Synthetic and Biological Exploration of (+)-Boldine - Identification of potential CNS receptor ligands

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Synthetic and Biological Exploration of (+)-Boldine – Identification of potential CNS receptor ligands

Sujay Joseph

Submitted in partial fulfillment
of the requirements for the degree of
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Signature
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The thesis is dedicated to my parents:

Joseph T. Chiramel and Daisy Abraham
Abstract

(+)-Boldine, an aporphine alkaloid, has been reported to be biologically active at various receptors: α-adrenergic and dopamine receptors, but very few Structure Activity Relationship (SAR) studies on (+)-boldine with respect to its activity on the serotonin receptors (5-HT) have been conducted. To this purpose, a library of novel analogs was synthesized from commercially available (+)-boldine via Mitsunobu reaction to assess the effect of bisbenzylation at the C2 and C9 positions on the affinity and selectivity at serotonin receptors.

As compared to (+)-boldine, the bisbenzylated analogues had diminished affinity at the 5HT1A, 5HT1D, 5HT2B, 5HT6, and 5HT7 receptors. Moderate improvement in the affinity was observed at 5HT2A and 5HT2C receptors. Thus, bisbenzylation of (+)-boldine at the C2 and C9 positions increased the selectivity and affinity to the 5HT2A, 5HT2C receptors over the 5HT1A, 5HT1D, 5HT2B, 5HT6, and 5HT7 receptors.

Our findings will be useful in the future design of high affinity 5HT2A and 5HT2C ligands based on the C2 and C9 benzylation of (+)-boldine’s aporphine core structure.
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Chapter 1: Introduction

1.1.1 Alkaloids

Natural products have played crucial roles in treating and preventing a plethora of human diseases. Medicines have come from various sources: plants, microorganisms, marine organisms among others. Licorice and myrrh – the earliest examples of natural products with medical relevance derived from plant sources, dates to 2600BC in Mesopotamia. Currently, the number of natural products with medical relevance is immense and increasing exponentially.\(^1\)

Alkaloids are a class of natural products which were discovered via systemic studies since the beginning of the 19\(^{th}\) century. In alkaloid plants, the primary function of the alkaloids predominantly involves protection from grazing animals and herbivorous insects.\(^2\) The first alkaloid synthesized and its structure elucidated was Coniine (Fig. 1). In the 1940’s the number of alkaloids was roughly around 800. In the last 70 years, this figure has increased exponentially to 12000 due to improvement in separation techniques and better equipment.\(^2\) Most alkaloids are basic in nature and also contain at least one nitrogen in a heterocyclic ring.\(^3\) Many of the alkaloids have potent biological activity and as a result these are used as pharmaceuticals, stimulants and poison.\(^4\)

![Coniine](image.png)

Fig. 1: Coniine
1.1.2 Aporphines: Occurrence, Structure and Biological Activity

Aporphines are one of the largest groups of the isoquinoline class of alkaloids. Currently, more than 500 aporphines structures have been reported. Aporphines have been classified into several subfamilies namely: aporphines, proaporphines, secoaporphines, oxoaporphines, dehydroaporphines, 7-hydroxyaporphines, aporphine dimers, and aristolactams.\(^5\),\(^6\) (Fig. 2) Aporphines are usually obtained from plants including members of the Annonaceae, Lauraceae, Monimiaceae, Menispermaceae, Hernandiaceae and Ranunculaceae families among others.\(^2\)

![Fig. 2: Members and subfamilies of aporphines](image)

Aporphine alkaloids have a tetracyclic skeleton which includes an isoquinoline core and one stereogenic center at C-6a.\(^7\) (Fig. 3)
Aporphine alkaloids and derivatives have been of great importance as these have antioxidative, malarial, viral and cytoprotective activity. Aporphines act on various receptors in the CNS both *in vitro* and *in vivo* as antagonists or agonists at the adrenergic, dopamine and serotonin receptors.⁶ Research has also suggested that some of the aporphines can be used are potent anti-cancer agents. Aporphines continue to be of great importance in the field of medicinal chemistry as they are very promising for the discovery of new ligands with high potency and selectivity for various CNS receptor subtypes.⁶

### 1.1.3 G-Protein Coupled Receptors (GPCR): Structure and Signaling

G-Protein coupled receptors (GPCRs) form the largest superfamily of receptors in the cell. GPCRs play pivotal role in regulating physiological processes such as sensory perception, immune defense, cell communication, chemotaxis and neurotransmission. Not surprisingly, 50% of the drugs in the market target/modulates the effects of GPCRs.⁸

GPCRs are integral proteins characterized by a 7TM (Transmembrane) helix because they contain seven transmembrane alpha-helices. These 7TM loops have hydrophobic domains tethered to the cell membrane, a carboxyl terminus, which is present on the cytosolic side of the cell and an amino terminus which is present on the extracellular side of the cell. There are three loops present on the outside of the cell that together form the ligand binding site. Three loops are
also present on the cytoplasmic side of the plasma membrane that provide binding site for intracellular signaling proteins.\textsuperscript{9} (Fig. 4)

\textbf{Fig. 4:} GPCR (G-Protein Coupled Receptor) structure

GPCRs are classified into 6 subfamilies (A-F) based on sequence homology. Alternatively, GPCRs are also categorized into numerical subfamilies (1-5). Class A/1 contains Rhodopsin like family, Class B/2 includes secretin like family, Class C/3 contains metabotropic glutamate and
pheromone family, Class D/4 encapsulates fungal pheromones, Class E includes cAMP receptor family and Class F/5 includes the frizzled/smoothened family. Class A/1 is the largest and includes the cationic neurotransmitters and cannabinoid and EDG (Endothelial Differentiation Gene) receptors which have lipid derived endogenous ligands. Additionally, serotonin (5-HT), dopamine and adrenergic receptors are also part of the Class A/1 Rhodopsin like family.\textsuperscript{10}

Members of the GPCRs are activated/deactivated by a wide range of molecules/ligands. Activated/deactivated GPCRs catalyze an exchange of Guanosine triphosphate (GTP) for Guanosine diphosphate (GDP) bound to the inactive G-protein α subunit, resulting in conformation change and dissociation of the complex. Consequently, separation of the G-protein α subunits from the β, γ is followed by a signaling cascade that passes information to the inside of the cell via interaction with the G-protein with the receptor.\textsuperscript{10} (Fig. 4).

In mammals, G protein α subunits are encoded by a multigene family containing at least 16 different subunit genes. The α subunit genes have been grouped into four classes: G\textsubscript{s}, G\textsubscript{i}, G\textsubscript{q}, and G\textsubscript{12}, based on sequence homology, gene structure, and regulation of specific effectors.\textsuperscript{11} G\textsubscript{s} subunit stimulates/activates the cAMP-dependent pathway by activating adenylyl cyclase. The signal can be further magnified by the interaction of adenylyl cyclase with a host of proteins. G\textsubscript{i} subunit inhibits/deactivates the cAMP-dependent pathway by deactivating adenylyl cyclase. G\textsubscript{q} proteins activate phospholipase C (PLC), which participates in a variety of cellular signaling pathways.\textsuperscript{12}

1.1.4 Overview pertinent receptors - Central Nervous System

There are variety of substances in the Central Nervous System that serve as neurotransmitters (NT) and neuromodulators. Some of the substances include amino acids and
amino acid derivatives, amines, peptides and purine derivatives. Glutamate, GABA, Glycine are few amino acids which act as NTs. Amine NTs include norepinephrine, epinephrine, dopamine, serotonin and histamine.\textsuperscript{13} Substance P and β-endorphin are peptides involved in the neurotransmission.\textsuperscript{14} Purines derivatives include ATP and Adenosine.\textsuperscript{15}

Aporphines are useful ligands for central nervous system receptors and possess high affinity for a number of dopamine receptors (D\textsubscript{1}/D\textsubscript{2}), serotonin (5-HT) and α-adrenergic receptors. Aporphines are known to be endowed with both agonist and antagonist activity at neuroreceptor sites.\textsuperscript{16} As such, a brief review of these receptors and subtypes merits discussion.

Dopamine (Fig. 5) is involved in a number of processes including motor control, autonomic function and motivation among others. Abnormal levels of dopamine are involved in a number of debilitating diseases including Schizophrenia, Parkinson’s disease, attention deficit hyperactivity disorder (ADHD), drug addiction and psychosis.\textsuperscript{17} Dopamine is synthesized in specific neurons, striatum and the limbic system and is released in dopaminergic synaptic clefts to elicit physiological effects after binding at dopamine receptors.\textsuperscript{24} Dopamine receptors in the CNS are grouped in two large families- D\textsubscript{1}, D\textsubscript{2} based on c-DNA studies, synaptic action and signaling mechanism of the receptors. The D\textsubscript{1} family consists of D\textsubscript{1} and D\textsubscript{5} receptors. Both of the receptors are coupled to G\textsubscript{α} which is involved in increasing cAMP levels and thus the excitability of the cell. D\textsubscript{1} receptors is the most abundant subtype in the mammalian forebrain and D\textsubscript{5} receptors are relatively sparse and less widely distributed compared to D\textsubscript{1} receptors.\textsuperscript{17} Doxanthrine and Dihydrexidine are selective ligands of D\textsubscript{1} and D\textsubscript{5} respectively.\textsuperscript{18} (Fig. 6)

The D\textsubscript{2} class includes D\textsubscript{2}, D\textsubscript{3}, D\textsubscript{4} receptors. The D\textsubscript{2} receptors are coupled to G\textsubscript{αi}, which leads to decreased cAMP levels. Subsequently, the excitability of cells is regulated.\textsuperscript{8} D\textsubscript{2} receptor
is second behind D₁ in abundance in the mammalian forebrain. Some of the drugs that decrease symptoms of Parkinson’s disease target D₂ receptors.¹⁷ Aripiprazole, Nafadotride and ABT 724 Trihydrochloride are some examples of selective ligands of the D₂, D₃, D₄ families respectively.¹⁹,²⁰,²¹ (Fig. 7)

**Fig. 5:** Dopamine – Native ligand of Dopamine receptors

![Dopamine](image)

**Fig. 6:** Selective D₁, D₅ Ligands

![Doxanthrene (D₁ agonist)](image) ![Dihydrexidine (D₅ agonist)](image)

![Aripiprazole (D₂ agonist)](image) ![Nafadotride (D₃ antagonist)](image)
Adrenergic receptors are also a class of GPCRs that are target of ligands of the catecholamine family. Adrenergic receptors are involved in circulation and the fight-flight response. There are two main groups of adrenergic receptors $\alpha$ and $\beta$. $\alpha$ adrenergic receptors are divided into two groups: $\alpha_1$ and $\alpha_2$. $\alpha_1$ subtypes include $\alpha_{1A}$, $\alpha_{1B}$ and $\alpha_{1D}$. $\alpha_2$ adrenoceptors are classified into $\alpha_{2A}$, $\alpha_{2B}$ and $\alpha_{2C}$. $\alpha_1$ adrenoceptors are found in the CNS and Peripheral Nervous System (PNS). In the CNS they are located post-synaptically and are involved in modulating excitation.\textsuperscript{22,23} In the PNS, $\alpha_1$ receptors are located on vascular and smooth muscle and are involved in contraction. $\alpha_1$ receptors are linked to G\textsubscript{q} GPCRs and $\alpha_2$ receptors are linked to G\textsubscript{i} GPCRs. $\alpha_2$ adrenoceptors are located on pre and post synaptic neurons and they mediate inhibitory role in the CNS and PNS. Phenylephrine is a selective agonist for $\alpha_1$-receptor.\textsuperscript{24} (Fig. 8) $\beta$-adrenoceptors are divided into subtypes: $\beta_1$, $\beta_2$, $\beta_3$ and $\beta_4$. The $\beta_1$-adrenoceptor is predominant in the striatum and heart and $\beta_2$ is predominant in vascular, uterine and smooth muscle in the airway. $\beta_1$ is coupled to G\textsubscript{s} GPCRs and is involved in stimulatory responses. However, other $\beta$-receptors can be either excitatory or inhibitory or a combination of both.\textsuperscript{22,23} Clenbuterol is a selective agonist of $\beta_2$ adrenoceptor.\textsuperscript{25} (Fig. 8)
Fig. 8: Selective $\alpha_1$ and $\beta_2$-receptor ligands

1.1.4.1 Serotonin Receptors: Structure and function and Clinical Implications

Serotonin (5-hydroxytryptamine, 5-HT) (Fig. 9) is another NT which is involved in wide array of functions including cognition and behavioral functions. In the brain serotonin is produced in the axon terminals and it diffuses out into the synaptic cleft to activate postsynaptic receptors. Any alteration in its neurochemistry is associated with a plethora of neuropsychiatric disorders. The serotonin family of receptors is larger than any other family of GPCRs: 13 distinct genes encode for each of the Serotonin GPCRs of the 7TM class. Studies on pharmacology, amino acid sequence similarity and signaling mechanisms suggest that there are at least 14 different receptor subtypes of serotonin receptors, which are divided into seven families - 5HT$_1$ to 5HT$_7$. All of the serotonin receptors are members of the type A/1 rhodopsin G-Protein Coupled Receptors (GPCRs) as described earlier, with one exception – the 5HT$_3$ receptor, which is a ligand gated ion channel. Serotonin agonists and antagonists for different 5-HT subfamilies are also useful drug targets for the treatment of numerous diseases and many of these ligands are readily available for clinical use.
The 5-HT₁ family is subdivided into five subfamilies: 5-HT₁A, 5-HT₁B, 5-HT₁D, 5-HT₁E and 5-HT₁F. 5-HT₁A receptors were the first serotonin 5-HT₁ receptors to be cloned and characterized. Like all other 5-HT₁ type receptors, the 5-HT₁A receptor is characterized by its high affinity to serotonin. 5HT₁A receptors are distributed pre and post synaptically within the brain. The activation of the 5-HT₁A receptors lead to neuronal hyperpolarization which in turn reduces firing rate of neurons. The presynaptic receptors are located in the raphe nuclei and are coupled to G-αᵢ/o receptors. Postsynaptic 5-HT₁A receptors are expressed densely in the limbic areas of the brain like the hippocampus and the septum. Preclinical studies show that the 5-HT₁A receptors play important roles in anxiety and depression. Additionally, the 5-HT₁A receptors are also linked to schizophrenia and symptoms related to Parkinson’s, neuroprotection and addiction to psychostimulants like amphetamine and cocaine. Agonists of these receptors have shown antidepressant properties while the antagonists are linked to weight reduction. The most known 5-HT₁A available compound is the agonist Buspirone. (Fig. 10)
**Fig. 10:** Selective 5-HT$_{1A}$ ligand

5-HT$_{1B}$ receptors are widely distributed throughout the CNS and its function varies depending on its location. Within the brain neurons, 5-HT$_{1B}$ receptors are presynaptic and are localized on axon terminals. Clinical studies indicate that the 5-HT$_{1B}$ receptors are involved in regulation of aggression, learning and memory processes and premature ejaculation.$^{27,28}$ 5-HT$_{1B}$ receptors are also thought to be involved in depression.$^{11}$ Ergotamine is a selective agonist of the 5-HT$_{1B}$ receptor.$^{30}$ (Fig. 11)

![Ergotamine](image)

Ergotamine
(5-HT$_{1B}$ agonist)

**Fig. 11:** Selective 5-HT$_{1B}$ ligand

5-HT$_{1D}$ receptors are located mostly within the basal ganglia and substantia nigra of the brain. 5-HT$_{1D}$ receptors are linked with migraines and coronary vasoconstriction.$^{27,28}$ Sumatriptan is a well-known 5-HT$_{1D}$ agonist which is used to treat migraines.$^{31}$ (Fig. 12)

![Sumatriptan](image)

Sumatriptan
(5-HT$_{1D}$ agonist)
**Fig. 12**: Selective 5-HT$_{1D}$ ligand

5-HT$_{1E}$ receptors are present mostly in the cortical layers II-VI and the hippocampus. Current understanding of 5-HT$_{1E}$ receptors function is poor, because there are no ligands specific for the 5-HT$_{1E}$ receptors. However, 5-HT$_{1E}$ receptors may play important roles in cognition and memory processes.$^{27,28}$

5-HT$_{1F}$ receptors are distributed widely in the brain and the spinal cord. 5-HT$_{1F}$ receptors are involved in acute migraines which are not accompanied by coronary vasoconstriction.$^{27,28}$ LY334370 is an agonist for 5-HT$_{1F}$ receptors.$^{32}$ (Fig. 13)

![LY334370 hydrochloride](image)

**Fig 13**: Selective 5-HT$_{1F}$ ligand

5-HT$_2$ receptor family is subdivided into three subfamilies: 5-HT$_{2A}$, 5-HT$_{2B}$ and 5-HT$_{2C}$. 5-HT$_2$ receptor family is coupled to G-α$_q$ GPCRs. 5-HT$_2$ receptor family use protein kinase C (PKC) and calcium as second messenger system.$^{27,28}$ (Fig. 14)
Fig 14: PKC, Calcium second messenger system

5-HT$_{2A}$ receptors are distributed at varying densities in the brain; the highest density is in the neocortex. 5-HT$_{2A}$ receptors are involved in closing/opening of channels in the brain, synaptic plasticity, analgesia in the spine and modulates various cardiovascular functions. Interestingly, 5-HT$_{2A}$ receptors are also key sites for hallucinogenic action. Psilocybin is an example of an agonist which has hallucinogenic properties. Ketanserin is an antagonist which prevents psilocybin from binding to the 5-HT$_{2A}$ receptor. (Fig. 15)

Fig. 15: Selective 5-HT$_{2A}$ ligand

5-HT$_{2B}$ receptors are mostly expressed in the kidneys, livers lungs. However, these are only weakly expressed in the brain. 5-HT$_{2B}$ receptor is the only 5-HT receptor that is necessary for viability. 5-HT$_{2B}$ receptors are linked to vulnerability to drug abuse, accurate functioning of
auditory system and proper formation of key structures in the heart and brain during development. Additionally, 5-HT$_{2B}$ receptors activation in the adult heart can at times lead to heart disease or fatality. Thus, antagonists of 5-HT$_{2B}$ receptors in the heart can be useful in treating cardiac hypertrophy.$^{27,28}$ RS-127445 is a selective antagonist of the 5-HT2B receptor.$^{34}$ (Fig. 16)

![RS-127445](image)

**Fig. 16:** Selective 5-HT$_{2B}$ ligand

5-HT$_{2C}$ receptors are mostly expressed in the choroid plexus region of the brain and it may help in regulating ion exchange between the brain and the CSF. 5-HT$_{2C}$ receptors also modulate dopaminergic function and might be involved in schizophrenia, depression, anxiety, appetite control and cardiovascular function.$^{27,28}$ Fenfluramine is a selective agonist of 5-HT$_{2C}$ receptor.$^{35}$ (Fig. 17)

![Fenfluramine](image)

**Fig. 17:** Selective 5-HT$_{2C}$ ligand
5-HT₃ receptors are mostly expressed in the peripheral and central nervous system at varying degrees. 5-HT₃ receptors don’t belong to the GPCR class of receptors and are non-selective cation (Na⁺/K⁺) ion channel receptors. These are closely related to the cysteine-loop ligand gated superfamily which includes Acetocholine nicotinic receptor, glycine and GABA⁰ receptor.²⁷,²⁸

5-HT₄ receptors are expressed both centrally and peripherally in the nervous system. 5-HT₄ receptors have low homology sequence to other 5-HT receptors and are involved in learning (long term potentiation), synaptic plasticity, and relaxation of the colon.²⁷,²⁸ Cisapride is an agonist of 5-HT₄ receptors.³⁶ (Fig. 18)

![Cisapride](image)

**Fig. 18:** Selective 5-HT₄ Ligand

5-HT₅ receptors have two isoforms: 5-HT₅₅A and 5-HT₅₅B. Currently, only 5-HT₅₅A is thought to be present in humans. 5-HT₅₅B receptors are broadly expressed in the CNS. Not much is known about the function as no selective ligand has been found, although 5-HT₅₅A receptors are implicated in control of circadian rhythm.²⁷,²⁸

5-HT₆ receptors are predominantly expressed in the CNS. However, not much is known about the function of 5-HT₆ receptors as very few selective agonists have been found. Nevertheless, this receptor may have potential therapeutic activity for anxiety, depression,
schizophrenia, epilepsy, obesity.\textsuperscript{27,28} EMD-386088 is a potent and selective 5-HT\textsubscript{6} agonist.\textsuperscript{37} (Fig. 19)

\begin{center}
\includegraphics[width=0.5\textwidth]{EMD-386088.png}
\end{center}

\textbf{Fig. 19: 5-HT\textsubscript{6} Ligand}

5-HT\textsubscript{7} receptors comprise of 5-HT\textsubscript{7A}, 5-HT\textsubscript{7B}, 5-HT\textsubscript{7C} and 5-HT\textsubscript{7D} receptors. 5-HT\textsubscript{7C} receptors are found only in rats. 5-HT\textsubscript{7} receptors are mainly found in the CNS. These receptors are believed to important in regulating sleep, hypertension, overall mood of an individual and regulating body temperature.\textsuperscript{27-28} Fluphenazine is a 5-HT\textsubscript{7} antagonist which is used to treat schizophrenia.\textsuperscript{38} (Fig 20)

\begin{center}
\includegraphics[width=0.5\textwidth]{Fluphenazine.png}
\end{center}

\textbf{Fig. 20: Selective 5-HT\textsubscript{7} ligand}
Table 1: Few examples of selective ligands used as therapeutics (NA- Not Applicable)

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Functional Activity</th>
<th>Therapeutic Use</th>
<th>Ligand</th>
</tr>
</thead>
<tbody>
<tr>
<td>D1</td>
<td>Agonist</td>
<td>Parkinsons</td>
<td>Doxanthrine</td>
</tr>
<tr>
<td>D2</td>
<td>Partial Agonist</td>
<td>Antipsychotic</td>
<td>Aripiprazole</td>
</tr>
<tr>
<td>D3</td>
<td>Antagonist</td>
<td>Antidepressant</td>
<td>Nafadotride</td>
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<td>D4</td>
<td>Agonist</td>
<td>Vasodilator</td>
<td>ABT 724 trihyrocychloride</td>
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<tr>
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<td>Agonist</td>
<td>Schizophrenia</td>
<td>Dihydrexidine</td>
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<td>Agonist</td>
<td>Decongestant</td>
<td>Phenylephrine</td>
</tr>
<tr>
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<td>Agonist</td>
<td>Broncodilator</td>
<td>Clenebuterol</td>
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<td>Agonist</td>
<td>Migraine</td>
<td>Sumatriptan</td>
</tr>
<tr>
<td>5-HT₁F</td>
<td>Agonist</td>
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<td>Antagonist</td>
<td>Antipsychotic</td>
<td>Fluphenazine</td>
</tr>
</tbody>
</table>

1.1.5 Structure Activity Relationships (SAR) studies of Aporphines at CNS

A plethora of studies can be found on the SAR of the aporphines because aporphines exhibit biological activity at various neuroreceptor sites. Various alkaloids containing the aporphine skeleton were found to have moderate activity towards dopamine receptors. Since many of the aporphine alkaloids have nonselective and weak/moderate activity, optimization studies may be done to enhance pharmacological activities as well as enhance understanding of ligand binding to receptors. As a result of these studies, new compounds can be identified which are selective towards a particular receptor. Furthermore, this approach can potentially lead to development of new and useful pharmacological tools and therapeutics.
A few studied aporphines are (S)-boldine, (R)-apomorphine and (S)-bulbocapnine. SAR studies on a series of substituted aporphines indicate that substitutions on ring A at C1 or C2 positions wherein hydroxy or methoxy groups in aporphine scaffold are replaced, increased affinity at dopamine receptors whereas, substituents on ring D at C9 position tended to have the opposite effect on dopamine receptors.\textsuperscript{40} (Fig. 21,22) The stereochemistry of the chiral center at C-6a was also an important factor which determines whether a ligand is an antagonist or an agonist. (R)-analogs were found to display agonist activity whereas the (S) molecules were found to display weak agonist, partial agonist and antagonist activity.\textsuperscript{41} It is known that (R)-apomorphine is a potent dopamine D\textsubscript{2} receptor agonist. Both (R) and (S) apomorphine were screened on various receptors and it was found that (S)-apomorphine binds to dopamine and \(\alpha\)-adrenoreceptors, while (R) form was active at \(\alpha\textsubscript{2}\)-adrenoreceptors.\textsuperscript{42} Furthermore, compounds that contained an electropositive or electronegative C2 substituent were found to display high D\textsubscript{2} binding potency and good D\textsubscript{2}/D\textsubscript{1} selectivity. Tolerance to variable C2 substituents might be indicative of a lipophilic cleft that could potentially interact with the substituents at C-2 position.\textsuperscript{43} Meta –OH substituent on ring A at C-2 position seemed to be of great importance for D\textsubscript{2} receptor affinity as it may be involved in hydrogen bonding with the hydroxyl group and peptides on the surface of D\textsubscript{2} proteins.\textsuperscript{44}
In additional studies, it was revealed that small structural changes to the aporphine core significantly affects the receptor binding profile in both \textit{in vivo} and \textit{in vitro} systems. For example: structural modification of the non-selective agonist aporphine (\textit{R})-apomorphine at C11 was done to study the SAR at \textit{D}$_1$, \textit{D}$_2$ and 5-HT$_{1A}$ receptors. It was discovered that the catechol functionality is not required for binding the DA receptors with high affinity. Hydrogen bond at C11 position was deemed as non-essential for high affinity binding to \textit{D}$_1$/\textit{D}$_2$ and 5-HT$_{1A}$ receptors.\textsuperscript{45} An increase in affinity and selectivity for 5-HT$_{1A}$ was observed when the size of the N-substituent was limited to methyl or hydrogen.\textsuperscript{46}

SAR studies on predicentrine (Fig. 23) show that iodination, bromination at C3 position on ring A increased selectivity potency compared to the parent compound at \textit{D}$_1$ receptor.\textsuperscript{47} Binding studies on C1-C11 bridged aporphines analogs show that these compounds have good affinity at 5-HT receptors: 5-HT$_{1A}$ and 5-HT$_7$ but have variable interaction with DA receptors.\textsuperscript{6,48} (Fig. 25) Claudi and colleagues synthesized and reported a series of C12-C13 bridged aporphines which had similar profiles as the C1-C11 bridged aporphines.\textsuperscript{49} (Fig. 26) SAR on the N-6 against DA receptors showed that the replacement of N-methyl with ethyl, propyl gave more potent agonists at \textit{D}$_2$ receptor.\textsuperscript{49,50}
Fig 23: Predicentrine, a DA ligand

Fig 24: Halogenated predicentrine analogue active at D₁ receptors

Fig 25: C₁, C₁₁ bridged aporphine active at 5-HT receptors

Fig 26: C₁₂, C₁₃ Bridged aporphine active at 5-HT receptors
SAR studies have been really useful tools in development of potent dopaminergic agents as a few of the aporphine derivatives of apomorphine have been used as potential therapeutics for Parkinson’s disease, Schizophrenia among other psychotic disorders.⁶

1.1.6 (+)-Boldine – History and Pharmacological Profile

![Fig. 27: Structure of (+)-boldine, 1](image)

(+)-Boldine 1 (1,10-dimethoxy-6-methyl-5,6,6a,7-tetrahydro-4H-dibenzo[de,g]quinoline-2,9-diol) (Fig. 27) has been isolated from the barks and leaves of the Boldo tree, *Peumus boldus* (Monimiaceae) which is indigenous to humid ecosystems of the Mediterranean climactic region of Central and South Chile.⁵¹ Boldo leaves contain between .4 to .5% of at least 17 different alkaloids belonging to the large benzylisoquinoline-derived family. (+)-Boldine is the major alkaloid, which accounts for 12-19% of the total alkaloid content. Boldo leaves contain tannins, essential oils like ascaridole, cineole and flavonoids like catechin. (+)-Boldine is also present as a minor constituent in a number of other species of the Monimiaceae, Magnoliaceae and Lauraceae. Interestingly, (+)-boldine content in the leaves of Boldo varies on a seasonal basis with maximum amounts present during summer.⁵²

Boldo leaves have been used by South American natives against diseases of the liver and treatment of gallstones.⁵³ The plant is still used as homeopathic medicine for digestive disorders
as laxatives, diuretics and problems associated with rheumatism among many other ailments. (+)-Boldine has been characterized as an antioxidant which protects the cells in different systems from the scavenging activity of free-radical induced peroxidation or enzyme in activation. (+)-Boldine exerts anti-inflammatory, anti-pyretic, anti-diabetic, anti-tumor promoting properties. (+)-Boldine has neuroleptic-like, anticonvulsant and antinociceptive actions. (+)-Boldine could also boost the immune system as it was found that it was a poor macrophage peroxide liberator in rats. In addition to a host of pharmacological properties, (+)-Boldine also exerts its effects on the smooth muscle as a relaxer. (+)-Boldine is a slightly selective α-adrenergic antagonist and non-selectively binding antagonist of D1 and D2 receptors.

(+)-Boldine has a plasma half-life of only 30 minutes and it may be due to (+)-boldine’s rapid glucuronidation in the liver. The presence of phenolic groups on (+)-boldine is associated with low metabolic stability. (+)-Boldine’s short systemic half-life is very disadvantageous in terms of its potential clinical usefulness. Additionally, it was indicated in studies that (+)-boldine has low toxicity. Research clearly indicates that (+)-boldine has clinically useful properties and so it is essential to find more stable analogues of (+)-boldine which retains its activity and these molecules could be useful for therapeutic purposes.

1.1.7 SAR studies on (+)-boldine

(+)-Boldine exhibits a wide range of pharmacological properties which makes it imperative to conduct SAR studies on (+)-boldine in a variety of assay systems. Only a few reports have been done on the SAR of (+)-boldine at CNS receptors. These SAR studies on (+)-boldine are discussed as they highlight the importance of structural changes on (+)-boldine and consequently its function.
Most of the bioactive aporphines like apomorphine and its congeners have \((R)\) antagonist stereochemistry at the 6α carbon, however, (+)-Boldine has \((S)\) stereochemistry and it’s an antagonist for the CNS receptors. The substitution patterns on (+)-boldine differs to a great extent to \((R)\)-apomorphine and derivatives which are DA agonists. Substitution patterns at 1, 2, 9 and 10 Carbon positions on (+)-boldine scaffold are responsible for the DA antagonist properties.\(^1\)

In order to increase the metabolic stability and retain/enhance biological activity of (+)-boldine, substituents were added to the readily available (+)-boldine scaffold. To that extent, (+)-boldine was brominated, chlorinated and iodinated at C-3 position. (Fig. 28) Radioligand binding studies of the halogenated analogues showed that halogenation at C-3 increased affinity to \(D_1\) but not \(D_2\) receptors. From this study, 3-iodoboldine was found to be the most potent derivative with a low nanomolar \(IC_{50}\) (2 nM). (Fig. 28) In the case of 3-iodoboldine, it lost its activity completely at the \(\alpha_1\) adrenergic receptors, while retaining its activity at \(D_1/D_2\) receptors. This was in contrast with the results of other halogenated derivatives, as they displayed both \(\alpha_1\) adrenergic and \(D_1/D_2\) affinity.\(^5\)

![Fig. 28: Halogenated C3 boldine analogues](image-url)

---

\(^1\) Boldine has (S) stereochemistry and it’s an antagonist for the CNS receptors. The substitution patterns on (+)-boldine differs to a great extent to (R)-apomorphine and derivatives which are DA agonists. Substitution patterns at 1, 2, 9 and 10 Carbon positions on (+)-boldine scaffold are responsible for the DA antagonist properties.

\(^5\) In the case of 3-iodoboldine, it lost its activity completely at the \(\alpha_1\) adrenergic receptors, while retaining its activity at \(D_1/D_2\) receptors. This was in contrast with the results of other halogenated derivatives, as they displayed both \(\alpha_1\) adrenergic and \(D_1/D_2\) affinity.
Another study which probed the SAR of (+)-boldine found that compared to (+)-boldine, bromine or chlorine substitution at C3 positions increases the $\alpha_{1A}$ adrenoreceptors subtype selectivity and also decreases the affinity of benzothiazepine site at the calcium channel in Rat cerebral cortex. Halogenation at C8 position did not significantly improve the activity with respect to 3-bromoboldine. (Fig. 29) Nitroso substitution (Fig. 30) at C3 position resulted in the loss of affinity and selectivity for the $\alpha_{1A}$ adrenoreceptors subtypes.\textsuperscript{55} 8-NH$_2$-boldine’s (Fig. 31) affinity was significantly decreased compared to (+)-boldine at $\alpha_{1D}$ adrenoreceptors. These results suggest that an amino group at C8 hinders molecules from binding to $\alpha_{1D}$ adrenoreceptors active site.\textsuperscript{57}

![Fig. 29: Halogenated C8 boldine analogues](image)

![Fig. 30: Nitroso substituted boldine analogue](image)
Fig. 31: Substituted C8 amine boldine analogue

SAR on (+)-boldine reveals that the phenol groups bonded to the aporphine ring system and the basic benzylic amine contribute heavily to the biological activity of aporphines and boldine. Martinez et. al tested (+)-boldine against several other aporphines and it was discovered that (+)-boldine displayed much pronounced activity possibly owing to the presence of free hydroxyl group on C-2 of the isoquinoline ring. The free hydroxyl group on C2 was also found to increase the affinity to D1 over D2, α1A and α1B adrenoreceptors subtypes in rats.55

Milian et. al. semi-synthesized halogenated phenanthrene alkaloids using (+)-boldine as the starting material. The SAR on (+)-boldine showed that the presence of the free phenolic groups was essential for the antioxidant activity. The phenanthrene alkaloids without the phenolic groups (Fig. 32) showed less potency than the alkaloids with the aporphine or the phenanthrene skeleton. Additionally, compounds with phenols in the phenanthrene skeleton were found to be more active compounds than the respective alkaloids with the aporphine skeleton. Absence of phenols in non-phenolic aporphines (Fig. 33) was found to be detrimental for stability as non-phenolic aporphines could be easily oxidized to dehydro- and oxo-aporphines.58
Fig. 32: C2, C9 substituted phenanthrene analogue

Fig. 33: C2, C9 substituted boldine with preserved aporphine skeleton

Thomet et. al. attached diphenylphosphinyl group at C3 position of (+)-boldine. (Fig. 34) In screening studies, it was found that with the introduction of phosphinyl group, the cytotoxicity, lipophilicity and intercalating behavior of (+)-boldine increased substantially. As a result of this, the diphenylphosphine derivative exhibited significant cytotoxic activity against two breast cancer lines (MDA-MB-231, MCF-7).  

Fig. 34: C3 Diphenylphosphinyl substituted boldine derivative
1.1.8 Objective of Study

(+)-Boldine is readily available commercially and has a chiral center at 6a. The total synthesis of alkylated derivatives of (+)-boldine at C2 and C9 requires several steps and often entails laborious purification. Semisynthetic studies on the readily available (+)-boldine scaffold for the SAR studies at CNS receptors offers considerable advantages as compared to total synthesis, with respect to yields and synthetic efficiency. Only a few reports have been done on the SAR studies of (+)-boldine at CNS Receptors. The primary objective of this study is to evaluate the potential of (+)-boldine derivatives as ligands for serotonin receptors: 5-HT\textsubscript{1A}, 5-HT\textsubscript{1D}, 5-HT\textsubscript{2A}, 5-HT\textsubscript{2B}, 5-HT\textsubscript{2C}, 5-HT\textsubscript{6} and 5-HT\textsubscript{7}. Secondary aim was to evaluate the extent to which structural changes on (+)-boldine are tolerated for affinity across the 5-HT (Serotonin) receptors. As mentioned before 5-HT receptors are implicated in several neuropsychiatric maladies including schizophrenia, depression, anxiety, insomnia and stimulant addiction.

Systemic studies on the benzylation at C2, C9 positions of (+)-boldine have not been reported. Study on the SAR of C2, C9 benzylated analogues could potentially lead to novel ligands that are valuable for understanding receptor–ligand interactions at the CNS that control receptor affinity, activity and selectivity.

1.1.9 Rationale for synthesis of analogs at C2 and C9 position in (+)-boldine

The central hypothesis of the project was that (+)-boldine can be structurally modified to give more potent antagonists at various 5-HT receptor subtypes: 5-HT\textsubscript{1A}, 5-HT\textsubscript{1D}, 5-HT\textsubscript{2A}, 5-HT\textsubscript{2B}, 5-HT\textsubscript{2C}, 5-HT\textsubscript{6} and 5-HT\textsubscript{7}. In order to test this hypothesis, compounds based on the (+)-boldine skeleton were semi-synthesized and screened in 5-HT receptor subtype assays for affinity and antagonist activity.
Systemic studies on the benzylolation at C2, C9 positions of (+)-boldine have not been reported. The information we obtain from the study might allow us to understand specific structural features of (+)-boldine that are necessary for its activity at the 5-HT receptors and provide more insights into the receptors that are involved in 5-HT agonism/antagonism. Minor modification to the (+)-boldine skeleton will result in better understanding of the pharmacophore part of (+)-boldine and could potentially result in identification of novel CNS receptors ligands which could be used for further studies of receptors and also aid in development of therapeutics.
Chapter 2

2.1 Results and Discussion:

2.1.1 Synthesis of C2 and C9 analogues from (+)-boldine

It was proposed that (+)-boldine could be functionalized to obtain a library of compounds using Williamson ether synthesis. However, it was found that the reaction of (+)-boldine under typical Williamson ether synthesis lead to the quaternization of the tertiary Nitrogen and subsequent cleavage of ring B to afford phenanthrene nucleus. (Scheme 1). The basic nature of the amine group was found to be problematic and it was deemed necessary to convert the amine group to a less basic functional group. To this purpose, the amine functionality was converted to N-carbamate moiety. During this reaction, it was discovered that Ring B of (+)-boldine opens up to give phenanthrene alkaloid nucleus similar to the products of Williamson ether synthesis. The Cl⁻ ion generated during the reaction was found to be problematic as it alone was able to convert (+)-boldine to its phenanthrene variety. Numerous unsuccessful attempts were made to sequester the chlorine ion from extracting acidic proton from ring C.

*Scheme 1*: Quarternization of boldine in typical Williamson ether conditions
Table 2: Typical conditions used for Williamson ether synthesis (Yield = % dibenzylated boldine)

<table>
<thead>
<tr>
<th>Entry</th>
<th>Benzylbromide</th>
<th>Base(K$_2$CO$_3$/LiCO$_3$)</th>
<th>Solvent</th>
<th>Temperature(°C)</th>
<th>Yield</th>
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<tbody>
<tr>
<td>1</td>
<td>1 eq.</td>
<td>1 eq.</td>
<td>Acetone</td>
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<td>2</td>
<td>DMF</td>
<td>25</td>
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</table>
Scheme 2: Alternate route for synthesis of dibenzylated boldine analogues

Table 3: Typical conditions used for amines’ N-carbamate protection (Yield = % dibenzylated boldine)

<table>
<thead>
<tr>
<th>Entry</th>
<th>Ethyl Carbamate</th>
<th>Base (K₂CO₃/LiCo₃)</th>
<th>Solvent</th>
<th>Temperature(°C)</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1 eq.</td>
<td>1 eq.</td>
<td>DCM</td>
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<td>0</td>
</tr>
<tr>
<td>2</td>
<td>1.4</td>
<td>1.4</td>
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<td>3</td>
<td>1.8</td>
<td>1.8</td>
<td>DCM</td>
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<td>0</td>
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<td>2</td>
<td>DCM</td>
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<td>0</td>
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<td>DCM</td>
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</tbody>
</table>
In order to avoid N-alkylation during phenolic alkylation, Mitsunobu reaction was tested as an alternative method. Phenolic alkylation on aporphines have been done only once, so exploration of phenolic alkylation is essential for functionalizing phenolic groups on aporphines like (+)-boldine.\textsuperscript{42} The route to synthesize (+)-boldine analogues is outlined in Fig. 35. (+)-Boldine was subjected to Mitsunobu alkylation to afford a mixture of mono and dialkylated (+)-boldine along with complex mixture of the reagents and minute amounts of starting material (+)-boldine. The complex mixture was subjected to column chromatography in order to separate the dialkylated benzyl ethers from the rest of the mixture. The (+)-boldine was reacted with Diisopropyl azodicarboxylate (DIAD), appropriate benzyl alcohol and polymer bound Triphenylphosphine (TPP/PPh\textsubscript{3}) and the following compounds 2-13 (Table 4) were afforded. Fortunately, (+)-boldine ethers are separable chromatographically, even though the separation is somewhat tedious owing to the similarities in R\textsubscript{f}.

It was found experimentally that 4 equivalents of the reagents were necessary to afford maximum yield of dialkylated benzyl ethers of (+)-boldine at C2, C9 positions. During purification, an acidic impurity was degrading the compounds so column chromatography was performed in basic alumina to separate the impurity. Additionally, it was very difficult to remove from PPh\textsubscript{3} from the dialkylated compounds as they appeared almost at the same R\textsubscript{f}. A solvent system could not be devised to separate PPh\textsubscript{3} from the products. To that extent, polymer bound PPh\textsubscript{3} was used to filter most of the PPh\textsubscript{3} from the reaction mixture. Subsequently, column chromatography in basic alumina and preparatory column in silica was performed to afford pure compounds 2-13 (Table 4) in moderate yields.
**Fig. 35:** Reaction scheme for Synthesis of C2 and C9 (+)-boldine analogues

**Table 4:** List of Benzyl alcohols used and the compounds made (N/A Not Applicable)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Benzyl Alcohol</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boldine (1)</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>2</td>
<td>4-Bromobenzyl alcohol</td>
<td>3-Bromo</td>
</tr>
<tr>
<td>3</td>
<td>4-Methoxybenzyl alcohol</td>
<td>4-Methoxy</td>
</tr>
<tr>
<td>4</td>
<td>2-Methoxybenzyl alcohol</td>
<td>2-Methoxy</td>
</tr>
<tr>
<td>5</td>
<td>3-Methoxybenzyl alcohol</td>
<td>3-Methoxy</td>
</tr>
<tr>
<td>6</td>
<td>4-(Trifluoromethyl)benzyl alcohol</td>
<td>4-(Trifluoromethyl)</td>
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<td>4-Methylbenzyl alcohol</td>
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</tr>
<tr>
<td>13</td>
<td>4-Iodobenzyl alcohol</td>
<td>4-Iodo</td>
</tr>
</tbody>
</table>
2.1.2 Reaction Mechanism – Mitsunobu reaction

The substitution of primary or secondary alcohols with nucleophiles mediated by a redox combination of triarylphosphine and Dialkyl azocarboxylate is popularly known as Mitsunobu reaction. Mitsunobu reaction was discovered in 1967 and has been widely used in synthetic organic chemistry. The Mitsunobu reaction is extensively used to prepare alkyl-aryl ethers under mild conditions. Apart from ethers, a wide range of compounds which includes esters, amines, azides, cyanides, thiocyanides, thioesters and thioethers can be synthesized using the Mitsunobu reaction. Common reagents include DEAD, DIAD, PPh₃ among others. The substrates of Mitsunobu reactions are usually primary or secondary alcohols. The nucleophile is usually an acidic compound with an –OH, -SH or an –NH group with pKₐ below 11, however, there have been cases where nucleophiles with pKₐ of 15 were also tolerated. Some of the common nucleophiles used in Mitsunobu reaction are phenols, carboxylic acids among a host of other acidic compounds. Common solvents for Mitsunobu reaction include THF, toluene, benzene, DMF, diethyl ether, acetonitrile, dichloromethane and 1,4-dioxane. Preferred P(III) component used is PPh₃ which is quite cheap and readily available commercially. Mitsunobu reaction produces triphenylphosphine oxide as a byproduct, and the unreacted PPh₃ is not water soluble, and very often these byproducts have to be separated from the desired products through column chromatography. This is one major disadvantage of Mitsunobu reaction.⁶²

Even though Mitsunobu reaction has been used for a long time, its mechanistic details particularly at the intermediate stages are still subject of debate and rigorous studies. The order of the steps greatly varies on the order the reagents are added.⁶¹ Studies reveal that in the beginning of the reaction, TPP forms an adduct with DIAD which leads to the formation DIAD-PPh₃ complex (Fig. 36). Adduct formation step is the fastest step in Mitsunobu reaction. The
DIAD-PPh₃ adduct subsequently, deprotonates/activates the phenol (+)-boldine to form the oxyphosphonium intermediate in step 3. The DIAD-PPh₃ can react with the alcohol instead of the phenol depending on the order of addition. Subsequently, appropriate benzylic alcohol attacks the DIAD-PPh₃ complex to give the PPh₃ and protonated DIAD as degradation products. If the reacting secondary alcohol in step 5 is chiral, it will lead to inversion products as the reaction occurs in an SN₂ manner. Finally, the boldo-phenoxide attacks the methylene attached to the aromatic ring to give the (+)-boldine ether. The free phenol on (+)-boldine goes through the same steps as indicated below (Fig. 36) to finally give the dialkylated (+)-boldine ether.⁶³,⁶⁴

![Chemical structure diagram](image)

**Fig. 36:** Current understanding of Mitsunobu reaction mechanism

### 2.1.3 Biological Results - Activity at CNS Receptors

(+)-Boldine analogues 2-13 were screened at a number of CNS receptors, ion channel and transporter sites. This screening was conducted by the Psychoactive Drug Screening Program (PDSP), at the NIH. (+)-Boldine was first screened at various receptor sites in primary radioligand binding assays that measure percent inhibition. If less than 50% inhibition was
obtained at a particular receptor in the primary assay, the compound was considered inactive and was not used for secondary assays. Secondary assays were only performed at those receptors where inhibition was greater than 50%. The secondary assays are used to calculate the binding affinity ($K_i$) values at serotonin receptor subtypes: 5-HT$_{1A}$, 5-HT$_{1D}$, 5-HT$_{2A}$, 5-HT$_{2B}$, 5-HT$_{2C}$, 5-HT$_{6}$ and 5-HT$_{7}$.$^{60}$

The data from the primary screen (Table 5) suggests that the percent inhibition by the parent compound (+)-boldine, 1 was much greater than its benzylated analogues (2-13) at the 5-HT$_{1A}$, 5-HT$_{1D}$, 5-HT$_{6}$, 5-HT$_{7}$ receptor subtypes. However, the percent inhibition at the 5-HT$_{2A}$ and 5-HT$_{2B}$ and 5-HT$_{2C}$ receptors sites is higher for the benzylated analogues (2-13) than the parent molecule (+)-boldine, 1. The only exceptions were compounds 4 and 10 at the 5-HT$_{2B}$ receptor subtype which had lower percent inhibition than (+)-boldine, 1. The primary screen data suggested that compounds 2, 9 were considered inactive at the 5-HT$_{6}$ and 5-HT$_{2B}$ receptor subtypes. Interestingly, compound 10’s inhibition was insignificant for all the receptors except for 5-HT$_{2A}$ and 5-HT$_{2C}$ receptor subtypes.

Table 5: Percent Inhibition of benzylated analogues 5-HT$_{1A}$, 5-HT$_{1D}$, 5-HT$_{2A}$, 5-HT$_{2B}$, 5-HT$_{2C}$, 5-HT$_{6}$ and 5-HT$_{7}$ receptors (NA: Not Applicable and N/A: Not Available)
### % Inhibition

<table>
<thead>
<tr>
<th>R</th>
<th>Compound</th>
<th>5-HT&lt;sub&gt;1A&lt;/sub&gt;</th>
<th>5-HT&lt;sub&gt;1D&lt;/sub&gt;</th>
<th>5-HT&lt;sub&gt;2A&lt;/sub&gt;</th>
<th>5-HT&lt;sub&gt;2B&lt;/sub&gt;</th>
<th>5-HT&lt;sub&gt;2C&lt;/sub&gt;</th>
<th>5-HT&lt;sub&gt;6&lt;/sub&gt;</th>
<th>5-HT&lt;sub&gt;7&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Boldine</strong></td>
<td>1</td>
<td>87.7</td>
<td>95.8</td>
<td>56.5</td>
<td>70.0</td>
<td>50.9</td>
<td>88.1</td>
<td>93.5</td>
</tr>
<tr>
<td><strong>p-Bromo</strong></td>
<td>2</td>
<td>86.7</td>
<td>72.8</td>
<td>79.3</td>
<td>70.8</td>
<td>90.6</td>
<td>19.3</td>
<td>81.3</td>
</tr>
<tr>
<td><strong>p-Chloro</strong></td>
<td>11</td>
<td>78.1</td>
<td>69.7</td>
<td>94.1</td>
<td>82</td>
<td>96.2</td>
<td>54.4</td>
<td>87.1</td>
</tr>
<tr>
<td><strong>p-Iodo</strong></td>
<td>13</td>
<td>70.1</td>
<td>79</td>
<td>91.2</td>
<td>89.2</td>
<td>95.8</td>
<td>64.1</td>
<td>81</td>
</tr>
<tr>
<td><strong>p-Fluoro</strong></td>
<td>7</td>
<td>81.1</td>
<td>80</td>
<td>90.1</td>
<td>87.9</td>
<td>94.1</td>
<td>64.9</td>
<td>93.5</td>
</tr>
<tr>
<td><strong>m-Fluoro</strong></td>
<td>9</td>
<td>73.2</td>
<td>55.8</td>
<td>71.8</td>
<td>44.8</td>
<td>83</td>
<td>49.6</td>
<td>76.6</td>
</tr>
<tr>
<td><strong>o-Fluoro</strong></td>
<td>8</td>
<td>65</td>
<td>79.3</td>
<td>91.9</td>
<td>84.5</td>
<td>94.2</td>
<td>67.8</td>
<td>N/A</td>
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<tr>
<td><strong>p-Trifluoromethyl</strong></td>
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<td>78.2</td>
<td>73.1</td>
<td>74.7</td>
<td>86</td>
<td>96.4</td>
<td>67.1</td>
<td>N/A</td>
</tr>
<tr>
<td><strong>p-Methyl</strong></td>
<td>12</td>
<td>83.9</td>
<td>81.8</td>
<td>95.3</td>
<td>90.8</td>
<td>96</td>
<td>69.4</td>
<td>N/A</td>
</tr>
<tr>
<td><strong>p-Benzylxy</strong></td>
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<td>25.7</td>
<td>41.1</td>
<td>70.1</td>
<td>24.9</td>
<td>88.5</td>
<td>7.4</td>
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</tr>
<tr>
<td><strong>p-Methoxy</strong></td>
<td>3</td>
<td>72.6</td>
<td>79.6</td>
<td>85.5</td>
<td>65.4</td>
<td>94.8</td>
<td>41.2</td>
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<tr>
<td><strong>m-Methoxy</strong></td>
<td>5</td>
<td>76.4</td>
<td>82.1</td>
<td>94.1</td>
<td>78.6</td>
<td>97.9</td>
<td>53</td>
<td>NA</td>
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<tr>
<td><strong>o-Methoxy</strong></td>
<td>4</td>
<td>72.3</td>
<td>63.3</td>
<td>80.9</td>
<td>50</td>
<td>93.1</td>
<td>15.6</td>
<td>NA</td>
</tr>
</tbody>
</table>

**SAR at 5-HT<sub>1A</sub> receptors:**

The affinity (Table 6) of most of the screened compounds at 5-HT<sub>1A</sub> is in the moderate range (200nM to 1000nM). The compound with the highest affinity at 5HT<sub>1A</sub> was **12**, p-Methylbenzyl ether with affinity of 289nM. However, the 5-HT<sub>1A</sub> site had low affinity values for compounds **4, 8, and 9**. It is interesting to note that Compound **10** is inactive at 5HT<sub>1A</sub>. Bisbenzylation of (+)-boldine was found to be disruptive at the 5-HT<sub>1A</sub> receptor, as the compounds **2-13** lost its affinity with respective to its parent compound (+)-boldine.
The electronic nature of compounds seems to be important for binding at the 5-HT\textsubscript{1A} receptors. This is evident from the loss of affinity from the para->meta->ortho analogues of fluorobenzyl and methoxybenzyl compounds. Halogens electronegativity seems to play an important role in the affinity for 5HT\textsubscript{1A}, as \textit{p}-Bromobenzyl ether had the highest affinity for 5HT\textsubscript{1A} and the most electron withdrawing compound \textit{p}-Trifluoromethyl-benzyl had the lowest affinity in the series of compounds. However, \textit{p}-Iodobenzyl ether contained the least electronegative element Iodine and the pattern of low electronegativity and high affinity does not hold true. The presence of bulky \textit{p}-Benzyloxybenzyl group might be hindering compound 10, from binding the binding pocket of the 5-HT\textsubscript{1A} active site. This might indirectly imply that the binding pocket in the active site for 5-HT\textsubscript{1A} is significantly smaller than the 5-HT\textsubscript{1A} 5-HT\textsubscript{1D} 5-HT\textsubscript{2B} and 5-HT\textsubscript{6} receptors.

**SAR at 5-HT\textsubscript{1D} receptors:**

The 5-HT\textsubscript{1D} receptors subtype had moderate to low activity for most of the compounds 2-13. However, compounds 9, 10 were inactive at the 5-HT\textsubscript{1D} receptors. Compound 7, \textit{p}-Fluorobenzyl ether, was the most active analogue in the series with affinity of 257nM. \textit{p}-Fluorobenzyl was more active than and \textit{p}-Iodobenzyl compound in the para series of the halogens. This suggests for halogens an increase in electronegativity is associated with an increase in affinity. There seems to be no other distinct affinity pattern for the compounds at 5-HT\textsubscript{1D} receptors. Benzylation of (+)-boldine was found to be disruptive at the 5-HT\textsubscript{1D} receptor, as the compounds 2-13 lost its affinity with respective to its parent compound (+)-boldine.
**SAR at 5-HT$_{2A}$ receptors:**

The benzylated compounds 2-13 had moderate activity at 5-HT$_{2A}$ receptors. The benzylated analogues increased (+)-boldine’s affinity at 5-HT$_{2A}$ receptor 3-13 times. Compound 7, $p$-Fluorobenzyl ether was the most active analogue with an affinity of 155$nM$. Compound 3, $p$-Methoxybenzyl ether had the lowest affinity (646$nM$) in the series. Interestingly compound 10 which was inactive at most subtypes is moderately active at 5-HT$_{2A}$. There is no pattern evident to suggest that the electronics or steric is play key roles in binding 5-HT$_{2A}$ receptors.

**SAR at 5-HT$_{2B}$ receptors**

Compounds 6, 10, 12 and 13 were found to be inactive at 5-HT$_{2B}$ receptors. Compounds 4, 5, 7, 8 and 9 had increased affinity to 5-HT$_{2B}$ receptors with respect to (+)-boldine. However, in the case of 2, 3 and 11 the binding affinity decreased compared to (+)-boldine. $o$-Methoxybenzyl, 4 was the most potent compound with an affinity of 37$nM$. There seems to be no distinct pattern in benzylation to suggest that steric or electronics play key roles in affecting binding affinity to 5-HT$_{2B}$ receptors.

**SAR at 5-HT$_{2C}$ receptors:**

The benzylated compounds 2-13 had high to moderate activity at 5-HT$_{2C}$ receptors. The benzylated analogues increased (+)-boldine’s affinity at 5-HT$_{2C}$ receptor 6-46 times. $p$-Methoxybenzyl 3 was the least active compound with an affinity of 329$nM$. Compound 8, $o$-Fluorobenzyl ether was the most active analogue with an affinity of 45$nM$. Compound 10, which was found to be inactive at most of the serotonin receptors was found to be moderately active at 5-HT$_{2C}$ receptor.
SAR at 5-HT6 receptors:

The 5-HT6 receptor had the least affinity (moderate to low) for the benzylated analogs of the two receptors subtypes. o-Methoxybenzyl ether was found to be the least disruptive substituent with an affinity of 453 nM. Benzylation significantly decreased the affinity of (+)-boldine at the 5-HT6 receptor.

Table 6: Affinity (Ki in nM) of benzylated analogues at 5-HT1A, 5-HT1D, 5-HT2A, 5-HT2B, 5-HT2C, 5-HT6 and 5-HT7 receptors (NA: Not Applicable and N/A: Not Available)

<table>
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<tr>
<th>R</th>
<th>Compound</th>
<th>5-HT1A</th>
<th>5-HT1D</th>
<th>5-HT2A</th>
<th>5-HT2B</th>
<th>5-HT2C</th>
<th>5-HT6</th>
<th>5-HT7</th>
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<tr>
<td>NA(Boldine)</td>
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<td>90</td>
<td>10</td>
<td>1960</td>
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<td>2</td>
<td>326</td>
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<td>1366</td>
<td>73</td>
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<td>273</td>
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<td>p-Chloro</td>
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<td>367</td>
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<td>226</td>
<td>953</td>
<td>105</td>
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<td>p-Iodo</td>
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<td>p-Fluoro</td>
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<td>516</td>
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<td>o-Fluorobenzyl</td>
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</tr>
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<td>p-Methyl</td>
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<td>540</td>
<td>199</td>
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<td>67</td>
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<td>Inactive</td>
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<td>630</td>
<td>329</td>
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<td>m-Methoxy</td>
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<td>557</td>
<td>261</td>
<td>78</td>
<td>179</td>
<td>589</td>
<td>230</td>
</tr>
<tr>
<td>o-Methoxy</td>
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<td>1213</td>
<td>578</td>
<td>453</td>
<td>37</td>
<td>64</td>
<td>453</td>
<td>557</td>
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</tbody>
</table>

### 2.1.4 Conclusions

Mitsunobu reactions were implemented that enabled for rapid, moderate yielding semi-synthesis of benzyl-alkylated ether analogues at the C2, C9 position of (+)-boldine. The synthetic procedure can be used to synthesize numerous alkylated ether analogues from various naturally occurring alkaloids as well as many synthetic compounds.

From the study it is evident that benzylation increases the affinity of the compounds 2-13 at the 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors. This supports our original hypothesis that benzylation at C2, C9 positions of (+)-boldine can be used to develop more potent analogs at two serotonin receptors: 5-HT$_{2A}$ and 5-HT$_{2C}$.

The phenolic groups present in (+)-boldine makes it difficult for drug development as the phenolic groups are glycosylated and sulfated, which subsequently leads to low bioavailability of the compound. Benzylation of (+)-boldine helps mask the phenol moiety without decreasing the potency of compounds 2-13 for the 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors. The retention of activity at 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors should engender more metabolically stable compounds in-vivo as benzylated compounds protect the labile phenolic groups seem to be essential for improving the pharmacokinetic profile of molecules like (+)-boldine.
The most potent benzylated compounds were \(\text{o-Methoxybenzyl ether, 4}\) with an affinity of 37nM and \(\text{o-Fluorobenzyl ether, 8}\) with an affinity of 45nM at the 5-HT\(_{2B}\) and 5-HT\(_{2C}\) receptors respectively. However, considering selectivity the most interesting ligand is \(\text{p-Benzylloxybenzyl, 10}\), which is selective only for the 5-HT\(_{2A}\) and 5-HT\(_{2C}\) receptors. It is interesting to note that \(\text{o-Fluorobenzyl ether, 8}\) had a 46-fold increase in affinity at the 5-HT\(_{2A}\) receptor compared to the parent compound (\(+\)-boldine, 1).

In the future, additional studies will be done to further query how the structural changes at C2, C9 position of (\(+\)-boldine affects antagonism at the 5-HT receptor subtypes: 5-HT\(_{1A}\), 5-HT\(_{1D}\), 5-HT\(_{2A}\), 5-HT\(_{2B}\), 5-HT\(_{2C}\), 5-HT\(_{6}\) and 5-HT\(_{7}\).

The study has positively impacted the field of (\(+\)-boldine research by identifying potent ligands based on the (\(+\)-boldine scaffold. The synthetic and biological work described herein sets the stage for future optimization studies.
2.1.5: References:


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31) Ottani, A; Ferraris, E; Giuliani, D; Mioni, C; Bertolini, A; Sternieri, E; Ferrari, A. Effect of sumatriptan in different models of pain in rats. *Eur J Pharmacol.* 2004, 497, 2, pp 181-6.


37) Mattsson, C; Sonesson, C; Sandahl, A; Greiner, HE; Gassen, M; Plaschke, J; Leibrock, J; Böttcher, H. 2-Alkyl-3-(1,2,3,6-tetrahydropyridin-4-yl)-1H-indoles as novel 5-HT6 receptor agonists. *Bioorg Med Chem Lett.* 2005, 15, 19, pp 4230-4.


2.1.6 Appendices:

Supporting Information:

General Information:

Commercial reagents from Santacruz chemicals, Acros organics, Sigma Aldrich and Maybridge were purchased at the highest commercial purity and used without further purification unless otherwise stated. Analytical TLC was performed on 254µm Whatman silica gel (Aluminum backing, UV 254 nm) plates using UV light and Cerium Molybdate in Sulfuric acid as visualizing agents. Column chromatography was carried out using Aluminum Oxide, basic Brockmann 1, 500-200 µm, 60A.

$^1$H NMR, $^{13}$C spectra were recorded on Bruker Avance III 400MHz or Varian INOVA 500MHz and $^{19}$F spectra were recorded on Bruker Avance III 400MHz. The samples were dissolved in CDCl$_3$, unless otherwise noted. The following abbreviations are used to explain the multiplicities: s, singlet; d, doublet; t, triplet; q, quartet; dd, doublet of doublets; dt, doublet of triplets; dq, doublet of quartets; ddd, doublet of doublet of doublets, m, multiplets. High-resolution mass spectra were obtained from the Mass Spectroscopy Facility at the Hunter College, The City University of New York.

General Procedure for benzylaion at C2, C9 positions on (+)-boldine scaffold

(+)-Boldine (1eq .200g), polymer bound TPP (4eq), benzylic alcohol (4eq) were added to a 2 neck RBF under argon. Anhydrous THF was added and the mixture was then cooled to 0ºC for 15 min. DIAD (4eq) was added dropwise slowly via a syringe. The reaction mixture was kept under ice for an additional hour and then at rt for 8 h. The progress of the reaction was monitored
via TLC in 5%MeOH/95%DCM solvent system. The solvent was evaporated in vacuo and the mixture was dissolved in DCM and filtered via Celite. Water (20mL) was added and the organic layer was then extracted with DCM (3x35mL). Saturated NaCl was added to aid in better separation. The combined organic layers were dried under Na$_2$SO$_4$. The solvent was removed under reduced pressure. The residue was purified by flash chromatography in basic alumina (80%EtOAc/20%Hexane followed by 98%EtOAc/2%MeOH). Subsequently, preparatory TLC was performed to further purify the compounds 2-13 (2-5%MeOH/DCM). The compounds were kept in freezer (-20°C) as it was found that moisture degrades the residues.

2,9-bis((4-bromobenzyl)oxy)-1,10-dimethoxy-6-methyl-5,6,6a,7-tetrahydro-4H-dibenzo[de,g]quinoline (2)

Yield 51.6%, Appearance: Amber brown

$^1$H NMR (400 MHz, CDCl$_3$) δ 8.10 (s, 1H), 7.52 (dd, $J$=2.8 ,4.8 Hz, 4H), 7.35 (d, 4H, $J$ = 8 Hz), 6.77 (s, 1H), 6.62 (s, 1H), 5.17-5.04 (m, 4H), 3.91 (s, 3H), 3.69 (s, 3H), 3.22-2.93 (m, 4H), 2.67-2.57 (m, 6H).

$^{13}$C NMR (125 MHz, CDCl$_3$): δ 150.9 (C), 148.1 (C), 147.1 (C), 144.8 (C), 136.3 (C), 136.2 (C), 131.7 (C), 129.3 (C), 129.0 (C), 129.0 (C), 127.1 (C), 125.3 (C), 121.8 (C), 121.8 (C), 113.4 (C), 112.5 (C), 112.3 (C), 70.3 (CH$_2$), 70.1 (CH$_2$), 62.5 (C), 60.3 (C), 56.1 (CH$_2$), 53.2 (CH$_2$), 44.0 (C), 34.4 (CH$_2$), 29.2 (C).

HRMS (EI) m/z calcd for [M]+: 664.069 found: 664.0693 Melting point: 74.6-76.6°C
1,10-dimethoxy-2,9-bis((4-methoxybenzyl)oxy)-6-methyl-5,6,6a,7-tetrahydro-4H-dibenzo[de,g]quinoline (3)

Yield 56.2%, Appearance: Brownish white

$^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.10 (s, 1H), 7.40 (dd, $J$=3.6, 4.8 Hz, 4H), 6.92 (d, 4H, $J$ = 8.4 Hz), 6.82 (s, 1H), 6.65 (s, 1H), 5.15-5.02 (m, 4H), 3.89 (s, 3H), 3.82 (s, 3H), 3.82 (s, 3H), 3.68 (s, 3H), 3.15-2.93(m, 4H), 2.66-2.47 (m, 6H).

$^{13}$C NMR (125 MHz, CDCl$_3$): $\delta$ 159.4 (C), 151.2 (C), 148.1 (C), 147.4 (C), 144.9 (C), 129.3 (C), 129.2 (C), 129.1 (C), 127.1 (C), 125.0 (C), 114.0 (C), 114.0 (C), 113.3 (C), 112.4 (C), 112.3 (C), 70.7 (CH$_2$), 70.6 (CH$_2$), 62.6 (CH$_2$), 60.2 (C), 56.2 (CH$_2$), 56.1 (CH$_2$), 55.3 (C), 53.3 (CH$_2$), 44.0 (C), 34.5 (C), 30.9 (C), 29.2 (C).

HRMS (EI) $m/z$ calcd for [M]+: 568.2698 found: 568.2694 Melting point: 90.2-93.8°C

1,10-dimethoxy-2,9-bis((2-methoxybenzyl)oxy)-6-methyl-5,6,6a,7-tetrahydro-4H-dibenzo[de,g]quinoline (4)

Yield 40.9%, Appearance: Light brown

$^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.13 (s, 1H), 7.54 (d, $J$=7.2 Hz, 2H), 7.32-7.26 (m, 2H), 7.00-6.98 (m, 2H), 6.91 (d, 2H $J$=8.4 Hz), 6.83 (s, 1H), 6.68 (s, 1H), 5.28-5.12 (m, 4H), 3.92 (s, 3H), 3.88 (s, 3H), 3.87 (s, 3H), 3.72 (s, 3H), 3.16-2.93(m, 4H), 2.67-2.46 (m, 6H).

$^{13}$C NMR (125 MHz, CDCl$_3$): $\delta$ 156.6 (C), 151.3 (C), 148.0 (C), 147.5 (C), 144.7 (C), 129.3 (C), 128.8 (C), 128.6 (C), 128.4 (C), 128.3 (C), 127.0 (C), 125.6 (C), 125.6 (C), 124.9 (C), 120.7 (C), 120.6 (C), 113.0 (C), 112.3 (C), 112.1 (C), 110.1 (C), 65.8 (C), 65.7 (C), 62.6 (C), 60.2 (C), 56.2 (C), 55.4 (C), 55.3 (C), 53.3 (C), 44.0 (C), 34.5 (C), 29.2 (C).

HRMS (EI) $m/z$ calcd for [M]+: 568.2688 found: 568.2694 Melting point: 57.0-60.5°C
1,10-dimethoxy-2,9-bis((3-methoxybenzyl)oxy)-6-methyl-5,6,6a,7-tetrahydro-4H-dibenzo[de,g]quinolone (5)

Yield 37.8%, Appearance: Brown

$^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.12 (s, 1H), 7.32-7.28 (m, 2H), 7.06-7.04 (m, 4H), 6.87-6.84 (m, 2H), 6.80 (s, 1H), 6.63 (s, 1H), 5.20-5.08 (m, 4H), 3.91 (s, 3H), 3.82 (s, 3H), 3.82 (s, 3H), 3.72 (s, 3H), 3.12-3.11 (m, 1H), 3.03-2.92 (m, 3H), 2.64 (d, 1H, J=2.4Hz), 2.62-2.49 (m, 6H).

$^{13}$C NMR (125 MHz, CDCl$_3$): $\delta$ 159.9 (C), 159.9 (C), 151.1 (C), 148.1 (C), 147.3 (C), 144.8 (C), 138.9 (C), 138.8 (C), 129.6 (C), 129.3 (C), 128.9 (C), 127.8 (C) 127.1 (C), 125.2 (C), 119.5 (C), 113.4 (C), 113.3 (C), 113.3 (C), 112.8 (C), 112.7 (C), 112.4 (C), 112.3 (C), 70.9 (CH$_2$), 70.6 (CH$_2$), 62.5 (CH$_2$), 60.3 (C), 56.1 (CH$_2$), 55.3 (CH$_2$), 55.2 (C), 53.3 (C), 44.0 (C), 34.5 (CH$_2$), 29.2 (C).

HRMS (EI) m/z calcld for [M]+: 568.2701 found: 568.2694 Melting point: 52.2-54.1°C

1,10-dimethoxy-6-methyl-2,9-bis((4-(trifluoromethyl)benzyl)oxy)-5,6,6a,7-tetrahydro-4H-dibenzo[de,g]quinoline (6)

Yield 49.2%, Appearance: Yellow brown

$^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.13 (s, 1H), 7.67-7.64 (m, 4H), 7.60 (d, 4H, J = 8 Hz), 6.79 (s, 1H), 6.63 (s, 1H), 5.29-5.15 (m, 4H), 3.93 (s, 3H), 3.71 (s, 3H), 3.16-2.93 (m, 4H), 2.66-2.46 (m, 6H).

$^{13}$C NMR (125 MHz, CDCl$_3$): $\delta$ 150.9 (C), 148.1 (C), 147.0 (C), 144.9 (C), 141.3 (C), 141.3 (C), 130.5 (C), 130.5 (C), 130.2 (C), 130.2 (C), 130.0 (C), 129.9 (C), 129.7 (C), 129.7 (C), 129.4 (C), 129.1 (C), 128.3 (C), 127.3 (C), 127.2 (C), 125.6 (C), 125.6 (C), 125.6 (C), 125.5 (C), 125.5 (C),
113.5 (C), 112.5 (C), 112.4 (C), 77.3 (C), 77.0 (C), 76.8 (C), 70.2 (C), 70.0 (C), 62.5 (C), 60.3 (C), 56.2 (C), 53.2 (C), 44.0 (C), 34.4 (C), 29.2 (C).

$^{19}$F NMR (470MHz) δ 62.51, 62.52

HRMS (EI) $m/z$ calcd for [M]+: 644.2239 found: 644.223 Melting point: 72.0-75.1°C

9-((3-fluorobenzyl)oxy)-2-((4-fluorobenzyl)oxy)-1,10-dimethoxy-6-methyl-5,6,6a,7-tetrahydro-4H-dibenzo[de,g]quinolone (7)

Yield 50.3%, Appearance: Yellow brown

$^1$H NMR (400 MHz, CDCl$_3$) δ 8.11 (s, 1H), 7.47-7.43 (m, 4H), 7.10-7.05 (m, 4H), 7.09-7.05 (m, 4H), 6.80 (s, 1H), 6.64 (s, 1H), 5.17-5.04 (m, 4H), 3.90 (s, 3H), 3.69 (s, 3H), 3.15-2.93 (m, 4H), 2.66-2.46 (m, 6H).

$^{13}$C NMR (125 MHz, CDCl$_3$): δ 163.5 (C), 161.5 (C), 161.5 (C), 151.0 (C), 148.2 (C), 147.2 (C), 144.9 (C), 132.9 (C), 132.9 (C), 129.3 (C), 129.3 (C), 129.2 (C), 129.0 (C), 128.0 (C), 127.1 (C), 125.3, 115.6 (C), 115.4 (C), 113.4 (C), 112.5 (C), 112.3 (C), 70.4 (C), 70.2 (C), 62.5 (C), 60.3 (C), 56.1 (C), 53.3 (C), 44.0 (C), 34.5 (C), 29.3 (C).

$^{19}$F NMR (470MHz) δ114.35, 114.45

HRMS (EI) $m/z$ calcd for [M]+: 544.2301 found: 544.2294 Melting point: 59.3-63.0°C
2,9-bis((2-fluorobenzyl)oxy)-1,10-dimethoxy-6-methyl-5,6,6a,7-tetrahydro-4H-
dibenzo[de,g]quinoline (8)

Yield 45.1%, Appearance: Amber brown

$^1$H NMR (400 MHz, CDCl$_3$) δ 8.12 (s, 1H), 7.61-7.55 (m, 2H), 7.32-7.27 (m, 2H), 7.19-7.15 (m,2H), 7.12-7.06 (m, 2H), 6.85 (s, 1H), 6.69 (s, 1H) 5.26-5.15 (m, 4H), 3.90 (s, 3H), 3.70 (s, 3H), 3.14-2.95 (m, 4H), 2.680-2.50 (m, 6H).

$^{13}$C NMR (125 MHz, CDCl$_3$): δ 161.3 (C), 159.5 (C), 159.4 (C), 150.9 (C), 148.2 (C), 147.1 (C), 144.8 (C), 129.8 (C), 129.7 (C), 129.7 (C), 129.6 (C), 129.6 (C), 129.5 (C), 129.4 (C), 129.0 (C), 128.0 (C), 127.1 (C), 125.3 (C), 124.4 (C), 124.4 (C), 124.3 (C), 124.3 (C), 124.3 (C), 124.3 (C), 115.4 (C), 115.3 (C), 115.2 (C), 115.1 (C), 113.3 (C), 112.4 (C), 112.3 (C), 64.6 (C), 64.6 (C), 64.5 (C), 62.5 (C), 60.3 (C), 56.2 (C), 53.3 (C), 44.0 (C), 34.4 (C), 29.2 (C).

$^{19}$F NMR (470MHz) δ118.62, 118.78

HRMS (EI) m/z calcd for [M]+: 544.2298 found: 544.2294 Melting point: 60.6-63.9°C

2,9-bis((3-fluorobenzyl)oxy)-1,10-dimethoxy-6-methyl-5,6,6a,7-tetrahydro-4H-
dibenzo[de,g]quinolone (9)

Yield 25.7%, Appearance: brown

$^1$H NMR (400 MHz, CDCl$_3$) δ 8.12 (s, 1H), 7.38-7.33 (m, 2H), 7.25-7.20 (m, 4H) 7.04-6.98 (m, 2H), 6.78 (s, 1H), 6.62 (s, 1H), 5.22-5.09 (m, 4H), 3.92 (s, 3H), 3.71 (s, 3H), 3.17-2.93 (m, 4H), 2.66-2.48 (s, 6H).

$^{13}$C NMR (125 MHz, CDCl$_3$): δ 164.0 (C), 162.1 (C), 162.0 (C), 150.9 (C), 148.2 (C), 147.1 (C), 144.8 (C), 139.9 (C), 139.8 (C), 139.8 (C), 139.7 (C), 130.2 (C), 130.1 (C), 127.2 (C), 125.3 (C),
122.6 (C), 122.6 (C), 122.6 (C), 114.9 (C), 114.8 (C), 114.8 (C), 114.7 (C), 114.7 (C), 114.7 (C), 114.2 (C), 114.0 (C), 113.4 (C), 112.4 (C), 112.3 (C), 70.2 (C), 70.2 (C), 70.0 (C), 70.0 (C), 62.5 (C), 60.3 (C), 56.2 (C), 56.1 (C), 53.2 (C), 44.0 (C), 34.4 (C), 29.7 (C), 29.1 (C), 21.8 (C), 21.7 (C), 21.7 (C).

$^{19}$F NMR (470MHz) δ112.82, 112.90

HRMS (EI) m/z calcd for [M]+: 664.069 found: 664.0693 Melting point: 51.7-54.3°C

2,9-bis((4-(benzyloxy)benzyl)oxy)-1,10-dimethoxy-6-methyl-5,6,6a,7-tetrahydro-4H-dibenzo[de,g]quinoline (10)

Yield 54.2%, Appearance: Yellow brown

$^1$H NMR (400 MHz, CDCl$_3$) δ 8.10 (s, 1H), 7.44-7.30 (m, 14H), 6.99 (d, 4H, $J$=8Hz), 6.81 (s, 1H), 6.65 (s, 1H), 5.14-5.01 (m, 4H), 3.88 (s, 3H), 3.67 (s, 3H), 3.13-2.93 (m, 4H), 2.66-2.48 (s, 6H).

$^{13}$C NMR (125 MHz, CDCl$_3$): δ 158.6 (C), 151.2 (C), 148.1 (C), 147.4 (C), 144.9 (C), 137.0 (C), 136.9 (C), 129.6 (C), 129.3 (C), 129.2 (C), 129.1 (C), 128.8 (C), 128.6 (C), 128.0 (C), 128.0 (C), 127.7 (C), 127.5 (C), 127.1 (C), 125.1 (C), 114.9 (C), 114.9 (C), 113.30 (C), 112.4 (C), 112.3 (C), 70.7 (C), 70.5 (C), 70.1 (C), 70.0 (C), 62.6 (C), 60.2 (C), 56.1 (C), 53.3 (C), 44.0 (C), 34.5 (C), 29.3 (C).

HRMS (EI) m/z calcd for [M]+: 720.3315 found: 720.332 Melting point: 62.7-64.8°C
2,9-bis((4-chlorobenzyl)oxy)-1,10-dimethoxy-6-methyl-5,6,6a,7-tetrahydro-4H-
dibenzo[de,g]quinolone (11)

Yield 53.5%, Appearance: Yellow brown

$^1$H NMR (400 MHz, CDCl$_3$) δ 8.10 (s, 1H), 7.42 (m, 4H), 7.38-7.35 (m, 4H), 6.78 (s, 1H), 6.62 (s, 1H), 5.16-5.08 (m, 4H), 3.91 (s, 3H), 3.69 (s, 3H), 3.15-2.92 (m, 4H), 2.65-2.47 (m, 6H).

$^{13}$C NMR (125 MHz, CDCl$_3$): δ 150.9 (C), 148.2 (C), 147.1 (C), 144.8 (C), 135.7 (C), 135.7 (C), 133.7 (C), 133.7 (C), 129.3 (C), 129.0 (C), 128.8 (C), 128.7 (C), 128.7 (C), 127.1 (C), 125.3 (C), 113.4 (C), 112.5 (C), 112.3 (C), 70.3 (C), 70.1 (C), 62.5 (C), 60.3 (C), 56.1 (C), 53.4 (C), 44.0 (C), 34.5 (C), 29.2 (C).

HRMS (EI) $m/z$ calcd for [M]+: 576.1703 found: 576.1703 Melting point: 69.1-72.5°C

1,10-dimethoxy-6-methyl-2,9-bis((4-methylbenzyl)oxy)-5,6,6a,7-tetrahydro-4H-
dibenzo[de,g]quinolone (12)

Yield 49%, Appearance: Brown

$^1$H NMR (400 MHz, CDCl$_3$) δ 8.10 (s, 1H), 7.37-7.35 (m, 4H), 7.19 (d, 4H, $J$ = 7.6 Hz), 6.80 (s, 1H), 6.65 (s, 1H), 5.18-5.05 (m, 4H), 3.906 (s, 3H), 3.69 (s, 3H), 3.30-2.94 (m, 4H), 2.67-2.36 (m, 6H).

$^{13}$C NMR (125 MHz, CDCl$_3$): δ 151.2 (C), 148.1 (C), 147.4 (C), 144.8 (C), 137.6 (C), 137.5 (C), 134.2 (C), 134.2 (C), 129.2 (C), 129.2 (C), 128.8 (C), 127.6 (C), 127.5 (C), 127.4 (C), 127.1 (C), 125.0 (C), 113.2 (C), 112.3 (C), 70.8 (C), 70.7 (C), 62.5 (C), 60.2 (C), 56.1 (C), 53.3 (C), 44.0 (C), 34.5 (C), 29.2 (C), 21.2 (C).

HRMS (EI) $m/z$ calcd for [M]+: 536.2799 found: 536.2795 Melting point: 64.7-67.9°C
2,9-bis((4-iodobenzyl)oxy)-1,10-dimethoxy-6-methyl-5,6,6a,7-tetrahydro-4H-
dibenzo[de,g]quinolone (13)

Yield 48.5%, Appearance: Yellow brown

$^1$H NMR (400 MHz, CDCl$_3$) δ 8.10 (s, 1H), 7.73-7.70 (m, 4H), 7.22 (d, 4H, $J = 8$ Hz), 6.76 (s, 1H), 6.61 (s, 1H), 5.16-5.03 (m, 4H), 3.90 (s, 3H), 3.68 (s, 3H), 3.15-2.92 (m, 4H), 2.64-2.53 (m, 6H).

$^{13}$C NMR (125 MHz, CDCl$_3$): δ 150.9 (C), 148.1 (C), 147.1 (C), 144.8 (C), 137.7 (C), 136.9 (C), 136.9 (C), 129.2 (C), 129.1 (C), 128.9 (C), 127.1 (C), 125.3 (C), 113.4 (C), 112.2 (C), 112.3 (C), 93.4 (C), 93.3 (C), 70.3 (C), 70.11 (C), 62.5 (C), 60.3 (C), 56.1 (C), 53.2 (C), 44.0 (C), 34.4 (C), 29.2 (C).

HRMS (EI) $m/z$ calcd for [M]+: 760.0417 found: 760.0415 Melting point: 80.9-84.4°C