g, yield 59%).

76: off-white solid; TLC (KMnO$_4$): R$_f$ = 0.33 (CH$_2$Cl$_2$/MeOH 75:25); m.p.: 43.0-44.6°C; HMRS [M+H]$^+$ (C$_{13}$H$_{21}$N$_2$$^+$) Calcd 205.1699 Found 205.1701.
^{1}H NMR (CD$_3$OD, 300 MHz) δ 7.13 – 7.06 (m, 2H), 6.68 – 6.57 (m, 3H), 3.41 – 3.32 (m, 1H), 3.11 – 3.00 (m, 2H), 2.59 (q, *J* = 7.3 Hz, 2H), 2.39 – 2.26 (m, 2H), 2.12 – 2.01 (m, 2H), 1.60 – 1.45 (m, 2H), 1.15 (t, *J* = 7.3 Hz, 3H).
^{13}C NMR (CD$_3$OD, 75 MHz) δ 148.9, 130.1, 118.2, 114.8, 53.2, 52.9, 50.6, 32.4, 11.7.
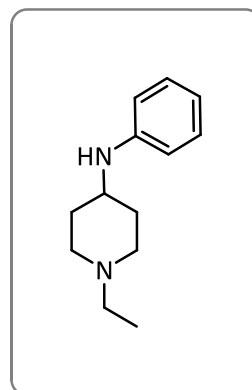
N-(1-ethylpiperidin-4-yl)-2,2,2-trifluoro-*N*-phenylacetamide (77)

In a flame-dried and argon-flushed flask, 1-ethyl-*N*-phenylpiperidin-4-amine **(76)** (1.53 g, 7.46 mmol, 1 equiv) was dissolved in dry CH_2Cl_2 (75 mL) and cooled 0 °C. Triethylamine (4.20 mL, 29.9 mmol, 4 equiv) was added, and then trifluoroacetic anhydride (3.20 mL, 22.4 mmol, 3 equiv) was slowly added dropwise. The resulting mixture was stirred at 0°C until complete consumption of the starting material (5h; TLC in CH_2Cl_2/MeOH 95:5). Upon completion, deionized water was then added to quench the reaction at 0°C. The organic layer was washed with saturated brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by silica gel column chromatography, eluting in CH_2Cl_2/MeOH 95:5 to 9:1. The isolated fraction was then further purified on a silica gel column chromatography eluting in EtOAc/MeOH 100:0 to 9:1 to afford the desired product as a yellow solid (911 mg, yield 41%).
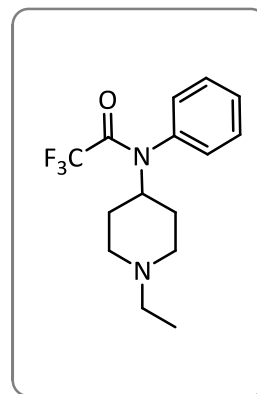
77: yellow solid; TLC ($KMnO_4$): R_f = 0.50 (CH_2Cl_2/MeOH 9:1); m.p.: 50.2-51.3°C; HMRS $[M+H]^+$ ($C_{15}H_{20}F_3N_2O^+$) Calcd 301.1522 Found 301.1531.

^1H NMR ($CDCl_3$, 300 MHz) δ 7.45 – 7.34 (m, 3H), 7.19 – 7.10 (m, 2H), 4.55 (tt, J = 12.2, 4.0 Hz, 1H), 3.03 – 2.92 (m, 2H), 2.37 (q, J = 7.2 Hz, 2H), 2.11 – 1.98 (m, 2H), 1.89 – 1.78 (m, 2H), 1.55 – 1.38 (m, 2H), 1.02 (t, J = 7.2 Hz, 3H).

^{13}C NMR ($CDCl_3$, 75 MHz) δ 156.7 (q, J = 35 Hz), 134.8, 130.6, 129.4, 128.9, 116.5 (q, J = 289 Hz), 55.3, 52.4, 52.1, 29.7, 12.1.

1,1-diethyl-4-(2,2,2-trifluoro-*N*-phenylacetamido)piperidinium iodide (78)

According to the general procedure **5.3.1.2**, EtI (782 µL, 9.73 mmol, 7 equiv) was added to a solution of *N*-(1-ethylpiperidin-4-yl)-2,2,2-trifluoro-*N*-phenylacetamide **(77)** (417 mg, 1.39 mmol, 1 equiv) in dry THF. The reaction was monitored by TLC in CH_2Cl_2/MeOH 95:5 and was complete after 22h at 90°C. Evaporation of the solvent under reduced pressure afforded the crude quaternary salt quantitatively that was purified by silica gel column chromatography, eluting in CH_2Cl_2/MeOH 94:6 to 9:1 to afford the pure final compound as a yellow solid (520 mg, yield 82%).
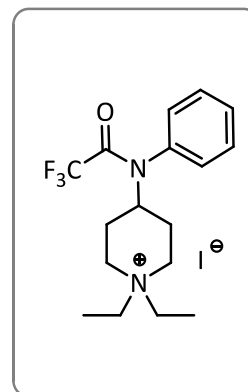
78: yellow solid; TLC ($KMnO_4$): R_f = 0.40 (CH_2Cl_2/MeOH 75:25); m.p.: 141.0-142.0°C; HMRS [M]$^+$ ($C_{17}H_{24}F_3N_2O^+$) Calcd 329.1835 Found 329.1849.

^{1}H NMR (CD_3OD, 300 MHz) δ 7.58 – 7.46 (m, 3H), 7.46 – 7.33 (m, 2H), 4.69 – 4.53 (m, 1H), 3.72 – 3.59 (m, 2H), 3.53 – 3.21 (m, 6H), 2.25 – 1.97 (m, 4H), 1.32 (t, *J* = 7.3 Hz, 3H), 1.22 (t, *J* = 7.3 Hz, 3H).

^{13}C NMR ((CD_3)$_2$SO, 75 MHz) δ 155.4 (q, *J* =34 Hz), 134.9, 130.0, 129.8, 129.2, 115.9 (q, *J* = 289 Hz), 57.4, 56.0, 54.9 (residual CH_2Cl_2), 53.2, 47.3, 22.7, 7.1, 6.7.

1,1-diethyl-4-(phenylamino)piperidinium iodide (7)

Under argon atmosphere, 1,1-diethyl-4-(2,2,2-trifluoro-*N*-phenylacetamido)piperidinium iodide **(78)** (400 mg, 0.88 mmol, 1 equiv) was dissolved in a mixture of MeOH/water 7:1 (49:7 mL). K_2CO_3 (1.46 g, 10.56 mmol, 12 equiv) was added at RT and the resulting mixture was heated at 65°C until complete consumption of the starting material, which occurred in 1h (TLC in CH_2Cl_2/MeOH 9:1). Upon completion, the mixture was cooled to RT and the solvents removed under reduced pressure. The crude was dissolved in MeOH and then filtered through cotton to remove precipitates. The filtrate was concentrated *in vacuo* and then purified on a silica gel column chromatography (CH_2Cl_2/MeOH 9:1 to 85:15) to afford the pure compound as a light yellow solid (173 mg). The compound was further purified by re-crystalization from hot EtOH to provide white crystals (79 mg, yield 25%).
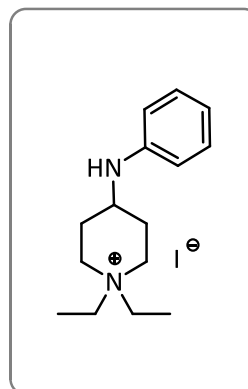
7: white crystals; TLC ($KMnO_4$): R_f = 0.37 (CH_2Cl_2/MeOH 75:25); m.p.: 203-204°C; HMRS [M]$^+$ ($C_{15}H_{25}N_2^+$) Calcd 233.2012 Found 233.2010.

^{1}H NMR (CD_3OD, 500 MHz) δ 7.12 (t, *J* = 7.5 Hz, 2H), 6.71 (d, *J* = 7.9 Hz, 2H), 6.65 (t, *J* = 7.4 Hz, 1H), 3.76 – 3.68 (m, 1H), 3.63 – 3.56 (m, 2H), 3.50 (q, *J* = 7.1 Hz, 2H), 3.46 – 3.36 (m, 4H), 2.25 – 2.15 (m, 2H), 1.94 – 1.83 (m, 2H), 1.35 (t, *J* = 7.3 Hz, 3H), 1.31 (t, *J* = 7.3 Hz, 3H).

^{13}C NMR ((CD_3)$_2$SO, 75 MHz) δ 147.2, 129.1, 116.3, 112.8, 55.6, 55.0, 49.5, 45.4, 25.0, 7.2, 6.7.

4-benzylidene-1-ethylpiperidine (79)

The reaction was carried out in anhydrous conditions and under argon atmosphere. Diethyl benzylphosphonate (1.2 mL, 5.57 mmol, 1.5 equiv) was dissolved in dry THF (17 mL) and 15-crown-5 (150 µL, 0.76 mmol, 0.2 equiv) was added. Then, at 0°C, sodium hydride 60% dispersion in mineral oil (223 mg, 5.57 mmol, 1.5 equiv) was added portionwise. The resulting mixture was stirred at 0°C for 40 min and then a solution of 1-ethyl-4-piperidone (500 ⍰L, 3.71 mmol, 1 equiv) in dry THF (30 mL) was slowly added dropwise at 0°C. The reaction mixture was stirred for 10 min at 0°C, then allowed to warm up and stir at RT until completion (TLC in CH$_2$Cl$_2$/MeOH 9:1), which occurred after 4 days. Upon completion, the mixture was cooled to 0°C, diluted with deionized water, and then extracted with EtOAc (x 3). The combined organic layers were washed with saturated aqueous solution of NaHCO$_3$ and saturated brine, then dried over MgSO$_4$, filtered and concentrated under reduced pressure to give a yellow oil. The crude was purified on a silica gel column chromatography eluting in CH$_2$Cl$_2$/MeOH 96:4 to afford the desired product as a pale yellowish oil (337 mg, yield 45 %).
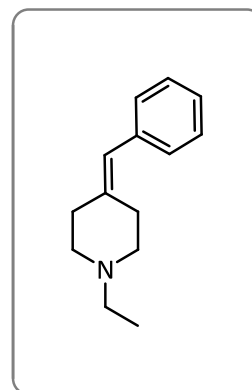
79: pale yellowish oil; TLC (KMnO$_4$; Dragendorff): R$_f$ = 0.33 (CH$_2$Cl$_2$/MeOH 85:15).
^{1}H NMR (CD$_3$OD, 300 MHz) δ 7.33 – 7.26 (m, 2H), 7.21 – 7.15 (m, 3H), 6.35 (s, 1H), 2.65 (dd, J = 6.5, 5.1 Hz, 2H), 2.58 – 2.49 (m, 6H), 2.48 – 2.42 (m, 2H), 1.15 (t, J = 7.3 Hz, 3H).
^{13}C NMR (CD$_3$OD, 75 MHz) δ 139.2, 138.8, 129.9, 129.2, 127.4, 125.1, 55.7, 54.9, 53.2, 36.4, 29.3, 11.8.

4-benzylidene-1,1-diethylpiperidinium iodide (8)

According to the general procedure **5.3.1.2**, EtI (940 ⧠L, 11.69 mmol, 7 equiv) was added to a solution of 4-benzylidene-1-ethylpiperidine **(79)** (337 mg, 1.67 mmol, 1 equiv) in EtOH. The reaction was monitored by TLC in CH_2Cl_2/MeOH 9:1 and was complete after 51h at 90°C. Evaporation of the solvent under reduced pressure afforded the crude quaternary salt quantitatively that was purified by silica gel column chromatography, eluting in CH_2Cl_2/MeOH 95:5 to 75:25. The product was then further purified by a second silica column eluting in CH_2Cl_2/MeOH 95:5 to 75:25 and by re-crystallization in EtOH/MeOH 1:1 to afford the pure product as white crystals (18 mg, yield 3%).
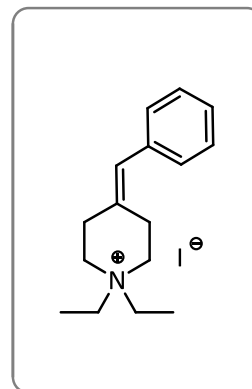
8: white crystals; TLC (KMnO$_4$; Dragendorff): R_f = 0.34 (CH_2Cl_2/MeOH 4:1); m.p.: 84.5-86.0°C; HMRS [M]$^+$ ($C_{16}H_{24}N^+$) Calcd 230.1903 Found 230.1900.

^{1}H NMR (CD$_3$OD, 300 MHz) δ 7.41 – 7.30 (m, 2H), 7.30 – 7.20 (m, 3H), 6.59 (s, 1H), 3.51 (q, J = 7.1 Hz, 6H), 3.41 – 3.34 (m, 2H), 2.91 – 2.81 (m, 2H), 2.81 – 2.73 (m, 2H), 1.34 (tt, J = 7.3, 1.9 Hz, 6H);

^{13}C NMR (CD$_3$OD, 126 MHz) δ 137.6, 132.2, 129.9, 129.5, 128.7, 128.2, 59.9, 59.2, 54.1, 30.3, 24.1, 7.5.

5.3.2 Experimental procedures for the synthesis of 9a-h, 10a-h

5.3.2.1 Synthesis of bromo-Δ²–isoxazoline 83

3-Methylene-1-azabicyclo[2.2.2]octane (80)

3-quinuclidinone hydrochloride, commercially available, was dissolved in a concentrated aqueous solution of K_2CO_3 (pH = 12) and the resulting mixture was extracted with CH_2Cl_2 (x 5). The organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to provide the free base as a colorless, amorphous solid. Under argon atmosphere, to a stirred suspension of potassium tert-butoxide (29.8 g, 265.2 mmol, 1.4 equiv) in dry Et_2O (500 mL) at 0°C, methyltriphenylphosphonium bromide (101.5 g, 284.1 mmol, 1.5 equiv) was added. The resulting mixture turned yellow during stirring and it was refluxed for 45 min. After cooling it down to 0°C, a solution of quinuclidin-3-one (23.7 g, 189.4 mmol, 1 equiv) in dry Et_2O was added dropwise. The final mixture was then stirred overnight at RT and monitored by TLC in CH_2Cl_2/MeOH 4:1 + 3 drops of aqueous NH_3. Upon completion, the reaction was quenched with acetone, filtered under vacuum and washed with Et_2O. The filtrate was concentrated under reduced pressure using a cold bath since the desired product was found to be volatile, affording the desired compound as a white solid (23.3 g, yield 100%).
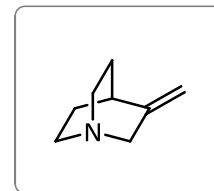
80: white solid; TLC (Dragendorff): R_f = 0.30 (CH_2Cl_2/MeOH 4:1); m.p.: 63.5-64.2°C.
^{1}H-NMR ($CDCl_3$, 300 MHz): δ 4.72 – 4.65 (m, 1H), 4.60 – 4.55 (m, 1H), 3.39 (br s, 2H), 2.87 – 2.69 (m, 4H), 2.44 – 2.28 (m, 1H), 1.70 – 1.52 (m, 4H).
^{13}C-NMR ($CDCl_3$, 75 MHz): δ 152.5, 103.8, 55.8, 47.6, 32.5, 31.6, 29.7, 28.3.

Borane-3-methylene-1-azabicyclo[2.2.2]octane complex (81)

Under argon atmosphere, 3-methylenequinuclidine **(80)** (23.3 g, 189.4 mmol, 1 equiv) was dissolved in dry THF (100 mL) and cooled to 0°C. A solution of 1.0 M BH_3 in THF (190 mL, 189.4 mmol, 1 equiv) was then added dropwise and the mixture was stirred at RT for 1.5h, monitored by TLC in CH_2Cl_2/MeOH 4:1 and cyclohexane/EtOAc 7:3. Upon completion, the reaction was concentrated in vacuum and the resulting crude was purified by silica gel column chromatography, eluting in cyclohexane/EtOAc 7:3 to give the pure *N*-boranyl product as colorless solid (19.6 g, yield 84%).
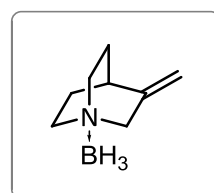
81: colorless solid; TLC ($KMnO_4$): R_f = 0.65 (cyclohexane/EtOAc 7:3); m.p.: 70-72°C.
^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.96 – 4.91 (m, 1H), 4.78 – 4.73 (m, 1H), 3.60 (br s, 2H), 3.13 – 2.91 (m, 4H), 2.61 – 2.53 (m, 1H), 1.93 – 1.74 (m, 4H).
^{13}C-NMR ($CDCl_3$, 75 MHz): δ 144.7, 106.7, 61.2, 54.1, 31.4, 26.4.

Dibromoformaldoxime (82)

A three-neck round-bottom flask was equipped with a mechanical stirrer, a thermometer and a dropper. Glyoxylic acid monohydrate (31.1 g, 338 mmol, 1 equiv) was dissolved in 200 mL of deionized water. Under vigorous mechanical stirring, hydroxylamine hydrochloride (23.5 g, 338 mmol, 1 equiv) was added and the resulting mixture was stirred at RT for 20h. Then, $NaHCO_3$ (58.8 g, 700 mmol, 2.07 equiv) was added portionwise at RT. A solution of bromine (24.1 mL, 470 mmol, 1.4 equiv) in CH_2Cl_2 (125 mL) was added dropwise to the biphasic system, cooled to 6°C with an ice bath, so that the system temperature would not go over 10°C. The final light-green mixture was stirred at RT for additional 3h, after which the organic layer was separated, and the water phase was extracted with CH_2Cl_2 (x 3). The combined organic phases were dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to reduce the volume of CH_2Cl_2, and then *n*-hexane was added till colorless crystals began to form. The solution was then cooled at -20°C till the crystallization was complete. The crystalline solid was filtered under vacuum and washed with *n*-hexane to provide the pure compound as colorless crystals (18.0 g, yield 26%).
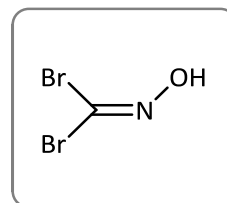
82: colorless crystals; TLC ($KMnO_4$): R_f = 0.65 (cyclohexane/EtOAc 7:3); m.p.: 67-68.5°C.
^{1}H-NMR ((CD_3)$_2$SO, 300 MHz): δ 12.67 (s, 1H).

(±)-3-Bromo-1-oxa-2,7-diaza-7-boranyl-7,10-ethanospiro[4.5]dec-2-ene (83)

Dibromoformaldoxime **(82)** (5.0 g, 24.6 mmol, 0.67 equiv) was added portionwise to a suspension of **81** (5.0 g, 36.5 mmol, 1 equiv) and K_2CO_3 (25.3 g, 182.7 mmol, 5 equiv) in EtOAc (120 mL). The reaction mixture was stirred at room temperature for 24h with further additions of dibromoformaldoxime to achieve complete consumption of the starting material **81**. Once the cycloaddition was completed, the mixture was filtered over a septum under vacuum and washed with EtOAc. The solvent was evaporated under reduced pressure and the residue was purified by silica gel column chromatography eluting in cyclohexane/EtOAc 1:1 to afford the desired cycloadduct **83**, which crystallized as colorless prisms from EtOAc (4.50 g, yield 48%).
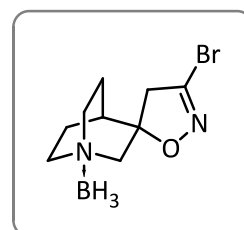
83: colorless prisms; TLC ($KMnO_4$; Dragendorff): R_f = 0.18 (cyclohexane/EtOAc 2:3); m.p.: 127-128°C.
^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.34 (dd, *J* = 14.6, 1.9 Hz, 1H), 3.26 (d, *J* = 17.5 Hz, 1H), 3.06 (d, *J* = 17.5 Hz, 1H), 3.14 – 2.87 (m, 5H), 2.35 – 2.21 (m, 1H), 2.21 – 2.13 (m, 1H), 1.97 – 1.84 (m, 1H), 1.75 – 1.60 (m, 2H).
^{13}C-NMR ($CDCl_3$, 75 MHz): δ 136.4, 85.8, 65.5, 52.5, 51.7, 51.1, 30.7, 22.1, 20.1.

5.3.2.2 General procedure for the coupling reaction between 83 and the appropriate phenyl alcohol (84-90)

In anhydrous conditions and under argon atmosphere, to a solution of the appropriate phenyl alcohol (1.5 equiv) in dry THF (3.5 mL/mmol **83**), cooled to 0°C, NaH 60% dispersion in mineral oil (1.65 equiv) was added portionwise. The mixture was stirred for 30 min at RT. After cooling to 0°C, a solution of bromo-Δ^2–isoxazoline **83** (1 equiv) in dry THF (3.5 mL/mmol **83**) was added dropwise and the final mixture was stirred at RT for 2h. Upon completion (TLC in cyclohexane/EtOAc 2:3), the reaction was quenched with saturated aqueous solution of $NaHCO_3$. The aqueous phase was then repeatedly extracted with CH_2Cl_2 (x 3). The combined organic phases were dried over anhydrous Na_2SO_4, filtered and concentrated in vacuum. The residue was then purified by silica gel column chromatography eluting in cyclohexane/EtOAc 2:3 to provide the pure product **84-90**.

5.3.2.3 General procedure for the cleavage of BH$_3$ group (91-98)

Under argon atmosphere, alkoxy *N*-boranyl Δ^2-isoxazoline derivatives **84-90** were dissolved in acetone (3.5 mL/mmol **84-90**) and cooled to 0°C. A solution of CF_3COOH (5 equiv) in acetone (3.5 mL/mmol **84-90**) was added dropwise and the resulting mixture was stirred at RT overnight (TLC in cyclohexane/EtOAc 2:3 or CH_2Cl_2/MeOH 95:5). Upon completion, the mixture was concentrated under reduced pressure and the residue was diluted with water. The aqueous solution (pH = 1-2) was extracted with Et_2O (x 1). The residual aqueous phase was basified with Na_2CO_3 and the desired product was extracted with CH_2Cl_2 (x 3), dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to provide compounds **91-98**, which were directly used in the next step without further purification.

5.3.2.4 General procedure for salification (9a-h)

The appropriate derivative **91-98** (1 equiv) was dissolved in MeOH (5 mL/mmol **91-98**), asolution of fumaric acid (1 equiv) in MeOH (2 mL/mmol **91-98**) was added and the mixture was stirred at RT for 2 h. The solvent was then removed under reduced pressure affording the crude salt quantitatively, followed by re-crystallization from a proper solvent to give the desired final compound **9a-h.**

5.3.2.5 General procedure for methylation (10a-h)

The appropriate derivative **91-98** (1 equiv) was dissolved in MeOH (5 mL/mmol **91-98**) and methyl iodide (8.0 equiv) was added. The mixture was stirred overnight at RT (TLC in CH_2Cl_2/MeOH 4:1). Upon complete consumption of the starting material, the solvent and the excess of methyl iodide were removed by evaporation under reduced pressure, providing the crude *N*-methylated analogues **10a-h** quantitatively. The pure compounds were then re-crystalized from a proper solvent.

(±)-3-(3-(trifluoromethyl)benzyl)oxy-1-oxa-2,7-diaza-7-boranyl-7,10-ethanospiro[4.5]dec-2-ene (84)

The title compound was prepared according to the general procedure described in **5.3.2.2**, by reacting **83** (200 mg, 0.77 mmol, 1 equiv), (3-(trifluoromethyl)phenyl)methanol (204 mg, 1.16 mmol, 1.5 equiv) and NaH 60% dispersion in mineral oil (51 mg, 1.28 mmol, 1.65 equiv). After standard workup, **84** was obtained as a yellow oil (219 mg, yield 81%).
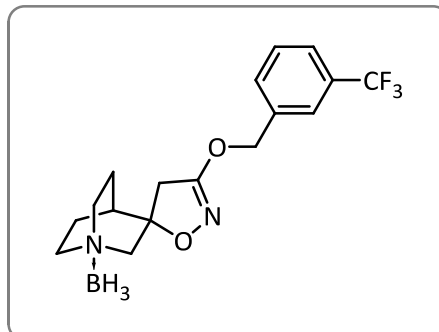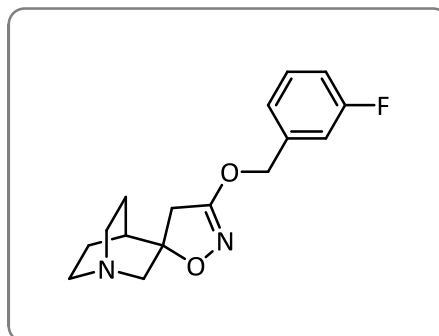
84: yellow oil; TLC (Dragendorff): R$_f$ = 0.38 (cyclohexane/EtOAc 2:3).
^{1}H NMR (CDCl$_3$, 300 MHz) δ 7.63 − 7.38 (m, 2H), 5.11 (s, 2H), 3.29 (dd, *J* = 14.4, 1.6 Hz, 1H), 3.06 (d, *J* = 16.8 Hz, 1H), 3.16 − 2.80 (m, 5H), 2.88 (d, *J* = 16.6 Hz, 1H), 2.38 − 2.14 (m, 2H), 1.95 − 1.77 (m, 1H), 1.77 − 1.51 (m, 2H).
^{13}C NMR (CDCl$_3$, 75 MHz) δ 165.6, 136.1, 131.5, 131.0 (q, *J* = 32.5 Hz), 129.2, 125.4 (q, *J* = 3.7 Hz), 124.8 (q, *J* = 3.6 Hz), 123.9 (q, *J* = 272.3 Hz), 84.5, 71.0, 65.4, 52.2, 51.5, 42.1, 30.2, 21.8, 19.7.

(±)-3-(3-(trifluoromethyl)benzyl)oxy-1-oxa-2,7-diaza-7,10-ethanospiro[4.5] dec-2-ene (91)

The title compound was obtained according to the general procedure **5.3.2.3** by reacting **84** (219 mg, 0.62 mmol, 1 equiv) and CF$_3$COOH (239 μL, 3.1 mmol, 5 equiv) in acetone. After disappearance of the starting material (TLC in cyclohexane/EtOAc 2:3 and CH$_2$Cl$_2$/MeOH 9:1), standard workup was applied to provide the desired compound (132 mg, yield 63%).
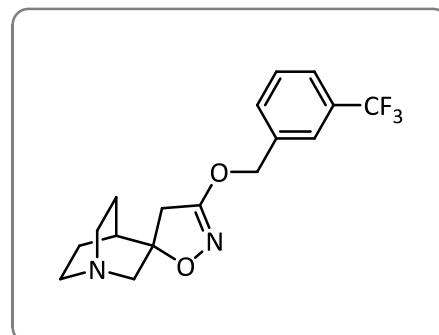
91: yellow oil; TLC (Dragendorff): R$_f$ = 0.41 (CH$_2$Cl$_2$/MeOH 4:1).
^{1}H NMR (CDCl$_3$, 300 MHz) δ 7.62 − 7.55 (m, 1H), 7.55 − 7.37 (m, 3H), 5.10 (s, 2H), 3.18 (dd, *J* = 14.6, 1.8 Hz, 1H), 3.04 (d, *J* = 16.4 Hz, 1H), 2.89 (dd, *J* = 12.1, 4.3 Hz, 1H), 2.84 − 2.58 (m, 5H), 2.13 − 1.99 (m, 1H), 1.99 − 1.93 (m, 1H), 1.66 − 1.53 (m, 1H), 1.53 − 1.41 (m, 1H), 1.41 − 1.28 (m, 1H).
^{13}C NMR (CDCl$_3$, 75 MHz) δ 165.8, 136.7, 131.5, 130.6 (d, *J* = 29.2 Hz), 129.1, 125.3 (d, *J* = 3.8 Hz), 124.8 (d, *J* = 3.1 Hz), 124.0 (d, *J* = 272.3 Hz), 86.9, 70.6, 62.9, 46.8, 46.5, 42.4, 30.8, 23.7, 21.0.

(±)-3-(3-(trifluoromethyl)benzyl)oxy-1-oxa-2,7-diaza-7,10-ethanospiro[4.5]dec-2-ene fumarate (9a)

The fumarate derivative **9a** was prepared according to the general procedure **5.3.2.4** by reacting **91** (132 mg, 0.39 mmol, 1 equiv) and fumaric acid (45 mg, 0.39 mmol, 1 equiv) in MeOH. The crude salt was recrystallized from *i*-PrOH to provide the pure compound as a white solid (166 mg, yield 93%).


x fumarate
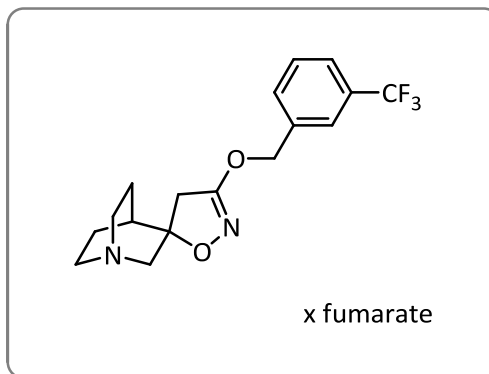
9a: white solid; TLC (alumina-Dragendorff): R_f = 0.40 (CH$_2$Cl$_2$/MeOH 98:2); m.p.: 114.3- 115.1°C; MS (ESI) m/z for C$_{17}$H$_{20}$F$_3$N$_2$O$_2^+$ [M+H]$^+$ calcd. 341.2, found 340.8.

^{1}H NMR (CD$_3$OD, 300 MHz) δ 7.77 – 7.71 (m, 1H), 7.71 – 7.54 (m, 3H), 6.69 (s, 2H), 5.22 (s, 2H), 3.57 (s, 2H), 3.42 – 3.21 (m, 5H), 3.16 (d, *J* = 16.9 Hz, 1H), 2.43 – 2.26 (m, 2H), 2.13 – 1.98 (m, 1H), 1.98 – 1.77 (m, 2H).

^{13}C NMR (CD$_3$OD, 75 MHz) δ 170.9, 167.7, 138.4, 136.0, 132.9, 131.9 (q, *J* = 32.3 Hz), 130.5, 126.2 (q, *J* = 3.6 Hz), 125.8 (q, *J* = 3.7 Hz), 125.5 (q, *J* = 271.8 Hz), 85.1, 71.9, 60.2, 47.4, 46.8, 42.4, 30.8, 20.9, 19.1.

(±)-3-(3-(trifluoromethyl)benzyl)oxy-1-oxa-2,7-diaza-7,10-ethanospiro[4.5]dec-2-ene methyl iodide (10a)

According to the general procedure **5.3.2.5**, CH$_3$I (135 μL, 2.16 mmol, 8 equiv) was added to a solution of the free base **91** (91 mg, 0.27 mmol, 1 equiv) in MeOH. Evaporation of the solvent under reduced pressure afforded the crude quaternary salt quantitatively and then it was re-crystalized from EtOAc/*i*-PrOH to give the pure final compound as a white solid (61 mg, yield 47%).
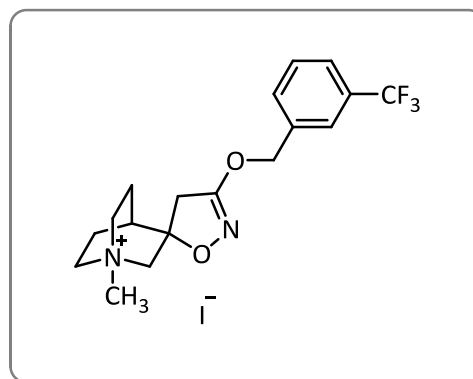

10a: white solids; TLC (alumina-Dragendorff): R_f = 0.33 (CH$_2$Cl$_2$/MeOH 9:1); m.p.: 141.3-142.0°C; MS (ESI) m/z for C$_{18}$H$_{22}$F$_3$N$_2$O$_2^+$ [M]$^+$ calcd. 355.2, found 355.0.

^{1}H NMR (CD$_3$OD, 300 MHz) δ 7.77 – 7.55 (m, 4H), 5.24 (s, 2H), 3.87 (dd, *J* = 13.8, 2.7 Hz, 1H), 3.73 (dd, *J* = 13.7, 2.7 Hz, 1H), 3.61 – 3.37 (m, 4H), 3.37 (d, *J* = 16.8 Hz, 1H), 3.22 (d, *J* = 16.9 Hz, 1H), 3.06 (s, 3H), 2.41 (dd, *J* = 11.5, 7.8 Hz, 2H), 2.22 – 2.07 (m, 1H), 2.07 – 1.91 (m, 2H).

^{13}C NMR (CD$_3$OD, 75 MHz) δ 167.6, 138.3, 133.0, 131.9 (q, *J* = 32.3 Hz), 130.5, 126.2 (q, *J* = 3.8 Hz), 125.8 (q, *J* = 3.9 Hz), 125.5 (q, *J* = 271.4 Hz), 85.4, 72.0, 69.6, 57.8, 57.2, 52.4, 42.4, 30.6, 22.1, 20.3.

(±)-3-(3-fluorobenzyl)oxy-1-oxa-2,7-diaza-7-boranyl-7,10-ethanospiro[4.5]dec-2-ene (85)

The title compound was prepared according to the general procedure described in **5.3.2.2**, by reacting **83** (200 mg, 0.77 mmol, 1 equiv), (3-fluorophenyl)methanol (146 mg, 1.16 mmol, 1.5 equiv) and NaH 60% dispersion in mineral oil (51 mg, 1.28 mmol, 1.65 equiv). After standard workup, **85** was obtained as a yellow oil (206 mg, yield 88%).
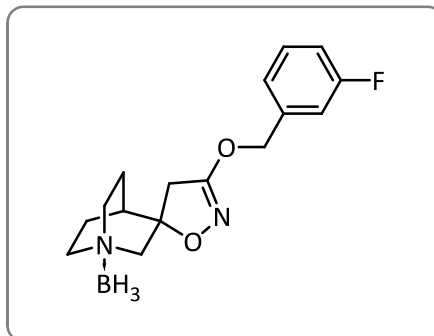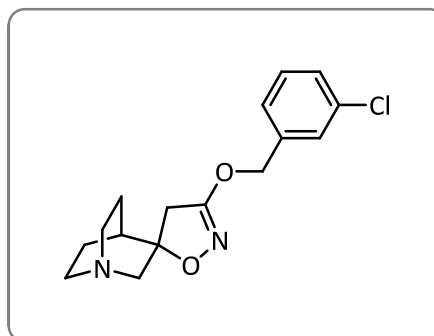
85: yellow oil; TLC (Dragendorff): R_f = 0.42 (cyclohexane/EtOAc 2:3).

^{1}H NMR (CDCl$_3$, 300 MHz) δ 7.28 (td, J = 9.3, 4.6 Hz, 1H), 7.08 (d, J = 7.8 Hz, 1H), 7.06 – 7.00 (m, 1H), 7.00 – 6.94 (m, 1H), 5.06 (s, 2H), 3.33 (dd, J = 14.6, 2.1 Hz, 1H), 3.11 – 2.89 (m, 6H), 2.85 (d, J = 16.5 Hz, 1H), 2.38 – 2.24 (m, 1H), 2.24 – 2.17 (m, 1H), 1.96 – 1.81 (m, 1H), 1.73 – 1.55 (m, 2H).

^{13}C NMR (CDCl$_3$, 75 MHz) δ 165.6, 162.8 (d, J = 246.5 Hz), 137.5 (d, J = 7.5 Hz), 130.3 (d, J = 8.4 Hz), 123.8 (d, J = 3.1 Hz), 115.6 (d, J = 21.1 Hz), 115.1 (d, J = 22.0 Hz), 84.4, 71.1, 71.1, 65.5, 52.2, 51.5, 42.2, 30.2, 21.9, 19.8.

(±)-3-(3-fluorobenzyl)oxy-1-oxa-2,7-diaza-7,10-ethanospiro[4.5]dec-2-ene (92)

The title compound was obtained according to the general procedure **5.3.2.3** by reacting **84** (180 mg, 0.59 mmol, 1 equiv) and CF$_3$COOH (227 μL, 2.95 mmol, 5 equiv) in acetone. After disappearance of the starting material (TLC in cyclohexane/EtOAc 2:3 and CH$_2$Cl$_2$/MeOH 9:1), standard workup was applied to provide the desired compound (132 mg, yield 77%).

92: yellow oil; TLC (Dragendorff): R_f = 0.44 (CH$_2$Cl$_2$/MeOH 95:5).

^{1}H NMR (CDCl$_3$, 300 MHz) δ 7.31 – 7.19 (m, 1H), 7.10 – 7.05 (m, 1H), 7.05 – 6.99 (m, 1H), 6.99 – 6.91 (m, 1H), 5.04 (s, 2H), 3.19 (dd, J = 14.6, 1.6 Hz, 1H), 3.03 (d, J = 16.4 Hz, 1H), 2.91 (dd, J = 14.6, 1.9 Hz, 1H), 2.85 – 2.57 (m, 5H), 2.15 – 1.99 (m, 1H), 1.99 – 1.91 (m, 1H), 1.66 – 1.52 (m, 1H), 1.52 – 1.41 (m, 1H), 1.41 – 1.28 (m, 1H).

^{13}C NMR (CDCl$_3$, 75 MHz) δ 165.8, 162.8 (d, J = 246.4 Hz), 138.0 (d, J = 7.5 Hz), 130.1 (d, J = 8.2 Hz), 123.6 (d, J = 3.1 Hz), 115.3 (d, J = 21.1 Hz), 115.0 (d, J = 22.0 Hz). 86.7, 70.6, 70.6, 62.8, 46.7, 46.4, 42.3, 30.7, 23.6, 20.9.

(±)-3-(3-fluorobenzyl)oxy-1-oxa-2,7-diaza-7,10-ethanospiro[4.5]dec-2-ene fumarate (9b)

The fumarate derivative **9b** was prepared according to the general procedure **5.3.2.4** by reacting **92** (110 mg, 0.38 mmol, 1 equiv) and fumaric acid (44 mg, 0.38 mmol, 1 equiv) in MeOH. The crude salt was recrystallized from *i*-PrOH to provide the pure compound as off-white solid (136 mg, yield 95%).

x 0.75 fumarate
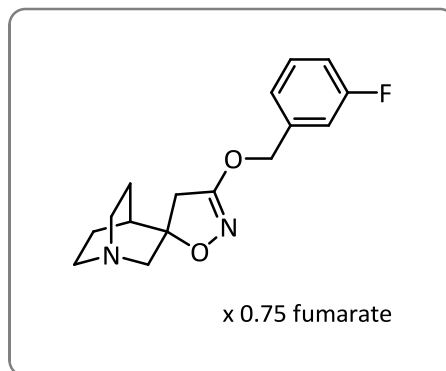
9b: off-white solid; TLC (alumina-Dragendorff): R_f = 0.33 (CH_2Cl_2/MeOH 98:2); m.p.: 171.8-172.3°C; MS (ESI) m/z for $C_{16}H_{20}FN_2O_2^+$ [M+H]$^+$ calcd. 291.2, found 291.1.

^{1}H NMR (CD3OD, 300 MHz) δ 7.44 – 7.34 (m, 1H), 7.25 – 7.19 (m, 1H), 7.19 – 7.13 (m, 1H), 7.13 – 7.03 (m, 1H), 6.68 (s, 1.5H), 5.14 (s, 2H), 3.56 (s, 2H), 3.42 – 3.19 (m, 5H), 3.16 (d, *J* = 16.7 Hz, 1H), 2.42 – 2.26 (m, 2H), 2.11 – 1.97 (m, 1H), 1.97 – 1.76 (m, 2H).

^{13}C NMR (CD3OD, 75 MHz) δ 169.5 (d, *J* = 260.7 Hz), 165.9, 139.7 (d, *J* = 7.5 Hz), 136.1, 131.4 (d, *J* = 8.2 Hz), 124.9 (d, *J* = 3.0 Hz), 116.2 (d, *J* = 21.4 Hz), 115.8 (d, *J* = 22.5 Hz), 85.0, 72.0, 60.3, 47.4, 46.8, 42.4, 30.8, 21.0, 19.1.

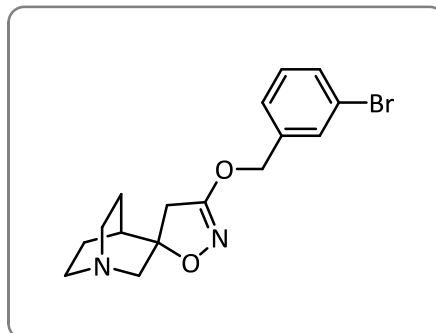## (±)-3-(3-fluorobenzyl)oxy-1-oxa-2,7-diaza-7,10-ethanospiro[4.5]dec-2-ene methyl iodide (10b)

According to the general procedure **5.3.2.5**, CH_3I (105 μL, 1.68 mmol, 8 equiv) was added to a solution of the free base **92** (60 mg, 0.21 mmol, 1 equiv) in MeOH. Evaporation of the solvent under reduced pressure afforded the crude quaternary salt quantitatively and then it was re-crystalized from acetone and Et$_2$O to give the pure final compound as a white solid (59 mg, yield 65%).
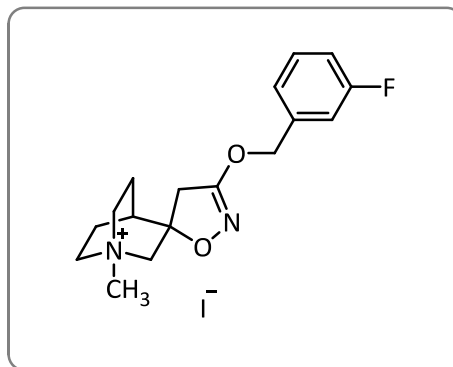
10b: white solid; TLC (alumina-Dragendorff): R_f = =.30 in CH_2Cl_2/MeOH 9:1; m.p.: 172.7-173.6°C; MS (ESI) m/z for $C_{17}H_{22}FN_2O_2^+$ [M]$^+$ calcd. 305.2, found 304.8.

^{1}H NMR (CD$_3$OD, 300 MHz) δ 7.40 (td, *J* = 8.0, 5.9 Hz, 1H), 7.22 (d, *J* = 7.9 Hz, 1H), 7.17 (dd, *J* = 9.7, 2.2 Hz, 1H), 7.08 (td, *J* = 8.3, 1.9 Hz, 1H), 5.16 (s, 2H), 3.85 (dd, *J* = 13.8, 2.6 Hz, 1H), 3.73 (dd, *J* = 13.7, 2.7 Hz, 1H), 3.61 – 3.33 (m, 4H), 3.36 (d, *J* = 16.9 Hz, 1H), 3.20 (d, *J* = 16.9 Hz, 1H), 3.06 (s, 3H), 2.48 – 2.33 (m, 2H), 2.21 – 2.07 (m, 1H), 2.07 – 1.91 (m, 2H).

^{13}C NMR (CD$_3$OD, 75 MHz) δ 167.7, 164.2 (d, *J* = 244.7 Hz), 139.6 (d, *J* = 7.4 Hz), 131.4 (d, *J* = 8.3 Hz), 124.9 (d, *J* = 3.0 Hz), 116.2 (d, *J* = 21.3 Hz), 115.8 (d, *J* = 22.4 Hz), 85.4, 72.0, 69.6, 57.8, 57.2, 52.4, 42.4, 30.6, 22.1, 20.3.

(±)-3-(3-chlorobenzyl)oxy-1-oxa-2,7-diaza-7-boranyl-7,10-ethanospiro[4.5]dec-2-ene (86)

The title compound was prepared according to the general procedure described in **5.3.2.2**, by reacting **83** (200 mg, 0.77 mmol, 1 equiv), (3-chlorophenyl)methanol (165 mg, 1.16 mmol, 1.5 equiv) and NaH 60% dispersion in mineral oil (51 mg, 1.28 mmol, 1.65 equiv). After standard workup, **86** was obtained as a yellow oil (222 mg, yield 90%).
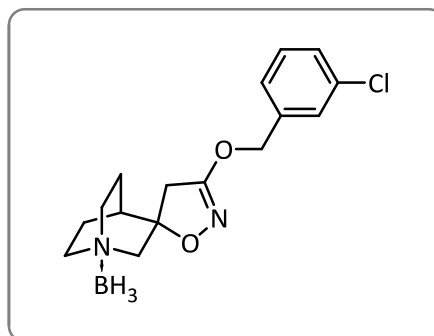
86: yellow oil¸ TLC (Dragendorff): R_f = 0.40 (cyclohexane/EtOAc 2:3).

^{1}H NMR (CDCl$_3$, 300 MHz) δ 7.33 – 7.24 (m, 2H), 7.23 – 7.16 (m, 2H), 5.04 (s, 2H), 3.33 (dd, J = 14.5, 2.0 Hz, 1H), 3.12 – 2.89 (m, 6H), 2.84 (d, J = 16.5 Hz, 1H), 2.38 – 2.23 (m, 1H), 2.23 – 2.16 (m, 1H), 1.95 – 1.80 (m, 1H), 1.72 – 1.54 (m, 2H).

^{13}C NMR (CDCl$_3$, 75 MHz) δ 165.6, 137.1, 134.6, 130.0, 128.9, 128.3, 126.4, 84.4, 71.1, 65.5, 52.2, 51.5, 42.3, 30.2, 21.9, 19.8.

(±)-3-(3-chlorobenzyl)oxy-1-oxa-2,7-diaza-7,10-ethanospiro[4.5]dec-2-ene (93)

The title compound was obtained according to the general procedure **5.3.2.3** by reacting **86** (110 mg, 0.34 mmol, 1 equiv) and CF$_3$COOH (131 μL, 1.7 mmol, 5 equiv) in acetone. After disappearance of the starting material (TLC in cyclohexane/EtOAc 2:3 and CH$_2$Cl$_2$/MeOH 9:1), standard workup was applied to provide the desired compound (84 mg, yield 81%).

93: yellow oil; TLC (Dragendorff): R_f = 0.38 (CH$_2$Cl$_2$/MeOH 9:1).

^{1}H NMR (CDCl$_3$, 300 MHz) δ 7.34 – 7.28 (m, 1H), 7.26 – 7.21 (m, 2H), 7.21 – 7.14 (m, 1H), 5.02 (s, 2H), 3.18 (dd, J = 14.6, 1.6 Hz, 1H), 3.03 (d, J = 16.4 Hz, 1H), 2.94 (dd, J = 14.6, 1.6 Hz, 1H), 2.85 – 2.58 (m, 5H), 2.14 – 2.00 (m, 1H), 2.00 – 1.93 (m, 1H), 1.68 – 1.53 (m, 1H), 1.53 – 1.27 (m, 2H).

^{13}C NMR (CDCl$_3$, 75 MHz) δ 165.8, 137.6, 134.5, 129.9, 128.6, 128.2, 126.3, 86.7, 70.6, 62.8, 46.7, 46.5, 42.4, 30.7, 23.6, 20.9.

(±)-3-(3-chlorobenzyl)oxy-1-oxa-2,7-diaza-7,10-ethanospiro[4.5]dec-2-ene fumarate (9c)

The fumarate derivative **9c** was prepared according to the general procedure **5.3.2.4** by reacting **93** (60 mg, 0.20 mmol, 1 equiv) and fumaric acid (23 mg, 0.20 mmol, 1 equiv) in MeOH. The crude salt was recrystallized from 1-PrOH to provide the pure compound as a white solid (73 mg, yield 93%).


x 0.75 fumarate
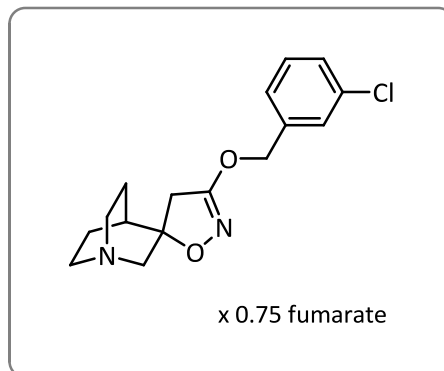
9c: white solid; TLC (alumina-Dragendorff): R_f = 0.36 (CH$_2$Cl$_2$/MeOH 98:2); m.p.: 164.6- 165.3°C; MS (ESI) m/z for C$_{16}$H$_{20}$ClN$_2$O$_2^+$ [M+H]$^+$ calcd. 307.1, found 307.9 [M+H]$^+$, 310.2 [M+2+H]$^+$.
^{1}H NMR (CD$_3$OD, 300 MHz) δ 7.47 – 7.42 (m, 1H), 7.40 – 7.30 (m, 3H), 6.69 (s, 1.5H), 5.13 (s, 2H), 3.55 (s, 2H), 3.41 – 3.18 (m, 5H), 3.13 (d, J = 16.9 Hz, 1H), 2.43 – 2.24 (m, 2H), 2.11 – 1.96 (m, 1H), 1.96 – 1.76 (m, 2H).
^{13}C NMR (CD$_3$OD, 75 MHz) δ 171.1, 167.7, 139.3, 136.1, 135.4, 131.2, 129.6, 129.2, 127.5, 85.1, 71.9, 60.3, 47.4, 46.8, 42.4, 30.8, 21.0, 19.1.

(±)-3-(3-chlorobenzyl)oxy-1-oxa-2,7-diaza-7,10-ethanospiro[4.5]dec-2-ene methyl iodide (10c)

According to the general procedure **5.3.2.5**, CH$_3$I (565 µL, 9.07 mmol, 8 equiv) was added to a solution of the free base **93** (348 mg, 1.13 mmol, 1 equiv) in MeOH. Evaporation of the solvent under reduced pressure afforded the crude quaternary salt quantitatively and then it was re-crystalized from acetone to give the pure final compound as a white solid (294 mg, yield 58%).
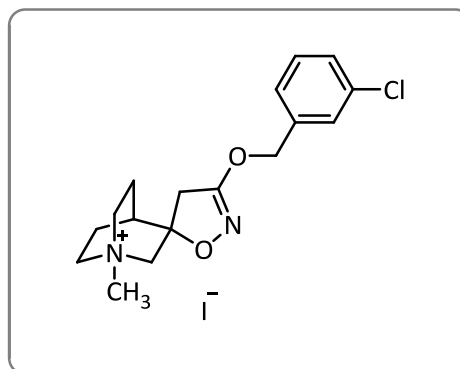

10c: white solid; TLC (alumina-Dragendorff): R_f = 0.34 (CH$_2$Cl$_2$/MeOH 9:1);   m.p.: 157.9-159.8°C; MS (ESI) m/z for C$_{17}$H$_{22}$ClN$_2$O$_2^+$ [M]$^+$ calcd. 321.1, found 321.2 [M+H]$^+$, 323.2 [M+2+H]$^+$.
^{1}H NMR (CD$_3$OD, 300 MHz) δ 7.47 – 7.43 (m, 1H), 7.39 – 7.31 (m, 3H), 5.14 (s, 2H), 3.87 (dd, J = 13.8, 2.7 Hz, 1H), 3.73 (dd, J = 13.7, 2.7 Hz, 1H), 3.60 – 3.36 (m, 4H), 3.36 (d, J = 16.9 Hz, 1H), 3.21 (d, J = 16.9 Hz, 1H), 3.06 (s, 3H), 2.49 – 2.31 (m, 2H), 2.21 – 2.07 (m, 1H), 2.07 – 1.91 (m, 2H).
^{13}C NMR (CD$_3$OD, 75 MHz) δ 167.6, 139.2, 135.4, 131.2, 129.6, 129.2, 127.5, 85.4, 72.0, 69.6, 57.8, 57.2, 52.3, 42.4, 30.6, 22.1, 20.3.

(±)-3-(3-bromobenzyl)oxy-1-oxa-2,7-diaza-7,10-ethanospiro[4.5]dec-2-ene (94)

The coupling reaction was performed following a procedure distinct from the general protocol **5.3.2.2**, which, in a single step, directly afforded the title compound since the cleavage of the BH$_3$ group occurred immediately. Intermediate **83** (400 mg, 1.55 mmol, 1 equiv) was dissolved in MeCN (6 mL), K$_2$CO$_3$ (641 mg, 4.64 mmol, 3 equiv) and then (3-bromophenyl)methanol (867 mg, 4.64 mmol, 3 equiv) were slowly added. The mixture was heated to 75°C and monitored by TLC (cyclohexane/EtOAc 2:3). Since no new spots were detected in TLC, NaH 60% dispersion in mineral oil (100 mg, 2.50 mmol) was added to induce the formation of the desired compound. After disappearance of the starting compound (2h), the reaction was quenched with satured aqueous solution of NaHCO$_3$, and extracted with CH$_2$Cl$_2$ (x 3). The combined organic phases were dryied over anhydrous Na$_2$SO$_4$, filtered and concentrated in vacuum. The residue was purified by silica gel column chromatography eluting in cyclohexane/EtOAc 7:3 to provide the pure desired compound (172 mg, yield 32%).

94: yellow oil; TLC (Dragendorff): R$_f$ = 0.21 in CH$_2$Cl$_2$/MeOH 9:1 (TLC stains: Dragendorff).
^{1}H NMR (CDCl$_3$, 300 MHz) δ 7.50 − 7.43 (m, 1H), 7.43 − 7.36 (m, 1H), 7.26 − 7.12 (m, 2H), 5.02 (s, 2H), 3.24 (d, J = 14.5 Hz, 1H), 3.05 (dd, J = 15.6, 3.8 Hz, 2H), 2.96 − 2.75 (m, 5H), 2.20 − 2.06 (m, 1H), 2.06 − 1.99 (m, 1H), 1.75 − 1.61 (m, 1H), 1.61 − 1.50 (m, 1H), 1.50 − 1.36 (m, 1H).
^{13}C NMR (CDCl$_3$, 75 MHz) δ 165.8, 137.7, 131.6, 131.1, 130.2, 126.8, 122.7, 86.1, 70.6, 62.0, 46.6, 46.3, 42.3, 30.6, 22.9, 20.4.

(±)-3-(3-bromobenzyl)oxy-1-oxa-2,7-diaza-7,10-ethanospiro[4.5]dec-2-ene fumarate (9d)

The fumarate derivative **9d** was prepared according to the general procedure **5.3.2.4** by reacting **94** (172 mg, 0.49 mmol, 1 equiv) and fumaric acid (57 mg, 0.49 mmol, 1 equiv) in MeOH. The crude salt was recrystallized from 1-PrOH to provide the pure compound as a off-white solid (208 mg, yield 91%).

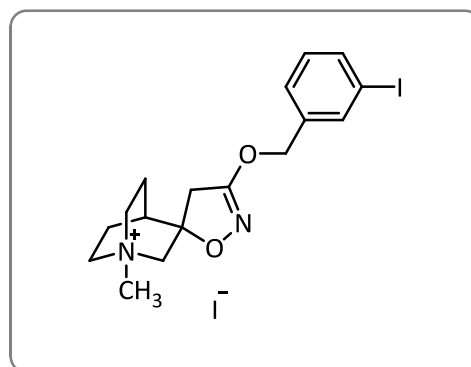**9d:** off-white solid; TLC (alumina-Dragendorff): R_f = 0.42 (CH$_2$Cl$_2$/MeOH 98:2); m.p.: 151.4-152.2°C; MS (ESI) m/z for C$_{16}$H$_{20}$BrN$_2$O$_2^+$ [M+H]$^+$ calcd. 351.1, found 351.4 [M+H]$^+$, 353.5 [M+2+H]$^+$.

^{1}H NMR (CD$_3$OD, 300 MHz) δ 7.58 (s, 1H), 7.53 − 7.45 (m, 1H), 7.37 (d, J = 7.7 Hz, 1H), 7.28 (t, J = 7.7 Hz, 1H), 6.68 (s, 2H), 5.11 (s, 2H), 3.57 (s, 2H), 3.42 − 3.21 (m, 5H), 3.15 (d, J = 16.9 Hz, 1H), 2.42 − 2.24 (m, 2H), 2.12 − 1.97 (m, 1H), 1.97 − 1.78 (m, 2H).

^{13}C NMR (CD$_3$OD, 75 MHz) δ 171.3, 167.7, 139.4, 136.1, 132.5, 132.1, 131.4, 127.9, 123.3, 85.0, 71.8, 60.0, 47.1, 46.5, 42.4, 30.8, 20.8, 19.0.

(±)-3-(3-bromobenzyl)oxy-1-oxa-2,7-diaza-7,10-ethanospiro[4.5]dec-2-ene methyl iodide (10d)

According to the general procedure **5.3.2.5**, CH$_3$I (169 μL, 2.72 mmol, 8 equiv) was added to a solution of the free base **94** (119 mg, 0.34 mmol, 1 equiv) in MeOH. Evaporation of the solvent under reduced pressure afforded the crude quaternary salt quantitatively and then it was re-crystalized from EtOH/*i*-PrOH to give the pure final compound as a yellow solid (77 mg, yield 46%).
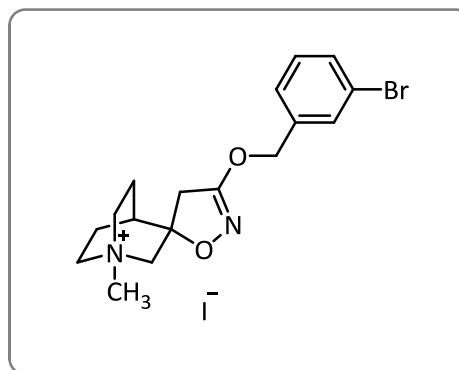

10d: yellow solid; TLC (alumina-Dragendorff): R_f = 0.33 (CH$_2$Cl$_2$/MeOH 9:1); m.p.: 171.3-172.5°C; MS (ESI) m/z for C$_{17}$H$_{22}$BrN$_2$O$_2^+$ [M]$^+$ calcd. 365.1, found 364.9 [M+H]$^+$, 367.2 [M+2+H]$^+$.

^{1}H NMR (CD$_3$OD, 300 MHz) δ 7.63 − 7.57 (m, 1H), 7.55 − 7.47 (m, 1H), 7.39 (d, J = 7.7 Hz, 1H), 7.30 (t, J = 7.8 Hz, 1H), 5.13 (s, 2H), 3.85 (dd, J = 13.8, 2.6 Hz, 1H), 3.72 (dd, J = 13.7, 2.7 Hz, 1H), 3.61 − 3.39 (m, 4H), 3.35 (d, J = 16.9 Hz, 1H), 3.20 (d, J = 16.9 Hz, 1H), 3.05 (s, 3H), 2.50 − 2.31 (m, 2H), 2.20 − 2.07 (m, 1H), 2.07 − 1.89 (m, 2H).

^{13}C NMR (CD$_3$OD, 75 MHz) δ 167.6, 139.4, 132.6, 132.2, 131.4, 128.0, 123.4, 85.4, 71.9, 69.6, 57.8, 57.2, 52.4, 42.4, 30.6, 22.1, 20.3.

(±)-3-(3-iodobenzyl)oxy-1-oxa-2,7-diaza-7-boranyl-7,10-ethanospiro[4.5]dec-2-ene (87)

The title compound was prepared according to the general procedure described in **5.3.2.2**, by reacting **83** (200 mg, 0.77 mmol, 1 equiv), (3-iodophenyl)methanol (272 mg, 1.16 mmol, 1.5 equiv) and NaH 60% dispersion in mineral oil (51 mg, 1.28 mmol, 1.65 equiv). After standard workup, **87** was obtained as a yellow oil (234 mg, yield 74%).
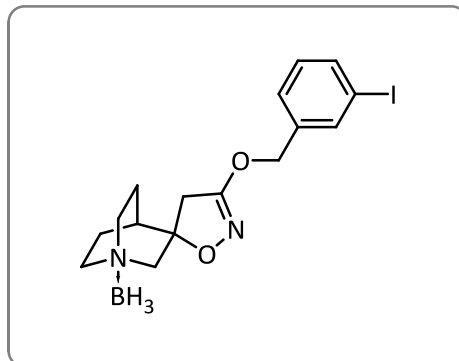

87: yellow oil; TLC (Dragendorff): R$_f$ = 0.44 (cyclohexane/EtOAc 2:3).

^{1}H NMR (CDCl$_3$, 300 MHz) δ 7.75 – 7.70 (m, 1H), 7.67 (dd, J = 7.9, 1.1 Hz, 1H), 7.32 (d, J = 7.7 Hz, 1H), 7.10 (t, J = 7.8 Hz, 1H), 5.05 (s, 2H), 3.35 (dd, J = 14.5, 1.9 Hz, 1H), 3.19 – 2.94 (m, 6H), 2.90 (d, J = 16.6 Hz, 1H), 2.40 – 2.21 (m, 2H), 2.00 – 1.84 (m, 1H), 1.78 – 1.60 (m, 2H).

^{13}C NMR (CDCl$_3$, 75 MHz) δ 165.5, 137.8, 137.4, 137.1, 130.4, 127.5, 94.4, 84.4, 70.9, 65.5, 52.2, 51.5, 42.3, 30.2, 21.9, 19.8.

(±)-3-(3-iodobenzyl)oxy-1-oxa-2,7-diaza-7,10-ethanospiro[4.5]dec-2-ene (95)

The title compound was obtained according to the general procedure **5.3.2.3** by reacting **87** (234 mg, 0.57 mmol, 1 equiv) and CF$_3$COOH (220 μL, 2.85 mmol, 5 equiv) in acetone. After disappearance of the starting material (TLC in cyclohexane/EtOAc 2:3 and CH$_2$Cl$_2$/MeOH 9:1), standard workup was applied to provide the desired compound (132 mg, yield 58%).
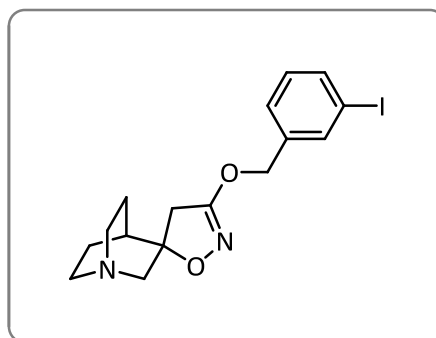
95: yellow oil; TLC (Dragendorff): R$_f$ = 0.32 (CH$_2$Cl$_2$/MeOH 4:1)

^{1}H NMR (CDCl$_3$, 300 MHz) δ 7.71 – 7.63 (m, 1H), 7.60 (dd, J = 7.9, 1.2 Hz, 1H), 7.26 (d, J = 7.7 Hz, 1H), 7.03 (t, J = 7.8 Hz, 1H), 4.99 (s, 2H), 3.18 (dd, J = 14.6, 1.8 Hz, 1H), 3.03 (d, J = 16.4 Hz, 1H), 2.88 (dd, J = 14.7, 1.8 Hz, 1H), 2.89 – 2.56 (m, 5H), 2.12 – 1.99 (m, 1H), 1.99 – 1.93 (m, 1H), 1.66 – 1.53 (m, 1H), 1.53 – 1.42 (m, 1H), 1.42 – 1.28 (m, 1H).

^{13}C NMR (CDCl$_3$, 75 MHz) δ 165.8, 137.9, 137.5, 137.1, 130.3, 127.4, 94.4, 86.8, 70.4, 62.9, 46.8, 46.5, 42.4, 30.8, 23.7, 21.0.

(±)-3-(3-iodobenzyl)oxy-1-oxa-2,7-diaza-7,10-ethanospiro[4.5]dec-2-ene fumarate (9e)

The fumarate derivative **9e** was prepared according to the general procedure **5.3.2.4** by reacting **95** (132 mg, 0.33 mmol, 1 equiv) and fumaric acid (28 mg, 0.33 mmol, 1 equiv) in MeOH. The crude salt was recrystallized from EtOH/*i*-PrOH to provide the pure compound as a white solid (139 mg, yield 88%).

x 0.75 fumarate
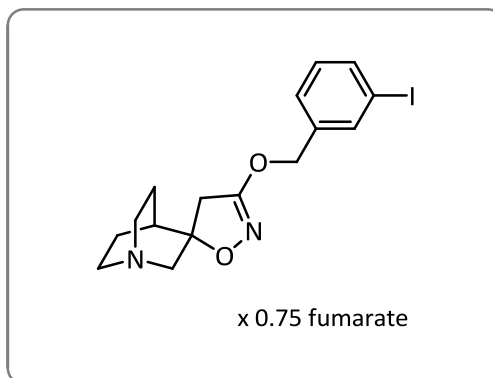
9e: white solid; TLC (alumina-Dragendorff): R_f = 0.42 (CH$_2$Cl$_2$/MeOH 98:2); m.p.: 154.1-155.3°C; MS (ESI) m/z for C$_{16}$H$_{20}$IN$_2$O$_2^+$ [M+H]$^+$ calcd. 399.1, found 398.6.

^{1}H NMR (CD$_3$OD, 300 MHz) δ 7.81 – 7.76 (m, 1H), 7.70 (d, *J* = 7.9 Hz, 1H), 7.40 (d, *J* = 7.7 Hz, 1H), 7.15 (t, *J* = 7.8 Hz, 1H), 6.68 (s, 1.5H), 5.09 (s, 2H), 3.55 (s, 2H), 3.41 – 3.18 (m, 5H), 3.14 (d, *J* = 16.9 Hz, 1H), 2.42 – 2.25 (m, 2H), 2.11 – 1.96 (m, 1H), 1.96 – 1.77 (m, 2H).

^{13}C NMR (CD$_3$OD, 75 MHz) δ 170.9, 167.7, 139.4, 138.6, 138.2, 136.0, 131.4, 128.5, 94.8, 84.9, 71.8, 60.0, 47.2, 46.6, 42.4, 30.8, 20.8, 19.0.

(±)-3-(3-iodobenzyl)oxy-1-oxa-2,7-diaza-7,10-ethanospiro[4.5]dec-2-ene methyl iodide (10e)

According to the general procedure **5.3.2.5**, CH$_3$I (140 µL, 2.24 mmol, 8 equiv) was added to a solution of the free base **95** (110 mg, 0.28 mmol, 1 equiv) in MeOH. Evaporation of the solvent under reduced pressure afforded the crude quaternary salt quantitatively and then it was re-crystalized from MeOH to give the pure final compound as a white solid (77 mg, yield 51%).

10e: white crystals; TLC (alumina-Dragendorff):R_f = 0.36 (CH$_2$Cl$_2$/MeOH 9:1); m.p.: 201.8-202.4°C (dec.); MS (ESI) m/z for C$_{17}$H$_{22}$IN$_2$O$_2^+$ [M]$^+$ calcd. 413.1, found 413.1.

^{1}H NMR ((CD$_3$)$_2$SO, 300 MHz) δ 7.83 – 7.79 (m, 1H), 7.77 – 7.71 (m, 1H), 7.48 – 7.42 (m, 1H), 7.21 (t, *J* = 7.8 Hz, 1H), 5.09 (s, 2H), 3.80 (dd, *J* = 13.7, 2.5 Hz, 1H), 3.68 (dd, *J* = 13.7, 2.5 Hz, 1H), 3.55 – 3.24 (m, 5H), 3.17 (d, *J* = 16.9 Hz, 1H), 2.97 (s, 3H), 2.35 – 2.25 (m, 1H), 2.23 – 2.08 (m, 1H), 2.06 – 1.92 (m, 1H), 1.92 – 1.77 (m, 2H).

^{13}C NMR ((CD$_3$)$_2$SO, 75 MHz) δ 166.0, 138.1, 137.0, 136.6, 130.6, 127.5, 94.7, 84.0, 70.0, 67.2, 55.3, 55.0, 50.9, 40.8, 28.6, 20.5, 18.8.

(±)-3-(3-methylbenzyl)oxy-1-oxa-2,7-diaza-7-boranyl-7,10-ethanospiro[4.5]dec-2-ene (88)

The title compound was prepared according to the general procedure described in **5.3.2.2**, by reacting **83** (200 mg, 0.77 mmol, 1 equiv), *m*-tolylmethanol (142 mg, 1.16 mmol, 1.5 equiv) and NaH 60% dispersion in mineral oil (51 mg, 1.28 mmol, 1.65 equiv). After standard workup, **88** was obtained as a pale yellow viscous oil (170 mg, yield 74%).
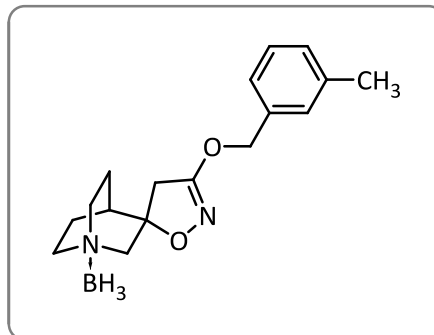
88: pale yellow viscous oil; TLC (Dragendorff): R_f = 0.53 (cyclohexane/EtOAc 2:3).
^{1}H NMR (CDCl$_3$, 300 MHz) δ 7.14 (t, *J* = 7.4 Hz, 1H), 7.09 – 6.96 (m, 3H), 4.95 (s, 2H), 3.22 (d, *J* = 14.3 Hz, 1H), 3.04 – 2.72 (m, 7H), 2.23 (s, 3H), 2.20 – 2.08 (m, 2H), 1.86 – 1.71 (m, 1H), 1.66 – 1.46 (m, 2H).
^{13}C NMR (CDCl$_3$, 75 MHz) δ 165.8, 138.4, 134.9, 129.5, 129.1, 128.5, 125.5, 84.1, 72.1, 65.4, 52.1, 51.5, 42.3, 30.1, 21.8, 21.3, 19.7.

(±)-3-(3-methylbenzyl)oxy-1-oxa-2,7-diaza-7,10-ethanospiro[4.5]dec-2-ene (96)

The title compound was obtained according to the general procedure **5.3.2.3** by reacting **96** (170 mg, 0.57 mmol, 1 equiv) and CF$_3$COOH (220 μL, 2.85 mmol, 5 equiv) in acetone. After disappearance of the starting material (TLC in cyclohexane/EtOAc 2:3 and CH$_2$Cl$_2$/MeOH 9:1), standard workup was applied to provide the desired compound (105 mg, yield 65%).
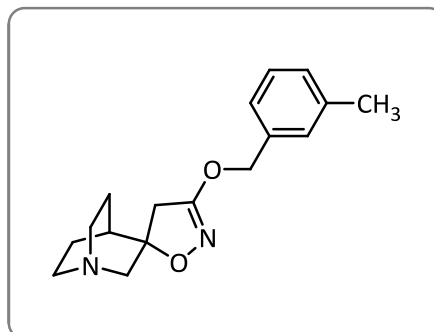
96: colorless viscous oil; TLC (Dragendorff): R_f = 0.26 (CH$_2$Cl$_2$/MeOH 4:1).
^{1}H NMR (CDCl3, 300 MHz) δ 7.25 – 7.14 (m, 1H), 7.14 – 7.02 (m, 3H), 5.01 (s, 2H), 3.18 (dd, *J* = 14.6, 1.9 Hz, 1H), 3.01 (d, *J* = 16.4 Hz, 1H), 2.93 – 2.84 (m, 1H), 2.84 – 2.57 (m, 5H), 2.29 (s, 3H), 2.14 – 1.99 (m, 1H), 1.99 – 1.93 (m, 1H), 1.68 – 1.51 (m, 1H), 1.51 – 1.40 (m, 1H), 1.40 – 1.26 (m, 1H).
^{13}C NMR (CDCl$_3$, 75 MHz) δ 166.1, 138.3, 135.5, 129.4, 129.2, 128.6, 125.5, 86.6, 71.7, 62.9, 46.8, 46.6, 42.5, 30.7, 23.7, 21.4, 21.0.

(±)-3-(3-methylbenzyl)oxy-1-oxa-2,7-diaza-7,10-ethanospiro[4.5]dec-2-ene fumarate (9f)

The fumarate derivative **9f** was prepared according to the general procedure **5.3.2.4** by reacting **96** (105 mg, 0.37 mmol, 1 equiv) and fumaric acid (43 mg, 0.37 mmol, 1 equiv) in MeOH. The crude salt was recrystallized from EtOH to provide the pure compound as a white solid (130 mg, yield 95%).

x 0.75 fumarate
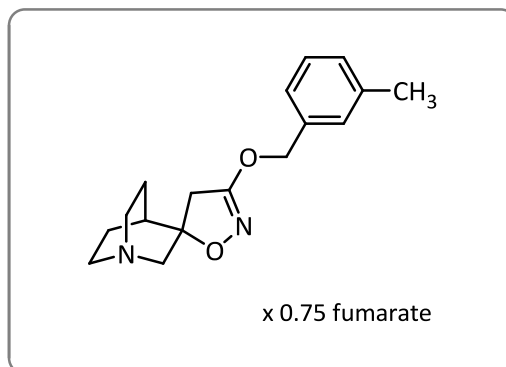
9f: white solid; TLC (alumina-Dragendorff): R_f = 0.36 (CH_2Cl_2/MeOH 98:2); m.p.: 123.4-124.8°C; MS (ESI) m/z for $C_{17}H_{23}N_2O_2^+$ [M+H]$^+$ calcd. 287.2, found 287.2.

^{1}H NMR (CD$_3$OD, 300 MHz) δ 7.30 – 7.11 (m, 4H), 6.68 (s, 1.5H), 5.08 (s, 2H), 3.56 (s, 2H), 3.43 – 3.19 (m, 5H), 3.13 (d, J = 16.8 Hz, 1H), 2.34 (s, 3H), 2.45 – 2.25 (m, 2H), 2.11 – 1.97 (m, 1H), 1.97 – 1.76 (m, 2H).

^{13}C NMR (CD$_3$OD, 75 MHz) δ 171.3, 168.0, 139.4, 136.8, 136.1, 130.2, 130.0, 129.5, 126.4, 84.8, 73.0, 60.2, 47.3, 46.7, 42.5, 30.8, 21.4, 20.9, 19.1.

(±)-3-(3-methylbenzyl)oxy-1-oxa-2,7-diaza-7,10-ethanospiro[4.5]dec-2-ene methyl iodide (10f)

According to the general procedure **5.3.2.5**, CH$_3$I (85 μL, 1.36 mmol, 8 equiv) was added to a solution of the free base **96** (46 mg, 0.17 mmol, 1 equiv) in MeOH. Evaporation of the solvent under reduced pressure afforded the crude quaternary salt quantitatively and then it was re-crystallized from EtOH/*i*-PrOH to give the pure final compound (42 mg, yield 57%).
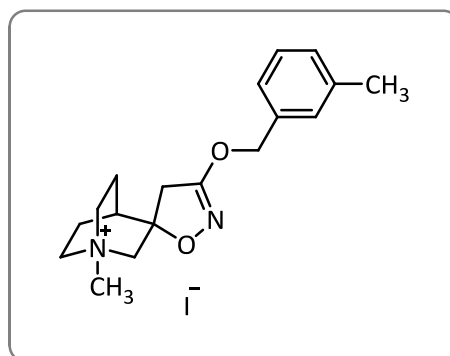
10f: white solid; TLC (alumina-Dragendorff): R_f = 0.34 (CH_2Cl_2/MeOH 9:1); m.p.: 173.6-174.4°C (dec.); MS (ESI) m/z for $C_{18}H_{25}N_2O_2^+$ [M]$^+$ calcd. 301.2, found 300.4.

^{1}H NMR (CD$_3$OD, 300 MHz) δ 7.32 – 7.10 (m, 4H), 5.09 (s, 2H), 3.83 (dd, J = 13.8, 2.4 Hz, 1H), 3.72 (dd, J = 13.7, 2.5 Hz, 1H), 3.64 – 3.32 (m, 5H), 3.17 (d, J = 16.9 Hz, 1H), 3.05 (s, 3H), 2.48 – 2.37 (m, 2H), 2.34 (s, 3H), 2.22 – 2.07 (m, 1H), 2.07 – 1.90 (m, 2H).

^{13}C NMR (CD$_3$OD, 75 MHz) δ 167.9, 139.4, 136.8, 130.3, 130.0, 129.5, 126.4, 85.2, 73.1, 69.6, 57.8, 57.2, 52.4, 42.5, 30.6, 22.1, 21.4, 20.4.

(±)-3-(3-methoxybenzyl)oxy-1-oxa-2,7-diaza-7-boranyl-7,10-ethanospiro[4.5]dec-2-ene (89)

The title compound was prepared according to the general procedure described in **5.3.2.2**, by reacting **83** (200 mg, 0.77 mmol, 1 equiv), (3-methoxyphenyl)methanol (160 mg, 1.16 mmol, 1.5 equiv) and NaH 60% dispersion in mineral oil (51 mg, 1.28 mmol, 1.65 equiv). After standard workup, **89** was obtained as a yellowish oil (219 mg, yield 90%).
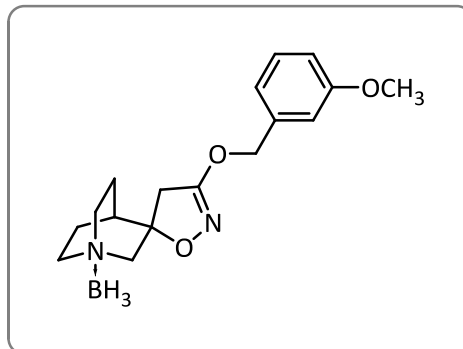
89: yellowish oil; TLC (Dragendorff): R_f = 0.38 (cyclohexane/EtOAc 2:3).

^{1}H NMR (CDCl$_3$, 300 MHz) δ 7.24 (td, J = 7.4, 1.4 Hz, 1H), 6.89 (d, J = 7.6 Hz, 1H), 6.87 − 6.81 (m, 2H), 5.04 (s, 2H), 3.76 (s, 3H), 3.33 (dd, J = 14.6, 2.2 Hz, 1H), 3.11 − 2.89 (m, 6H), 2.84 (d, J = 16.5 Hz, 1H), 2.39 − 2.24 (m, 1H), 2.24 − 2.17 (m, 1H), 1.96 − 1.80 (m, 1H), 1.73 − 1.54 (m, 2H).

^{13}C NMR (CDCl$_3$, 75 MHz) δ 165.7, 159.6, 136.5, 129.6, 120.3, 114.1, 113.6, 84.1, 71.7, 65.3, 55.1, 52.0, 51.3, 42.0, 30.0, 21.6, 19.6.

(±)-3-(3-methoxybenzyl)oxy-1-oxa-2,7-diaza-7,10-ethanospiro[4.5]dec-2-ene (97)

The title compound was obtained according to the general procedure **5.3.2.3** by reacting **89** (170 mg, 0.54 mmol, 1 equiv) and CF$_3$COOH (208 μL, 2.7 mmol, 5 equiv) in acetone. After disappearance of the starting material (TLC in cyclohexane/EtOAc 2:3 and CH$_2$Cl$_2$/MeOH 9:1), standard workup was applied to provide the desired compound (124 mg, yield 76%).
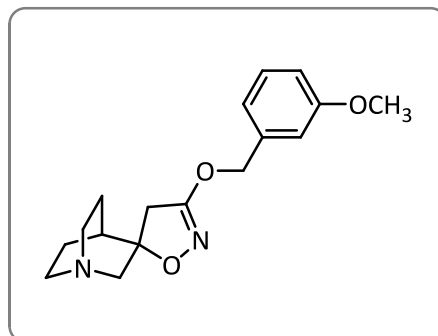
97: yellow oil; TLC (Dragendorff): R_f = 0.24 (CH$_2$Cl$_2$/MeOH 4:1).

^{1}H NMR (CDCl$_3$, 300 MHz) δ 7.27 − 7.19 (m, 1H), 6.93 − 6.87 (m, 1H), 6.87 − 6.84 (m, 1H), 6.82 (ddd, J = 8.2, 2.6, 0.8 Hz, 1H), 5.03 (s, 2H), 3.75 (s, 3H), 3.20 (dd, J = 14.6, 2.0 Hz, 1H), 3.03 (d, J = 16.4 Hz, 1H), 2.89 (dd, J = 14.7, 2.1 Hz, 1H), 2.85 − 2.56 (m, 5H), 2.15 − 2.01 (m, 1H), 2.01 − 1.94 (m, 1H), 1.67 − 1.52 (m, 1H), 1.53 − 1.28 (m, 2H).

^{13}C NMR (CDCl$_3$, 75 MHz) δ 165.9, 159.7, 137.0, 129.6, 120.4, 114.0, 113.6, 86.5, 71.3, 62.7, 55.2, 46.7, 46.4, 42.4, 30.6, 23.5, 20.9.

(±)-3-(3-methoxybenzyl)oxy-1-oxa-2,7-diaza-7,10-ethanospiro[4.5]dec-2-ene fumarate (9g)

The fumarate derivative **9g** was prepared according to the general procedure **5.3.2.4** by reacting **97** (105 mg, 0.35 mmol, 1 equiv) and fumaric acid (40 mg, 0.35 mmol, 1 equiv) in MeOH. The crude salt was recrystallized from *i*-PrOH to provide the pure compound as a white solid (134 mg, yield 98%).

x 0.75 fumarate
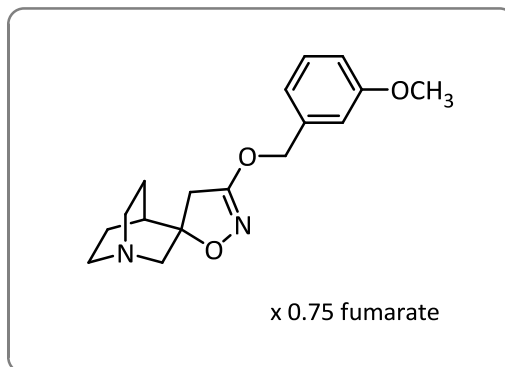
9g: white solid; TLC (alumina-Dragendorff): R_f = 0.32 (CH_2Cl_2/MeOH 98:2); m.p.: 136.4-137.2°C; MS (ESI) m/z for $C_{17}H_{23}N_2O_3^+$ [M+H]$^+$ calcd. 303.2, found 302.8.

^{1}H NMR (CD$_3$OD, 300 MHz) δ 7.28 (t, *J* = 8.1 Hz, 1H), 6.99 – 6.93 (m, 2H), 6.93 – 6.87 (m, 1H), 6.69 (s, 1.5H), 5.10 (s, 2H), 3.79 (s, 3H), 3.53 (s, 2H), 3.43 – 3.16 (m, 5H), 3.12 (d, *J* = 16.8 Hz, 1H), 2.41 – 2.26 (m, 2H), 2.09 – 1.96 (m, 1H), 1.95 – 1.76 (m, 2H).

^{13}C NMR (CD$_3$OD, 75 MHz) δ 171.0, 167.9, 161.3, 138.4, 136.1, 130.7, 121.4, 115.0, 114.8, 84.9, 72.8, 60.4, 55.7, 47.4, 46.8, 42.5, 30.8, 21.0, 19.2.

(±)-3-(3-methoxybenzyl)oxy-1-oxa-2,7-diaza-7,10-ethanospiro[4.5]dec-2-ene methyl iodide (10g)

According to the general procedure **5.3.2.5**, CH$_3$I (90 μL, 1.44 mmol, 8 equiv) was added to a solution of the free base **97** (54 mg, 0.18 mmol, 1 equiv) in MeOH. Evaporation of the solvent under reduced pressure afforded the crude quaternary salt quantitatively and then it was treated with *n*-hexanes to give the pure final compound as a white solid (72 mg, yield 90%).
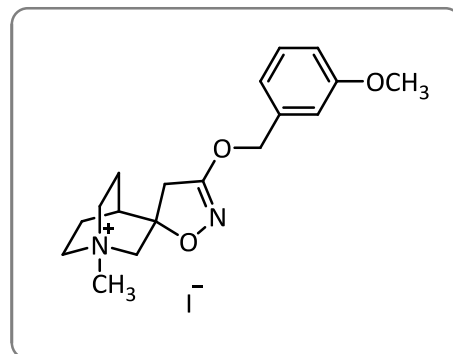
10g: white solid; TLC (alumina-Dragendorff): R_f = 0.30 (CH_2Cl_2/MeOH 9:1); m.p.: 59.3-61.8°C; MS (ESI) m/z for $C_{18}H_{25}N_2O_3^+$ [M]$^+$ calcd. 317.2, found 317.1.

^{1}H NMR (CD$_3$OD, 300 MHz) δ 7.33 – 7.25 (m, 1H), 7.00 – 6.94 (m, 2H), 6.91 (dd, *J* = 8.2, 2.4 Hz, 1H), 5.11 (s, 2H), 3.85 (dd, *J* = 13.8, 2.6 Hz, 1H), 3.80 (s, *J* = 1.7 Hz, 3H), 3.72 (dd, *J* = 13.7, 2.7 Hz, 1H), 3.61 – 3.39 (m, 4H), 3.35 (d, *J* = 16.9 Hz, 1H), 3.19 (d, *J* = 16.9 Hz, 1H), 3.06 (s, 3H), 2.48 – 2.32 (m, 2H), 2.21 – 2.07 (m, 1H), 2.07 – 1.90 (m, 2H).

^{13}C NMR (CD$_3$OD, 75 MHz) δ 167.8, 161.3, 138.3, 130.7, 121.4, 115.0, 114.8, 85.2, 72.9, 69.6, 57.8, 57.2, 55.8, 52.3, 42.5, 30.6, 22.1, 20.4.

(±)-3-(naphthalen-2-ylmethoxy)-1-oxa-2,7-diaza-7-boranyl-7,10-ethanospiro[4.5]dec-2-ene (90)

The title compound was prepared according to the general procedure described in **5.3.2.2**, by reacting **83** (300 mg, 1.16 mmol, 1 equiv), 2-naphthylmethanol (275 mg, 1.74 mmol, 1.5 equiv) and NaH 60% dispersion in mineral oil (76 mg, 1.91 mmol, 1.65 equiv). After standard workup, **90** was obtained as a pale yellow oil (305 mg, yield 78%).
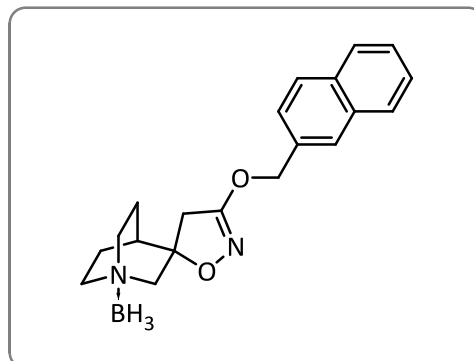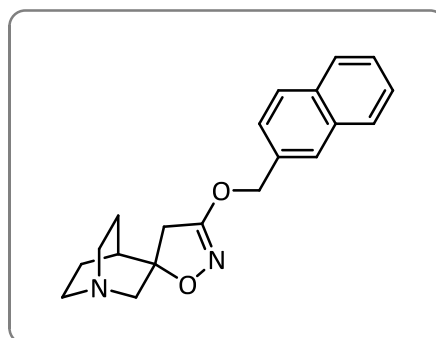
90: pale yellow oil; TLC (Dragendorff): R_f = 0.47 (cyclohexane/EtOAc 2:3).

^{1}H NMR (CDCl$_3$, 300 MHz) δ 7.82 – 7.71 (m, 4H), 7.46 – 7.36 (m, 3H), 5.20 (s, 2H), 3.25 (dd, J = 14.5, 1.8 Hz, 1H), 3.04 – 2.74 (m, 7H), 2.29 – 2.16 (m, 1H), 2.16 – 2.09 (m, 1H), 1.84 – 1.69 (m, 1H), 1.63 – 1.46 (m, 2H).

^{13}C NMR (CDCl$_3$, 75 MHz) δ 165.7, 133.3, 133.1, 132.5, 128.5, 128.1, 127.7, 127.7, 126.6, 126.5, 125.8, 84.2, 72.1, 65.4, 52.1, 51.4, 42.3, 30.1, 21.8, 19.7.

(±)-3-(naphthalen-2-ylmethoxy)-1-oxa-2,7-diaza-7,10-ethanospiro[4.5]dec-2-ene (98)

The title compound was obtained according to the general procedure **5.3.2.3** by reacting **90** (305 mg, 0.90 mmol, 1 equiv) and CF$_3$COOH (347 μL, 4.50 mmol, 5 equiv) in acetone. After disappearance of the starting material (TLC in cyclohexane/EtOAc 2:3 and CH$_2$Cl$_2$/MeOH 9:1), standard workup was applied to provide the desired compound (164 mg, yield 57%).

98: yellow oil; TLC (Dragendorff): R_f = 0.46 (CH$_2$Cl$_2$/MeOH 4:1).

^{1}H NMR (CDCl$_3$, 300 MHz) δ 7.83 – 7.68 (m, 4H), 7.46 – 7.34 (m, 3H), 5.20 (s, 2H), 3.25 – 3.12 (m, 1H), 3.01 (d, J = 16.4 Hz, 1H), 2.93 – 2.54 (m, 6H), 2.14 – 2.00 (m, 1H), 2.00 – 1.91 (m, 1H), 1.63 – 1.49 (m, 1H), 1.49 – 1.25 (m, 2H).

^{13}C NMR (CDCl$_3$, 75 MHz) δ 166.0, 133.3, 133.2, 133.0, 128.4, 128.1, 127.7, 127.6, 126.4, 126.4, 125.9, 86.5, 71.7, 62.8, 46.8, 46.5, 42.5, 30.7, 23.6, 20.9.

(±)-3-(naphthalen-2-ylmethoxy)-1-oxa-2,7-diaza-7,10-ethanospiro[4.5]dec-2-ene fumarate (9h)

The fumarate derivative **9h** was prepared according to the general procedure **5.3.2.4** by reacting **98** (164 mg, 0.51 mmol, 1 equiv) and fumaric acid (59 mg, 0.51 mmol, 1 equiv) in MeOH. The crude salt was recrystallized from EtOH to provide the pure compound as a white solid (173 mg, yield 83%).

x 0.75 fumarate
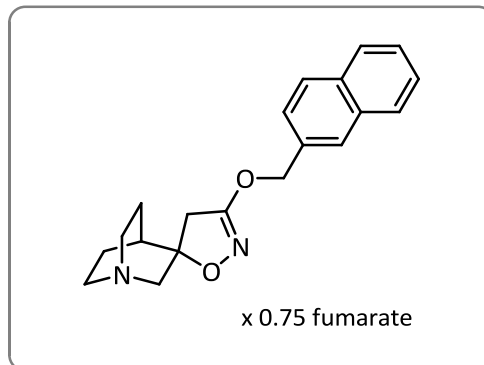
9h: white solids; TLC (alumina-Dragendorff): R_f = 0.40 (CH$_2$Cl$_2$/MeOH 98:2); m.p.: 192.6-193.4°C; MS (ESI) m/z for C$_{20}$H$_{23}$N$_2$O$_2^+$ [M+H]$^+$ calcd. 323.2, found 322.8.

^{1}H NMR (CD$_3$OD, 300 MHz) δ 7.92 – 7.81 (m, 4H), 7.55 – 7.45 (m, 3H), 6.69 (s, 1.5H), 5.30 (s, 2H), 3.57 (s, 2H), 3.40 – 3.20 (m, 5H), 3.16 (d, J = 16.9 Hz, 1H), 2.42 – 2.25 (m, 2H), 2.12 – 1.96 (m, 1H), 1.96 – 1.76 (m, 2H).

^{13}C NMR (CD$_3$OD, 75 MHz) δ 171.3, 168.0, 136.2, 134.8, 134.6, 134.3, 129.4, 129.0, 128.7, 128.5, 127.5, 127.4, 126.8, 84.9, 73.1, 60.2, 47.3, 46.7, 42.5, 30.8, 20.9, 19.1.

(±)-3-(naphthalen-2-ylmethoxy)-1-oxa-2,7-diaza-7,10-ethanospiro[4.5]dec-2-ene methyl iodide (10h)

According to the general procedure **5.3.2.5**, CH$_3$I (125 µL, 2.00 mmol, 8 equiv) was added to a solution of the free base **98** (81 mg, 0.25 mmol, 1 equiv) in MeOH. Evaporation of the solvent under reduced pressure afforded the crude quaternary salt quantitatively and then it was re-crystalized from acetone to give the pure final compound as a white solid (73 mg, yield 63%).
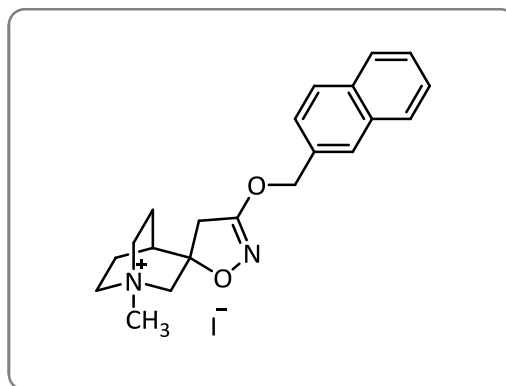
10h: white solid; TLC (alumina-Dragendorff): R_f = 0.30 (CH$_2$Cl$_2$/MeOH 9:1); m.p.: 154.8-156.2°C; MS (ESI) m/z for C$_{21}$H$_{25}$N$_2$O$_2^+$ [M]$^+$ calcd. 337.2, found 337.1.

^{1}H NMR (CD$_3$OD, 300 MHz) δ 7.93 – 7.83 (m, 4H), 7.56 – 7.47 (m, 3H), 5.31 (s, 2H), 3.81 (dd, J = 14.0, 2.5 Hz, 1H), 3.73 (dd, J = 13.8, 2.5 Hz, 1H), 3.60 – 3.33 (m, 5H), 3.20 (d, J = 16.9 Hz, 1H), 3.04 (s, 3H), 2.51 – 2.33 (m, 2H), 2.21 – 2.06 (m, 1H), 2.06 – 1.88 (m, 2H).

^{13}C NMR (CD$_3$OD, 75 MHz) δ 167.9, 134.8, 134.6, 134.3, 129.4, 129.0, 128.7, 128.6, 127.5, 127.4, 126.8, 85.2, 73.2, 69.6, 57.8, 57.2, 52.3, 42.5, 30.6, 22.1, 20.4.

5.3.3 Experimental procedures for the synthesis of 11a-m, 12a-g

5.3.3.1 Synthesis of borane methyl 2-(quinuclidin-3-yl)acetate complex (101)

2-(1-azabicyclo[2.2.2]oct-3-ylidene)-acetic acid methyl ester (99)

In a flame dried flask and under argon atmosphere, trimethyl phosphonoacetate (7.74 mL, 48.0 mmol, 1.2 equiv) was dissolved in dry THF (750 mL). The solution was cooled to 0°C with an ice bath and sodium hydride 60% dispersion in mineral oil (1.92 g, 48.0 mmol, 1.2 equiv) was added portionwise. After one hour of stirring at room temperature, quinuclidin-3-one (5.00 g, 40.0 mmol, 1 equiv) was added and the resulting mixture was stirred overnight at RT. The reaction was monitored by TLC (CH$_2$Cl$_2$/MeOH 9:1). Upon complete consumption of the starting material, the reaction was quenched with deionized water at 0°C (100 mL) and then the mixture was extracted once with CH$_2$Cl$_2$. The organic layer underwent an acid-base extraction: 1N HCl was added (pH = 1) and the organic phase removed, the acid water phase was then basified with K$_2$CO$_3$ (pH = 9) and extracted with CH$_2$Cl$_2$. The combined organic layers dried over anhydrous Na$_2$SO$_4$, filtered and concentrated under reduced pressure. Methyl 2-(quinuclidin-3-ylidene)acetate (**99***)* was obtained as a mixture of 1:1 (*E)* and (*Z)* stereoisomers (see ^{1}H and ^{13}C NMR) and was directly reduced in the next reaction step (7.25 g, yield 100%).
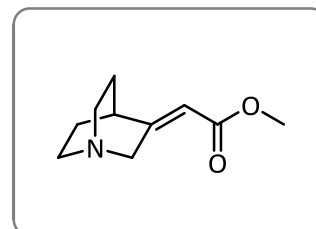
99: yellow and very viscous oil; TLC (Dragendorff): R$_f$ = 0.40 (CH$_2$Cl$_2$/MeOH 9:1).
^{1}H NMR (CDCl$_3$, 300 MHz) δ 5.63 (t, *J* = 2.5 Hz, 1H), 5.61 (t, *J* = 1.7 Hz, 1H), 3.94 – 3.85 (m, 3H), 3.66 (s, 3H), 3.65 (s, 3H), 3.47 – 3.41 (m, 2H), 2.98 – 2.71 (m, 8H), 2.48 – 2.38 (m, 1H), 1.84 – 1.56 (m, 8H).
^{13}C NMR (CDCl$_3$, 75 MHz) δ 169.6, 167.9, 166.8, 166.3, 110.6, 110.3, 56.4, 50.7, 47.4, 47.2, 33.5, 27.1, 26.1, 26.1.
Note: in the ^{13}C NMR, the following pairs of peaks belong to the same carbon in the two different stereoisomers (*E* and *Z*): 169.6 and 167.9, 166.8 and 166.3, 110.6 and 110.3.

1-azabicyclo[2.2.2]octane-3-acetic acid methyl ester (100)

Methyl 2-(quinuclidin-3-ylidene)acetate (**99**) (7.25 g, 40.0 mmol, 1 equiv) was dissolved in MeOH (500 mL) and Pd/C (725 mg, 10% w/w). The mixture was fluxed with argon and then it was stirred under H_2 at room temperature for 1h. The reaction solution was filtered through a Celite pad, washed with MeOH, and the filtrate was concentrated under reduced pressure. The residue was dissolve in CH_2Cl_2, 1N HCl was added and the mixture was extracted three times with CH_2Cl_2. The aqueous phase was basified with sodium carbonate (pH = 9) and extracted three times with CH_2Cl_2. The combined organic phase were dried over anhydrous Na_2SO_4, filtered and the solvent evaporated *in vacuum* to provide the desired compound methyl 2-(quinuclidin-3-yl)acetate (**100**) in quantitative yield (7.33 g, yield 100%).
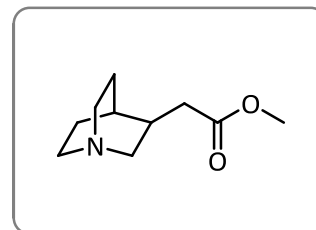
100: colorless oil; TLC (Dragendorff): R_f = 0.19 (CH_2Cl_2/MeOH 7:3).
^{1}H NMR (CDCl$_3$, 300 MHz) δ 3.67 (s, 3H), 3.22 – 3.07 (m, 1H), 2.91 – 2.71 (m, 4H), 2.43 – 2.29 (m, 3H), 2.19 – 2.02 (m, 1H), 1.72 – 1.52 (m, 4H), 1.48 – 1.32 (m, 1H).
^{13}C NMR (CDCl$_3$, 75 MHz) δ 173.3, 54.7, 51.6, 47.7, 46.8, 38.5, 32.6, 28.1, 25.2, 21.2.

Borane 1-azabicyclo[2.2.2]octane-3-acetic acid methyl ester complex (101)

Under argon atmosphere, methyl 2-(quinuclidin-3-yl)acetate **(100)** (7.33 g, 40.0 mmol, 1 equiv) was dissolved in dry THF (500 mL) and cooled to 0°C. A solution of 1.0 M BH_3 in THF (40 mL, 40.0 mmol, 1 equiv) was then slowly added dropwise. The resulting mixture was stirred at RT for 1.5h and monitored by TLC in CH_2Cl_2/MeOH 7:3 or cyclohexane/EtOAc 4:1. Upon completion, the reaction was quenched with saturated aqueous solution of $NaHCO_3$ and then extracted three times with CH_2Cl_2. The collected organic phases were dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure affording the pure *N*-boranyl derivative as a white solid (7.65 g, yield 97%).
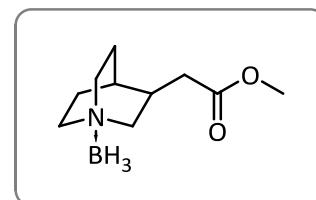
101: white solid; TLC (Dragendorff): R_f = 0.30 (cyclohexane/EtOAc 4:1); m.p.: 86.8-87.4°C.
^{1}H NMR (CDCl$_3$, 300 MHz) δ 3.62 (s, 3H), 3.31 – 3.14 (m, 1H), 3.03 – 2.82 (m, 4H), 2.54 – 2.41 (m, 1H), 2.41 – 2.22 (m, 3H), 1.85 – 1.67 (m, 4H), 1.67 – 1.47 (m, 1H).
^{13}C NMR (CDCl$_3$, 75 MHz) δ 172.1, 59.7, 53.5, 52.9, 51.8, 37.8, 31.5, 26.3, 24.8, 20.1.

5.3.3.2 General procedure for the synthesis of amidoximes (102-114)

In a sealed vial, to a stirred solution of the corresponding carbonitrile (1 equiv) in EtOH, aqueous hydroxylamine 50% w/w (4 equiv) was added and the resulting mixture was heated at 90°C till complete consumption of the starting material, which usually occurred within 1h. Once cooled to RT, the solvent was evaporated under reduced pressure, providing, without further purification, the pure amidoxime quantitatively.

5.3.3.3 General procedure for the synthesis of 115-126 and 135

The title compound was obtained by reacting methyl ester **100** or **101** and the appropriate amidoxime **102-114**.

5.3.3.3.1: in anhydrous conditions and under inert (argon) atmosphere, the appropriate amidoxime (3 equiv) was suspended in dry THF and molecular sieves were added. After stirring at RT for 30 min, NaH 60% dispersion in mineral oil (3 equiv) was added portionwise at RT and the resulting mixture was heated at 50°C for 20 min. After cooling it down at room temperature, a solution of methyl ester **100** or **101** (1 equiv) in dry THF was added dropwise. The final suspension was stirred for 2h at 80°C and monitored by TLC in CH_2Cl_2/MeOH 7:3. Upon complete consumption of the starting material, standard workup was applied.

5.3.3.3.2: the appropriate amidoxime (3 equiv) was suspended in THF, Cs_2CO_3 (3 equiv) was added and the mixture was heated at 50°C for 20 min. After cooling it down at room temperature, a solution of methyl ester **100** or **101** (1 equiv) in dry THF was added dropwise. The final suspension was stirred for 12h at 80°C and monitored by TLC in CH_2Cl_2/MeOH 7:3. Upon complete consumption of the starting material, standard workup was applied.

Standard workup for 5.3.3.3: upon complete consumption of the starting material, the reaction was quenched with saturated aqueous solution of $NaHCO_3$ and diluted with CH_2Cl_2.Depending on the starting material used, the mixture underwent different treatment. **101**: in a separatory funnel, the mixture was acidified with 1N HCl (pH = 1) and the organic phase removed. The organic phase was then extracted two more times with 1N HCl. The aqueous layers were combined, basified with sodium carbonate and extracted three times with CH_2Cl_2. **102**: the mixture was extracted three times with CH_2Cl_2. In both cases, the combined organic phases were dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residue obtained was then purified by a silica gel column chromatography eluting in a proper solvent mixture to afford the pure desired compound.

5.3.3.4 General procedure for the cleavage of BH₃ group (127-134 and 136-139)

Under argon atmosphere, the 1,2,4-oxadiazole derivative **115-126** (1 equiv) was dissolved in acetone acetone (3.5 mL/mmol **115-126**) and cooled to 0°C (ice bath). A solution of CF_3COOH (5 equiv) in acetone (3.5 mL/mmol **115-126**) was then added dropwise and the resulting mixture was stirred at room temperature overnight. The reaction was monitored by silica gel TLC (cyclohexane/EtOAc 2:3 or CH_2Cl_2/MeOH 95:5). Once completed, the solvent was removed under reduced pressure and the residue was diluted with deionized water. The aqueous solution (pH = 1-2) was extracted with Et_2O (x 1). The residual aqueous phase was basified with sodium carbonate and extracted with CH_2Cl_2 (x 3). The combined organic phases were dried over anhydrous Na_2SO_4, filtered and evaporated *in vacuum* to afford the pure desired compound, which was directly used in the next step without further purification. When, in the protocol **5.3.3.3**, **100** instead of **101** was used, procedure **5.3.3.4** was not necessary since the derivative was already achieved as a free base.

5.3.3.5 General procedure for salification (11a-m)

The appropriate derivative **127-139** (1 equiv) was dissolved in MeOH (5 mL/mmol **127-139**) and a solution of fumaric acid (1 equiv) in MeOH (2 mL/mmol **127-139**) was added. The mixture was stirred at RT for 2h (TLC in CH_2Cl_2/MeOH). Additional amounts of fumaric acid (0.5 equiv) were then added when no improvement was observed in order to achieve complete consumption of the starting material. Once completed, the solvent was removed under reduced pressure affording the crude salt quantitatively, followed by re-crystallization from a proper solvent to give the desired final compound **11a-m**.

5.3.3.6 General procedure for methylation (12a-g)

The appropriate derivative **127-139** (1 equiv) was dissolved in MeOH (5 mL/mmol **127-139**) and methyl iodide (8.0 equiv) was added. The mixture was stirred overnight at RT and monitored by silica gel TLC (CH_2Cl_2/MeOH 4:1). Upon complete consumption of the starting material, the solvent and the excess of methyl iodide were removed by evaporation under reduced pressure, thus providing the crude *N*-methylated analogues **12a-m** quantitatively. The pure compounds were then re-crystalized from a proper solvent.

N'-hydroxy-3-(trifluoromethyl)benzimidamide (102)

According to the general procedure **5.3.3.2**, hydroxylamine (50% w/w in water, 430 µL, 7.01 mmol, 4 equiv) was added to a stirred solution of 3-(trifluoromethyl)benzonitrile (234 µL, 1.75 mmol, 1 equiv) and ethanol (6 mL) at RT. The resulting mixture was heated at 90°C for 1.5h. After concentration under reduced pressure, the desired *N'*-hydroxy-3-(trifluoromethyl)-benzimidamide (**102**) was obtained as a pure pale yellow oil (350 mg, yield 98%).
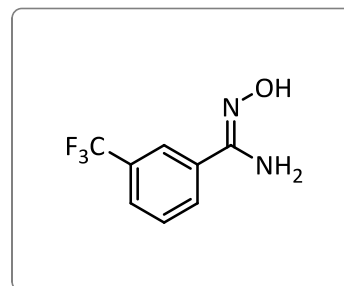
102: pale yellow oil; TLC (PMA): R_f = 0.45 (cyclohexane/EtOAc 1:1).
^{1}H NMR (CDCl$_3$, 300 MHz) δ 8.77 (br s, 1H), 7.87 − 7.80 (m, 1H), 7.78 − 7.70 (m, 1H), 7.65 − 7.57 (m, 1H), 7.50 − 7.41 (m, 1H), 4.87 (br s, 2H).
^{13}C NMR (CDCl$_3$, 75 MHz) δ 151.8, 133.4, 131.3 (d, *J* = 32.6 Hz), 129.4, 129.3, 126.8 (q, *J* = 3.7 Hz), 124.0 (q, *J* = 272.3 Hz), 123.1 (q, *J* = 3.9 Hz).

Borane 5-(4-quinuclidin-3-ylmethyl)-3-(3-(trifluoromethyl)phenyl)-1,2,4-oxadiazole complex (115)

The title compound was prepared according to general procedure **5.3.3.3.1** by reacting methyl ester **101** (421 mg, 2.14 mmol, 1 equiv), *N'*-hydroxy-3-(trifluoromethyl)-benzimidamide (**102**) (1.31 g, 6.41 mmol, 3 equiv) and NaH 60% dispersion in mineral oil (256 mg, 6.41 mmol, 3 equiv) in dry THF (10

mL). After standard workup and silica gel column chromatography (cyclohexane/EtOAc 3:2 to 1:1), the desired compound **115** was obtained as a pure pale yellow oil (355 mg, yield 47%).
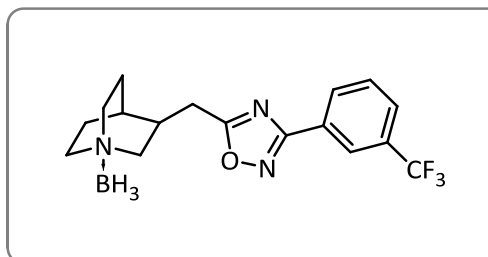
115: pale yellow oil; TLC (Dragendorff): R_f = 0.63 (cyclohexane/EtOAc 1:1).
^{1}H NMR (CDCl$_3$, 300 MHz) δ 8.29 − 8.22 (m, 1H), 8.21 − 8.14 (m, 1H), 7.69 (dd, *J* = 7.9, 0.5 Hz, 1H), 7.55 (t, *J* = 7.8 Hz, 1H), 3.37 − 3.23 (m, 1H), 3.07 − 2.86 (m, 6H), 2.61 (ddd, *J* = 13.5, 6.9, 1.9 Hz, 1H), 2.57 − 2.43 (m, 1H), 1.97 − 1.85 (m, 2H), 1.85 − 1.73 (m, 2H), 1.73 − 1.58 (m, 1H).
^{13}C NMR (CDCl$_3$, 75 MHz) δ 178.1, 167.5, 131.6 (q, *J* = 32.9 Hz), 130.7, 129.6, 128.0 (q, *J* = 3.8 Hz), 127.6, 124.5 (q, *J* = 3.9 Hz), 123.8 (q, *J* = 272.6 Hz), 59.5, 53.6, 53.0, 33.2, 30.2, 26.3, 24.7, 20.1.

5-(4-quinuclidin-3-ylmethyl)-3-(3(trifluoromethyl)phenyl)-1,2,4-oxadiazole (127)

The title compound was obtained according to the general procedure **5.3.3.4** by reacting **115** (355 mg, 1.01 mmol, 1 equiv) and CF$_3$COOH (389 µL, 5.05 mmol, 5 equiv) in acetone. After disappearance of the starting material (TLC in cyclohexane/EtOAc 1:1), standard workup was applied to provide the desired compound **127** (340 mg, yield 100%).
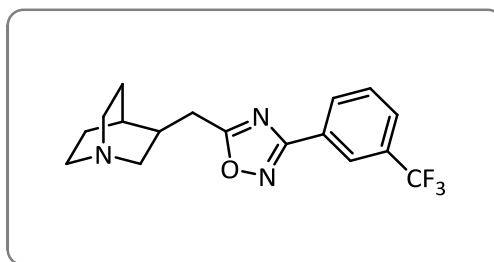
127: yellow oil; TLC (alumina-Dragendorff): R$_f$ = 0.43 (CH$_2$Cl$_2$/MeOH 9:1).
^{1}H NMR (CDCl$_3$, 300 MHz) δ 8.30 – 8.22 (m, 1H), 8.17 (d, *J* = 7.8 Hz, 1H), 7.66 (d, *J* = 7.9 Hz, 1H), 7.51 (t, *J* = 7.8 Hz, 1H), 3.13 (dd, *J* = 13.5, 9.9 Hz, 1H), 2.94 (d, *J* = 7.8 Hz, 2H), 2.87 – 2.66 (m, 4H), 2.43 (ddd, *J* = 13.7, 6.2, 1.7 Hz, 1H), 2.31 – 2.14 (m, 1H), 1.78 – 1.48 (m, 4H), 1.48 – 1.30 (m, 1H).
^{13}C NMR (CDCl$_3$, 75 MHz) δ 179.4, 167.3, 131.5 (q, *J* = 32.9 Hz), 130.6, 129.5, 127.8, 127.7 (q, *J* = 3.7 Hz), 124.4 (q, *J* = 3.8 Hz), 123.8 (q, *J* = 272.4 Hz), 54.5, 47.6, 46.8, 34.3, 30.6, 27.9, 25.0, 20.9.

5-(4-quinuclidin-3-ylmethyl)-3-(3(trifluoromethyl)phenyl)-1,2,4-oxadiazole fumarate (11a)

The fumarate derivative **11a** was prepared according to the general procedure **5.3.3.4** by reacting **127** (180 mg, 0.53 mmol, 1 equiv) and fumaric acid (62 mg, 0.53 mmol, 1 equiv) in MeOH. After 2h, additional fumaric acid (31 mg, 0.27 mmol, 0.5 equiv) was added to achieve complete consumption of the starting material. The crude salt was recrystallized from EtOAc and *n*-hexane to provide the pure compound as a white solid (111 mg, yield 41%).

x 1.50 fumarate
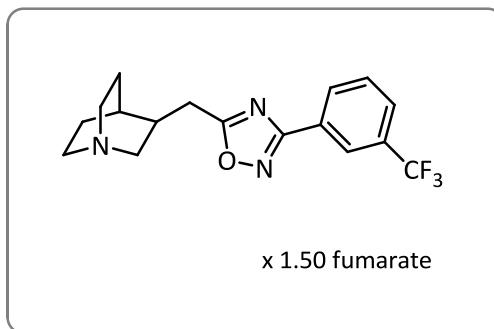
11a: white solid; TLC (alumina-Dragendorff): R$_f$ = 0.38 (CH$_2$Cl$_2$/MeOH 96:4); m.p.: 128.8-130.4°; MS (ESI) m/z for C$_{17}$H$_{19}$F$_3$N$_3$O$^+$ [M+H]$^+$ calcd. 338.2, found 338.1.
^{1}H NMR (CD$_3$OD, 300 MHz) δ 8.30 – 8.19 (m, 2H), 7.84 – 7.74 (m, 1H), 7.73 – 7.61 (m, 1H), 6.63 (s, 3H), 3.75 – 3.58 (m, 1H), 3.40 – 3.11 (m, 6H), 3.11 – 2.98 (m, 1H), 2.85 – 2.66 (m, 1H), 2.07 (s, 2H), 1.98 (s, 2H), 1.87 (s, 1H).
^{13}C NMR (CD$_3$OD, 75 MHz) δ 180.2, 170.0, 168.5, 135.8, 132.5 (q, *J* = 32.8 Hz), 131.8, 131.2, 129.1, 129.0 (q, *J* = 3.6 Hz), 125.3 (q, *J* = 271.6 Hz), 124.9 (q, *J* = 3.9 Hz), 53.0, 47.6, 47.1, 32.6, 30.1, 25.3, 24.9, 19.0.

5-(4-quinuclidin-3-ylmethyl)-3-(3(trifluoromethyl)phenyl)-1,2,4-oxadiazole methyl iodide (12a)

According to the general procedure **5.3.3.6**, CH$_3$I (224 μL, 3.60 mmol, 8 equiv) was added to a solution of the free base **127** (150 mg, 0.45 mmol, 1 equiv) in MeOH. Evaporation of the solvent under reduced pressure afforded the crude quaternary salt quantitatively and then it was re-crystalized from MeOH and Et$_2$O to give the pure final compound as a white solid (147 mg, yield 68%).
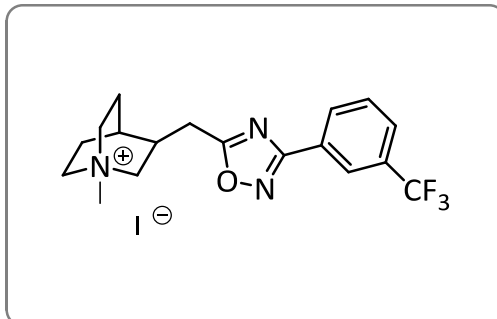
12a: white solid; TLC (alumina-Dragendorff): R$_f$ = 0.30 (CH$_2$Cl$_2$/MeOH 9:1); m.p.: 170.3-172.0°C (dec.); MS (ESI) m/z for C$_{18}$H$_{21}$F$_3$N$_3$O$^+$ [M]$^+$ calcd. 352.2, found 352.4.

^{1}H NMR (CD$_3$OD, 300 MHz) δ 8.37 − 8.29 (m, 2H), 7.90 − 7.83 (m, 1H), 7.79 − 7.70 (m, 1H), 3.96 − 3.81 (m, 1H), 3.61 − 3.47 (m, 4H), 3.43 − 3.21 (m, 3H), 3.03 (s, 3H), 3.00 − 2.85 (m, 1H), 2.37 − 2.19 (m, 2H), 2.19 − 2.08 (m, 2H), 2.08 − 1.93 (m, 1H).

^{13}C NMR (CD$_3$OD, 75 MHz) δ 179.97, 168.50, 132.48 (q, J = 32.7 Hz), 131.88, 131.23, 129.10, 129.01 (q, J = 3.8 Hz), 125.25 (q, J = 271.5 Hz), 124.92 (q, J = 3.9 Hz), 63.01, 58.03, 57.58, 52.65, 33.62, 30.18, 25.91, 25.22, 20.26.

3-fluoro-*N*'-hydroxybenzimidamide (103)

According to the general procedure **5.3.3.2**, hydroxylamine (50% w/w in water, 2.29 mL, 37.42 mmol, 4 equiv) was added to a stirred solution of 3-fluorobenzonitrile (1.0 mL, 9.35 mmol, 1 equiv) and ethanol (8 mL) at RT. The resulting mixture was heated at 90°C for 1h. After concentration under reduced pressure, the desired 3-fluoro-*N*'-hydroxybenzimidamide **(103)** was obtained as a pure colorless oil (1.44 g, yield 100%).
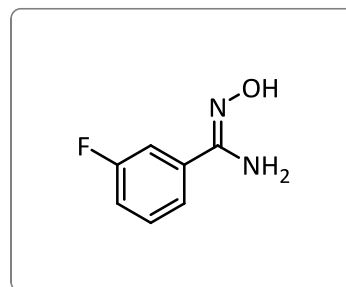
103: colorless oil; TLC (PMA): R$_f$ = 0.51 (cyclohexane/EtOAc 1:1).
^{1}H NMR (CDCl$_3$, 300 MHz) δ 7.64 (br s, 1H), 7.43 – 7.28 (m, 3H), 7.18 – 6.99 (m, 1H), 4.96 (br s, 2H).
^{13}C NMR (CDCl$_3$, 75 MHz) δ 162.8 (d, *J* = 246.4 Hz), 151.9, 134.7 (d, *J* = 8.1 Hz), 130.4 (d, *J* = 8.2 Hz), 121.7 (d, *J* = 3.0 Hz), 117.0 (d, *J* = 21.2 Hz), 113.2 (d, *J* = 23.4 Hz).

Borane 3-(3-fluorophenyl)-5-(4-quinuclidin-3-ylmethyl)-1,2,4-oxadiazole complex (116)

The title compound was prepared according to general procedure **5.3.3.3.2** by reacting methyl ester **101** (500 mg, 2.54 mmol, 1 equiv), 3-fluoro-*N*'-hydroxy-benzimidamide **(103)** (1.17 g, 7.61 mmol, 3 equiv) and Cs$_2$CO$_3$ (2.48 g, 7.61 mmol, 3 equiv) in THF (15 mL). After standard workup and silica gel column chromatography (cyclohexane/EtOAc 7:3), the desired compound **116** was obtained as a pure pale yellow oil (589 mg, yield 77%).
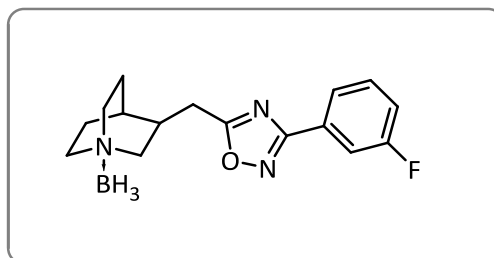
116: pale yellow oil; TLC (Dragendorff): R$_f$ = 0.43 (cyclohexane/EtOAc 7:3).
^{1}H NMR (CDCl$_3$, 300 MHz) δ 7.82 – 7.75 (m, 1H), 7.69 (ddd, *J* = 9.4, 2.6, 1.5 Hz, 1H), 7.39 (td, *J* = 8.0, 5.7 Hz, 1H), 7.14 (tdd, *J* = 8.4, 2.6, 1.0 Hz, 1H), 3.39 – 3.24 (m, 1H), 3.09 – 2.87 (m, 6H), 2.62 (ddd, *J* = 13.6, 6.9, 2.0 Hz, 1H), 2.57 – 2.43 (m, 1H), 1.97 – 1.85 (m, 2H), 1.85 – 1.74 (m, 2H), 1.74 – 1.59 (m, 1H).
^{13}C NMR (CDCl$_3$, 75 MHz) δ 177.8, 167.6, 162.9 (d, *J* = 246.7 Hz), 130.7 (d, *J* = 8.1 Hz), 128.6 (d, *J* = 8.6 Hz), 123.2 (d, *J* = 3.2 Hz), 118.4 (d, *J* = 21.2 Hz), 114.5 (d, *J* = 23.7 Hz), 59.4, 53.6, 52.9, 33.1, 30.1, 26.2, 24.6, 20.0.

3-(3-fluorophenyl)-5-(4-quinuclidin-3-ylmethyl)-1,2,4-oxadiazole (128)

The title compound was obtained according to the general procedure **5.3.3.4** by reacting **116** (589 mg, 1.96 mmol, 1 equiv) and CF$_3$COOH (753 µL, 9.78 mmol, 5 equiv) in acetone. After disappearance of the starting material (TLC in cyclohexane/EtOAc 1:1), standard workup was applied to provide the desired compound **128** (529 mg, yield 94%).
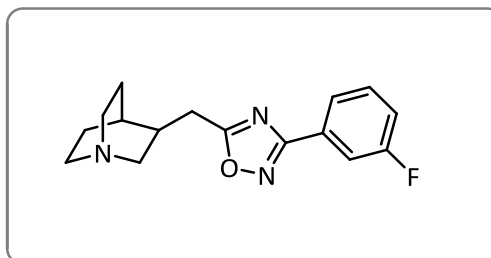
128: yellow oil; TLC (alumina-Dragendorff): R$_f$ = 0.48 (CH$_2$Cl$_2$/MeOH 95:5).
^{1}H NMR (CDCl$_3$, 300 MHz) δ 7.79 – 7.72 (m, 1H), 7.65 (ddd, J = 9.5, 2.5, 1.5 Hz, 1H), 7.33 (td, J = 8.1, 5.8 Hz, 1H), 7.07 (tdd, J = 8.4, 2.6, 0.9 Hz, 1H), 3.17 – 3.03 (m, 1H), 2.89 (d, J = 7.9 Hz, 2H), 2.84 – 2.62 (m, 4H), 2.39 (ddd, J = 13.7, 6.2, 1.8 Hz, 1H), 2.25 – 2.09 (m, 1H), 1.73 – 1.57 (m, 2H), 1.57 – 1.43 (m, 2H), 1.43 – 1.28 (m, 1H).
^{13}C NMR (CDCl$_3$, 75 MHz) δ 179.00, 167.17, 162.63 (d, J = 246.7 Hz), 130.35 (d, J = 8.2 Hz), 128.73 (d, J = 8.6 Hz), 122.89 (d, J = 3.1 Hz), 117.84 (d, J = 21.2 Hz), 114.20 (d, J = 23.6 Hz), 54.31, 47.41, 46.60, 34.00, 30.37, 27.74, 24.76, 20.67.

3-(3-fluorophenyl)-5-(4-quinuclidin-3-ylmethyl)-1,2,4-oxadiazole fumarate (11b)

The fumarate derivative **11b** was prepared according to the general procedure **5.3.3.5** by reacting **128** (220 mg, 0.77 mmol, 1 equiv) and fumaric acid (89 mg, 0.77 mmol, 1 equiv) in MeOH. The crude salt was recrystallized from EtOAc and *n*-hexane to provide the pure compound as a white solid (89 mg, yield 29%).
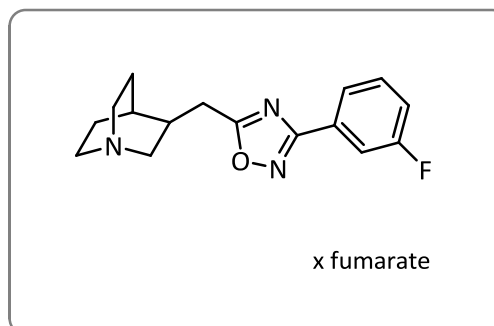
11b: white solid; TLC (alumina-Dragendorff): R$_f$ = 0.57 (CH$_2$Cl$_2$/MeOH 9:1); m.p.: 158.1-159.7°C; MS (ESI) m/z for C$_{16}$H$_{19}$FN$_3$O$^+$ [M+H]$^+$ calcd. 288.2, found 288.0.
^{1}H NMR (CD$_3$OD, 300 MHz) δ 7.92 – 7.85 (m, 1H), 7.76 (ddd, J = 9.6, 2.6, 1.5 Hz, 1H), 7.55 (td, J = 8.0, 5.7 Hz, 1H), 7.30 (tdd, J = 8.5, 2.7, 1.0 Hz, 1H), 6.68 (s, 2H), 3.72 (ddd, J = 12.8, 10.2, 2.4 Hz, 1H), 3.46 – 3.17 (m, 6H), 3.12 (ddd, J = 13.0, 7.1, 1.8 Hz, 1H), 2.89 – 2.72 (m, 1H), 2.26 – 2.09 (m, 2H), 2.09 – 1.99 (m, 2H), 1.99 – 1.84 (m, 1H).
^{13}C NMR (CD$_3$OD, 75 MHz) δ 179.91, 171.38, 168.64, 164.33 (d, J = 245.4 Hz), 136.18, 132.19 (d, J = 8.2 Hz), 130.17 (d, J = 8.5 Hz), 124.23 (d, J = 3.1 Hz), 119.26 (d, J = 21.5 Hz), 115.03 (d, J = 24.1 Hz), 52.95, 47.54, 47.02, 32.61, 30.09, 25.29, 24.93, 18.97.

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3-(3-fluorophenyl)-5-(4-quinuclidin-3-ylmethyl)-1,2,4-oxadiazole methyl iodide (12b)

According to the general procedure **5.3.3.6**, CH$_3$I (433 µL, 6.96 mmol, 8 equiv) was added to a solution of the free base **128** (250 mg, 0.87 mmol, 1 equiv) in MeOH. Evaporation of the solvent under reduced pressure afforded the crude quaternary salt quantitatively and then it was re-crystalized from MeOH and EtOH to give the pure final compound as a white solid (128 mg, yield 34%).
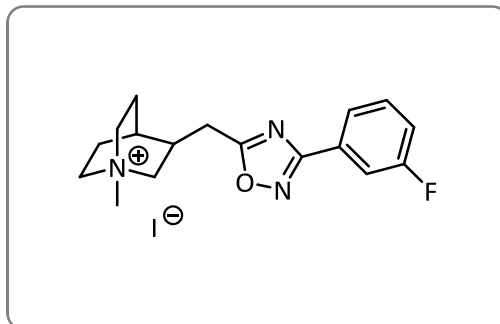

12b: white solid; TLC (alumina-Dragendorff): R$_f$ = 0.35 (CH$_2$Cl$_2$/MeOH 9:1); m.p.: 149.6-151.1°C; MS (ESI) m/z for C$_{17}$H$_{21}$FN$_3$O$^+$ [M]$^+$ calcd. 302.2, found 302.4.

^{1}H NMR (CD$_3$OD, 300 MHz) δ 7.92 – 7.86 (m, 1H), 7.80 – 7.74 (m, 1H), 7.55 (td, J = 8.0, 5.9 Hz, 1H), 7.35 – 7.26 (m, 1H), 3.94 – 3.82 (m, 1H), 3.59 – 3.46 (m, 4H), 3.40 – 3.19 (m, 3H), 3.02 (s, 3H), 2.99 – 2.83 (m, 1H), 2.34 – 2.17 (m, 2H), 2.17 – 2.07 (m, 2H), 2.07 – 1.92 (m, 1H).

^{13}C NMR (CD$_3$OD, 75 MHz) δ 179.71, 168.65, 164.34 (d, J = 245.4 Hz), 132.20 (d, J = 8.4 Hz), 130.14 (d, J = 8.4 Hz), 124.26 (d, J = 3.2 Hz), 119.29 (d, J = 21.5 Hz), 115.07 (d, J = 24.1 Hz), 63.03, 58.00, 57.56, 52.64, 33.62, 30.12, 25.92, 25.22, 20.25.

3-chloro-*N'*-hydroxybenzimidamide (104)

According to the general procedure **5.3.3.2**, hydroxylamine (50% w/w in water, 2.67 mL, 43.62 mmol, 4 equiv) was added to a stirred solution of 3-chlorobenzonitrile (1.50 g, 10.90 mmol, 1 equiv) and ethanol (8 mL) at RT. The resulting mixture was heated at 90°C for 1h. After concentration under reduced pressure, the desired 3-chloro-*N'*-hydroxybenzimidamide **(104)** was obtained as a pure colorless oil (1.86 g, yield 100%).
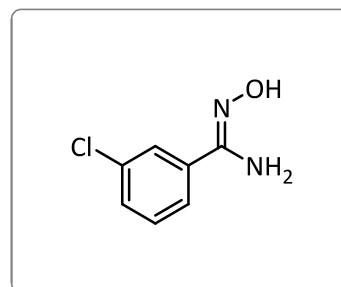
104: colorless oil; TLC (PMA): R$_f$ = 0.51 (cyclohexane/EtOAc 1:1).
^{1}H NMR ((CD$_3$)$_2$SO, 300 MHz) δ 9.77 (s, 1H), 7.73 – 7.69 (m, 1H), 7.65 (dt, *J* = 6.7, 1.8 Hz, 1H), 7.44 – 7.35 (m, 2H), 5.88 (s, 2H).
^{13}C NMR ((CD$_3$)$_2$SO, 75 MHz) δ 149.6, 135.4, 132.9, 130.0, 128.6, 125.1, 123.9.

Borane 3-(3-chlorophenyl)-5-(4-quinuclidin-3-ylmethyl)-1,2,4-oxadiazole complex (117)

The title compound was prepared according to general procedure **5.3.3.3.2** by reacting methyl ester **101** (400 mg, 2.03 mmol, 1 equiv), 3-chloro-*N'*-hydroxybenzimidamide **(104)** (1.04 g, 6.09 mmol, 3 equiv) and Cs$_2$CO$_3$ (1.98 g, 6.09 mmol, 3 equiv) in THF (10 mL). After standard workup and silica gel column

chromatography (cyclohexane/EtOAc 7:3), the desired compound **117** was obtained as a pure pale yellow oil (609 mg, yield 95%).
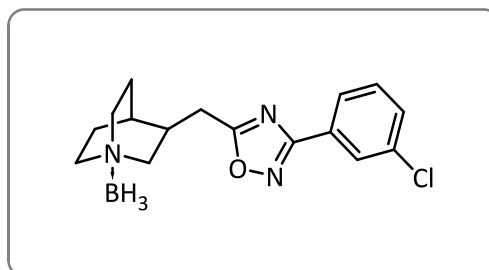
117: white solid; TLC (Dragendorff): R$_f$ = 0.42 (cyclohexane/EtOAc 7:3); m.p.: 159.0-160.3°C.
^{1}H NMR (CDCl$_3$, 300 MHz) δ 8.01 – 7.97 (m, 1H), 7.88 (dt, *J* = 7.5, 1.5 Hz, 1H), 7.42 (ddd, *J* = 8.1, 2.0, 1.4 Hz, 1H), 7.35 (t, *J* = 7.8 Hz, 1H), 3.39 – 3.24 (m, 1H), 3.09 – 2.86 (m, 6H), 2.61 (ddd, *J* = 13.6, 6.9, 2.1 Hz, 1H), 2.57 – 2.42 (m, 1H), 1.99 – 1.85 (m, 2H), 1.85 – 1.73 (m, 2H), 1.73 – 1.59 (m, 1H).
^{13}C NMR (CDCl$_3$, 75 MHz) δ 177.9, 167.6, 135.1, 131.5, 130.4, 128.4, 127.7, 125.6, 59.5, 53.7, 53.0, 33.2, 30.2, 26.3, 24.7, 20.1.

3-(3-chlorophenyl)-5-(4-quinuclidin-3-ylmethyl)-1,2,4-oxadiazole (129)

The title compound was obtained according to the general procedure **5.3.3.4** by reacting **118** (609 mg, 1.92 mmol, 1 equiv) and CF$_3$COOH (740 μL, 9.59 mmol, 5 equiv) in acetone. After disappearance of the starting material (TLC in cyclohexane/EtOAc 1:1), standard workup was applied to provide the desired compound **129** (531 mg, yield 91%).
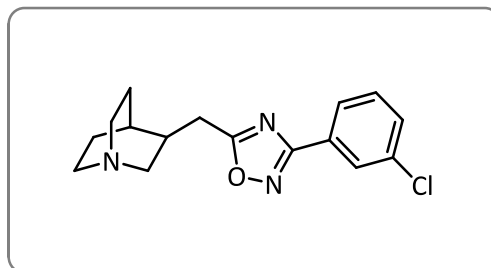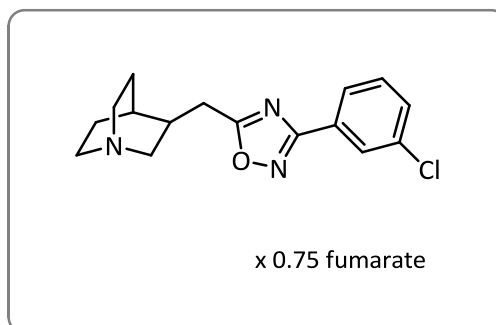
129: white solid; TLC (alumina-Dragendorff): R$_f$ = 0.57 (CH$_2$Cl$_2$/MeOH 9:1); m.p.: 90.7-93.1°C.

^{1}H NMR (CDCl$_3$, 300 MHz) δ 8.04 − 7.96 (m, 1H), 7.94 − 7.84 (m, 1H), 7.45 − 7.28 (m, 2H), 3.21 − 3.06 (m, 1H), 2.94 (d, *J* = 7.8 Hz, 2H), 2.88 − 2.67 (m, 4H), 2.50 − 2.37 (m, 1H), 2.23 (dd, *J* = 15.4, 7.4 Hz, 1H), 1.80 − 1.49 (m, 4H), 1.49 − 1.32 (m, 1H).

^{13}C NMR (CDCl$_3$, 75 MHz) δ 179.2, 167.3, 134.9, 131.2, 130.2, 128.6, 127.5, 125.4, 54.6, 47.7, 46.8, 34.3, 30.6, 28.0, 25.0, 20.9.

3-(3-chlorophenyl)-5-(4-quinuclidin-3-ylmethyl)-1,2,4-oxadiazole fumarate

(11c)

The fumarate derivative **11c** was prepared according to the general procedure **5.3.3.5** by reacting **129** (250 mg, 0.82 mmol, 1 equiv) and fumaric acid (96 mg, 0.82 mmol, 1 equiv) in MeOH. The crude salt was recrystallized from EtOH and acetone to provide the pure compound as a white solid (153 mg, yield 48%).

x 0.75 fumarate

11c: white solid; TLC (alumina-Dragendorff): R$_f$ = 0.47 (CH$_2$Cl$_2$/MeOH 9:1); m.p.: 152.2-153.6°C; MS (ESI) m/z for C$_{16}$H$_{19}$ClN$_3$O$^+$ [M+H]$^+$ calcd. 304.1, found 304.1 [M+H]$^+$, 306.3 [M+2+H]$^+$.

^{1}H NMR (CD$_3$OD, 300 MHz) δ 8.04 (dd, *J* = 2.4, 1.1 Hz, 1H), 7.98 (dt, *J* = 7.2, 1.5 Hz, 1H), 7.59 − 7.54 (m, 1H), 7.54 − 7.47 (m, 1H), 6.67 (s, 1.5H), 3.72 (ddd, *J* = 12.8, 10.2, 2.4 Hz, 1H), 3.46 − 3.17 (m, 6H), 3.11 (ddd, *J* = 13.1, 7.1, 1.6 Hz, 1H), 2.88 − 2.72 (m, 1H), 2.27 − 2.09 (m, 2H), 2.09 − 1.99 (m, 2H), 1.99 − 1.85 (m, 1H).

^{13}C NMR (CD$_3$OD, 75 MHz) δ 179.9, 171.5, 168.5, 136.2, 136.0, 132.4, 131.8, 129.9, 128.2, 126.6, 53.0, 47.6, 47.0, 32.6, 30.1, 25.3, 25.0, 19.0.

3-(3-chlorophenyl)-5-(4-quinuclidin-3-ylmethyl)-1,2,4-oxadiazole methyl iodide (12c)

According to the general procedure **5.3.3.6**, CH$_3$I (466 μL, 7.48 mmol, 8 equiv) was added to a solution of the free base **129** (284 mg, 0.94 mmol, 1 equiv) in MeOH. Evaporation of the solvent under reduced pressure afforded the crude quaternary salt quantitatively and then it was re-crystalized from MeOH and EtOH to give the pure final compound as a white solid (194 mg, yield 46%).
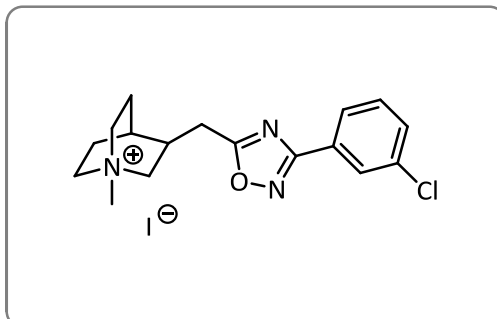
12c: white solid; TLC (alumina-Dragendorff): R$_f$ = 0.37 (CH$_2$Cl$_2$/MeOH 85:15); m.p.: 118.6-120.1°C; MS (ESI**)** m/z for C$_{17}$H$_{21}$ClN$_3$O$^+$ [M]$^+$ calcd. 318.1, found 318.5 [M+H]$^+$, 320.3 [M+2+H]$^+$.

^{1}H NMR (CD$_3$OD, 300 MHz) δ 8.06 – 8.03 (m, 1H), 8.02 – 7.97 (m, 1H), 7.59 – 7.54 (m, 1H), 7.54 – 7.47 (m, 1H), 3.95 – 3.81 (m, 1H), 3.61 – 3.46 (m, 4H), 3.42 – 3.19 (m, 3H), 3.03 (s, 3H), 2.98 – 2.84 (m, 1H), 2.36 – 2.17 (m, 2H), 2.17 – 2.08 (m, 2H), 2.08 – 1.92 (m, 1H).

^{13}C NMR (CD$_3$OD, 75 MHz) δ 179.7, 168.5, 136.0, 132.4, 131.8, 129.9, 128.2, 126.7, 63.0, 58.0, 57.6, 52.7, 33.6, 30.2, 25.9, 25.2, 20.3.

3-bromo-*N*'-hydroxybenzimidamide (105)

According to the general procedure **5.3.3.2**, hydroxylamine (50% w/w in water, 2.02 mL, 32.96 mmol, 4 equiv) was added to a stirred solution of 3-bromobenzonitrile (1.50 g, 8.24 mmol, 1 equiv) and ethanol (8 mL) at RT. The resulting mixture was heated at 90°C for 1h. After concentration under reduced pressure, the desired 3-fluoro-*N*'-hydroxybenzimidamide **(105)** was obtained as a pure colorless oil (1.77 g, yield 100%).
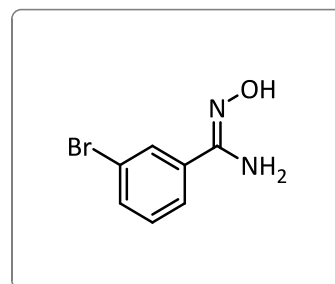
105: colorless oil; TLC (PMA): R$_f$ = 0.51 (cyclohexane/EtOAc 1:1).
^{1}H NMR (CDCl$_3$, 300 MHz) δ 7.75 – 7.69 (m, 1H), 7.53 – 7.45 (m, 2H), 7.20 (dd, *J* = 9.4, 6.4 Hz, 1H), 4.79 (br s, 2H).
^{13}C NMR (CDCl$_3$, 75 MHz) δ 151.6, 134.6, 133.2, 130.3, 129.2, 124.6, 122.9.

Borane 3-(3-bromophenyl)-5-(4-quinuclidin-3-ylmethyl)-1,2,4-oxadiazole complex (118)

The title compound was prepared according to general procedure **5.3.3.3.2** by reacting methyl ester **101** (300 mg, 1.52 mmol, 1 equiv), 3-bromo-*N*'-hydroxybenzimidamide **(105)** (982 mg, 4.57 mmol, 3 equiv) and Cs$_2$CO$_3$ (1.49 g, 4.57 mmol, 3 equiv) in THF (10 mL). After standard workup and silica gel column chromatography (cyclohexane/EtOAc 7:3), the desired compound **118** was obtained as a pure pale yellow oil (490 mg, yield 89%).
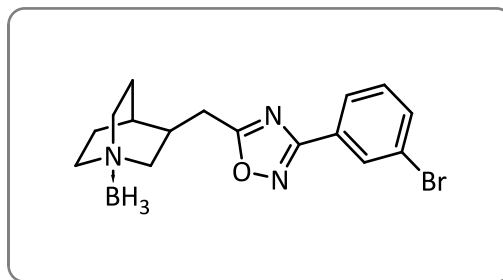
118: white solid; TLC (Dragendorff): R$_f$ = 0.42 (cyclohexane/EtOAc 7:3); m.p.: 155.2-155.8°C.
^{1}H NMR (CDCl$_3$, 300 MHz) δ 8.15 (t, *J* = 1.8 Hz, 1H), 7.96 – 7.89 (m, 1H), 7.57 (ddd, *J* = 8.0, 2.0, 1.0 Hz, 1H), 7.29 (t, *J* = 7.9 Hz, 1H), 3.38 – 3.23 (m, 1H), 3.07 – 2.87 (m, 6H), 2.62 (ddd, *J* = 13.7, 6.9, 2.1 Hz, 1H), 2.57 – 2.41 (m, 1H), 1.99 – 1.85 (m, 2H), 1.85 – 1.73 (m, 2H), 1.73 – 1.58 (m, 1H).
^{13}C NMR (CDCl$_3$, 75 MHz) δ 177.9, 167.5, 134.4, 130.6, 128.7, 126.1, 123.1, 59.6, 53.7, 53.1, 33.3, 30.3, 26.4, 24.8, 20.2.

3-(3-bromophenyl)-5-(4-quinuclidin-3-ylmethyl)-1,2,4-oxadiazole (130)

The title compound was obtained according to the general procedure **5.3.3.4** by reacting **118** (490 mg, 1.35 mmol, 1 equiv) and CF$_3$COOH (521 µL, 6.77 mmol, 5 equiv) in acetone. After disappearance of the starting material (TLC in cyclohexane/EtOAc 1:1), standard workup was applied to provide the desired compound **130** (400 mg, yield 85%).
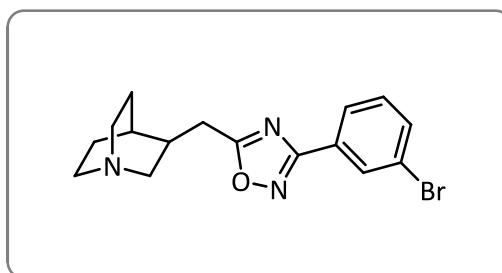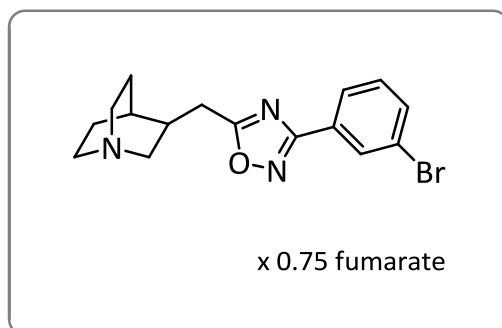
130: white solid; TLC (alumina-Dragendorff): R$_f$ = 0.51 (CH$_2$Cl$_2$/MeOH 9:1); m.p.: 107.6-108.8°C.

^{1}H NMR (CDCl$_3$, 300 MHz) δ 8.19 – 8.14 (m, 1H), 7.97 – 7.90 (m, 1H), 7.60 – 7.52 (m, 1H), 7.28 (t, J = 7.9 Hz, 1H), 3.21 – 3.07 (m, 1H), 2.94 (d, J = 7.9 Hz, 2H), 2.89 – 2.68 (m, 4H), 2.43 (ddd, J = 13.7, 6.2, 1.1 Hz, 1H), 2.30 – 2.15 (m, 1H), 1.86 – 1.48 (m, 4H), 1.49 – 1.33 (m, 1H).

^{13}C NMR (CDCl$_3$, 75 MHz) δ 179.3, 167.3, 134.2, 130.5, 128.9, 126.0, 123.0, 54.6, 47.7, 46.9, 34.3, 30.7, 28.1, 25.0, 21.0.

3-(3-bromophenyl)-5-(4-quinuclidin-3-ylmethyl)-1,2,4-oxadiazole fumarate (11d)

The fumarate derivative **11d** was prepared according to the general procedure **5.3.3.5** by reacting **130** (200 mg, 0.57 mmol, 1 equiv) and fumaric acid (67 mg, 0.57 mmol, 1 equiv) in MeOH. The crude salt was recrystallized from EtOH and acetone to provide the pure compound as a white solid (137 mg, yield 55%).

x 0.75 fumarate

11d: white solid; TLC (alumina-Dragendorff): R$_f$ = 0.42 (CH$_2$Cl$_2$/MeOH 95:5); m.p.: 141.9-143.8°C; MS (ESI) m/z for C$_{16}$H$_{19}$BrN$_3$O$^+$ [M+H]$^+$ calcd. 348.1, found 348.3. [M+H]$^+$, 350.2 [M+2+H]$^+$.

^{1}H NMR (CD$_3$OD, 300 MHz) δ 8.19 (t, J = 1.8 Hz, 1H), 8.03 (dt, J = 7.8, 1.2 Hz, 1H), 7.71 (ddd, J = 8.0, 1.9, 1.0 Hz, 1H), 7.44 (t, J = 7.9 Hz, 1H), 6.67 (s, 1.5H), 3.72 (ddd, J = 12.8, 10.2, 2.4 Hz, 1H), 3.45 – 3.17 (m, 6H), 3.11 (ddd, J = 13.0, 7.0, 1.6 Hz, 1H), 2.88 – 2.71 (m, 1H), 2.26 – 2.10 (m, 2H), 2.10 – 1.99 (m, 2H), 1.99 – 1.85 (m, 1H).

^{13}C NMR (CD$_3$OD, 75 MHz) δ 180.0, 171.5, 168.4, 136.2, 135.4, 132.0, 131.1, 130.1, 127.0, 123.9, 53.0, 47.6, 47.1, 32.6, 30.1, 25.3, 24.9, 19.0.

3-(3-bromophenyl)-5-(4-quinuclidin-3-ylmethyl)-1,2,4-oxadiazole methyl iodide (12d)

According to the general procedure **5.3.3.6**, CH$_3$I (286 μL, 4.59 mmol, 8 equiv) was added to a solution of the free base **130** (200 mg, 0.57 mmol, 1 equiv) in MeOH. Evaporation of the solvent under reduced pressure afforded the crude quaternary salt quantitatively and then it was re-crystalized from EtOH and acetone to give the pure final compound as a white solid (160 mg, yield 57%).
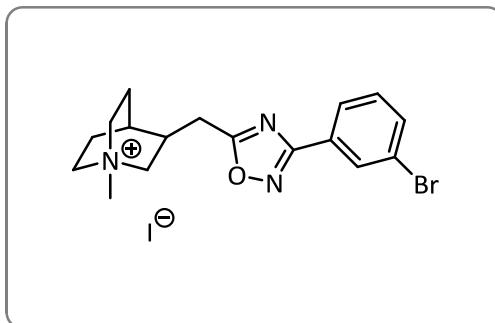

12d: white solid; TLC (alumina-Dragendorff): R$_f$ = 0.51 (CH$_2$Cl$_2$/MeOH 85:15); m.p.: 171.1-172.8°C (dec.); MS (ESI) m/z for C$_{17}$H$_{21}$BrN$_3$O$^+$ [M]$^+$ calcd. 362.1, found 362.3 [M+H]$^+$, 364.2 [M+2+H]$^+$.

^{1}H NMR (CD$_3$OD, 300 MHz) δ 8.19 (t, *J* = 1.7 Hz, 1H), 8.06 − 8.01 (m, 1H), 7.71 (ddd, *J* = 8.1, 2.0, 1.0 Hz, 1H), 7.45 (t, *J* = 7.9 Hz, 1H), 3.94 − 3.81 (m, 1H), 3.59 − 3.47 (m, 4H), 3.42 − 3.19 (m, 3H), 3.03 (s, 3H), 3.00 − 2.83 (m, 1H), 2.34 − 2.18 (m, 2H), 2.18 − 2.08 (m, 2H), 2.08 − 1.92 (m, 1H).

^{13}C NMR (CD$_3$OD, 75 MHz) δ 179.7, 168.4, 135.4, 132.0, 131.1, 130.1, 127.1, 123.8, 63.0, 58.0, 57.6, 52.7, 33.6, 30.2, 25.9, 25.2, 20.3.

N'-hydroxy-3-iodobenzimidamide (106)

According to the general procedure **5.3.3.2**, hydroxylamine (50% w/w in water, 1.60 mL, 26.2 mmol, 4 equiv) was added to a stirred solution of 3-iodobenzonitrile (1.50 g, 6.55 mmol, 1 equiv) and ethanol (8 mL) at RT. The resulting mixture was heated at 90°C for 1h. After concentration under reduced pressure, the desired *N'*-hydroxy-3-iodobenzimidamide (**106**) was obtained as a pure colorless oil (1.72 g, yield 100%).
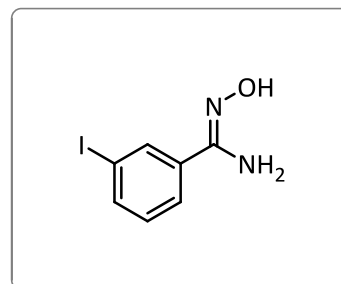

106: colorless oil; TLC (PMA): R_f = 0.51 (cyclohexane/EtOAc 1:1).
^{1}H NMR (CDCl$_3$, 300 MHz) δ 8.57 (br s, 1H), 7.97 (t, *J* = 1.7 Hz, 1H), 7.74 (ddd, *J* = 7.9, 1.7, 1.0 Hz, 1H), 7.58 (ddd, *J* = 7.8, 1.5, 1.1 Hz, 1H), 7.12 (t, *J* = 7.9 Hz, 1H), 4.91 (br s, 2H).
^{13}C NMR (CDCl$_3$, 75 MHz) δ 151.6, 139.1, 135.0, 134.5, 130.4, 125.3, 94.5.

Borane 3-(3-iodophenyl)-5-(4-quinuclidin-3-ylmethyl)-1,2,4-oxadiazole complex (119)

The title compound was prepared according to general procedure **5.3.3.3.2** by reacting methyl ester **101** (500 mg, 2.54 mmol, 1 equiv), *N'*-hydroxy-3-iodobenzimidamide **(106)** (1.99 g, 7.61 mmol, 3 equiv) and Cs$_2$CO$_3$ (2.48 g, 7.61 mmol, 3 equiv) in THF (15 mL). After standard workup

and silica gel column chromatography (cyclohexane/EtOAc 7:3), the desired compound **119** was obtained as a pure pale yellow oil (873 mg, yield 84%).
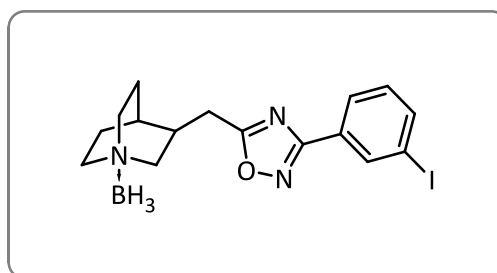
119: pale yellow oil; TLC (Dragendorff): R_f = 0.43 (cyclohexane/EtOAc 7:3).
^{1}H NMR (CDCl$_3$, 300 MHz) δ 8.37 − 8.32 (m, 1H), 7.99 − 7.93 (m, 1H), 7.82 − 7.73 (m, 1H), 7.19 − 7.11 (m, 1H), 3.38 − 3.23 (m, 1H), 3.09 − 2.86 (m, 6H), 2.61 (ddd, *J* = 13.6, 6.9, 1.7 Hz, 1H), 2.56 − 2.42 (m, 1H), 1.98 − 1.85 (m, 2H), 1.85 − 1.74 (m, 2H), 1.74 − 1.59 (m, 1H).
^{13}C NMR (CDCl$_3$, 75 MHz) δ 177.8, 167.2, 140.3, 136.3, 130.6, 128.6, 126.6, 94.5, 59.5, 53.6, 53.0, 33.2, 30.2, 26.3, 24.7, 20.1.

3-(3-iodophenyl)-5-(4-quinuclidin-3-ylmethyl)-1,2,4-oxadiazole (131)

The title compound was obtained according to the general procedure **5.3.3.4** by reacting **119** (873 mg, 2.13 mmol, 1 equiv) and CF$_3$COOH (822 µL, 10.67 mmol, 5 equiv) in acetone. After disappearance of the starting material (TLC in cyclohexane/EtOAc 1:1), standard workup was applied to provide the desired compound **131** (768 mg, yield 91%).
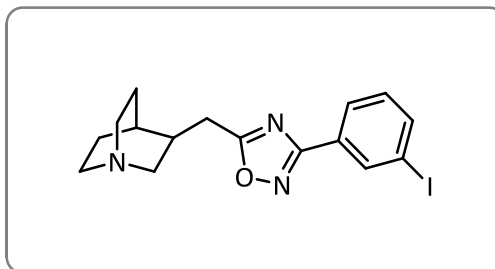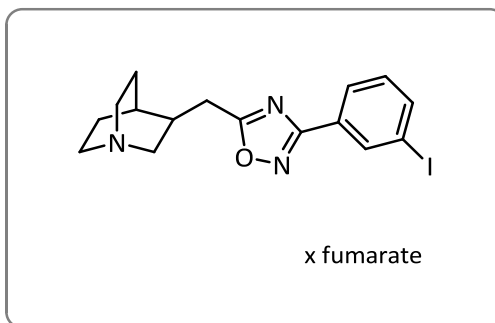

131: white solid; TLC (alumina-Dragendorff): R$_f$ = 0.50 (CH$_2$Cl$_2$/MeOH 95:5); m.p.: 149.4-150.5°C.

^{1}H NMR (CDCl$_3$, 300 MHz) δ 8.44 − 8.40 (m, 1H), 8.06 − 7.99 (m, 1H), 7.85 − 7.79 (m, 1H), 7.24 − 7.16 (m, 1H), 3.20 (dd, J = 13.5, 9.8 Hz, 1H), 3.00 (d, J = 7.9 Hz, 2H), 2.95 − 2.74 (m, 4H), 2.49 (ddd, J = 13.7, 6.2, 1.8 Hz, 1H), 2.35 − 2.20 (m, 1H), 1.84 − 1.72 (m, 1H), 1.72 − 1.55 (m, 3H), 1.55 − 1.39 (m, 1H).

^{13}C NMR (CDCl$_3$, 75 MHz) δ 179.3, 167.1, 140.2, 136.3, 130.6, 128.9, 126.6, 94.5, 54.7, 47.8, 47.0, 34.4, 30.8, 28.1, 25.1, 21.1.

3-(3-iodophenyl)-5-(4-quinuclidin-3-ylmethyl)-1,2,4-oxadiazole fumarate (11e)

The fumarate derivative **11e** was prepared according to the general procedure **5.3.3.5** by reacting **131** (294 mg, 0.74 mmol, 1 equiv) and fumaric acid (86 mg, 0.74 mmol, 1 equiv) in MeOH. The crude salt was recrystallized from MeOH and *n*-hexane to provide the pure compound as a white solid (321 mg, yield 85%).


x fumarate

11e: white solid; TLC (alumina-Dragendorff): R$_f$ = 0.59 (CH$_2$Cl$_2$/MeOH 9:1); m.p.: 154.4-156.3°C; MS (ESI) m/z for C$_{16}$H$_{19}$IN$_3$O$^+$ [M+H]$^+$ calcd. 396.1, found 396.0.

^{1}H NMR (CD$_3$OD, 300 MHz) δ 8.38 (t, J = 1.7 Hz, 1H), 8.07 − 8.01 (m, 1H), 7.93 − 7.87 (m, 1H), 7.29 (t, J = 7.9 Hz, 1H), 6.69 (s, 2H), 3.72 (ddd, J = 12.8, 10.2, 2.4 Hz, 1H), 3.45 − 3.16 (m, 6H), 3.11 (ddd, J = 13.1, 7.0, 1.7 Hz, 1H), 2.88 − 2.72 (m, 1H), 2.26 − 2.09 (m, 2H), 2.09 − 1.98 (m, 2H), 1.98 − 1.84 (m, 1H).

^{13}C NMR (CD$_3$OD, 75 MHz) δ 179.9, 169.9, 168.2, 141.4, 137.1, 135.7, 131.9, 129.9, 127.5, 95.0, 52.9, 47.5, 47.0, 32.6, 30.1, 25.3, 24.9, 19.0.

3-(3-iodophenyl)-5-(4-quinuclidin-3-ylmethyl)-1,2,4-oxadiazole methyl iodide (12e)

According to the general procedure **5.3.3.6**, CH$_3$I (344 μL, 5.52 mmol, 8 equiv) was added to a solution of the free base **131** (271 mg, 0.69 mmol, 1 equiv) in MeOH. Evaporation of the solvent under reduced pressure afforded the crude quaternary salt quantitatively and then it was re-crystalized from MeOH to give the pure final compound as a bright white crystals (167 mg, yield 45%).
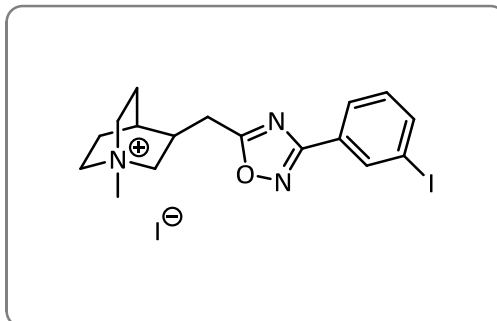

12e: bright white crystals; TLC (alumina-Dragendorff): R$_f$ = 0.39 (CH$_2$Cl$_2$/MeOH 85:15); m.p.: 233.9-236.4°C (dec.); MS (ESI) m/z for C$_{17}$H$_{21}$IN$_3$O$^+$ [M]$^+$ calcd. 410.1, found 410.3.

^{1}H NMR (CD$_3$OD, 300 MHz) δ 8.39 (t, *J* = 1.6 Hz, 1H), 8.09 − 8.03 (m, 1H), 7.91 (ddd, *J* = 7.9, 1.8, 1.1 Hz, 1H), 7.29 (t, *J* = 7.9 Hz, 1H), 3.95 − 3.76 (m, 1H), 3.58 − 3.42 (m, 4H), 3.40 − 3.15 (m, 3H), 3.01 (s, 3H), 2.97 − 2.82 (m, 1H), 2.35 − 2.18 (m, 2H), 2.18 − 2.08 (m, 2H), 2.08 − 1.92 (m, 1H).

^{13}C NMR (CD$_3$OD, 75 MHz) δ 179.7, 168.3, 141.5, 137.1, 131.9, 129.9, 127.5, 95.0, 63.1, 58.0, 57.6, 52.7, 33.6, 30.2, 25.9, 25.2, 20.3.

Borane 3-phenyl-5-(4-quinuclidin-3-ylmethyl)-1,2,4-oxadiazole complex (120)

The title compound was prepared according to general procedure **5.3.3.3.2** by reacting methyl ester **101** (500 mg, 2.54 mmol, 1 equiv), benzamidoxime (**107**) (1.04 g, 7.61 mmol, 3 equiv) and Cs_2CO_3 (2.48 g, 7.61 mmol, 3 equiv) in THF (15 mL). After standard workup and silica gel column chromatography (cyclohexane/EtOAc 7:3), the desired compound **120** was obtained as a pure white solid (370 mg, yield 52%).
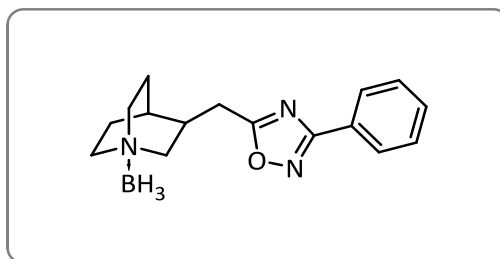
120: white solid; TLC (Dragendorff): R_f = 0.42 (cyclohexane/EtOAc 7:3); m.p.: 129.2-130.6°C.
^{1}H NMR (CDCl$_3$, 300 MHz) δ 8.03 – 7.95 (m, 2H), 7.48 – 7.37 (m, 3H), 3.36 – 3.24 (m, 1H), 3.06 – 2.87 (m, 6H), 2.62 (ddd, *J* = 13.7, 7.0, 2.0 Hz, 1H), 2.57 – 2.43 (m, 1H), 1.95 – 1.84 (m, 2H), 1.84 – 1.72 (m, 2H), 1.72 – 1.57 (m, 1H).
^{13}C NMR (CDCl$_3$, 75 MHz) δ 177.5, 168.2, 131.2, 128.8, 127.3, 126.5, 59.3, 53.4, 52.8, 32.9, 30.00, 26.0, 24.5, 19.8.

### 3-phenyl-5-(4-quinuclidin-3-ylmethyl)-1,2,4-oxadiazole (132)

The title compound was obtained according to the general procedure **5.3.3.4** by reacting **120** (370 mg, 1.31 mmol, 1 equiv) and CF_3COOH (503 μL, 6.53 mmol, 5 equiv) in acetone. After disappearance of the starting material (TLC in cyclohexane/EtOAc 1:1), standard workup was applied to provide the desired compound **132** (327 mg, yield 92%).
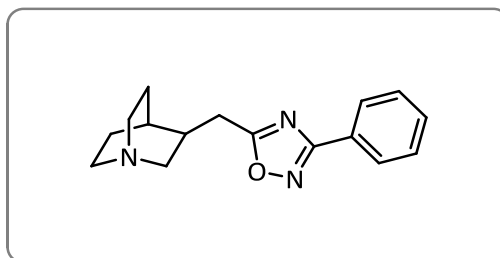
132: yellow oil; TLC (Dragendorff): R_f = 0.51 (CH$_2$Cl$_2$/MeOH 95:5).
^{1}H NMR (CDCl$_3$, 300 MHz) δ 8.04 – 7.95 (m, 2H), 7.47 – 7.35 (m, 3H), 3.21 – 3.08 (m, 1H), 2.94 (d, *J* = 7.9 Hz, 2H), 2.89 – 2.69 (m, 4H), 2.45 (ddd, *J* = 13.7, 6.3, 1.9 Hz, 1H), 2.31 – 2.15 (m, 1H), 1.79 – 1.63 (m, 2H), 1.63 – 1.49 (m, 2H), 1.49 – 1.35 (m, 1H).
^{13}C NMR (CDCl$_3$, 75 MHz) δ 178.9, 168.4, 131.2, 128.9, 127.5, 126.9, 54.6, 47.7, 46.9, 34.3, 30.7, 28.0, 25.0, 21.0.

3-phenyl-5-(4-quinuclidin-3-ylmethyl)-1,2,4-oxadiazole fumarate (11f)

The fumarate derivative **11f** was prepared according to the general procedure **5.3.3.5** by reacting **132** (327 mg, 1.21 mmol, 1 equiv) and fumaric acid (141 mg, 1.21 mmol, 1 equiv) in MeOH. The crude salt was recrystallized from MeOH and Et_2O to provide the pure compound as a white solid (308 mg, yield 66%).

x fumarate
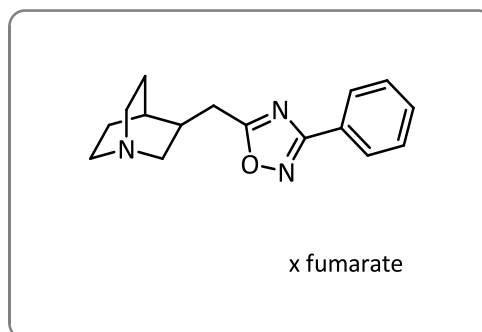
11f: white solid; TLC (alumina-Dragendorff): R_f = 0.41 (CH_2Cl_2/MeOH 95:5); m.p.: 141.8-143.4°C; MS (ESI) m/z for $C_{16}H_{20}N_3O^+$ [M+H]$^+$ calcd. 270.2, found 270.5.
^{1}H NMR (CD_3OD, 300 MHz) δ 8.08 – 8.01 (m, 2H), 7.58 – 7.46 (m, 3H), 6.68 (s, 2H), 3.71 (ddd, J = 12.8, 10.2, 2.4 Hz, 1H), 3.43 – 3.16 (m, 6H), 3.11 (ddd, J = 13.1, 7.0, 1.9 Hz, 1H), 2.86 – 2.72 (m, 1H), 2.25 – 2.09 (m, 2H), 2.09 – 1.98 (m, 2H), 1.98 – 1.84 (m, 1H).
^{13}C NMR (CD_3OD, 75 MHz) δ 179.6, 171.4, 169.5, 136.2, 132.5, 130.1, 128.3, 128.0, 53.0, 47.5, 47.0, 32.7, 30.1, 25.3, 24.9, 19.0.

3-phenyl-5-(4-quinuclidin-3-ylmethyl)-1,2,4-oxadiazole methyl iodide (12f)

According to the general procedure **5.3.3.6**, CH_3I (351 μL, 5.64 mmol, 8 equiv) was added to a solution of the free base **132** (190 mg, 0.71 mmol, 1 equiv) in MeOH. Evaporation of the solvent under reduced pressure afforded the crude quaternary salt quantitatively and then it was re-crystalized from acetone to give the pure final compound as a white solid (158 mg, yield 54%).
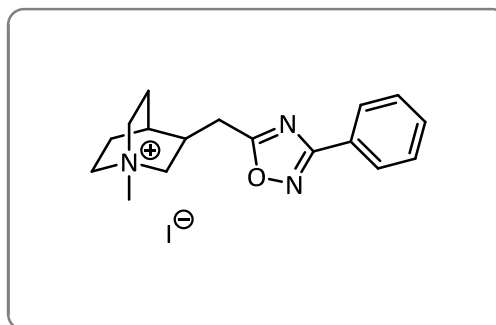
12f: white solid; TLC (alumina-Dragendorff): R_f = 0.43 (CH_2Cl_2/MeOH 85:15); m.p.: 159.4-161.2°C; MS (ESI) m/z for $C_{17}H_{22}N_3O^+$ [M]$^+$ calcd. 284.2, found 284.1.
^{1}H NMR (CD_3OD, 300 MHz) δ 8.09 – 8.03 (m, 2H), 7.58 – 7.47 (m, 3H), 3.93 – 3.80 (m, 1H), 3.58 – 3.47 (m, 4H), 3.40 – 3.18 (m, 3H), 3.02 (s, 3H), 2.99 – 2.83 (m, 1H), 2.34 – 2.17 (m, 2H), 2.17 – 2.07 (m, 2H), 2.07 – 1.93 (m, 1H).
^{13}C NMR (CD_3OD, 75 MHz) δ 179.3, 169.5, 132.5, 130.1, 128.4, 127.9, 63.0, 58.0, 57.6, 52.7, 33.6, 30.1, 25.9, 25.2, 20.3.

N'-hydroxy-3-methylbenzimidamide (108)

According to the general procedure **5.3.3.2**, hydroxylamine (50% w/w in water, 3.06 mL, 50.0 mmol, 4 equiv) was added to a stirred solution of 3-methylbenzonitrile (1.50 mL, 12.50 mmol, 1 equiv) and ethanol (8 mL) at RT. The resulting mixture was heated at 90°C for 1h. After concentration under reduced pressure, the desired *N'*-hydroxy-3-methylbenzimidamide (**108**) was obtained as a colorless oil (1.80 g, yield 96%).
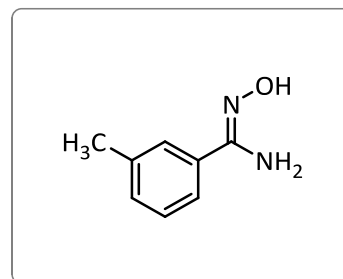
108: colorless oil; TLC (PMA): R$_f$ = 0.46 (cyclohexane/EtOAc 1:1).
^{1}H NMR (CDCl$_3$, 300 MHz) δ 7.50 − 7.38 (m, 2H), 7.34 − 7.18 (m, 2H), 4.94 (br s, 2H), 2.37 (s, 3H).
^{13}C NMR (CDCl$_3$, 75 MHz) δ 152.9, 138.4, 132.5, 130.8, 128.6, 126.7, 123.1, 21.5.

Borane 5-(4-quinuclidin-3-ylmethyl)-3-*m*-tolyl-1,2,4-oxadiazole complex (121)

The title compound was prepared according to general procedure **5.3.3.3.2** by reacting methyl ester **101** (300 mg, 1.52 mmol, 1 equiv), *N'*-hydroxy-3-methylbenzimidamide (**108**) (686 mg, 4.57 mmol, 3 equiv) and Cs$_2$CO$_3$ (1.49 g, 4.57 mmol, 3 equiv) in THF (10 mL). After standard workup and silica gel column chromatography (cyclohexane/EtOAc 3:2 to 1:1), the desired compound **121** was obtained as a pure pale yellow oil (406 mg, yield, 90%).
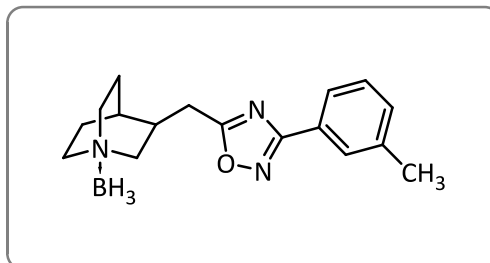
121: pale yellow oil; TLC (Dragendorff): R$_f$ = 0.51 (cyclohexane/EtOAc 7:3).
^{1}H NMR (CDCl$_3$, 300 MHz) δ 7.83 − 7.74 (m, 2H), 7.34 − 7.21 (m, 2H), 3.37 − 3.22 (m, 1H), 3.06 − 2.86 (m, 6H), 2.61 (ddd, *J* = 13.7, 6.9, 2.0 Hz, 1H), 2.56 − 2.42 (m, 1H), 2.35 (s, 3H), 1.96 − 1.81 (m, 2H), 1.81 − 1.71 (m, 2H), 1.71 − 1.55 (m, 1H).
^{13}C NMR (CDCl$_3$, 75 MHz) δ 177.4, 168.6, 138.8, 132.2, 128.9, 128.1, 126.5, 124.7, 59.5, 53.6, 53.0, 33.3, 30.2, 26.3, 24.7, 21.4, 20.1.

5-(4-quinuclidin-3-ylmethyl)-3-m-tolyl-1,2,4-oxadiazole (133)

The title compound was obtained according to the general procedure **5.3.3.4** by reacting **121** (406 mg, 1.37 mmol, 1 equiv) and CF$_3$COOH (526 µL, 6.83 mmol, 5 equiv) in acetone. After disappearance of the starting material (TLC in cyclohexane/EtOAc 1:1), standard workup was applied to provide the desired compound **133** (357 mg, yield 92%).
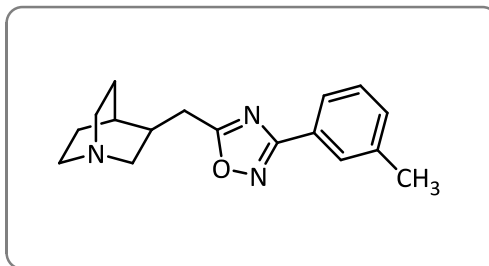
133: yellow oil; TLC (alumina-Dragendorff): R$_f$ = 0.33 (CH$_2$Cl$_2$/MeOH 94:6).
^{1}H NMR (CDCl$_3$, 300 MHz) δ 7.76 (d, J = 9.1 Hz, 2H), 7.33 – 7.20 (m, 2H), 3.72 – 3.57 (m, 1H), 3.49 – 2.97 (m, 7H), 2.75 – 2.58 (m, 1H), 2.34 (s, 3H), 2.18 – 2.01 (m, 2H), 2.01 – 1.89 (m, 2H), 1.89 – 1.74 (m, 1H).
^{13}C NMR (CDCl$_3$, 75 MHz) δ 176.9, 168.5, 138.8, 132.2, 128.9, 128.0, 126.2, 124.5, 51.8, 46.4, 45.8, 31.7, 29.6, 24.2, 24.2, 21.4, 18.3.

5-(4-quinuclidin-3-ylmethyl)-3-*m*-tolyl-1,2,4-oxadiazole fumarate (11g)

The fumarate derivative **11g** was prepared according to the general procedure **5.3.3.5** by reacting **133** (68 mg, 0.24 mmol, 1 equiv) and fumaric acid (28 mg, 0.24 mmol, 1 equiv) in MeOH. After 2h, additional fumaric acid (14 mg, 0.12 mmol, 1 equiv) was added (two times) to achieve complete consumption of the starting material. The crude salt was recrystallized from acetone to provide the pure compound as a white solid (109 mg, yield 88%).
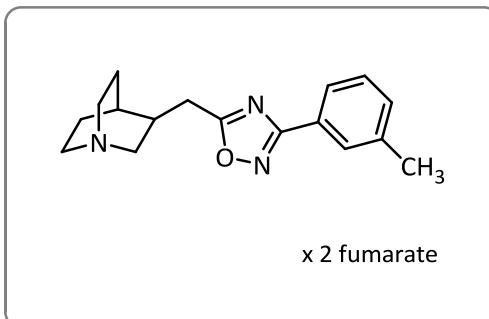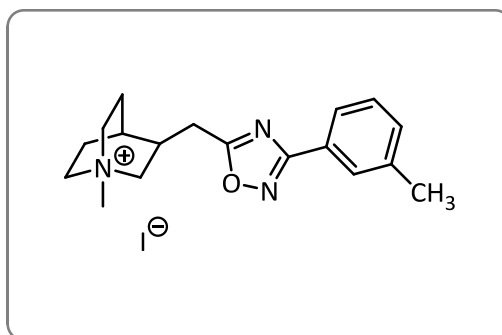
11g: white solid; TLC (alumina-Dragendorff): R$_f$ = 0.38 (CH$_2$Cl$_2$/MeOH 96:4); m.p.: 210-215°C (dec.); MS (ESI) m/z for C$_{17}$H$_{22}$N$_3$O$^+$ [M+H]$^+$ calcd. 284.2, found 284.4.
^{1}H NMR (CD$_3$OD, 300 MHz) δ 7.91 – 7.86 (m, 1H), 7.86 – 7.80 (m, 1H), 7.44 – 7.34 (m, 2H), 6.76 (s, 4H), 3.74 (ddd, J = 12.8, 10.2, 2.5 Hz, 1H), 3.48 – 3.33 (m, 4H), 3.25 (dd, J = 7.7, 5.7 Hz, 2H), 3.14 (ddd, J = 13.0, 7.1, 1.8 Hz, 1H), 2.89 – 2.74 (m, 1H), 2.42 (s, 3H), 2.28 – 2.11 (m, 2H), 2.11 – 2.01 (m, 2H), 2.01 – 1.85 (m, 1H).
^{13}C NMR (CD$_3$OD, 75 MHz) δ 179.4, 169.6, 168.1, 140.1, 135.2, 133.2, 130.0, 128.8, 127.8, 125.5, 53.1, 47.7, 47.2, 32.6, 30.1, 25.2, 24.9, 21.4, 18.9.

5-(4-quinuclidin-3-ylmethyl)-3-*m*-tolyl-1,2,4-oxadiazole methyl iodide (12g)

According to the general procedure **5.3.3.6**, CH$_3$I (557 µL, 8.95 mmol, 8 equiv) was added to a solution of the free base **133** (317 mg, 1.12 mmol, 1 equiv) in MeOH. Evaporation of the solvent under reduced pressure afforded the crude quaternary salt quantitatively and then it was re-crystalized from MeOH and EtOH to give the pure final compound as a white solid (191 mg, yield 40%).

12g: white solid: TLC (alumina-Dragendorff): R$_f$ = 0.30 (CH$_2$Cl$_2$/MeOH 9:1); m.p.: 162.1-164.2°C; MS (ESI) m/z for C$_{18}$H$_{24}$N$_3$O$^+$ [M]$^+$ calcd. 298.2, found 298.1.

^{1}H NMR (CD$_3$OD, 300 MHz) δ 7.90 – 7.81 (m, 2H), 7.42 – 7.33 (m, 2H), 3.95 – 3.82 (m, 1H), 3.60 – 3.49 (m, 4H), 3.41 – 3.18 (m, 3H), 3.03 (s, 3H), 3.00 – 2.83 (m, 1H), 2.41 (s, 3H), 2.35 – 2.22 (m, 1H), 2.22 – 2.17 (m,1), 2.17 – 2.08 (m, 2H), 2.08 – 1.95 (m, 1H).

^{13}C NMR (CD$_3$OD, 75 MHz) δ 179.2, 169.6, 140.1, 133.2, 130.0, 128.8, 127.8, 125.5, 63.1, 58.0, 57.6, 52.6, 33.6, 30.2, 25.9, 25.2, 21.4, 20.3.

N'-hydroxy-4-methoxybenzimidamide (109)

According to the general procedure **5.3.3.2**, hydroxylamine (50% w/w in water, 1.84 mL, 30.04 mmol, 4 equiv) was added to a stirred solution of 4-methoxybenzonitrile (1.0 g, 7.51 mmol, 1 equiv) and ethanol (8 mL) at RT. The resulting mixture was heated at 90°C for 1.5h. After concentration under reduced pressure, the desired *N'*-hydroxy-4-methoxybenzimidamide **(109)** was obtained as a pure white solid (1.21 g, yield 97%).
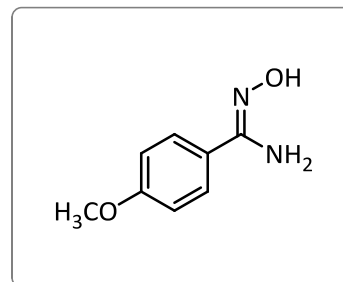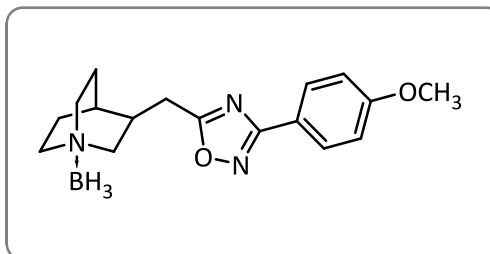
109: white solid; TLC (PMA): R$_f$ = 0.30 (cyclohexane/EtOAc 1:4); m.p.: 121.0-121.5°C.
^{1}H NMR (CDCl$_3$, 300 MHz) δ 7.57 (d, *J* = 8.9 Hz, 2H), 6.91 (d, *J* = 8.9 Hz, 2H), 4.90 (s, 2H), 3.83 (s, 3H).
^{13}C NMR (CDCl$_3$, 75 MHz) δ 161.1, 152.7, 127.4, 125.1, 114.2, 55.5.

Borane 3-(4-methoxyphenyl)-5-(4-quinuclidin-3-ylmethyl)-1,2,4-oxadiazole complex (122)

The title compound was prepared according to general procedure **5.3.3.3.2** by reacting methyl ester **101** (152 mg, 0.77 mmol, 1 equiv), *N'*-hydroxy-4-methoxybenzimidamide (**109**) (384 mg, 2.31 mmol, 3 equiv) and Cs$_2$CO$_3$ (753 mg, 2.31 mmol, 3 equiv) in THF (5 mL). After standard workup and silica gel column chromatography (cyclohexane/EtOAc 4:1 to 3:2), the desired compound **122** was obtained as a pure yellow oil (75 mg, yield 31%).

122: yellow oil; TLC (Dragendorff): R$_f$ = 0.43 in (cyclohexane/EtOAc 1:4).
^{1}H NMR (CDCl$_3$, 300 MHz) δ 7.92 (d, *J* = 8.9 Hz, 2H), 6.92 (d, *J* = 9.0 Hz, 2H), 3.80 (s, 3H), 3.36 – 3.24 (m, 1H), 3.04 – 2.88 (m, 6H), 2.62 (ddd, *J* = 13.7, 7.0, 2.1 Hz, 1H), 2.56 – 2.43 (m, 1H), 1.99 – 1.82 (m, 2H), 1.82 – 1.72 (m, 2H), 1.72 – 1.57 (m, 1H).
^{13}C NMR (CDCl$_3$, 75 MHz) δ 177.2, 168.2, 162.2, 129.2, 119.1, 114.4, 59.6, 55.5, 53.7, 53.0, 33.3, 30.2, 26.3, 24.7, 20.1.

3-(4-methoxyphenyl)-5-(4-quinuclidin-3-ylmethyl)-1,2,4-oxadiazole (134)

The title compound was obtained according to the general procedure **5.3.3.4** by reacting **123** (61 mg, 0.19 mmol, 1 equiv) and CF$_3$COOH (73 μL, 0.95 mmol, 5 equiv) in acetone. After disappearance of the starting material (TLC in cyclohexane/EtOAc 3:2), standard workup was applied to provide the desired compound **134** (57 mg, yield 100%).
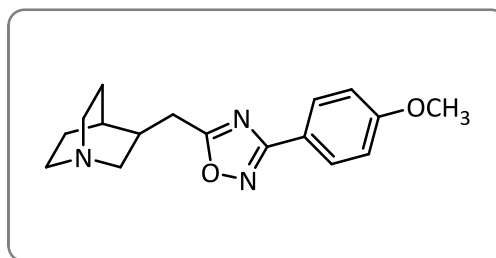

134: yellow oil; TLC (alumina-Dragendorff): R$_f$ = 0.30 (CH$_2$Cl$_2$/MeOH 95:5).
^{1}H NMR (CDCl$_3$, 300 MHz) δ 7.93 (d, J = 8.9 Hz, 2H), 6.91 (d, J = 8.9 Hz, 2H), 3.79 (s, 3H), 3.24 – 3.07 (m, 1H), 2.93 (d, J = 7.9 Hz, 2H), 2.88 – 2.67 (m, 4H), 2.52 – 2.41 (m, 1H), 2.32 – 2.18 (m, 1H), 1.82 – 1.64 (m, 2H), 1.64 – 1.36 (m, 3H).
^{13}C NMR (CDCl$_3$, 75 MHz) δ 178.5, 168.1, 162.0, 129.1, 119.3, 114.4, 55.5, 54.2, 47.5, 46.7, 34.1, 30.6, 27.7, 24.9, 20.7.

3-(4-methoxyphenyl)-5-(4-quinuclidin-3-ylmethyl)-1,2,4-oxadiazole fumarate 11h)

The fumarate derivative **11h** was prepared according to the general procedure **5.3.3.5** by reacting **134** (57 mg, 0.19 mmol, 1 equiv) and fumaric acid (22 mg, 0.19 mmol, 1 equiv) in MeOH. After 2h, additional fumaric acid (11 mg, 0.10 mmol, 0.5 equiv) was added to achieve complete consumption of the starting material. The crude salt was recrystallized from MeOH/n-hexane to provide the pure compound as a white solid (25 mg, yield 30%).


x 1.25 fumarate
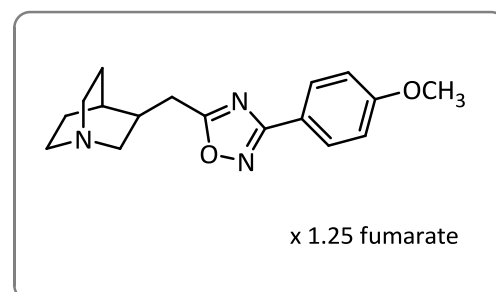
11h: white solid; TLC (alumina-Dragendorff): R$_f$ = 0.33 (CH$_2$Cl$_2$/MeOH 96:4); m.p.: 174.7-175.5°C; MS (ESI) m/z for C$_{17}$H$_{22}$N$_3$O$_2$$^+$ [M+H]$^+$ calcd. 300.2, found 300.0.
^{1}H NMR (CD$_3$OD, 300 MHz) δ 7.97 (d, J = 9.0 Hz, 2H), 7.04 (d, J = 9.0 Hz, 2H), 6.69 (s, 2.5H), 3.86 (s, 3H), 3.77 – 3.64 (m, 1H), 3.46 – 3.26 (m, 4H), 3.22 (dd, J = 7.7, 5.5 Hz, 2H), 3.18 – 3.05 (m, 1H), 2.87 – 2.71 (m, 1H), 2.27 – 2.09 (m, 2H), 2.09 – 1.99 (m, 2H), 1.99 – 1.84 (m, 1H).
^{13}C NMR (CD$_3$OD, 75 MHz) δ 179.2, 170.6, 169.3, 163.8, 136.0, 130.0, 120.2, 115.4, 56.0, 53.1, 47.7, 47.1, 32.7, 30.1, 25.2, 24.9, 19.0.

N'-hydroxyacetimidamide (110)

According to the general procedure **5.3.3.2**, hydroxylamine (50% w/w in water, 25.0 mL, 384.0 mmol, 4 equiv) was added to a stirred solution of acetonitrile (5.0 mL, 96.0 mmol, 1 equiv) and ethanol (50 mL) at RT. The resulting mixture was heated at 90°C for 3h. After concentration under reduced pressure, the desired acetamidoxime (**110**) was re-crystallized from *i*-PrOH (7.10 g, yield 100%).
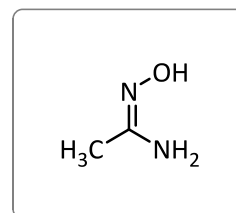
110: white solid; TLC (PMA): R_f = 0.54 (CH$_2$Cl$_2$/MeOH 4:1); m.p.: 134-136°C.
^{1}H NMR ((CD$_3$)$_2$SO, 300 MHz): δ 8.76 (br s, 1H), 5.33 (br s, 2H), 1.62 (s, 3H).
^{13}C NMR ((CD$_3$)$_2$SO, 75 MHz): δ 149.6, 16.7.

### 3-methyl-5-(4-quinuclidin-3-ylmethyl)-1,2,4-oxadiazole (135)

The title compound was prepared according to general procedure **5.3.3.3.1** by reacting methyl ester **100** (50 mg, 0.27 mmol, 1 equiv), acetamidoxime **(110)** (61 mg, 0.82 mmol, 3 equiv) and NaH 60% dispersion in mineral oil (33 mg, 0.82 mmol, 3 equiv) in dry THF (2 mL). After standard extraction, **135** was obtained as a pure viscous oil, therefore column chromatography was not necessary (55 mg, yield 100%).
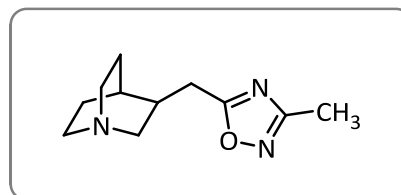
135: light brown oil; TLC (Dragendorff): R_f = 0.34 (CH$_2$Cl$_2$/MeOH 7:3).
^{1}H NMR (CDCl$_3$, 300 MHz) δ 3.17 – 3.01 (m, 1H), 2.84 (d, *J* = 7.9 Hz, 2H), 2.81 – 2.65 (m, 4H), 2.37 (ddd, *J* = 13.7, 6.3, 1.9 Hz, 1H), 2.30 (s, 3H), 2.23 – 2.07 (m, 1H), 1.73 – 1.46 (m, 4H), 1.46 – 1.31 (m, 1H).
^{13}C NMR (CDCl$_3$, 75 MHz) δ 178.5, 167.0, 54.5, 47.6, 46.8, 34.1, 30.5, 27.9, 24.9, 20.9, 11.5.

3-methyl-5-(4-quinuclidin-3-ylmethyl)-1,2,4-oxadiazole fumarate (11i)

The fumarate derivative **11i** was prepared according to the general procedure **5.3.3.5** by reacting **135** (55 mg, 0.27 mmol, 1 equiv) and fumaric acid (31 mg, 0.27 mmol, 1 equiv) in MeOH. After 2h, additional fumaric acid (16 mg, 0.14 mmol, 0.5 equiv) was added to achieve complete consumption of the starting material. The crude salt was recrystallized from *i*-PrOH to provide the pure compound as a white solid (76 mg, yield 80%).


x 1.25 fumarate
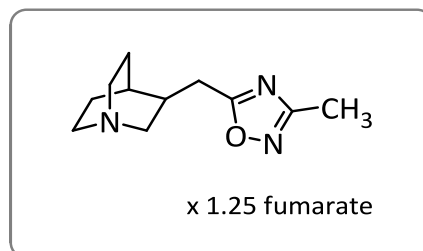
11i: white solid; TLC (alumina-Dragendorff): R_f = 0.30 (CH$_2$Cl$_2$/MeOH 96:4); m.p.: 158.2-158.8°C. MS (ESI) m/z for C$_{11}$H$_{18}$N$_3$O$^+$ [M+H]$^+$ calcd. 208.1, found 208.2.

^{1}H NMR (CD$_3$OD, 300 MHz) δ 6.70 (s, 2.5H), 3.64 (ddd, *J* = 12.8, 10.2, 2.4 Hz, 1H), 3.43 − 3.18 (m, 4H), 3.14 (dd, *J* = 7.7, 4.4 Hz, 2H), 3.04 (ddd, *J* = 13.0, 7.0, 2.0 Hz, 1H), 2.77 − 2.62 (m, 1H), 2.34 (s, 3H), 2.22 − 2.10 (m, 1H), 2.10 − 1.96 (m, 3H), 1.96 − 1.82 (m, 1H).

^{13}C NMR (CD$_3$OD, 75 MHz) δ 179.1, 170.6, 168.5, 136.0, 52.9, 47.5, 47.0, 32.6, 29.9, 25.1, 24.9, 18.9, 11.3.

Borane 3-(pyridin-3-yl)-5-(4-quinuclidin-3-ylmethyl)-1,2,4-oxadiazole complex (123)

The title compound was prepared according to general procedure **5.3.3.3.1** by reacting methyl ester **101** (350 mg, 1.78 mmol, 1 equiv), 3-pyridylamidoxime (**111**) (731 mg, 5.33 mmol, 3 equiv) and NaH 60% dispersion in mineral oil (213 mg, 5.33 mmol, 3 equiv) in dry THF (10 mL). After standard workup and silica gel column chromatography (cyclohexane/EtOAc 1:1), the desired compound **123** was obtained as a pure yellow oil (258 mg, yield 51%).
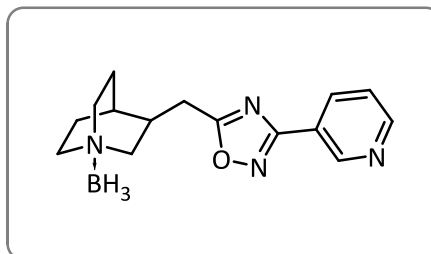
123: yellow oil; TLC (Dragendorff): R$_f$ = 0.28 (cyclohexane/EtOAc 1:1).
^{1}H NMR (CDCl$_3$, 300 MHz) δ 9.22 (d, *J* = 2.1 Hz, 1H), 8.68 (dd, *J* = 4.9, 1.7 Hz, 1H), 8.26 (dt, *J* = 8.0, 1.9 Hz, 1H), 7.37 (dd, *J* = 7.9, 4.9 Hz, 1H), 3.38 – 3.25 (m, 1H), 3.10 – 2.87 (m, 6H), 2.62 (ddd, *J* = 13.5, 6.9, 2.0 Hz, 1H), 2.57 – 2.43 (m, 1H), 1.99 – 1.86 (m, 2H), 1.86 – 1.75 (m, 2H), 1.75 – 1.56 (m, 1H).
^{13}C NMR (CDCl$_3$, 75 MHz) δ 178.0, 166.4, 152.1, 148.5, 134.6, 123.7, 122.8, 59.3, 53.5, 52.8, 33.0, 30.0, 26.1, 24.6, 19.9.

3-(pyridin-3-yl)-5-(4-quinuclidin-3-ylmethyl)-1,2,4-oxadiazole (136)

The title compound was obtained according to the general procedure **5.3.3.4** by reacting **124** (258 mg, 0.91 mmol, 1 equiv) and CF$_3$COOH (351 μL, 4.55 mmol, 5 equiv) in acetone. After disappearance of the starting material (TLC in cyclohexane/EtOAc 1:1), standard workup was applied to provide the desired compound **136** (225 mg, yield 92%).
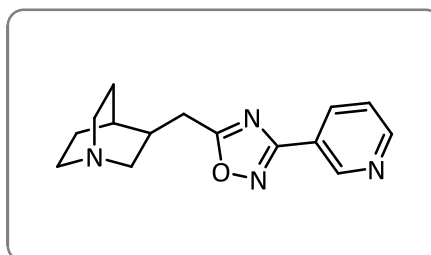
136: yellow oil; TLC (alumina-Dragendorff): R$_f$ = 0.30 (CH$_2$Cl$_2$/MeOH 85:15).
^{1}H NMR (CDCl$_3$, 300 MHz) δ 9.23 (d, *J* = 2.0 Hz, 1H), 8.70 – 8.63 (m, 1H), 8.27 (dt, *J* = 8.0, 2.0 Hz, 1H), 7.35 (ddd, *J* = 8.1, 4.9, 0.8 Hz, 1H), 3.21 – 3.07 (m, 1H), 2.96 (d, *J* = 7.9 Hz, 2H), 2.89 – 2.67 (m, 4H), 2.44 (ddd, *J* = 13.7, 6.2, 1.9 Hz, 1H), 2.32 – 2.15 (m, 1H), 1.79 – 1.67 (m, 1H), 1.67 – 1.63 (m, 1H), 1.63 – 1.49 (m, 2H), 1.49 – 1.34 (m, 1H).
^{13}C NMR (CDCl$_3$, 75 MHz) δ 179.5, 166.5, 152.1, 148.7, 134.7, 123.7, 123.2, 54.6, 47.7, 46.9, 34.4, 30.7, 28.1, 25.1, 21.0.

3-(pyridine-3-yl)-5-(4-quinuclidin-3-ylmethyl)-1,2,4-oxadiazole fumarate (11j)

The fumarate derivative **11j** was prepared according to the general procedure **5.3.3.5** by reacting **136** (225 mg, 0.83 mmol, 1 equiv) and fumaric acid (97 mg, 0.83 mmol, 1 equiv) in MeOH. After 2h, additional fumaric acid (48 mg, 0.42 mmol, 0.5 equiv) was added to achieve complete consumption of the starting material. The crude salt was recrystallized from MeOH/*i*-PrOH to provide the pure compound as a white solid (185 mg, yield 54%).

x 1.25 fumarate
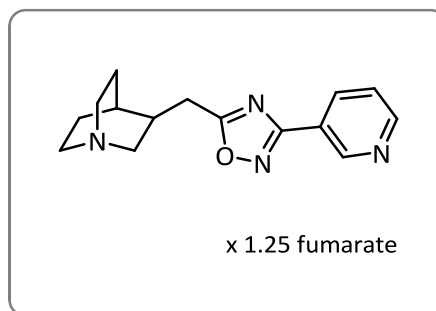
11j: white solid; TLC (alumina-Dragendorff): R_f = 0.39 (CH$_2$Cl$_2$/MeOH 94:6); m.p.: 182.1-183.4°C; MS (ESI) m/z for C$_{15}$H$_{19}$N$_4$O$^+$ [M+H]$^+$ calcd. 271.2, found 271.4.

^{1}H NMR (CD$_3$OD, 300 MHz) δ 9.20 (d, *J* = 1.5 Hz, 1H), 8.72 (dd, *J* = 4.9, 1.5 Hz, 1H), 8.51 – 8.42 (m, 1H), 7.61 (ddd, *J* = 8.0, 5.0, 0.8 Hz, 1H), 6.71 (s, 2.5H), 3.74 (ddd, *J* = 12.8, 10.2, 2.4 Hz, 1H), 3.47 – 3.32 (m, 4H), 3.29 (dd, *J* = 5.2, 2.8 Hz, 2H), 3.14 (ddd, *J* = 13.1, 7.0, 1.9 Hz, 1H), 2.91 – 2.74 (m, 1H), 2.31 – 2.11 (m, 2H), 2.11 – 2.00 (m, 2H), 2.00 – 1.85 (m, 1H).

^{13}C NMR (CD$_3$OD, 75 MHz) δ 180.3, 169.9, 167.5, 152.7, 148.8, 136.6, 135.7, 125.7, 124.9, 53.0, 47.6, 47.1, 32.6, 30.1, 25.3, 24.9, 19.0.

Borane 3-(pyridin-4-yl)-5-(4-quinuclidin-3-ylmethyl)-1,2,4-oxadiazole complex (124)

The title compound was prepared according to general procedure **5.3.3.3.1** by reacting methyl ester **101** (350 mg, 1.78 mmol, 1 equiv), 4-pyridylamidoxime (**112**) (731 mg, 5.33 mmol, 3 equiv) and NaH 60% dispersion in mineral oil (213 mg, 5.33 mmol, 3 equiv) in dry THF (10 mL). After standard workup and silica gel column chromatography (cyclohexane/EtOAc 1:1), the desired compound **124** was obtained as a pure yellow oil (392 mg, yield 78%).
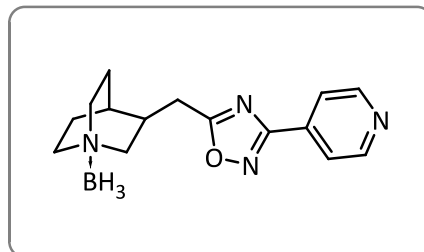
124: yellow oil; TLC (Dragendorff): R$_f$ = 0.33 (cyclohexane/EtOAc 3:7).
^{1}H NMR ((CD$_3$)$_2$SO, 300 MHz) δ 8.79 (dd, J = 4.5, 1.6 Hz, 2H), 7.91 (dd, J = 4.4, 1.7 Hz, 2H), 3.29 – 3.08 (m, 3H), 2.99 – 2.74 (m, 4H), 2.66 – 2.54 (m, 1H), 2.53 – 2.38 (m, 1H), 1.98 – 1.78 (m, 1H), 1.78 – 1.65 (m, 2H), 1.64 – 1.42 (m, 2H).
^{13}C NMR ((CD$_3$)$_2$SO, 75 MHz) δ 179.9, 166.2, 150.9, 133.5, 120.9, 58.7, 53.0, 52.4, 32.3, 29.1, 25.4, 24.1, 19.3.

3-(pyridin-4-yl)-5-(4-quinuclidin-3-ylmethyl)-1,2,4-oxadiazole (137)

The title compound was obtained according to the general procedure **5.3.3.4** by reacting **125** (392 mg, 1.38 mmol, 1 equiv) and CF$_3$COOH (532 µL, 6.90 mmol, 5 equiv) in acetone. After disappearance of the starting material (TLC in cyclohexane/EtOAc 1:1), standard workup was applied to provide the desired compound **137** (340 mg, yield 91%).
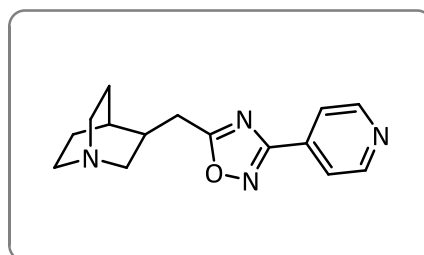
137: yellow oil; TLC (alumina-Dragendorff): R$_f$ = 0.47 (CH$_2$Cl$_2$/MeOH 95:5).
^{1}H NMR (CDCl$_3$, 300 MHz) δ 8.69 (dd, J = 4.4, 1.7 Hz, 2H), 7.85 (dd, J = 4.5, 1.7 Hz, 2H), 3.13 (dd, J = 13.6, 9.8 Hz, 1H), 2.96 (d, J = 7.9 Hz, 2H), 2.86 – 2.67 (m, 4H), 2.42 (ddd, J = 13.8, 6.2, 1.9 Hz, 1H), 2.28 – 2.14 (m, 1H), 1.77 – 1.47 (m, 4H), 1.47 – 1.33 (m, 1H).
^{13}C NMR (CDCl$_3$, 75 MHz) δ 179.8, 166.9, 150.6, 134.2, 121.2, 54.5, 47.6, 46.8, 34.2, 30.6, 27.9, 24.9, 20.9.

3-(pyridin-4-yl)-5-(4-quinuclidin-3-ylmethyl)-1,2,4-oxadiazole fumarate (11k)

The fumarate derivative **11k** was prepared according to the general procedure **5.3.3.5** by reacting **137** (340 mg, 1.26 mmol, 1 equiv) and fumaric acid (146 mg, 1.26 mmol, 1 equiv) in MeOH. After 2h, additional fumaric acid (73 mg, 0.63 mmol, 0.5 equiv) was added to achieve complete consumption of the starting material. The crude salt was recrystallized from MeOH and EtOH to provide the pure compound as a white solid (168 mg, yield 30%).

x 1.5 fumarate
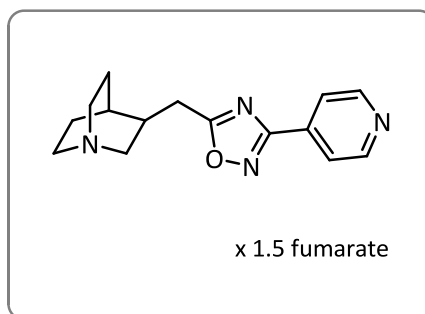
11k: white solid; TLC (alumina-Dragendorff): R_f = 0.30 in CH_2Cl_2/MeOH 95:5; m.p.: 187.8-190.2°C; MS (ESI) m/z for $C_{15}H_{19}N_4O^+$ [M+H]$^+$ calcd. 271.2, found 271.4.

^{1}H NMR (CD$_3$OD, 300 MHz) δ 8.74 (dd, J = 4.7, 1.5 Hz, 2H), 8.04 (dd, J = 4.6, 1.6 Hz, 2H), 6.71 (s, 3H), 3.73 (ddd, J = 12.8, 10.2, 2.4 Hz, 1H), 3.46 – 3.19 (m, 6H), 3.12 (ddd, J = 13.0, 7.0, 1.8 Hz, 1H), 2.89 – 2.73 (m, 1H), 2.26 – 2.10 (m, 2H), 2.10 – 1.99 (m, 2H), 1.99 – 1.84 (m, 1H).

^{13}C NMR (CD$_3$OD, 75 MHz) δ 180.6, 169.9, 167.9, 151.3, 136.4, 135.7, 122.8, 52.9, 47.5, 47.0, 32.6, 30.1, 25.3, 24.9, 19.0.

N'-hydroxyfuran-2-carboximidamide (113)

According to the general procedure **5.3.3.2**, hydroxylamine (50% w/w in water, 790 µL, 12.89 mmol, 4 equiv) was added to a stirred solution of 2-furonitrile (282 µL, 3.22 mmol, 1 equiv) and ethanol (6 mL) at RT. The resulting mixture was heated at 90°C for 1.5h. After concentration under reduced pressure, the desired *N'*-hydroxyfuran-2-carboxyimidamide (**113**) was obtained as a pure white solid (400 mg, yield 99%).
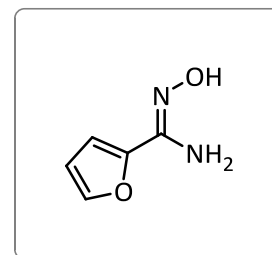
113: white solid; TLC (PMA): R$_f$ = 0.32 (cyclohexane/EtOAc 1:1). m.p.: 57.2 – 57.5°C.
^{1}H NMR (CDCl$_3$, 300 MHz) δ 8.67 (br s, 1H), 7.30 (dd, *J* = 1.7, 0.7 Hz, 1H), 6.69 (dd, *J* = 3.4, 0.6 Hz, 1H), 6.29 (dd, *J* = 3.5, 1.8 Hz, 1H), 5.05 (br s, 2H).
^{13}C NMR (CDCl$_3$, 75 MHz) δ 145.9, 145.5, 142.9, 111.6, 108.4.

Borane 3-(furan-2-yl)-5-(4-quinuclidin-3-ylmethyl)-1,2,4-oxadiazole complex (125)

The title compound was prepared according to general procedure **5.3.3.3.2** by reacting methyl ester **101** (200 mg, 1.01 mmol, 1 equiv), *N'*-hydroxyfuran-2-carboxyimidamide (**113**) (384 mg, 3.04 mmol, 3 equiv) and Cs$_2$CO$_3$ (992 mg, 3.04 mmol, 3 equiv) in dry THF (10 mL). After standard workup and silica gel column chromatography (cyclohexane/EtOAc 7:3 to 3:2), the desired compound **125** was obtained as a pure yellow oil (158 mg, yield 57%).
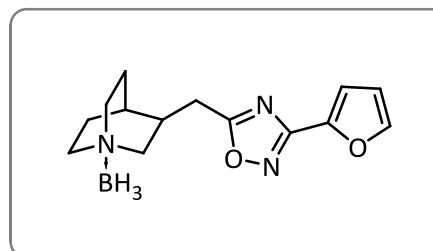
125: yellow oil; TLC (Dragendorff): R$_f$ = 0.24 (cyclohexane/EtOAc 7:3).
^{1}H NMR (CDCl$_3$, 300 MHz) δ 7.55 (dd, *J* = 1.8, 0.8 Hz, 1H), 7.05 (dd, *J* = 3.5, 0.5 Hz, 1H), 6.50 (dd, *J* = 3.3, 1.8 Hz, 1H), 3.36 – 3.22 (m, 1H), 3.06 – 2.86 (m, 6H), 2.59 (ddd, *J* = 13.5, 7.0, 2.1 Hz, 1H), 2.55 – 2.41 (m, 1H), 1.95 – 1.83 (m, 2H), 1.83 – 1.72 (m, 2H), 1.72 – 1.57 (m, 1H).
^{13}C NMR (CDCl$_3$, 75 MHz) δ 177.6, 161.5, 145.4, 142.1, 114.1, 112.0, 59.4, 53.6, 53.0, 33.2, 30.1, 26.3, 24.7, 20.1.

3-(furan-2-yl)-5-(4-quinuclidin-3-ylmethyl)-1,2,4-oxadiazole (138)

The title compound was obtained according to the general procedure **5.3.3.4** by reacting **125** (158 mg, 0.58 mmol, 1 equiv) and CF$_3$COOH (223 µL, 2.89 mmol, 5 equiv) in acetone. After disappearance of the starting material (TLC in cyclohexane/EtOAc 7:3), standard workup was applied to provide the desired compound **138** (146 mg, yield 97%).
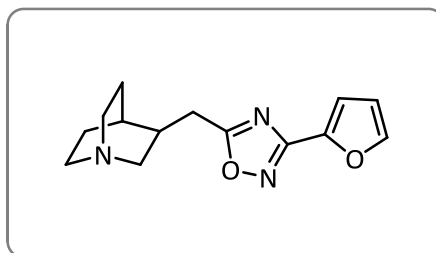

138: yellow oil; TLC (alumina-Dragendorff): R$_f$ = 0.39 (CH$_2$Cl$_2$/MeOH 85:15).
^{1}H NMR (CDCl$_3$, 300 MHz) δ 7.54 (dd, *J* = 1.7, 0.8 Hz, 1H), 7.05 (dd, *J* = 3.5, 0.7 Hz, 1H), 6.49 (ddd, *J* = 3.4, 1.8, 0.7 Hz, 1H), 3.13 (dd, *J* = 13.0, 10.3 Hz, 1H), 2.93 (d, *J* = 7.9 Hz, 2H), 2.89 – 2.67 (m, 4H), 2.43 (dd, *J* = 13.6, 6.3 Hz, 1H), 2.32 – 2.14 (m, 1H), 1.80 – 1.66 (m, 1H), 1.66 – 1.48 (m, 3H), 1.48 – 1.34 (m, 1H).
^{13}C NMR (CDCl$_3$, 75 MHz) δ 179.0, 161.4, 145.2, 142.4, 113.7, 111.9, 54.5, 47.7, 46.9, 34.3, 30.6, 27.9, 25.0, 20.9.

3-(furan-2-yl)-5-(4-quinuclidin-3-ylmethyl)-1,2,4-oxadiazole fumarate (11l)

The fumarate derivative **11l** was prepared according to the general procedure **5.3.3.5** by reacting **138** (78 mg, 0.30 mmol, 1 equiv) and fumaric acid (35 mg, 0.30 mmol, 1 equiv) in MeOH. The crude salt was recrystallized from MeOH/*n*-hexane to provide the pure compound as a white solid (93 mg, yield 83%).

x fumarate
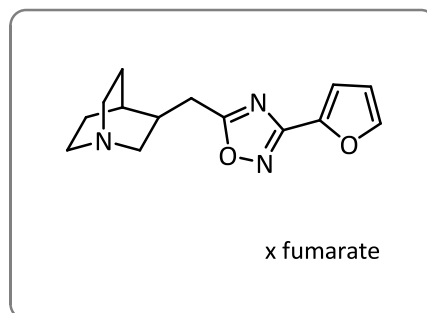
11l: white solid; TLC (alumina-Dragendorff): R$_f$ = 0.44 (CH$_2$Cl$_2$/MeOH 94:6); m.p.: 158.5-159.3°C; MS (ESI) m/z for C$_{14}$H$_{18}$N$_3$O$_2^+$ [M+H]$^+$ calcd. 260.1, found 260.0.
^{1}H NMR (CD$_3$OD, 300 MHz) δ 7.75 (dd, *J* = 1.8, 0.8 Hz, 1H), 7.15 (dd, *J* = 3.5, 0.6 Hz, 1H), 6.68 (s, 2H), 6.65 (dd, *J* = 3.5, 1.8 Hz, 1H), 3.69 (ddd, *J* = 12.8, 10.2, 2.3 Hz, 1H), 3.60 (q, *J* = 7.1 Hz, 0.11H, 5% Et$_2$O residual), 3.45 – 3.27 (m, 4H), 3.23 (dd, *J* = 7.7, 5.1 Hz, 2H), 3.09 (ddd, *J* = 13.0, 7.0, 1.8 Hz, 1H), 2.87 – 2.69 (m, 1H), 2.25 – 2.08 (m, 2H), 2.08 – 1.98 (m, 2H), 1.98 – 1.82 (m, 1H), 1.17 (t, *J* = 7.0 Hz, 0.12H, 5% Et$_2$O residual).
^{13}C NMR (CD$_3$OD, 75 MHz) δ 179.5, 171.3, 162.4, 147.0, 143.5, 136.2, 115.1, 113.0, 52.9, 47.6, 47.1, 32.6, 30.0, 25.2, 24.9, 18.9.

Thiophene-2-amidoxime (114)

According to the general procedure **5.3.3.2**, hydroxylamine (50% w/w in water, 2.63 mL, 42.96 mmol, 4 equiv) was added to a stirred solution of 2-thiophenecarbonitrile (1.0 mL, 10.74 mmol, 1 equiv) and ethanol (8 mL) at RT. The resulting mixture was heated at 90°C for 1.5h. After concentration under reduced pressure, the desired thiophene-2-amidoxime (**114**) was obtained as a pure white solid (1.53 g, yield 100%).
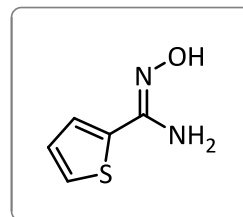
114: white solid; TLC (PMA): R$_f$ = 0.52 (cyclohexane/EtOAc 1:1); m.p.: 90-96°C.
^{1}H NMR (CDCl$_3$, 300 MHz) δ 7.52 – 7.08 (m, 4H), 7.01 (dd, J = 4.9, 3.9 Hz, 1H), 4.98 (br s, 2H).
^{13}C NMR (CDCl$_3$, 75 MHz) δ 148.6, 134.9, 127.2, 126.8, 125.3.

Borane 5-(4-quinuclidin-3-ylmethyl)-3-(thiophen-2-yl)-1,2,4-oxadiazole complex (126)

The title compound was prepared according to general procedure **5.3.3.3.1** by reacting methyl ester **101** (350 mg, 1.78 mmol, 1 equiv), thiophene-2-amidoxime (**114**) (758 mg, 5.33 mmol, 3 equiv) and NaH 60% dispersion in mineral oil (213 mg, 5.33 mmol, 3 equiv) in dry THF (10 mL). After standard workup and silica gel column chromatography (cyclohexane/EtOAc 75:15), the desired compound **126** was obtained as a pure pale yellow oil (336 mg, yield 65%).
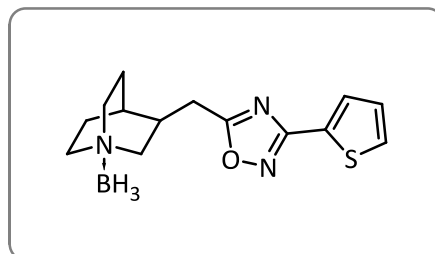
126: pale yellow oil; TLC (Dragendorff): R$_f$ = 0.39 (cyclohexane/EtOAc 7:3).
^{1}H NMR (CDCl$_3$, 300 MHz) δ 7.71 (dd, J = 3.7, 1.2 Hz, 1H), 7.44 (dd, J = 5.0, 1.2 Hz, 1H), 7.09 (dd, J = 5.0, 3.7 Hz, 1H), 3.37 – 3.21 (m, 1H), 3.07 – 2.85 (m, 6H), 2.60 (ddd, J = 13.6, 7.0, 2.1 Hz, 1H), 2.55 – 2.41 (m, 1H), 1.98 – 1.84 (m, 2H), 1.84 – 1.72 (m, 2H), 1.72 – 1.53 (m, 1H).
^{13}C NMR (CDCl$_3$, 75 MHz) δ 177.5, 164.5, 129.7, 129.5, 128.1, 128.0, 59.3, 53.5, 52.9, 33.1, 30.0, 26.1, 24.6, 20.0.

5-(4-quinuclidin-3-ylmethyl)-3-(thiophen-2-yl)-1,2,4-oxadiazole (139)

The title compound was obtained according to the general procedure **5.3.3.4** by reacting **126** (336 mg, 1.16 mmol, 1 equiv) and CF$_3$COOH (447 μL, 5.80 mmol, 5 equiv) in acetone. After disappearance of the starting material (TLC in cyclohexane/EtOAc 3:2 and CH$_2$Cl$_2$/MeOH 9:1), standard workup was applied to provide the desired compound **139** (320 mg, yield 100%).
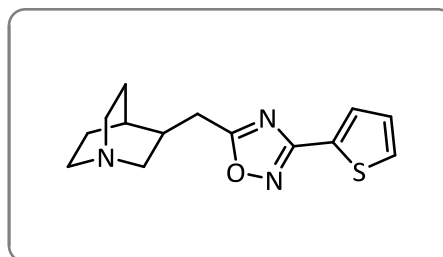
142: yellow oil; TLC (alumina-Dragendorff): R$_f$ = 0.38 (CH$_2$Cl$_2$/MeOH 85:15).
^{1}H NMR (CDCl$_3$, 300 MHz) δ 7.71 (d, *J* = 3.7 Hz, 1H), 7.42 (d, *J* = 5.0 Hz, 1H), 7.08 (dd, *J* = 5.0, 3.7 Hz, 1H), 3.19 – 3.03 (m, 1H), 2.92 (d, *J* = 7.8 Hz, 2H), 2.86 – 2.65 (m, 5H), 2.41 (ddd, *J* = 13.6, 6.3, 1.8 Hz, 2H), 2.29 – 2.13 (m, 1H), 1.77 – 1.47 (m, 5H), 1.47 – 1.31 (m, 1H).
^{13}C NMR (CDCl$_3$, 75 MHz) δ 179.0, 164.5, 129.6, 129.3, 128.5, 128.1, 54.6, 47.8, 46.9, 34.4, 30.7, 28.1, 25.1, 21.0.

5-(4-quinuclidin-3-ylmethyl)-3-(thiophen-2-yl)-1,2,4-oxadiazole fumarate (11m)

The fumarate derivative **11m** was prepared according to the general procedure **5.3.3.5** by reacting **139** (320 mg, 1.16 mmol, 1 equiv) and fumaric acid (135 mg, 1.16 mmol, 1 equiv) in MeOH. The crude salt was recrystallized from acetone to provide the pure compound as a white solid (303 mg, yield 72%).

x 0.75 fumarate
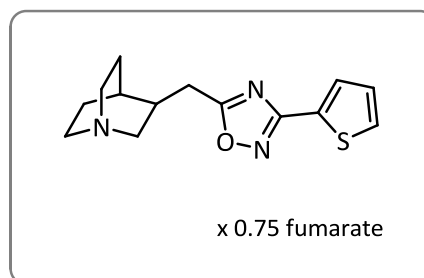
11m: white solid; TLC (alumina-Dragendorff): R$_f$ = 0.35 (CH$_2$Cl$_2$/MeOH 96:4); m.p.:145.3-146.8°C; MS (ESI) m/z for C$_{14}$H$_{18}$N$_3$OS$^+$ [M+H]$^+$ calcd. 276.1, found 276.6.
^{1}H NMR (CD$_3$OD, 300 MHz) δ 7.79 (dd, *J* = 3.6, 1.2 Hz, 1H), 7.68 (dd, *J* = 5.0, 1.1 Hz, 1H), 7.20 (dd, *J* = 5.0, 3.7 Hz, 1H), 6.68 (s, 1.5H), 3.70 (ddd, *J* = 12.8, 10.2, 2.4 Hz, 1H), 3.46 – 3.27 (m, 4H), 3.23 (dd, *J* = 7.7, 5.8 Hz, 2H), 3.11 (ddd, *J* = 13.0, 7.0, 1.7 Hz, 1H), 2.86 – 2.71 (m, 1H), 2.27 – 2.09 (m, 2H), 2.09 – 1.99 (m, 2H), 1.99 – 1.85 (m, 1H).
^{13}C NMR (CD$_3$OD, 75 MHz) δ 179.5, 171.4, 165.7, 136.2, 130.9, 130.8, 129.2, 129.1, 53.0, 47.6, 47.1, 32.6, 30.0, 25.3, 24.9, 19.0.

CHAPTER VI

Conclusions

The experimental activity of this PhD thesis focused on the design, synthesis and pharmacological evaluation of several classes of compounds, PAMs and silent agonists, to in depth study the mechanism of activation of the alpha7 nAChR involved in the cholinergic inflammatory pathway.

➢ **Chemistry**

- Synthesis of alpha7 **PAMs derivatives**
  Through a hybridization approach two sets of 5-HI compounds and were synthesized by connecting aromatic moiety to the nitrogen atom (**1a**-**f**) or to the carbon atom (**2a**-**m**, **3**, **4a**-**b**) of the indole nucleus.

- Synthesis of alpha7 **Silent agonists**
  Three different series of new potential alpha7 silent agonists were designed and prepared:
  ▪ diEPP analogues (**6a**-**u**, **7**, **8**)
  ▪ quinuclidine spirocyclic-Δ^2-isoxazoline nucleus (**9a**-**h**, **10a**-**h**)
  ▪ quinuclidin-oxadiazole derivatives (**11a**-**m**, **12a**-**g**)

➢ **Pharmacology**

The target compounds obtained were evaluated in electrophysiological experiments in *Xenopus* oocytes expressing human alpha7 nAChRs. To date only a few data are available because many derivatives are still under investigation.

- Three compounds **2b**,**2f** and **2g** of the PAM indole series, displayed enhanced PAM activity with respect to the parent compund. In particular, **2b** showed a potentiated activity two folds higher than 5-HI and comparable to the one of genistein. From the CRCs were generated at different concentrations, compound **2g** resulted more potent and about twice as efficacious as 5-HI, whereas derivatives **2b** and **2f** turned out to be far more efficacious than the parent compound, but not much more potent.
- The electrophysiological profile of the novel diEPP derivatives **6a**-**u** was greatly influenced by the nature and sensitive to the position of the aryl substituent. **6a**-**u** exhibited a broad range of activities, including partial agonism and various degrees of silent agonism at the alpha7 receptor subtype, while some compounds were effectively inactive, displaying no agonism and very weak silent agonism. In particular, compounds **6g**, **6i**, **6r** and **6s** showed the best silent agonism profile of the series. the enhanced silent agonism achieved with trifluoromethyl, fluoro and carboxamide groups arise from a combination of multifactorial effects, polarity, lipophilicity, hydrogen and fluorine bonding, which bear in common the ability to selectively stabilize the desensitized state of the alpha7 receptor sensitive to PNU-120596. Besides, target derivatives **7** and **8** showed enhanced PNU-120596 potentiation compared to the parent compound and in particular **7** appeared to be superior for the better ratio of desensitization to residual partial agonism compared to **8**.

Abbreviations

Solvents

DCM or CH_2Cl_2: dichloromethane; $CHCl_3$: chloroform; MeOH: methanol; EtOH: ethanol; EtOAc: ethyl acetate; MeCN: acetonitrile; THF: tetrahydrofuran; Et_2O: diethyl ether; H_2O: water; DMSO: dimethyl sulfoxide; DMF: dimethylformamide; $CDCl_3$: deuterated chloroform; CD_3OD: deuterated methanol; $(CD_3)_2SO$: deuterated dimethyl sulfoxide; $(CD_3)_2CO$: deuterated acetone.

Reagents

NaH: sodium hydride; Na_5O_4: sodium sulfate, $NaHCO_3$: sodium bicarbonate; Na_2CO_3: sodium carbonate; K_2CO_3: potassium carbonate; CF_3COOH: trifluoroacetic acid; CH_3I: methyl iodide; HCl: hydrochloric acid; H_2: hydrogen; Pd/C: palladium on carbon; BH_3 in THF: borane tetrahydrofuran complex; $KMnO_4$: potassium permanganate; PMA: phosphomolybdic acid; $Na_2S_2O_3$: sodium thiosulfate; $CuSO_4$: copper sulfate; *n*-BuLi: *n*-butyllithium; NH_4Cl: ammonium chloride; CuI: copper iodide; Na_2SO_3: sodium sulfite; $Pd(PPh_3)_2Cl_2$: bis(triphenylphosphine)palladium(II) dichloride; Et_3N: triethylamine; Boc: *tert*-butoxycarbonyl; NIS: *N*-iodosucinimide; BTMA ICl_4: benzyl(trimethyl)ammonium tetrachloroiodate; DMAP: 4-(Dimethylamino)pyridine; BBr_3: boron tribromide; MEM: methoxyethoxymethyl; Cs_2CO_3: cesium carbonate; EtI: iodoethane; MeI: iodomethane; TBAF: tetrabutylammonium fluoride; DIPEA: *N,N*-Diisopropylethylamine; DMF/DMA: *N,N*-Dimethylformamide dimethyl acetal.

Analytical characteristics:

GENERAL – m.p.: melting point; °C: Celsius degree; RT: room temperature; h: hours; min: minutes; equiv.: equivalents;

NMR Spectroscopy – MHz: megaHertz; δ: chemical shift (ppm); ppm: parts per million; *J:* NMR coupling constant; Hz: Hertz; s: singlet; br s: broad singlet; d: doublet; dd: double of doublets; ddd: double of doublet of doublet; dt: double of triplets; t: triplet; q: quartet; m: multiplet.

MASS Spectroscopy – *m/z*: mass to charge ratio; M^+: molecular ion.

CHROMATOGRAPHY – TLC: thin layer chromatography; R_f : retention factor.

References

[1] A. Chatzidaki, J. M. D'Oyley, J. K. Gill-Thind and T. D. Sheppard, "The influence of allosteric modulators and transmembrane mutations on desensitisation and activation of a7 nicotinic acetylcholine receptors," *Neuropharmacology,* vol. 97, pp. 75-85, 2015.

[2] A. Chatzidaki and N. S. Millar, "Allosteric modulation of nicotinic acetylcholine receptors," *Biochemical Pharmacology,* vol. 97, pp. 408-417, 2015.

[3] K. T. Dineley, A. A. Pandya and J. L. Yakel, "Nicotinic ACh receptors as therapeutic targets in CNS disorders," *Trends in Pharmacological Sciences,* vol. 36, no. 2, pp. 96-108, 2015.

[4] V. V. Uteshev, "The therapeutic promise of positive allosteric modulation of nicotinic receptors," *European Journal of Pharmacology,* vol. 727, pp. 181-185, 2014.

[5] D. Paterson and A. Nordberg, "Neuronal nicotinic receptors in the human brain," *Progress in Neurobiology ,* vol. 61, pp. 75-111, 2000.

[6] D. C. Kombo, A. Mazurov, K. Tallapragada, P. S. Philip S. Hammond, J. Chewning, T. A. Terry A. Hauser, M. Vasquez-Valdivieso, D. Yohannes, T. T. Talley, P. Taylor and W. S. Caldwell, "Docking studies of benzylidene anabaseine interactions with a7 nicotinic acetylcholine receptor (nAChR) and acetylcholine binding proteins (AChBPs): Application to the design of related a7 selective ligands," *European Journal of Medicinal Chemistry,* vol. 46, pp. 5625-5635, 2011.

[7] R. L. Papke, C. Stokes, D. Williams, J. Wang and N. A. Horenstein, "Cysteine accessibility analysis of the human alpha7 nicotinic acetylcholine receptor ligand-binding domain identifies L119 as a gatekeeper," *Neuropharmacology,* vol. 60, no. 1, pp. 159-171, 2011.

[8] A. A. Jensen, B. Frølund, T. Liljefors and P. Krogsgaard-Larsen, "Neuronal Nicotinic Acetylcholine Receptors: Structural Revelations, Target Identifications, and Therapeutic Inspirations," *Journal of Medicinal Chemistry,* vol. 48, no. 15, pp. 4705-4745, 2005.

[9] R. Exley and S. J. Cragg, "Presynaptic nicotinic receptors: a dynamic and diverse cholinergic filter of striatal dopamine neurotransmission," *British Journal of Pharmacology,* vol. 153, p. S283–S297, 2008.

[10] M. N. Romanelli and F. Gualtieri, "Cholinergic Nicotinic Receptors: Competitive Ligands, Allosteric Modulators, and Their Potential Applications," *Medicinal Research Reviews,* vol. 23, no. 4, pp. 393-426, 2003.

[11] J. Dani and D. Bertrand, "Nicotinic Acetylcholine Receptors and Nicotinic Cholinergic Mechanisms of the Central Nervous System," *Annual Review of Pharmacology and Toxicology,* vol. 47, pp. 699-729, 2007.

[12] R. J. Lukas, J. P. Changeux, N. Le Novère, E. X. Albuquerque, D. Balfour, D. K. Berg, D. Bertrand, V. A. Chiappinelli, P. B. Clarke, A. C. Collins, J. A. Dani, S. R. Grady, K. J. Kellar, J. M. Lindstrom, M. J. Marks, M. Quik, P. Taylor and S. Wonnacott, "International Union of Pharmacology. XX. Current status of the nomenclature for nicotinic acetylcholine receptors and their subunits," *Pharmacological Reviews,* vol. 51, no. 2, pp. 397-401, 1999.

[13] P. van Nierop, S. Bertrand, D. W. Munno, Y. Gouwenberg, J. van Minnen, J. D. Spafford, N. I. Syed, D. Bertrand and A. B. Smit, "Identification and Functional Expression of a

Family of Nicotinic Acetylcholine Receptor Subunits in the Central Nervous System of the Mollusc Lymnaea stagnalis," *The Journal of Biological Chemistry,* vol. 281, pp. 1680-1691, 2006.

[14] N. Bocquet, L. Prado de Carvalho, J. Cartaud, J. Neyton, C. Le Poupon, A. Taly, T. Grutter, J. Changeux and P. J. Corringer, "A prokaryotic proton-gated ion channel from the nicotinic acetylcholine receptor family," *Nature,* vol. 445, no. 7123, pp. 116-119, 2007.

[15] R. J. Hilf and R. Dutzler, "A prokaryotic perspective on pentameric ligand-gated ion channel structure.," *Current Opinion in Structural Biology,* vol. 19, no. 4, pp. 418-424, 2009.

[16] P. Newhouse, A. Singh and A. Potter, "Nicotine and nicotinic receptor involvement in neuropsychiatric disorders," *Current Topics in Medicinal Chemistry ,* vol. 4, no. 3, pp. 267-282, 2004.

[17] N. Ripoll, M. Bronnec and M. Bourin, "Nicotinic receptors and schizophrenia.," *Current Medical Research and Opinion ,* vol. 20, pp. 1057 - 1074, 2004.

[18] R. Shytle, A. A. R. J. Silver, M. B. Newman, D. V. Sheehan and P. R. Sanberg, "The Nicotinic Acetylcholine Receptor as a Target for Antidepressant Drug Development," *Molecular Psychiatry,* vol. 7, pp. 525 - 535, 2002.

[19] S. Wonnacott, "Presynaptic nicotinic ACh receptors," *Trends in Neurosciences,* vol. 20, no. 2, pp. 92-98, 1997.

[20] R. L. Papke, "Merging old and new perspectives on nicotinic acetlycholine receptors," *Biochemical Pharmacology,* vol. 89, pp. 1-11, 2014.

[21] R. Faghih, M. Gopalakrishnan and C. A. Briggs, "Allosteric modulators of the alpha7 nicotinic acetylcholine receptor," *Journal of Medicinal Chemistry,* vol. 51, no. 4, pp. 701-712, 2008.

[22] K. Krisztina Pesti, A. K. Szabo, A. Mike and E. S. Vizi, "Kinetic properties and open probability of a7 nicotinic acetylcholine receptors," *Neuropharmacology,* vol. 81, pp. 101-115, 2014.

[23] R. L. Papke and J. K. Porter Papke, "Comparative pharmacology of rat and human a7 nAChR conducted with net charge analysis," *British Journal of Pharmacology ,* vol. 137, pp. 49-61, 2002.

[24] D. K. Williams, J. Wang and R. L. Papke, "Investigation of the Molecular Mechanism of the a7 Nicotinic Acetylcholine Receptor Positive Allosteric Modulator PNU-120596 Provides Evidence for Two Distinct Desensitized States," *Molecular Pharmacology,* vol. 80, pp. 1013-1032, 2011.

[25] D. Williams, C. Peng, M. Kimbrell and R. Papke, "Intrinsically Low Open Probability of a7 Nicotinic Acetylcholine Receptors Can Be Overcome by Positive Allosteric Modulation and Serum Factors Leading to the Generation of Excitotoxic Currents at Physiological Temperatures," *Molecular Pharmacology,* vol. 82, pp. 746-759, 2012.

[26] D. K. Williams, J. Wang and R. L. Papke, "Positive allosteric modulators as an approach to nicotinic acetylcholine receptor-targeted therapeutics: advantages and limitations," *Biochemical Pharmacology,* vol. 82, no. 8, pp. 915-930, 2011.

[27] D. Bertrand and M. Gopalakrishnan, "Allosteric modulation of nicotinic acetylcholine receptors," *Biochemical Pharmacology ,* vol. 74, no. 8, pp. 1155-1163, 2007.

[28] M. D. Isaacson, N. A. Horenstein, C. Stokes, W. R. Kem and R. L. Papke, "Point-to-point ligand-receptor interactions across the subunit interface modulate the induction and stabilization of conformational states of alpha7 nAChR by benzylidene anabaseines," *Biochemical Pharmacology,* vol. 85, no. 6, pp. 817-828, 2013.

[29] M. S. Thomsen and J. D. Mikkelsen, "The α7 nicotinic acetylcholine receptor ligands methyllycaconitine, NS6740 and GTS-21 reduce lipopolysaccharide-induced TNF-α release from microglia," *Journal of Neuroimmunology,* vol. 251, pp. 65-72, 2012.

[30] K. Chojnacka, R. L. Papke and N. A. Horenstein, "Synthesis and evaluation of a conditionally-silent agonist for the a7 nicotinic acetylcholine receptor," *Bioorganic & Medicinal Chemistry Letters,* vol. 23, no. 14, pp. 4145-4149, 2013.

[31] M. L. Sinkus, S. Graw, R. Freedman, R. G. Ross, H. A. Lester and S. Leonard, "The human CHRNA7 and CHRFAM7A genes: A review of the genetics, regulation, and function," *Neuropharmacology,* vol. 96, pp. 274-288, 2015.

[32] J. Gault, M. Robinson, R. Berger, C. Drebing, J. Logel, J. Hopkins, T. Moore, S. Jacobs, J. Meriwether, M. Choi, E. Kim, K. Walton, K. Buiting, A. Davis, C. Breese, R. Freedman and S. Leonard, "Genomic organization and partial duplication of the human alpha7 neuronal nicotinic acetylcholine receptor gene (CHRNA7)," *Genomics,* vol. 52, pp. 173 - 185, 1998.

[33] T. Costantini, X. Dang, R. Coimbra, B. Eliceiri and A. Baird, "CHRFAM7A, a human-specific and partially duplicated α7-nicotinic acetylcholine receptor gene with the potential to specify a human-specific inflammatory response to injury," *Journal of Leukocyte Biology,* vol. 97, pp. 1 - 11, 2015.

[34] R. Clark, D. Lamppu, L. Libertine, A. McDonough, A. Kumar, G. LaRosa, R. Rush and D. Elbaum, "Discovery of Novel 2-((Pyridin-3-yloxy)methyl)piperazines as α7 Nicotinic Acetylcholine Receptor Modulators for the Treatment of Inflammatory Disorders," *Journal of Medicinal Chemistry,* vol. 57, no. 10, pp. 3966-3983, 2014.

[35] W. J. de Jonge and L. Ulloa, "The alpha7 nicotinic acetylcholine receptor as a pharmacological target for inflammation," *British Journal of Pharmacology,* vol. 151, p. 915 – 929, 2007.

[36] M. B. Marrero, R. Lucas, C. Salet, T. A. Hauser and A. Mazurov, "An α7 Nicotinic Acetylcholine Receptor-Selective Agonist Reduces Weight Gain and Metabolic Changes in a Mouse Model of Diabetes," *The Journal of Pharmacology and Experimental Therapeutics,* vol. 332, pp. 173-180, 2010.

[37] H. Wang, M. Yu, M. Ochani, C. Amella, M. Tanovic, S. Susarla, J. Li, H. Wang, H. Yang, L. Ulloa, Y. Al-Abed, C. Czura and K. Tracey, "Nicotinic acetylcholine receptor α7 subunit is an essential regulator of inflammation," *Nature,* vol. 421, pp. 384 - 388, 2003.

[38] D. Martelli, M. J. McKinley and R. M. McAllen, "The cholinergic anti-inflammatory pathway: A critical review," *Autonomic Neuroscience: Basic and Clinical,* vol. 182, pp. 65-69, 2014.

[39] C. A. Báez-Pagán, M. Manuel Delgado-Vélez and J. A. Lasalde-Dominicci, "Activation of the Macrophage α7 Nicotinic Acetylcholine Receptor and Control of Inflammation," *Journal of Neuroimmune Pharmacology,* vol. 10, p. 468–476, 2015.

[40] P. Filippini, A. Cesario, M. Fini, F. Locatelli and S. Rutella, "The Yin and Yang of Non-Neuronal α7-Nicotinic Receptors in Inflammation and Autoimmunity," *Current Drug Targets,* vol. 13, no. 5, pp. 644-655, 2012.

[41] D. Wang, Zhou, R. and Y. Yao, "Role of cholinergic anti-inflammatory pathway in regulating host response and its interventional strategy for inflammatory diseases," *Chinese Journal of Traumatology* , vol. 12, no. 6, pp. 355-364, 2009.

[42] H. Yoshikawa, M. Kurokawa, N. Ozaki, K. Nara, K. Atou, E. Takada, H. Kamochi and N. Suzuki, "Nicotine inhibits the production of proinflammatory mediators in human monocytes by suppression of I-kB phosphorylation and nuclear factor-kB transcriptional activity through nicotinic acetylcholine receptor a7," *Clinical and Experimental Immunology,* vol. 146, pp. 116-123, 2006.

[43] T. Li, S. Wu, H. Zhang, Y. Wang, H. Luo, X. Zuo and X. Xiao, "Activation of Nicotinic Receptors Inhibits TNF-α-Induced Production of Pro-inflammatory Mediators Through the JAK2/STAT3 Signaling Pathway in Fibroblast-Like Synoviocytes," *Inflammation,* vol. 38, no. 4, pp. 1424-1433, 2015.

[44] G. Peña, B. Cai, J. Liu, E. P. van der Zanden, E. A. Deitch, W. J. de Jonge and L. Ulloa, "Unphosphorylated STAT3 modulates alpha7 nicotinic receptor signaling and cytokine production in sepsis," *European Journal of Immunology,* vol. 40, no. 9, p. 2580–2589, 2010.

[45] W. J. de Jonge, E. P. van der Zanden, F. O. The, M. F. Bijlsma, D. J. van Westerloo, R. J. Roelof J Bennink, H. Berthoud, S. Uematsu, S. Akira, R. M. van den Wijngaard and G. E. Boeckxstaens, "Stimulation of the vagus nerve attenuates macrophage activation by activating the Jak2-STAT3 signaling pathway," *Nature Immunology,* vol. 6, no. 8, pp. 844-851, 2005.

[46] M. V. Skok, "Editorial: To channel or not to channel? Functioning of nicotinic acetylcholine receptors in leukocytes," *Journal of Leukocyte Biology,* vol. 86, pp. 1-3, 2009.

[47] B. J. Melancon, C. R. Hopkins, M. R. Wood, K. A. Emmitte, C. M. Niswender, A. Christopoulos, J. Conn and C. W. Lindsley, "Allosteric Modulation of Seven Transmembrane Spanning Receptors: Theory, Practice, and Opportunities for Central Nervous System Drug Discovery," *Journal of Medicinal Chemistry,* vol. 55, no. 4, p. 1445–1464, 2012.

[48] M. S. Thomsen and J. D. Mikkelsen, "Type I and II positive allosteric modulators differentially modulate agonist-induced up-regulation of α7 nicotinic acetylcholine receptors," *Journal of Neurochemistry,* vol. 123, no. 1, pp. 73-83, 2012.

[49] K. Freitas, S. Ghosh, F. I. Carroll, A. H. Lichtman and M. I. Damaj, "Effects of alpha 7 positive allosteric modulators in murine inflammatory and chronic neuropathic pain models," *Neuropharmacology,* vol. 65, p. 156–164, 2013.

[50] S. Banerjee, Y. Li, Z. Wang and F. H. Sarkar, "Multi-targeted therapy of cancer by genistein," *Cancer Letters,* vol. 269, no. 2, pp. 226-242, 2008.

[51] G. Ji, Y. Zhang, Q. Yang, S. Cheng, J. Hao, X. Zhao and Z. Jiang, "Genistein suppresses LPS-induced inflammatory response through inhibiting NF-κB following AMP kinase activation in RAW 264.7 macrophages," *PLOS ONE,* vol. 7, no. 12, pp. 1-7, 2012.

[52] R. Cancello, A. Zulian, S. Maestrini, M. Mencarelli, A. Della Barba, C. Invitti, A. Liuzzi and A. M. Di Blasio, "The nicotinic acetylcholine receptor α7 in subcutaneous mature adipocytes: downregulation in human obesity and modulation by diet-induced weight loss," *International Journal of Obesity,* vol. 36, no. 12, pp. 1552-1557, 2012.

[53] K. Freitas, S. S. Negus, F. I. Carroll and M. I. Damaj, "In vivo pharmacological

interactions between a type II positive allosteric modulator of a7 nicotinicACh receptors and nicotinic agonists in a murine tonic pain model," *British Journal of Pharmacology,* vol. 169, p. 567–579, 2013.

[54] K. Freitas, F. I. Carroll and M. I. Damaj, "The Antinociceptive Effects of Nicotinic Receptors a7-Positive Allosteric Modulators in Murine Acute and Tonic Pain Models," *The Journal of Pharmacology and Experimental Therapeutics,* vol. 344, p. 264–275, 2013.

[55] G. Munro, R. R. Hansen, H. K. Erichsen, D. B. Timmermann and J. K. Christensen, "The a7 nicotinic ACh receptor agonist compound B and positive allosteric modulator PNU-120596 both alleviate inflammatory hyperalgesia and cytokine release in the rat," *British Journal of Pharmacology ,* vol. 167, p. 421–435, 2012.

[56] R. L. Papke, D. Bagdas, A. R. Kulkarni, T. Gould, S. D. AlSharari, G. A. Thakur and M. I. Damaj, "The analgesic-like properties of the alpha7 nAChR silent agonist NS6740 is associated with non-conducting conformations of the receptor," *Neuropharmacology,* vol. 91, pp. 34-42, 2015.

[57] R. L. Papke, K. Chojnacka and N. A. Horenstein, "The Minimal Pharmacophore for Silent Agonism of the a7 Nicotinic Acetylcholine Receptor," *The Journal of Pharmacology and Experimental Therapeutics ,* vol. 350, pp. 665-680, 2014.

[58] R. Zwart, G. De Filippi, L. M. Broad, G. I. McPhie, K. H. Pearson and T. Baldwinson, "5-Hydroxyindole potentiates human α7 nicotinic receptor-mediated responses and enhances acetylcholine-induced glutamate release incerebellar slices," *Neuropharmacology ,* vol. 43, pp. 374-384, 2002.

[59] M. Grilli, L. Raiteri, L. Patti, M. Parodi, F. Robino, M. Raiteri and M. Marchi, "Modulation of the function of presynaptic a7 and non-a7 nicotinic receptors by the tryptophan metabolites, 5-hydroxyindole and kynurenate in mouse brain," *British Journal of Pharmacology ,* vol. 149, pp. 724-732, 2006.

[60] J. A. van Hooft and H. P. M. Henk P.M. Vijverberg, "Selection of distinct conformational states of the 5-HT3 receptor by full and partial agonists," *British Journal of Pharmacology ,* vol. 117, pp. 839-846, 1996.

[61] A. Rusin, Z. Krawczyk, G. Grynkiewicz, A. Gogler, J. Zawisza-Puchałka and W. Szeja, "Synthetic derivatives of genistein, their properties and possible applications," *Acta Biochimica Polonica,* vol. 57, no. 1, pp. 23-34, 2010.

[62] A. A. Ganai and H. Farooqi, "Bioactivity of genistein: A review of in vitro and in vivo studies," *Biomedicine & Pharmacotherapy ,* vol. 76, pp. 30-38, 2015.

[63] B. C. Barlaam and T. M. Piser, "ESTROGEN RECEPTOR-B LIGANDS," *WO 2000062765,* 2000.

[64] C. Dallanoce, P. Magrone, C. Matera, F. Frigerio, G. Grazioso, M. De Amici, S. Fucile, V. Piccari, K. Frydenvang, L. Pucci, C. Gotti, F. Clementi and C. De Micheli, "Design, Synthesis, and Pharmacological Characterization of Novel Spirocyclic Quinuclidinyl-Δ2-Isoxazoline Derivatives as Potent and Selective Agonists of α7 Nicotinic Acetylcholine Receptors," *ChemMedChem,* vol. 6, p. 889 – 903, 2011.

[65] C. Dallanoce, P. Magrone, P. Bazza, G. Grazioso, L. Rizzi, L. Riganti, C. Gotti, F. Clementi, K. Frydenvang and M. De Amici, "New Analogues of Epiboxidine Incorporating the 4,5-Dihydroisoxazole Nucleus: Synthesis, Binding Affinity at Neuronal Nicotinic Acetylcholine Receptors, and Molecular Modeling Investigations," *Chemistry and*

Biodiversity, vol. 6, pp. 244-259, 2009.

[66] H. Zhang, Q. Qian Cai and D. Ma, "Amino Acid Promoted CuI-Catalyzed C-N Bond Formation between Aryl Halides and Amines or N-Containing Heterocycles," *Journal of Organic Chemistry,* vol. 70, pp. 5164-5173, 2005.

[67] D. Ma, Q. Qian Cai and H. Zhang, "Mild Method for Ullmann Coupling Reaction of Amines and Aryl Halides," *Organic Letters,* vol. 5, no. 14, pp. 2453-2455, 2003.

[68] D. Ma and Q. Qian Cai, "L-Proline Promoted Ullmann-Type Coupling Reactions of Aryl Iodides with Indoles, Pyrroles, Imidazoles or Pyrazoles," *Synlett ,* vol. 1, pp. 128-130, 2004.

[69] E. Elliott, E. R. Bowkett, J. L. Maggs and J. Bacsa, "Convenient Syntheses of Benzo-Fluorinated Dibenz[b,f]azepines: Rearrangements of Isatins, Acridines, and Indoles," *Organic Letters,* vol. 13, no. 20, pp. 5592-5595, 2011.

[70] J. C. K. A. Antilla and S. L. Buchwald, "The Copper-Catalyzed N-Arylation of Indoles," *Journal of American Chemical Society,* vol. 124, no. 39, p. 11684–11688, 2002.

[71] M. Frotscher, E. Ziegler, S. Marchais-Oberwinkler, P. Kruchten, A. Neugebauer, L. Fetzer, C. Scherer, U. Müller-Vieira, J. Messinger, H. Thole and H. R. W., "Design, synthesis, and biological evaluation of (hydroxyphenyl)naphthalene and -quinoline derivatives: potent and selective nonsteroidal inhibitors of 17beta-hydroxysteroid dehydrogenase type 1 (17beta-HSD1) for the treatment of estrogen-dependent disease," *Journal of Medicinal Chemistry ,* vol. 51, no. 7, pp. 2158-2169, 2008.

[72] D. K. Cashion, G. Chen, D. Kasi, H. C. Kolb, C. Liu, A. Sinha, A. K. Szardenings, E. Wang, C. Yu, W. Zhang, U. B. Gangadharmath and J. C. Walsh, "Preparation of aromatic and heteroaromatic derivatives as imaging agents for detecting neurological disorders," *WO 2011119565 ,* 2011.

[73] S. Beaumont, P. Retailleau, P. Dauban and R. H. Dodd, "Synthesis of Indolobenzazepinones by Application of an Isocyanide-Based Multicomponent Reaction," *European Journal Organic Chemistry,* vol. 2008, no. 30, pp. 5162-5175, 2008.

[74] E. Nuti, F. Casalini, S. I. Avramova, S. Santamaria, M. Fabbi and S. Silvano Ferrini, "Potent Arylsulfonamide Inhibitors of Tumor Necrosis Factor-r Converting Enzyme Able to Reduce Activated Leukocyte Cell Adhesion Molecule Shedding in Cancer Cell Models," *Journal of Medicinal Chemistry,* vol. 53, p. 2622–2635, 2010.

[75] N. M. Padial, E. Quartapelle Procopio, C. Montoro, E. Lopez, J. E. Oltra, V. Colombo, A. Maspero, N. Masciocchi, S. Simona Galli, I. Senkovska, S. Kaskel, E. Barea and J. A. R. Navarro, "Highly Hydrophobic Isoreticular Porous Metal–Organic Frameworks for the Capture of Harmful Volatile Organic Compounds," *Angewandte Chemie, International Edition,* vol. 52, no. 32, pp. 8290-8294, 2013.

[76] K. Ghosh, D. Kar, S. Joardar, D. Sahu and B. Ganguly, "Azaindole-1,2,3-triazole conjugate as selective fluorometric sensor for dihydrogenphosphate," *Royal Society of Chemistry Advances,* vol. 3, pp. 16144-16151, 2013.

[77] S. Hamri, J. Rodríguez, J. Basset, G. Guillaumet and M. D. Pujol, "A convenient iodination of indoles and derivatives," *Tetrahedron ,* vol. 68, pp. 6269-6275, 2012.

[78] S. D. Kuduk, R. K. Chang, J. M.-C. Wai, C. N. Di Marco, V. Cofre, R. M. DiPardo, S. P. Cook, M. J. Cato, A. Jovanovska, M. O. Urban, M. Leitl, R. H. Spencer, S. A. Kane, G. D. Hartman and M. T. Bilodeau, "Amidine derived inhibitors of acid-sensing ion channel-3

(ASIC3)," *Bioorganic & Medicinal Chemistry Letters ,* vol. 19, p. 4059–4063, 2009.

[79] C. Thiebes, G. K. S. Prakash, N. A. Petasis and G. A. Olah, "Mild Preparation of Haloarenes by Ipso-Substitution of Arylboronic Acids with N-Halosuccinimides," *Synlett,* vol. 2, pp. 141-142, 1998.

[80] B. Willy and T. J. J. J. J. Müller, "Consecutive multi-component syntheses of heterocycles via palladium-copper catalyzed generation of alkynones," *ARKIVOC ,* vol. i, pp. 195-208, 2008.

[81] J. D. S. Denis, J. S. I. Gordon, V. M. Carroll and R. Priefer, "Novel Synthesis of the Isoflavone Genistein," *Synthesis,* no. 10, pp. 1590-1592, 2010.

[82] Y. B. Sinkevich, A. E. Shchekotikhin and Y. N. Luzikov, "SYNTHESIS OF THIOPHENOQUINIZARINE DERIVATIVES," *Chemistry of Heterocyclic Compounds,* vol. 43, no. 10, 2007.

[83] A. Matin, N. G. Moon, M. S., N. X. Yang, N. K. Salam and J. R. Hanrahan, "7-Hydroxy-benzopyran-4-one Derivatives: A Novel Pharmacophore of Peroxisome Proliferator-Activated Receptor r and -γ (PPARr and γ) Dual Agonists," *Journal of Medicinal Chemistry,* vol. 52, no. 21, pp. 6835-6850, 2009.

[84] J. V. Mello and N. S. Finney, "Convenient Synthesis and Transformation of 2,6-Dichloro-4-iodopyridine," *Organic letters, 3(26), , 2001.,* vol. 3, no. 26, pp. 4263-4265.

[85] E. S. Kim, H. S. Kim and J. N. Kim, "An efficient Pd-catalyzed hydration of nitrile with acetaldoxime," *Tetrahedron Letters ,* vol. 50, p. 2973–2975, 2009.

[86] P. E. Fleming, Z. Shi, S. Chen, J. F. Schmidt, J. C. Reader, N. D. Hone and J. P. Ciavarri, "Preparation of aryl pyrimidines as protein kinase C inhibitors," *WO 2005066139 A2,* 2005.

[87] R. F. Borch, M. D. Bernstein, Durst and H. D., "The cyanohydridoborate anion as a selective reducing agent," *Journal of American Chemical Society,* vol. 93, no. 12, p. 2897–2904, 1971.

[88] A. Lombardi Borgia, M. Menichincheri, P. Orsini, A. Panzeri, E. Perrone, E. Vanotti, M. Nesi and C. Marchionni, "Preparation of substituted indazole derivatives for use as kinase inhibitors," *WO 2009013126 A1,* 2009.

[89] K.-Y. Ye, H. He, W.-B. Liu, L.-X. Dai, G. Helmchen and S.-L. Shu-Li You, "Iridium-Catalyzed Allylic Vinylation and Asymmetric Allylic Amination Reactions with o-Aminostyrenes," *Journal of American Chemical Society,* vol. 133, no. 46, p. 19006–19014, 2011.

[90] P. K. Ruprah, K. J. Merchant, L. M. Walsh, C. M. Kerr, C. Fieldhouse, D. Harrisson, S. Maine and K. Hazel, "Preparation of piperidinylalkene and analogs derivatives as modulators of histamine H3 receptor for the treatment of H3 related disorders, especially central nervous system disorders," *WO 2013027001 A1,* 2013.

[91] A. Kivrak and M. Zora, "A novel synthesis of 1,2,4-oxadiazoles and isoxazoles," *Tetrahedron,* vol. 70, p. 817 – 831, 2014.

[92] S. Chiou and H. J. Shine, "A simplified procedure for preparing 3,5-disubstituted-1,2,4-oxadiazoles by reaction of amidoximes with Acyl chlorides in pyridine solution," *Journal of Heterocyclic Chemistry,* vol. 26, pp. 125-128, 1989.

[93] R. L. Papke, "Estimation of both the potency and efficacy of a7 nAChR agonists from single-concentration responses," *Life Sciences,* vol. 78, p. 2812 – 2819, 2006.

[94] H.-J. Bohm, D. Banner, S. Bendels, M. Kansy, B. Kuhn, K. Muller, U. Obst-Sander and M. Stahl, "Fluorine in Medicinal Chemistry," *ChemBioChem,* vol. 5, no. 5, pp. 637-643, 2004.

[95] K. Eskandari and M. Lesani, "Does Fluorine Participate in Halogen Bonding?," *Chemistry A European Journal,* vol. 21, pp. 4739-4746, 2015.

[96] K. E. Riley and P. Hobza, "Strength and Character of Halogen Bonds in Protein-Ligand Complexes," *Crystal Growth and Design,* vol. 11, p. 4272–4278, 2011.

[97] P. Politzer, P. Lane, M. C. Concha, Y. Ma and J. S. Murray, "An overview of halogen bonding," *J. Mol. Modeling ,* vol. 13, no. 2, pp. 305-311, 2007.

[98] y. Lu, Y. Wang and W. Zhu, "Nonbonding interactions of organic halogens in biological systems: implications for drug discovery and biomolecular design," *Physical Chemistry Chemical Physics,* vol. 12, p. 4543–4551, 2010.