Synthesis of Novel Aporphine-Inspired Neuroreceptor Ligands

Nirav R. Kapadia

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SYNTHESIS OF NOVEL APORPHINE-INSPIRED NEURORECEPTOR LIGANDS

by

Nirav R Kapadia

A dissertation submitted to the Graduate Faculty in Chemistry in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York.

2016
This manuscript has been read and accepted by the Graduate Faculty in Chemistry in satisfaction of the dissertation requirement for the degree of Doctor of Philosophy

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Supervisory Committee

THE CITY UNIVERSITY OF NEW YORK
ABSTRACT

Synthesis of Novel Aporphine-Inspired Neuroreceptor Ligands

by

Nirav R Kapadia

Advisor: Dr. Wayne Harding

Aporphines are a group of tetracyclic alkaloids that belong to the ubiquitous tetrahydroisoquinoline family. The aporphine template is known to be associated with a range of biological activities. Aporphines have been explored as antioxidants, anti-tuberculosis, antimicrobial and anticancer agents. Within the Central Nervous Systems (CNS), aporphine alkaloids are known to possess high affinity for several clinically valuable targets including dopamine receptors (predominantly D₁ and D₂), serotonin receptors (5-HT₁A and 5-HT₇) and α-adrenergic receptors. Aporphines are also inhibitors of the acetylcholinesterase enzyme – a clinical target for the treatment of Alzheimer’s disease. Considering the diverse profile of aporphine alkaloids at CNS receptors they can be considered as “privileged scaffold” for the design of CNS drugs.

The aporphine alkaloid nantenine is a 5-HT₂A receptor antagonist and has moderate affinity for the 5-HT₂A receptor. Selective 5-HT₂A antagonists have therapeutic potential for the treatment of a number of neuropsychiatric disorders including depression, schizophrenia and sleep disorders. The aporphine core of nantenine serves as a valuable lead for the identification of selective 5-HT₂A antagonists.

In order to understand the structural tolerance of the aporphine core required for 5-HT₂A antagonism an exhaustive Structure Activity Relationship (SAR) study was designed. Accordingly, a diverse library of nantenine analogues was synthesized and evaluated for affinity at the 5-HT₂A
receptor. Results from the SAR studies demonstrate that the nitrogen atom of nantenine is required for affinity and that introduction of a phenyl ring at the C4 position is detrimental for 5-HT$_{2A}$ receptor affinity. At the C3 position, introduction of halogen atoms is beneficial for 5-HT$_{2A}$ antagonistic activity. Furthermore, a library of C3 analogues having hydrophobic substituents as well as ring D indole analogues is currently being evaluated for affinity at the 5-HT$_{2A}$ receptors. These compounds will further expand our understanding of the tolerance of the aporphine core required for 5-HT$_{2A}$ antagonism.

In order to rationalize the affinity of certain high affinity ligands, molecular docking studies were conducted. Selected compounds were docked into a homology model of the 5-HT$_{2A}$ receptor to extract information about possible binding modes. Based on results of these studies, it is concluded that the interaction of C3 halogenated aporphine analogues with Phe339/Phe340 residues might be responsible for their enhanced affinity. Information obtained from molecular docking studies is being utilized for design of advanced generations of analogues.

Finally, a novel series of flexible tris-(phenylalkyl)amines were synthesized and evaluated to test the importance of a rigid aporphine core as well as incorporation of N-phenylalkyl substituents. These compounds featuring a halogen substituent in ring C, were found to have high affinity and selectivity for the 5-HT$_{2B}$ receptor, with some of the compounds being more potent than the selective 5-HT$_{2B}$ antagonist SB200646. Results from this study indicate that ring C of these compounds is generally tolerant for halogen substitution. The synthetic feasibility of this newly identified template (4 high-yielding synthetic steps from commercially available materials) makes this scaffold attractive for the synthesis of larger libraries of analogs and promise for optimization of 5-HT$_{2B}$ affinity and selectivity.
ACKNOWLEDGEMENTS

Although the process of PhD terminates at the completion of a thesis, it also marks the commencement of a new scientific journey. I would like to take this opportunity to recognize and thank many people who have directly and indirectly contributed in this journey.

First and foremost, I would like to thank my PhD mentor, Prof. Wayne W Harding for his mentorship. Your guidance has enabled me to successfully complete this thesis as well as helped me to evolve as a scientist. You displayed patience and confidence in me, whenever I encountered any problems. You also showed immense excitement and supported me when I had positive results. Under your mentorship, I have learned a lot about the application of modern organic chemistry in the field of drug discovery and drug design. Your discipline and passion for organic chemistry continue to inspire me. I will be forever grateful to be a part of your research group.

I also thank my committee members, Prof. Shengping Zheng and Prof. Adam Profit for always providing their valuable feedback which help shaped the course of this thesis.

I was fortunate to be trained by and to work alongside two highly talented post-doctoral researchers – Dr. Shashikanth Ponnala and Dr. Sudarshan Madapa. I thank both of them for having taught me as well as master me many synthetic chemistry and literature search techniques. I will be forever indebted to your assistance.

I also thank the Dept. of Chemistry at Hunter College for setting up a unique and congenial research environment. A special thanks to Dr. Matthew Devany for maintaining a state of the art NMR facility. I would also like to thank Late. Dr. Cliff Soll, Dr. Srinivas Chakravartula as well as Dr. Barney Yoo for providing Mass spectrometry services. I thank Prof. Mootoo and his research
group as well as Prof. Zheng and his research group for providing generous access to chemicals. I also appreciate the efforts put in by the Chemistry Department’s administrative and teaching staff: Ms. Mirela Settenhofer, Ms. Shontel Housten and Ms. Olena Shport - your consistent efforts always helped to reduce the impact of non-research activities on my research.

This journey although difficult, was made to look easy because of the constant support of my friends. First I would like to thank my lab colleagues - Satish Gadhiya, Sujay Joseph and Sharear Ahmed for always providing me your assistance, feedback and lighter moments in the lab. A special thanks to Kriti Kalpana – you made me believe in me and gave me beautiful memories outside the lab to cherish. I thank Ashwini Goghare and Deven Patel for your help and encouragement. I also thank Zhwei Yin and Junior Gonzales for their support.

Finally I am indebted to my family without whose love, support and motivation this work was impossible. I would like to thank my father, Mr. Raman Kapadia and mother Mrs. Saroj Kapadia for always instilling the importance of hard work and determination in my life. I also thank Nilesh, Hiren and Kalpana for all their help, moral support and encouragement. I could not have accomplished this without you.
I dedicate this thesis to my parents and family.

आ धीरिस हु मारा माता-पिता अने परिवार ने अर्पण कर छु

कर्मण्येवाधिकारस्ते मा फलेषु कदाचन।
मा कर्मफलहेतुर्भूमिः ते सहुगृहस्त्वकर्मणिः ॥ २-४७॥

You have the right to work only but never to its fruits.
Let not the fruits of action be your motive, nor let your attachment be to inaction.

(Bhagwad Gita, Chapter 2, verse 47)
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<tr>
<td>$^1$H NMR</td>
<td>Proton Nuclear Magnetic Resonance</td>
</tr>
<tr>
<td>$^{13}$C NMR</td>
<td>Carbon Nuclear Magnetic Resonance</td>
</tr>
<tr>
<td>5-HT</td>
<td>5-hydroxytryptamine</td>
</tr>
<tr>
<td>5-HT$_{1A}$</td>
<td>Serotonin receptor - subtype 1A</td>
</tr>
<tr>
<td>5-HT$_{2A}$</td>
<td>Serotonin receptor - subtype 2A</td>
</tr>
<tr>
<td>5-HT$_{2B}$</td>
<td>Serotonin receptor - subtype 2B</td>
</tr>
<tr>
<td>5-HT$_{2C}$</td>
<td>Serotonin receptor - subtype 2C</td>
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<tr>
<td>5-HT$_{7}$</td>
<td>Serotonin receptor - subtype 7</td>
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<td>$\alpha_{1A}$</td>
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</tr>
<tr>
<td>$\alpha_{1D}$</td>
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<td>Alpha 2A adrenergic receptor</td>
</tr>
<tr>
<td>$\alpha_{2B}$</td>
<td>Alpha 2B adrenergic receptor</td>
</tr>
<tr>
<td>$\alpha_{2C}$</td>
<td>Alpha 2C adrenergic receptor</td>
</tr>
<tr>
<td>$\delta$</td>
<td>Chemical shift in ppm</td>
</tr>
<tr>
<td>AChE</td>
<td>AcetylCholinesterase</td>
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<tr>
<td>AcOH</td>
<td>Acetic acid</td>
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<tr>
<td>ADHD</td>
<td>Attention Deficit Hyperactivity Disorder</td>
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<tr>
<td>AIBN</td>
<td>Azobisisobutyronitrile</td>
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<tr>
<td>AMDA</td>
<td>9-aminomethyl-9-10-dihydroanthracene</td>
</tr>
<tr>
<td>BBB</td>
<td>Blood Brain Barrier</td>
</tr>
<tr>
<td>BF$_3$.Et$_2$O</td>
<td>Boron trifluoride etherate</td>
</tr>
<tr>
<td>BH$_3$.THF</td>
<td>Borane tetrahydrofuran</td>
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<td>BnBr</td>
<td>Benzyl bromide</td>
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<tr>
<td>(BoC)$_2$O</td>
<td>Di-tert-butyl dicarbonate</td>
</tr>
<tr>
<td>BuChE</td>
<td>Butyrylcholinesterase</td>
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<tr>
<td>$n$-Bu$_3$SnH</td>
<td>Tributyltin hydride</td>
</tr>
<tr>
<td>C$_2$H$_5$OH</td>
<td>Ethanol</td>
</tr>
<tr>
<td>CaCl$_2$</td>
<td>Calcium chloride</td>
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</table>
calcd.  Calculated
CDCl$_3$  Deuterated chloroform
CDI  1,1’-Carbonyldiimidazole
CH$_3$COOH  Acetic acid
CH$_3$NO$_2$  Nitromethane
CNS  Central Nervous System
CsF  Cesium Fluoride
d  Doublet
D$_1$  Dopamine receptor - subtype D$_1$
D$_2$  Dopamine receptor - subtype D$_2$
DCM  Dichloromethane
DIAD  Diisopropyl azodicarboxylate
DIPEA  $N,N$-Diisopropylethylamine
DMAP  4-Dimethylaminopyridine
DMF  Dimethyl formamide
DMP  Dess-Martin Periodinane
DMSO  Dimethyl sulfoxide
DOI  2,5-Dimethoxy-4-iodoamphetamine
EDCI  1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
ESP  Electostatic potential
Et  Ethyl
EtOH  Ethanol
FeCl$_3$  Ferric chloride
FLIPR  Fluorescence Imaging Plate Reader
g  Gram
GPCR  G-Protein Coupled Receptor
h  Hour
H$_1$  Histamine receptor - subtype H$_1$
H$_3$  Histamine receptor - subtype H$_3$
H$_2$SO$_4$  Sulfuric acid
HBr  Hydrogen bromide
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<tr>
<td>HCl</td>
<td>Hydrogen chloride</td>
</tr>
<tr>
<td>HCOOH</td>
<td>Formic acid</td>
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<tr>
<td>HCT-116</td>
<td>Human colon cancer cell line</td>
</tr>
<tr>
<td>HIV-1</td>
<td>Human Immunodeficiency Virus-1</td>
</tr>
<tr>
<td>hNET</td>
<td>Human Norepinephrine Transporter</td>
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<tr>
<td>HOBt</td>
<td>Hydroxybenzotriazole</td>
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<tr>
<td>HRESIMS</td>
<td>High Resolution Electrospray Ionisation Mass Spectroscopy</td>
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<tr>
<td>HY</td>
<td>Hydrophobic group</td>
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<td>HYA</td>
<td>Hydrophobic Aromatic Rings</td>
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<tr>
<td>Hz</td>
<td>Hertz</td>
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<td>IBS</td>
<td>Irritable Bowel Syndrome</td>
</tr>
<tr>
<td>IC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>Half maximal inhibitory concentration</td>
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<tr>
<td>IGROV</td>
<td>An ovarian cancer cell line</td>
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<td>J</td>
<td>Coupling constant</td>
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<td>K&lt;sub&gt;2&lt;/sub&gt;CO&lt;sub&gt;3&lt;/sub&gt;</td>
<td>Potassium carbonate</td>
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<tr>
<td>K&lt;sub&gt;e&lt;/sub&gt;</td>
<td>Apparent binding affinity</td>
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<tr>
<td>K&lt;sub&gt;i&lt;/sub&gt;</td>
<td>Binding affinity</td>
</tr>
<tr>
<td>KNH&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Potassium amide</td>
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<td>l-5-HTP</td>
<td>5-hydroxy-L-tryptophan</td>
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<tr>
<td>LAH</td>
<td>Lithium aluminum hydride</td>
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<tr>
<td>LDA</td>
<td>Lithium diisopropylamide</td>
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<tr>
<td>LiBH&lt;sub&gt;4&lt;/sub&gt;</td>
<td>Lithium borohydride</td>
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<tr>
<td>LSD</td>
<td>Lysergic acid diethylamide</td>
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<td>m</td>
<td>Multiplet</td>
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<tr>
<td>M</td>
<td>Molar</td>
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<tr>
<td>MDMA</td>
<td>3,4-methylenedioxy methamphetamine</td>
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<td>Me</td>
<td>Methyl</td>
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<tr>
<td>MeI</td>
<td>Methyl Iodide</td>
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<td>MeOH</td>
<td>Methanol</td>
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<tr>
<td>mg</td>
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<tr>
<td>Abbreviation</td>
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<td>MHz</td>
<td>MegaHertz</td>
</tr>
<tr>
<td>min</td>
<td>Minute</td>
</tr>
<tr>
<td>mL</td>
<td>Milliliter</td>
</tr>
<tr>
<td>mmol</td>
<td>Millimoles</td>
</tr>
<tr>
<td>mp</td>
<td>Melting point</td>
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<tr>
<td>Na(AcO)$_3$BH</td>
<td>Sodium triacetoxyborohydride</td>
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<td>Na$_2$CO$_3$</td>
<td>Sodium carbonate</td>
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<td>NaBH$_4$</td>
<td>Sodium borohydride</td>
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<td>NaO$_2$CCH$_3$</td>
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<td>Sodium Hydroxide</td>
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<td>NBS</td>
<td>N-Bromosuccinimide</td>
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<td>NCI</td>
<td>National Cancer Institute</td>
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<td>nd</td>
<td>Not determined</td>
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<tr>
<td>NH$_4$OAC</td>
<td>Ammonium acetate</td>
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<tr>
<td>NIH</td>
<td>National Institutes of Health</td>
</tr>
<tr>
<td>nM</td>
<td>Nanomolar</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear Magnetic Resonance</td>
</tr>
<tr>
<td>NRI</td>
<td>Norepinephrine Reuptake Inhibitor</td>
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<td>PCl$_5$</td>
<td>Phosphorus pentachloride</td>
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<td>Pd(dppf)Cl$_2$</td>
<td>[1,1’-Bis(diphenylphosphino)ferrocene]dichloropalladium (II)</td>
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<td>Pd(OAc)$_2$</td>
<td>Palladium (II) acetate</td>
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<td>Pd(PPh$_3$)$_4$</td>
<td>Tetrakis(triphenylphosphine)palladium (0)</td>
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<td>Protein Data Bank</td>
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<td>PDE5</td>
<td>Phosphodiesterase Type 5</td>
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<td>PDSP</td>
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<td>POCl$_3$</td>
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<tr>
<td>PPA</td>
<td>Polyphosphoric acid</td>
</tr>
<tr>
<td>Pr</td>
<td>Propyl</td>
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</table>
PTSA  \( p \)-Toluenesulfonic acid

s  singlet

SAR  Structure Activity Relationship

SEM  Standard Error of the Mean

SnCl\(_2\)  Stannous chloride

SPECT  Single Photon Emission Computed Tomography

t  triplet

T-47D  A breast cancer cell line

TBAF  Tetra-\(n\)-butylammonium fluoride

TBSCI  \( tert \)-Butyldimethylsilyl chloride

TEA  Triethylamine

\( tert \)  tertiary

TFA  Trifluoroacetic acid

TFAA  Trifluoroacetic anhydride

THF  Tetrahydrofuran

TLC  Thin Layer Chromatography

TMSCl  Trimethylsilyl chloride

VHD  Valvular Heart Disease

ZnBr\(_2\)  Zinc Bromide
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CHAPTER I: INTRODUCTION
1.1 Aporphine Alkaloids

1.1.1 Background & Occurrence

Aporphine alkaloids are natural and synthetic alkaloids that possess a tetracyclic framework. Chemically they incorporate a tetrahydroisoquinoline substructure and belong to the isoquinoline class of alkaloids. More than 500 members of this class of alkaloids have been isolated. Aporphine alkaloids are widely distributed in *Annonaceae, Lauraceae, Monimiaceae, Menispermaceae, Hernandiaceae* and other plant families.¹ Both naturally occurring and synthetic aporphine alkaloids possess diverse range of pharmacological actions. Figure 1 shows the basic aporphine skeleton.²

![Figure 1: Basic aporphine skeleton](image)

1.1.2 Biosynthesis of Aporphine Alkaloids

Extensive research has indicated that a majority of aporphine alkaloids are biosynthesized from the tetrahydrobenzylisoquinoline alkaloid *(S)-reticuline* (2).³⁻⁵ The biosynthesis of *(S)-reticuline* from L-tyrosine is shown in Figure 2. In the first step the amino acid L-tyrosine (3) undergoes a phenolic oxidation to generate catechol 4, which then undergoes decarboxylation to generate amine 5. Alternatively 3 can also undergo transamination to produce ketoacid 6 followed by decarboxylation to produce aldehyde 7. Amine 5 and aldehyde 7 are then subjected to an enzymatic Pictet-Spengler reaction to furnish the tetrahydroisoquinoline compound 8 which undergoes
subsequent transformations to produce (S)-reticuline (2). (S)-reticuline can then be transformed to aporphines via a direct phenolic oxidation as shown in Figure 3. An alternate biosynthetic route for the synthesis of aporphine alkaloids involves dienone intermediate 14 as shown in Figure 4.1,4

Figure 2: Biosynthetic route of (S)-reticuline (2)

Figure 3: Biotransformation of 2 to aporphines 11 and 12
Several groups have synthesized aporphine alkaloids using biomimetic strategies that involve either oxidative coupling or dienone intermediates, thus validating the above mentioned biosynthetic routes.\textsuperscript{6-10}

\textbf{Figure 4}: Alternate biosynthetic route for aporphines via dienone intermediate (R\textsubscript{1} or R\textsubscript{2} = appropriate group)
1.1.3 Common Synthetic Approaches to Aporphine Alkaloids:

There are several synthetic routes to aporphine alkaloids reported. A summary of some of the common approaches is mentioned here. Pioneering work done by Neumeyer and co-workers as well as Zhang and co-workers involved the use of an acid catalyzed rearrangement of morphine (23) (Figure 5).\textsuperscript{11-16} This route offers easy access for the synthesis of C10 and C11 aporphine analogues.

Another common approach involves the synthesis of aporphine alkaloids from a benzyl-tetrahydroisoquinoline type precursor. The synthesis of the benzyl-tetrahydroisoquinoline precursor (28) is typically accomplished through a Pictet-Spengler reaction or a Bischler-Napieralski reaction (Figure 6).\textsuperscript{17-20} Recent development of chiral Pictet-Spengler catalysts and chiral imine reducing agents has allowed the construction of the requisite precursor compounds with the desired stereochemistry.\textsuperscript{21,22}
Transformation of benzyl tetrahydroisoquinoline derivatives to aporphine alkaloids has been reported using several conditions. An earlier approach involves a Pschorr cyclization for the formation of the biaryl bond (Figure 7). The requisite aryl radical (32) can be generated through the decomposition of a diazonium salt (31) in the presence of a copper or zinc catalyst.\textsuperscript{23,24}

Another approach involves the use of an oxidative photocyclization reaction on a stilbene-phenanthrene (34) type of system (Figure 8).\textsuperscript{25}
Recent advances in palladium catalyzed cross coupling reactions has led to its application in the synthesis of aporphine alkaloids (Figure 9). Several groups including ours have reported optimized conditions for this reaction.\textsuperscript{26-29}

**Figure 9:** Palladium catalyzed synthesis of aporphine alkaloids

Alternative methods for the construction of the biaryl bond involves i) intramolecular oxidation through the use of oxidation reagents such as ferric chloride and hypervalent iodine reagents (Figure 10) and ii) aryl-aryl coupling via a benzyne intermediate (Figure 11).\textsuperscript{30-32}

**Figure 10:** Synthesis of aporphine alkaloid (40) via oxidative cyclization

**Figure 11:** Synthesis of aporphine alkaloid (43) via a benzyne intermediate
Another less commonly used approach for the synthesis of aporphines involves their synthesis via phenanthrene alkaloids.\textsuperscript{33,34}

1.1.4 Pharmacological Effects of Aporphine Alkaloids:
Aporphine alkaloids exhibit a plethora of effects within the CNS. There are a number of aporphine alkaloids reported as ligands at dopamine and serotonin receptors.\textsuperscript{1,35} Ligands at the D\textsubscript{1} and D\textsubscript{2} dopamine receptor subtypes have a potential role in the treatment of Parkinson’s disease, schizophrenia, Attention Deficit Hyperactivity Disorder (ADHD), depression, and drug abuse.\textsuperscript{36-38} Ligands at the 5-HT\textsubscript{1A} serotonin receptor subtype have been useful in the treatment of anxiety, schizophrenia and depression.\textsuperscript{39-42}

Aporphines are also ligands at the 5-HT\textsubscript{2A} and 5-HT\textsubscript{7} receptors. Selective 5-HT\textsubscript{2A} ligands have promising applications in the treatment of drug abuse and insomnia. Mixed dopamine/5-HT\textsubscript{2A} ligands have potential for the alleviation of symptoms of depression and schizophrenia.\textsuperscript{43,44} Ligands at the 5-HT\textsubscript{7} receptor have shown promising results for the treatment of sleep disorders, migraine and depression.\textsuperscript{45-47} Moreover aporphines possessing affinity at the dopamine and serotonin receptors have potential use as PET (Positron Emission Tomography) and SPECT (Single Photon Emission Computed Tomography) radiotracers for brain imaging studies.\textsuperscript{48} Aporphines are also reported as inhibitors of the enzyme Acetylcholinesterase and as antagonists of the \textalpha\textsubscript{1}-adrenergic receptor and thus have potential therapeutic role in the treatment of Alzheimer’s disease and hypertension respectively.\textsuperscript{49-52}

SAR at Dopamine receptors:
Extensive Structure Activity Relationship (SAR) studies done on aporphine alkaloids have produced selective D\textsubscript{1} and D\textsubscript{2} ligands.
Apomorphine (44, Apokyn®) which is an FDA approved drug for the treatment of advanced Parkinson’s disease is a non-selective dopamine agonist. 44 has affinity for both D₁ (Ki = 250 nM) and D₂ (Ki = 13 nM) receptors.53,54 Apomorphine analogues with R-configuration at C6a (compounds 45 and 46) displayed dopamine agonist activity whereas analogues with S-configuration at C6a (compound 47) displayed dopamine antagonistic activity.55,56 Typically an N-propyl group enhances selectivity for the D₂ receptor (compare 44 with 45).57 A C11 OH group is important for binding at the D₁ receptor (compare 48 and 44).12,58 Molecular docking studies have suggested this OH group to be involved in a hydrogen bond interaction with the D₁ receptor.59 Replacement of the C10 OH group with a methyl group results in significant loss of affinity at the dopamine receptors (compare 49 and 44).13 Ortho-dihydroxyl substitution at C10-C11 enhances affinity at dopamine receptors; shifting this unit to other positions resulted in inactive compounds.60 Small lipophilic groups at the C2 position enhance selectivity at D₂ receptor (compare 50, 51 with 44) indicating the presence of a lipophilic pocket in the receptor interacting with this part of the molecule.16,61,62
SAR at Serotonin receptors:

As discussed earlier, a C10 methyl group significantly diminishes affinity for dopamine receptors; however this also results in increased affinity for the serotonin 5-HT$_{1A}$ receptor subtype (compound 49).$^{63,64}$ This observation resulted in the proposal of a methyl pocket which has been further validated by synthesis and evaluation of aporphines possessing small alkyl groups at C10 position.$^{65}$ Analogues with R-configuration at C6a (compound 49) displayed 5-HT$_{1A}$ agonist activity whereas analogues with S-configuration at C6a (compound 52) displayed 5-HT$_{1A}$ antagonistic activity. Moreover it has been observed that aporphine analogs lacking hydroxyl groups in ring D possess good affinity and selectivity for the 5-HT$_{1A}$ receptor (compound 53a and 53b).$^{59,66}$

**Figure 13:** Structures of monohydroxylated compounds 48, 49 and C2 analogues 50 and 51

<table>
<thead>
<tr>
<th>Compound</th>
<th>$K_i$: $D_1$</th>
<th>$K_i$: $D_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>48</td>
<td>26 nM</td>
<td>108 nM</td>
</tr>
<tr>
<td>49</td>
<td>9650 nM</td>
<td>1150 nM</td>
</tr>
<tr>
<td>50</td>
<td>$&gt;5000$ nM</td>
<td>54 nM</td>
</tr>
<tr>
<td>51</td>
<td>1780 nM</td>
<td>170 nM</td>
</tr>
</tbody>
</table>

**Figure 14:** Structures of 5-HT$_{1A}$ ligands 49, 52 and 53a-b

<table>
<thead>
<tr>
<th>Compound</th>
<th>$K_i$: 5-HT$_{1A}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>49</td>
<td>0.45 nM</td>
</tr>
<tr>
<td>52</td>
<td></td>
</tr>
<tr>
<td>53a</td>
<td>4.5 nM</td>
</tr>
<tr>
<td>53b</td>
<td>1.8 nM</td>
</tr>
</tbody>
</table>
Smaller substituents on the nitrogen atom (methyl or hydrogen) increase affinity and selectivity for the 5-HT$_{1A}$ receptor.\textsuperscript{65} At the C2 position, a methoxy group seems to increase selectivity towards the 5-HT$_{1A}$ type.\textsuperscript{13}

![Figure 15: Structures of N-methyllaurotenanine (54) and (±)-nantenine (55)](image)

Compound 54 (N-methyllaurotenanine) is a naturally occurring aporphine alkaloid that possesses good affinity at both 5-HT$_{1A}$ and 5-HT$_7$ receptor.\textsuperscript{67} Recent SAR studies show that modification of the phenolic OH at C9 is detrimental for affinity at 5-HT$_{2A}$ receptor, however it is not essential for affinity at 5-HT$_{1A}$ and 5-HT$_7$ receptors.\textsuperscript{68} Similarly Nantenine (55) is a naturally occurring aporphine alkaloid reported to have dual antagonist effects at the 5-HT$_{2A}$ and $\alpha_{1A}$ receptors.\textsuperscript{69-72}

**SAR at Alpha-adrenergic receptors:**

The aporphine alkaloid Boldine (56) is an $\alpha_1$ antagonist having higher affinity for the $\alpha_{1A}$ subtype. SAR studies indicate that the presence of a hydroxyl group at the C2 position increases affinity at the $\alpha_{1A}$ and $\alpha_{1B}$ receptor subtypes. Introduction of a halogen atom at the C3 position resulted in increased affinity for the $\alpha_{1A}$ receptor. Moreover, the presence of a catechol moiety at C10/C11 led to increased selectivity for the $\alpha_{1D}$ receptor subtype. At the $\alpha_2$ receptor, boldine displays undistinguishable affinity for the $\alpha_{2A}$, $\alpha_{2B}$ and $\alpha_{2C}$ subtypes.\textsuperscript{73}
Similar to the trend seen for dopamine receptors, an \( N \)-methyl group is best tolerated across all alpha adrenergic receptor subtypes (when compared to \( N \)-propyl and NH). Moreover, aporphines having an \( S \)-configuration at C6a have greater affinity across all alpha adrenergic subtypes compared to \( R \)-configuration.\(^5\)\(^1\)

**SAR at Acetylcholinesterase enzyme:**

Naturally occurring alkaloids Nuciferine (57), \( N \)-methylasimilobine (58) and Magnoflorine (59) possess acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) inhibitory activity, which are clinical targets for the treatment of Alzheimer’s disease.\(^4\)\(^9\)\(^,\)\(^5\)\(^0\) SAR studies of nuciferine have shown that the presence of a C2 OH increases AChE inhibitory activity. This observation has been validated by molecular docking studies, where the C2 OH group is involved in a hydrogen bond interaction with Ser 293 residue of the AChE enzyme.\(^4\)\(^9\)
Apart from CNS effects, aporphine alkaloids also possess several non-CNS activities including inhibition of platelet aggregation, anti-Human Immunodeficiency Virus 1 (HIV-1) integrase activity, anthelmintic, leishmanicidal and cytotoxicity. 74-78

A summary of the pharmacological action of aporphines within the CNS is shown in Table 1.

**Table 1: Summary of clinical significance of aporphines as CNS agents**

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Activity</th>
<th>Clinical Significance</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>D1</td>
<td>Agonist</td>
<td>Parkinson’s disease</td>
<td>12,53, 54</td>
</tr>
<tr>
<td>D1</td>
<td>Antagonist</td>
<td>Schizophrenia, Obesity</td>
<td>58</td>
</tr>
<tr>
<td>D2</td>
<td>Agonist</td>
<td>Parkinson’s disease, Restless Leg Syndrome</td>
<td>61, 62</td>
</tr>
<tr>
<td>D2</td>
<td>Antagonist</td>
<td>Schizophrenia, Bipolar disorder</td>
<td>57</td>
</tr>
<tr>
<td>5-HT_{1A}</td>
<td>Agonist</td>
<td>Anxiety, Parkinson’s disease</td>
<td>59, 63-66</td>
</tr>
<tr>
<td>5-HT_{1A}</td>
<td>Antagonist</td>
<td>Schizophrenia, Cognitive dysfunction</td>
<td>63, 64</td>
</tr>
<tr>
<td>5-HT_{2A}</td>
<td>Antagonist</td>
<td>Sleep disorder, Drug abuse, Depression</td>
<td>69-72</td>
</tr>
<tr>
<td>5-HT_{7}</td>
<td>Antagonist</td>
<td>Depression, Migraine, Sleep disorder</td>
<td>67, 68</td>
</tr>
<tr>
<td>α_{1A}</td>
<td>Antagonist</td>
<td>High blood pressure</td>
<td>51, 73</td>
</tr>
</tbody>
</table>
1.2 5-HT$_{2A}$ Antagonists:

1.2.1 Background:

The human 5-HT$_{2A}$ receptor, first cloned in 1990 is a subtype of the 5-HT$_2$ receptors, the latter being a subfamily of the serotonin 5-HT (5-hydroxytryptamine) receptor.$^{79}$ This receptor is an excitatory receptor and belongs to the G-Protein Coupled Receptor (GPCRs) superfamily of receptors.

![Figure 18: Structure of serotonin](image)

Like other 5-HT receptors, serotonin (60) is an endogenous ligand at the 5-HT$_{2A}$ receptor. The 5-HT$_{2A}$ receptor is distributed mainly in the cerebral cortex, neocortex, hippocampus and thalamus regions of the brain. In the periphery, 5-HT$_{2A}$ receptors are located in platelets, vascular smooth muscles and uterine smooth muscles. In the CNS 5-HT$_{2A}$ receptor signaling is associated with the pathogenesis of migraine headaches, depression, anxiety and cognitive impairment of various diseases. It is also associated with various other processes such as vascular smooth muscle contraction, uterine contraction and platelet aggregation.$^{80}$

1.2.2 Clinical Significance of 5-HT$_{2A}$ Antagonists:

Antagonists at the 5-HT$_{2A}$ receptor are heavily pursued due to their great clinical significance. Selective and non-selective 5-HT$_{2A}$ antagonists find applications in a wide array of CNS disorders such as insomnia, anxiety, schizophrenia, depression and memory loss. Selective blockade of 5-HT$_{2A}$ receptor is a recently discovered strategy for the treatment of insomnia.
The selective 5-HT$_{2A}$ antagonist volinanserin (M100907) (61) was shown to increase total sleep time in elderly human patients.\textsuperscript{81} Similarly when the 5-HT$_{2A}$ antagonist eplivanserin (SR-46,349) (62) was co-administered with the clinically available drug zolpidem, beneficial synergistic effects were observed in animal models of insomnia.\textsuperscript{82} Compound 61, 62 and other selective 5-HT$_{2A}$ antagonists such as pruvanserin (EMD 281014) (63), nelotanserin (APD-125) (64) and pimavanserin (ACP-103) (65) were evaluated in Phase III clinical trials for the treatment of insomnia.\textsuperscript{83}

Another important therapeutic potential of 5-HT$_{2A}$ antagonists is in the treatment of drug abuse symptoms. There is ample evidence of 5-HT$_{2A}$ antagonists showing promising results for treatment of drug abuse symptoms.

For example the popular drug of abuse MDMA (ecstasy) is believed to cause some of its adverse effects through the 5-HT$_{2A}$ receptor. Accordingly volinanserin (61) and ketanserin (66), a mixed 5-HT$_{2A}$/2C antagonist were found to attenuate hyperthermia - one of the most profound side
effects of MDMA.\textsuperscript{84} Ketanserin (66) was also shown to reduce MDMA induced neuronal apoptosis. \textsuperscript{85,86} Furthermore, MDMA induced perceptual changes and emotional excitation were found to be attenuated by ketanserin in healthy humans.\textsuperscript{87}

\textbf{Figure 21: Structures of Ketanserin (66) and Amperozide (67)}

Furthermore, volinanserin (61) reduced reinstatement of cocaine seeking behavior and cocaine induced hyperlocomotor activity in rats.\textsuperscript{88} Co-administration of ketanserin (66) with pergolide (D\textsubscript{2} receptor agonist) was shown to reduce methaphetamine sensitization in rats.\textsuperscript{89} Similarly the 5-HT\textsubscript{2A} antagonist amperozide (67) was shown to suppress alcohol abuse in rat models.\textsuperscript{90}

5-HT\textsubscript{2A} antagonists also have therapeutic potential for the treatment of depression. Currently all atypical antipsychotics have 5-HT\textsubscript{2A} antagonistic properties, which may contribute to their antidepressant efficacy.\textsuperscript{91} 5-HT\textsubscript{2A} antagonist by themselves also have been shown to cause antidepressant effects. For example the highly selective 5-HT\textsubscript{2A} antagonist pruvanserin (63) increased swimming and reduced immobility in rats in the forced swim test-a direct measure of its ability to decrease depression.\textsuperscript{92} Similarly volinanserin (61) decreased immobility time in rats that were withdrawn from chronic nicotine exposure.\textsuperscript{93} BIP-1 (68) a 5-HT\textsubscript{2A} antagonist was found to shown positive results for chronic models of depression.\textsuperscript{94}
5-HT$_{2A}$ antagonists also have therapeutic potential as anti-anxiety drugs and for the treatment of schizophrenia. Eplivanserin (62) was shown to antagonize anxiolytic actions of DOI (69, 2,5-Dimethoxy-4-iodoamphetamine) in mice using the elevated plus maze model.\textsuperscript{95}

Similarly administration of ketanserin (66) and volinanserin (61) resulted in blockage of postnatal fluoxetine induced anxiety in rat.\textsuperscript{96} Co-administration of risperidone (a drug approved for treatment of schizophrenia) with ritanserin (70), a mixed 5-HT$_{2A}$/2C antagonist, produced superior results in decreasing negative symptoms of schizophrenia in human patients.\textsuperscript{97} Similarly improvement in cognitive abilities of schizophrenic patients followed by the administration volinanserin (61) has been reported.\textsuperscript{98}

5-HT$_{2A}$ antagonists also have promising application in the treatment of Parkinson’s disease. Administration of ritanserin (70) resulted in reduced motor symptoms in a mouse model of Parkinson’s disease.\textsuperscript{99} Similarly administration of volinanserin (61) produced anti-Parkinson’s effects in mice.\textsuperscript{100}
5-HT$_{2A}$ antagonists also have a potential for the therapeutic intervention of other conditions such as cardiac ischemia, atherosclerotic lesions, diabetes and asthma.$^{101-104}$

1.2.3 Structural Classification of 5-HT$_{2A}$ Antagonists:

Based on their structures 5-HT$_{2A}$ antagonist can be broadly classified as follows:

a. Indole-alkylamines

b. Aryl-piperazines

c. Alkyl-piperidines/alkyl-piperazines

d. Polycyclic/tricyclic agents

e. Miscellaneous

A) Indole-alkylamines:

![Indole-alkylamines](image1)

B) Aryl-piperazines:

![Aryl-piperazines](image2)

**Figure 24:** Structures of indolealkylamines (71 and 72) and arylpiperazines (73, 74 and 75)
Amongst the above mentioned structural categories, alkyl-piperazines/alkyl-piperazines and polycyclic agents are the largest categories of 5-HT$_{2A}$ antagonists.

**Figure 25:** Structures of alkylpiperidines (66 and 76), alkyl piperazine (77) and polycyclic/tricyclic agents (78, 79 and 80)

Amongst the above mentioned structural categories, alkyl-piperazines/alkyl-piperidines and polycyclic agents are the largest categories of 5-HT$_{2A}$ antagonists.

**Figure 26:** Structures of compounds 81 and 82 - classified into miscellaneous class of 5-HT$_{2A}$ antagonists
1.2.4 5-HT$_{2A}$ Antagonist Pharmacophore:

In order to design better and selective 5-HT$_{2A}$ antagonists several groups have proposed 5-HT$_{2A}$ antagonist pharmacophore models through the conformational analysis of several 5-HT$_{2A}$ antagonists. Considering the wide structural variations amongst available 5-HT$_{2A}$ antagonists, it is difficult to have a single pharmacophore. A brief summary is mentioned below. Figure 27 shows the pharmacophore model proposed by Glennon and co-workers.$^{105}$ This model which is based on the structure of (+)-LSD, does not differentiate between agonist and antagonist.

![Pharmacophore model based on (+)-LSD. Ligand properties important for affinity are shown.](Recept. Biochem. Methodol. 1991, 15, 19)

Another model proposed by Holtje-Jendretzki included several ligand sites that could be involved in hydrogen bonding and hydrophobic interactions and one site that could be involved in an ionic interaction.$^{106}$ Compounds included in this model had a phenyl-methyl-piperidine unit in common, and it was considered to be a pivotal feature for the design of 5-HT$_{2A}$ antagonists. Moreover this antagonist model was found to partially overlap with an agonist model (proposed by the same authors) and hence it was suggested that agonist and antagonist of the 5-HT$_{2A}$ receptor could have some common binding features.
Pharmacophore models independently proposed by Andersen and Mokrosz were found to possess striking similarities despite studying different classes of 5-HT\textsubscript{2A} antagonists\textsuperscript{107,108}. Figure 28 shows the models proposed by Andersen (Model I) and Mokrosz (Model II).

![Figure 28: Pharmacophore models proposed by Andersen (I) and Mokrosz (II) (J. Med. Chem. 1994, 37, 950; Arch. Pharm. 1995, 328, 659)](image)

Both the groups proposed a triangular arrangement of groups in their respective models. The distance between the center of two aromatic rings as well as the distance between the center of each aromatic ring and the basic nitrogen atom was found to be critical for binding affinity. As per the Andersen model, ideal distance between each aromatic ring is 5.1 Å and the distance between the basic nitrogen atom and each aromatic ring was 7.1 Å (ring A) and 8.1 Å (ring B). On the other hand as per the Mokrosz model, the distance between the centers of two aromatic ring was between 4.6 Å to 7.3 Å, while the distance between the basic nitrogen atom and each aromatic ring was 5.2 Å-8.4 Å (ring A) and 5.7 Å to 8.5 Å (ring B). It is to be noted that none of the above models predicted a perfect triangular arrangement of groups, in other words the distance between the basic nitrogen atom from one aromatic ring is different from the distance between nitrogen atom and other aromatic ring.
Klabunde and Evers proposed two pharmacophore models: Class I (linear) and Class II (triangular). Class I pharmacophore defines four required features for binding: i) protonated nitrogen atom (PI) ii) two Hydrophobic Aromatic Rings (HYA) iii) Hydrogen Bond Acceptor (HBA) (Figure 29, Model I). In Class II pharmacophore there are three essential features: i) protonated nitrogen atom (PI) ii) aromatic ring (AR) and iii) hydrophobic group (HY) (Figure 29, Model II)

A similar classification of pharmacophore model into linear and triangular fashion has been reported by Rowley and coworkers.

Figures 27, 28 and 29 shows different proposed pharmacophore models for 5-HT2A antagonists. Looking at the different models, it can be concluded that 5-HT2A antagonists could possibly have more than one binding mode. Also it is possible to have some common binding region for different 5-HT2A antagonists.
1.2.5 Recent Review of 5-HT$_2$A Antagonists:

A recent review of 5-HT$_2$A antagonists is described here. (For the sake of brevity, only work published after 2005 is discussed.)

Xiong and co-workers reported the synthesis and evaluation of phenethyl-piperazine based selective 5-HT$_2$A antagonists (83-87) for the treatment of insomnia (Figure 30).

![Figure 30: Structure of aryl piperazines 83-87](image)

<table>
<thead>
<tr>
<th>Compound</th>
<th>5-HT$_2$A</th>
<th>5-HT$_2$C</th>
</tr>
</thead>
<tbody>
<tr>
<td>83. $R_1 = H$, $R_2 = Br$, $X = CH_2$,</td>
<td>$K_i = 68$ nM</td>
<td>$K_i &gt; 10000$ nM</td>
</tr>
<tr>
<td>84. $R_1 = 4$-F, $R_2 = Br$, $X = CH_2$,</td>
<td>$K_i = 3.6$ nM</td>
<td>$K_i = 4146$ nM</td>
</tr>
<tr>
<td>85. $R_1 = 2,4$-di-F, $R_2 = Br$, $X = CH_2$,</td>
<td>$K_i = 1.1$ nM</td>
<td>$K_i = 2820$ nM</td>
</tr>
<tr>
<td>86. $R_1 = 4$-F, $R_2 = Br$, $X = CO$,</td>
<td>$K_i = 3.9$ nM</td>
<td>$K_i = 2120$ nM</td>
</tr>
<tr>
<td>87. $R_1 = 2,4$-di-F, $R_2 = Br$, $X = CO$,</td>
<td>$K_i = 70$ nM</td>
<td>$K_i &gt; 10000$ nM</td>
</tr>
</tbody>
</table>

SAR data from this study indicated that the presence of lipophilic substituents in the phenyl ring enhanced 5-HT$_2$A antagonistic affinity (compare 84 with 83). Compound 85 having a 2,4-difluoro substitution in the phenyl ring was the most potent compound identified in this series. However stability studies indicated that 85 has a poor half-life of 9 min. Replacement of the methylene linker in 85 with a carbonyl linker (compound 86) resulted in extension of the half-life to 60 min, while still retaining the 5-HT$_2$A antagonist potency.

In a separate study, the same group investigated the effect of replacing the pyrazole ring in compound 83 with various fused arylpyrazole rings (compounds 88-92, Figure 31). Compounds having a fused phenylpyrazole ring were found to have comparable affinity to their mono-cyclic pyrazole analogue (compare 81 and 85). Although the fused phenylpyrazole containing
compounds had similar 5-HT$_{2A}$ affinity, they displayed less selectivity for the 5-HT$_{2A}$ receptor compared to their mono-cyclic pyrazole analogues.

![Figure 31: Structure of aryl piperazines 88-92](image)

Yoo and co-workers reported the synthesis and evaluation of aryl sulfonamides (93-100) as 5-HT$_{2A}$ antagonists. These compounds feature either a cyclohexylmethyl (Figure 32) or a cyclopropylmethyl group (Figure 33) on the nitrogen of the sulfonamide functionality. Compounds with the N-cyclohexylmethyl group (93-96) displayed enhanced affinity at the 5-HT$_{2A}$ receptor compared to compounds having the N-cyclopropylmethyl group (97-100).

![Figure 32: Structures of N-cyclohexylmethyl substituted aryl sulfonamides 93-96](image)
These compounds also displayed better selectivity for the 5-HT$_{2A}$ receptor (over the 5-HT$_7$ receptor but not over 5-HT$_{2C}$ receptor). Thus compound 93 has an affinity of 0.5 nM (K$_i$) for the 5-HT$_{2A}$ receptor and showed 3000 times higher affinity for the 5-HT$_{2A}$ receptor over 5-HT$_7$ receptor. Modifications of the phenyl piperazine group revealed that a 4-fluoro phenyl ring improved the 5-HT$_{2A}$ receptor affinity the most, which is consistent with other reported 5-HT$_{2A}$ antagonists. Moreover modification of the aryl part of the aryl sulfonamide moiety suggested that the 2-naphthyl ring was better tolerated for the 5-HT$_{2A}$ receptor affinity.

![Chemical Structure]

<table>
<thead>
<tr>
<th></th>
<th>5-HT$_{2A}$</th>
<th>5-HT$_{2C}$</th>
<th>5-HT$_7$</th>
</tr>
</thead>
<tbody>
<tr>
<td>97. Ar = 2-Naphthyl, R = 4-F</td>
<td>IC$_{50}$ = 51 nM</td>
<td>IC$_{50}$ = 344 nM</td>
<td>IC$_{50}$ = 49.6 nM</td>
</tr>
<tr>
<td>98. Ar = 2-Naphthyl, R = 3-CF$_3$</td>
<td>IC$_{50}$ = 221 nM</td>
<td>IC$_{50}$ = 304 nM</td>
<td>IC$_{50}$ = 342 nM</td>
</tr>
<tr>
<td>99. Ar = 1-Naphthyl, R = H</td>
<td>IC$_{50}$ = 171 nM</td>
<td>IC$_{50}$ = 1459 nM</td>
<td>IC$_{50}$ = 152 nM</td>
</tr>
<tr>
<td>100. Ar = 8-Quinolinyl, R = H</td>
<td>IC$_{50}$ = 292 nM</td>
<td>IC$_{50}$ = 2159 nM</td>
<td>IC$_{50}$ = 749 nM</td>
</tr>
</tbody>
</table>

**Figure 33**: Structures of N-cyclohexylmethyl substituted aryl sulfonamides 97-100

The synthesis and evaluation of novel imidazobenzazepine compounds as dual H$_1$/5-HT$_{2A}$ antagonists (101-105) for the treatment of sleep disorders was reported by Gianotti and co-workers. In this novel zwitterionic series of compounds, the affinity at both H$_1$ and 5-HT$_{2A}$ receptors was found to be affected by modification of the acid side chain. Only compound 103 that incorporates a 2,2-dimethylpropanoic acid side chain substitution on the piperazine ring was found to have a balanced affinity for both H$_1$ and 5-HT$_{2A}$ receptor (Figure 34). Substitution with other acid side chains resulted in compounds having increased affinity for H$_1$ but not 5-HT$_{2A}$ receptor.
Modifications of the imidazole ring by addition of small lipophilic or small electron withdrawing groups resulted in compounds that displayed poor selectivity over the 5-HT_{2C} receptor.

Heffernan and co-workers reported the design and synthesis of novel indole containing compounds as dual Norepinephrine Reuptake Inhibitors (NRIs) and 5-HT_{2A} receptor antagonists (Figure 35). Their approach was to first generate a common pharmacophore for NRIs using Montel Carlo conformational analysis, followed by virtual screening to identify dual NRIs and 5-HT_{2A} antagonist ligands that fit in the given pharmacophore model. A library of analogues (106-111) based on this hit, was synthesized and evaluated for binding affinity at human Norepinephrine Transporter (hNET) and 5-HT_{2A} receptor. In this series of compounds, cyclic tertiary amine analogues (110 and 111) were found to have superior potency at the 5-HT_{2A} receptor, compared to their acyclic counterparts. Increasing the alkyl chain length between the basic amine and indole nucleus resulted in decreased affinity for both hNET and 5-HT_{2A} receptor.
Enzensperger and co-workers reported the synthesis and evaluation of novel azecine ligands (112-117) at the 5-HT$_{2A}$ and Dopamine D$_1$ and D$_2$ receptor systems with the goal of improving selectivity for the 5-HT$_{2A}$ receptor.$^{116}$

Evaluation of analogues revealed that expansion of the central 10 membered azecine ring (112) to regioisomeric 11 membered central ring containing compounds (113 and 114) is tolerated
for affinity at 5-HT$_{2A}$ and D$_1$ receptor, but does not enhance affinity at these targets. Furthermore replacement of the $N$-methyl group with an $N$-ethyl group or NH group resulted in decreased or complete loss of affinity respectively.

Swain and co-workers reported the synthesis and evaluation of low molecular weight aryl diazepines (118-123) as 5-HT$_{2A}$ ligands. SAR results indicated that small lipophilic groups were tolerated at the para position of the aromatic ring (118 and 119). Replacement of the secondary amine (of the tert-butyl amine moiety) with tertiary amines resulted in dramatic loss in 5-HT$_{2A}$ affinity indicating the necessity of a hydrogen bond donor (compare 120 and 122). Incorporation of 5 or 6 membered cyclic alkyl groups also resulted in small increase in affinity (123).

Shireman and co-workers reported the synthesis of aryl pyrimidine derivatives (124-127) as 5-HT$_{2A}$ antagonists. Compound 124 identified through a high throughput screen is a non-selective 5-HT$_{2A}$ antagonist. In order to decrease the basicity of the pyrimidine ring and hence increase the selectivity towards 5-HT$_{2A}$ receptor, the amino group was replaced with small lipophilic groups to produce 5-HT$_{2A}$ selective compounds 126 and 127.

<table>
<thead>
<tr>
<th>Compound</th>
<th>$R$</th>
<th>$IC_{50}$ (nM)</th>
<th>$IC_{50}$ (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>118</td>
<td>4-Me</td>
<td>11</td>
<td>113</td>
</tr>
<tr>
<td>119</td>
<td>4-F</td>
<td>21</td>
<td>113</td>
</tr>
<tr>
<td>120</td>
<td>2-F</td>
<td>18</td>
<td>113</td>
</tr>
<tr>
<td>121</td>
<td>2,4-di-F</td>
<td>11</td>
<td>113</td>
</tr>
<tr>
<td>122</td>
<td>Et$_2$N</td>
<td>&gt;1000</td>
<td>113</td>
</tr>
<tr>
<td>123</td>
<td>HN</td>
<td>3.6</td>
<td>113</td>
</tr>
</tbody>
</table>

**Figure 37**: Structures of aryl pyrimidine compounds 118-123
Ladduwahetty and co-workers reported the synthesis and evaluation of non-basic 5-HT\textsubscript{2A} receptor antagonists.\textsuperscript{119} Initially discovered compound 128 displays poor 5-HT\textsubscript{2A} receptor affinity. It is thought that although the amide in 128 can act as a hydrogen bond acceptor, the restricted rotation of 128 prevents it from reaching a suitable binding position. Subsequent SAR studies led to the discovery of 129-131 that displayed 24-73 times selectivity over the 5-HT\textsubscript{2C} receptor.

Overall, diverse structural classes of compounds have been pursued as 5-HT\textsubscript{2A} antagonists. As discussed previously due to their wide structural variations, more than one pharmacophore...
model is needed to guide the design of 5-HT$_{2A}$ antagonists. Furthermore the requirement of a basic nitrogen atom proposed by these pharmacophore models is now questionable following the discovery of non-basic 5-HT$_{2A}$ antagonists. As can be inferred from the above examples, a common problem that lies in the development of 5-HT$_{2A}$ antagonists is the issue of selectivity over other subtypes. A truly selective 5-HT$_{2A}$ antagonist with desirable pharmacokinetic properties would be valuable for clinical applications.

1.3 Conclusions

Aporphine alkaloids are a group of natural as well as synthetic alkaloids that possess a diverse range of pharmacological actions. At the CNS, aporphine alkaloids are ligands at several clinically valuable targets such as the dopamine, serotonin, alpha adrenergic receptors and at the acetylcholinesterase enzyme. From the several SAR studies done on aporphine alkaloids it can be inferred that small structural changes on the aporphine scaffold can generate ligands that have selective affinity for a particular receptor as well as ligands with unique multi receptor profile. Such ligands have a potential to be developed as a clinical agent for treatment/mitigation of neuropsychiatric disorders as seen with apomorphine. Moreover, such ligands can also serve as unique pharmacological tools for the study of receptor functions. Considering the diverse profile of aporphine alkaloids at the CNS receptors, they can be considered as “privileged scaffolds” for CNS drug design.

Known 5-HT$_{2A}$ antagonists are a structurally diverse class of compounds. 5-HT$_{2A}$ antagonists have therapeutic potential for the treatment of several CNS maladies such as insomnia, drug abuse, depression, anxiety, schizophrenia, Parkinson’s disease and memory loss. Several 5-HT$_{2A}$ antagonist pharmacophore models have been proposed to facilitate and guide future design
of 5-HT$_2$A antagonists. Although different, these models share some common features such as: i) a basic nitrogen atom, ii) one or two aromatic rings and iii) hydrophobic binding region.

Furthermore, no truly selective 5-HT$_2$A antagonist is clinically available. Considering the widespread role of 5-HT$_2$A receptor in the progression of several neuropsychiatric disorders there is an unmet need for selective and potent 5-HT$_2$A antagonists.

There is limited information available on aporphines as ligands at the 5-HT$_2$A receptor. The rich CNS pharmacology of aporphine alkaloids makes them an attractive starting point for being developed as 5-HT$_2$A antagonists. Such an endeavor would allow us to understand the structural features of an aporphine scaffold that are required for 5-HT$_2$A antagonism. Moreover this study would also produce ligands that will be useful as valuable pharmacological tools. Overall such an effort would be beneficial from both synthetic and medicinal chemistry point of view.
CHAPTER II: SYNTHESIS AND EVALUATION OF NANTENINE ANALOGUES
2.1 Background

As discussed in the previous chapter, aporphine alkaloids have a diverse range of pharmacological actions. Specifically at the CNS, aporphine alkaloids are ligands at dopamine, serotonin and alpha adrenergic receptors. At serotonin receptors, aporphine alkaloids have been studied in depth as ligands at the 5-HT$_{1A}$ and 5-HT$_7$ receptors. However not much work has been done in studying aporphines as ligands at the 5-HT$_{2A}$ receptor. Ligands at the 5-HT$_{2A}$ receptor have valuable applications in the treatment of insomnia, drug abuse and several neuropsychiatric disorders such as depression, anxiety, schizophrenia and Parkinson’s disease. The aporphine scaffold can be considered as a privileged scaffold for CNS drug design and can be used for the development of novel 5-HT$_{2A}$ ligands.

2.2 Nantenine As A Lead For The Identification of New 5-HT$_{2A}$ Antagonists

Nantenine (55) is an aporphine alkaloid isolated from the fruit of *Nandina domestica* Thunberg. Indra and co-workers in 2002 showed that nantenine inhibited 5-hydroxy-L-tryptophan (5-HTP) induced head-twitch response by blocking 5-HT$_{2A}$ receptors in mice. Later the same group reported SAR studies showing nantenine as an antagonist at the 5-HT$_{2A}$ and $\alpha_1$ receptors. Studies done by Fantegrossi revealed the ability of nantenine to block and reverse MDMA induced physiological effects such as hyperthermia, locomotor stimulation and head-twitch responses in mice. These anti-MDMA effects of nantenine were attributed to its antagonism at 5-HT$_{2A}$ and $\alpha_1$ receptors.

Research in our group has focused on the synthesis and evaluation of nantenine analogues as 5-HT$_{2A}$ antagonists. The first step in this direction that was accomplished previously, was to
synthesize nantenine and screen it across available CNS receptors via the Psychoactive Drug Screening Program (PDSP) of the NIH. Results from this screening showed that nantenine is highly selective for the $\alpha_{1A}$ receptor ($K_i = 2$ nM) compared to other $\alpha_{1A}$ subtypes. However, at the 5-HT$_{2A}$ receptor nantenine was found to have moderate affinity ($K_i = 850$ nM). In order to improve the potency and selectivity of nantenine analogues for the 5-HT$_{2A}$ receptor, a systematic SAR study was initiated. A brief discussion of our previous findings is described.

2.2.1 Previous Structure Activity Relationship (SAR) Studies$^{28,122,123}$

Table 2 shows the apparent binding affinity ($K_e$) values for a series of C1 (132-138), C2 (139-143) and N6 (144-146) nantenine analogues. At the C1 position the effect of alkyl group substitution was studied through compounds 132-138. Progressive increase in the alkyl chain length at this position, resulted in increased affinity. As seen in Table 2, the C1 ethyl analogue (132, $K_e = 890$ nM) was found equipotent to nantenine. Substitution with propyl (133, $K_e = 297$ nM) and butyl (134, $K_e = 274$ nM) groups resulted in three times increase in potency. The $n$-hexyloxy analogue (136, $K_e = 71$ nM) which was the most potent compound identified in this series, was 11 times more potent than nantenine. Other potent compounds identified in this series had a cyclopropylmethoxy group (137, $K_e = 68$ nM) and allyloxy group (138, $K_e = 70$ nM).
Table 2: Previous Structure Activity Relationship (SAR) data of nantenine.

<table>
<thead>
<tr>
<th>Compound</th>
<th>R¹</th>
<th>R²</th>
<th>R³</th>
<th>5-HT₂A-Kₑ (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>132</td>
<td>Et</td>
<td>OMe</td>
<td>Me</td>
<td>890</td>
</tr>
<tr>
<td>133</td>
<td>n-Pr</td>
<td>OMe</td>
<td>Me</td>
<td>297</td>
</tr>
<tr>
<td>134</td>
<td>n-Bu</td>
<td>OMe</td>
<td>Me</td>
<td>274</td>
</tr>
<tr>
<td>135</td>
<td>n-Pen</td>
<td>OMe</td>
<td>Me</td>
<td>171</td>
</tr>
<tr>
<td>136</td>
<td>n-hex</td>
<td>OMe</td>
<td>Me</td>
<td>71</td>
</tr>
<tr>
<td>137</td>
<td>Cyclopropylmethyl</td>
<td>OMe</td>
<td>Me</td>
<td>68</td>
</tr>
<tr>
<td>138</td>
<td>Allyl</td>
<td>OMe</td>
<td>Me</td>
<td>70</td>
</tr>
<tr>
<td>139</td>
<td>Me</td>
<td>OEt</td>
<td>Me</td>
<td>378</td>
</tr>
<tr>
<td>140</td>
<td>Me</td>
<td>OºPr</td>
<td>Me</td>
<td>485</td>
</tr>
<tr>
<td>141</td>
<td>Me</td>
<td>OºBu</td>
<td>Me</td>
<td>943</td>
</tr>
<tr>
<td>142</td>
<td>Me</td>
<td>OºPen</td>
<td>Me</td>
<td>&gt; 10,000</td>
</tr>
<tr>
<td>143</td>
<td>Me</td>
<td>OBn</td>
<td>Me</td>
<td>154</td>
</tr>
<tr>
<td>144</td>
<td>Me</td>
<td>OMe</td>
<td>H</td>
<td>&gt; 10,000</td>
</tr>
<tr>
<td>145</td>
<td>Me</td>
<td>OMe</td>
<td>Et</td>
<td>&gt; 10,000</td>
</tr>
<tr>
<td>146</td>
<td>Me</td>
<td>OMe</td>
<td>Pr</td>
<td>&gt; 10,000</td>
</tr>
<tr>
<td>Nantenine (55)</td>
<td>Me</td>
<td>OMe</td>
<td>Me</td>
<td>850</td>
</tr>
</tbody>
</table>

At the C2 position, replacement of the methoxy group with small alkoxy groups was tolerated. Thus replacement with ethoxy (139, Kₑ = 378 nM) and propyloxy (140, Kₑ = 485 nM) resulted in 2 and 1.7 times increase in potency respectively. However replacements with larger alkoxy groups were detrimental for 5-HT₂A receptor affinity (141, Kₑ = 943 nM; and 142, Kₑ > 10,000 nM). Compound 143 (Kₑ = 154 nM) with a benzyloxy group at the C2 position was the most potent compound in this series.
None of the N6 analogues (144-146) had affinity for the 5-HT<sub>2A</sub> receptor. This suggests that the N-Methyl group is important for affinity at the 5-HT<sub>2A</sub> receptor.

Flexible analogues 147-150 (Figure 40) were synthesized and evaluated for 5-HT<sub>2A</sub> receptor affinity in order to examine the importance of molecular rigidity. None of the flexible compounds had affinity for the 5-HT<sub>2A</sub> receptor, thus suggesting that an intact aporphine scaffold is critical for 5-HT<sub>2A</sub> receptor affinity.

![Figure 40: Structures of flexible compounds 147-150](image)

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
<th>K&lt;sub&gt;e&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>147</td>
<td>H</td>
<td>&gt; 10,000 nM</td>
</tr>
<tr>
<td>148</td>
<td>Me</td>
<td>&gt; 10,000 nM</td>
</tr>
<tr>
<td>149</td>
<td></td>
<td>5180 nM</td>
</tr>
<tr>
<td>150</td>
<td></td>
<td>&gt; 10,000 nM</td>
</tr>
</tbody>
</table>

2.2.2 Summary of Previous Findings:

The following conclusions were drawn from previous SAR studies:

1. Intact aporphine core is required for 5-HT<sub>2A</sub> receptor affinity.
2. At the C1 position, progressive increase in alkyl chain length results in increased affinity for the 5-HT<sub>2A</sub> receptor.
3. Only small alkyl groups are tolerated at the C2 position.
4. The N-methyl group is critical for affinity.

A summary of previous findings is shown in Figure 41
2.3 Rationale & Central Hypothesis:

**Rationale:** Previous SAR studies of nantenine have focused on modifications at the C1, C2 and N6 position. However, in order to understand the structural features of nantenine required for 5-HT$_{2A}$ antagonism there is a need to carry out an exhaustive SAR study. Such a study will provide information which will be valuable for the design of selective and potent 5-HT$_{2A}$ ligands. Moreover it is highly likely that such studies will generate novel ligands having selective 5-HT$_{2A}$ receptor affinity as well as ligands with unique multi-receptor affinities. In order to make this ultimate goal a reality, we expanded our SAR study to relatively unexplored positions of the nantenine template. Accordingly it was decided to study structural modifications at the C3, C4 and ring D positions of nantenine.

C3 analogues were designed to test the effect of hydrophobic substitution at these position on 5-HT$_{2A}$ receptor affinity. The design of C4 phenyl analogues was predicated on the hypothesis that the addition of a phenyl moiety at the C4 position would enhance the 5-HT$_{2A}$ receptor affinity (given the pharmacophoric similarity to known 5-HT$_{2A}$ ligands). The synthesis of ring D analogues was based on the idea of replacing the “phenyl methylenedioxy” unit of nantenine with
heteroaromatic rings as potential bioisosteres. Moreover oxygen containing isochroman analogues were also synthesized to test the effect of replacing the N6 nitrogen atom with an oxygen atom.

**Central Hypothesis:** The central hypothesis tested behind this work is that structural modifications of nantenine will generate potent and selective 5-HT$_{2A}$ antagonists.

### 2.4 Results:

#### 2.4.1 C4 Phenyl Analogues:

**Rationale:** 9-aminomethyl-9,10-dihydroanthracene (AMDA, 151a) is a structurally unique high affinity selective 5-HT$_{2A}$ antagonist ($K_i = 20$ nM).$^{124}$ The 3-methoxy analogue of AMDA compound 151b is 2.5 times more potent than AMDA via hydrogen bonding interactions between the methoxy group and 5-HT$_{2A}$ receptor side chains.$^{125}$ AMDA is speculated to have a different binding mode than other existing 5-HT$_{2A}$ ligands.$^{126}$

![Chemical Structures](image.png)

*Figure 42: Structures of AMDA (151a), 151b and C4 phenyl analogues (152)*

151a. $R = H$ (AMDA), $K_i = 20$ nM
151b. $R = Me, K_i = 7.5$ nM
We envisaged that incorporation of a phenyl group at the C4 position of the aporphine nucleus would engender improved affinity for the 5-HT$_{2A}$ receptor, based on its structural resemblance to the AMDA template.

As discussed earlier, in previous SAR studies it was observed that C1 alkoxy substituents increased affinity at the 5-HT$_{2A}$ receptor.$^{28,122}$ As a result it was decided to retain C1 alkoxy substituents for a series of C4 phenyl analogues. The synthesis of C1 alkoxy/C4 phenyl aporphine analogues is shown in Schemes 1 and 2.

![Scheme 1: Reagents and conditions: a) BuBr, K$_2$CO$_3$, acetonitrile, reflux, 4h, 94%; b) CH$_3$NO$_2$, NH$_4$OAc, CH$_3$COOH, reflux, 4h, 82%; c) phenyl magnesium bromide, THF, 0 °C-rt, 18h, 32%; d) SnCl$_2$, EtOH, reflux, 16h, 65%; e) CDI, THF, 0 °C-rt, 16h, 60%; f) PCl$_5$, DCM, 0 °C-rt, 16h; g) NaBH$_4$,MeOH, 0 °C-rt, 4h, 88% over 2 steps; h) (BoC)$_2$O, DIPEA, DMAP, DCM, rt, 16h, 85%; i) Pd(OAc)$_2$, Di-tert-butyl (methyl) phosphonium tetrafluoroborate, K$_2$CO$_3$, DMSO, 135 °C, microwaves, 200W, 88%; j) H$_2$, Pd/C, THF:MeOH 1:1, rt, 6h, 90%;]
Thus as shown in Scheme 1, the synthesis started with commercially available compound 153 (vanillin) which was protected as a benzyl ether to afford compound 154. Reaction of 154 under Henry Reaction conditions provided the Michael acceptor compound 155. Treatment of 155 with phenyl magnesium bromide furnished the Michael adduct 156, which after reduction with SnCl₂, gave primary amine 157. Amine 157 was then coupled with readily available acid 158 (6-bromo-3,4-(methylenedioxy)phenylacetic acid) in presence of CDI to afford amide 159. Bischler-Napieralski reaction of amide 159, followed by reduction of the intermittent imine with NaBH₄, gave the secondary amine 160. Compound 161, which was the precursor for the intramolecular biaryl coupling reaction was easily obtained from 160 after protection with a Boc group.

163

164a: R = Me; 94 %
164b: R = Et; 90 %
164c: R = Pr; 80 %
164d: R = n-butyl; 87 %
164e: R = hexyl; 79 %
164f: R = cyclopropylmethyl; 64%
164g: R = Allyl; 77 %
164h: R = p-bromobenzyl; 96 %

165a: R = Me; 56 %
165b: R = Et; 45 %
165c: R = Pr; 36 %
165d: R = n-butyl; 26 %
165e: R = hexyl; 40 %
165f: R = cyclopropylmethyl; 58%
165g: R = Allyl; 60 %
165h: R = p-bromobenzyl; 45 %

**Scheme 2: Reagents and conditions:**
- a) appropriate alkyl halide, K₂CO₃, acetonitrile, 16h;
- b) ZnBr₂, DCM, rt, 18 h;
- c) HCHO, Na(AcO)₂BH, DCM, rt, 16 h.

With compound 161 in hand an intramolecular C-H activation reaction was attempted using conditions that were previously optimized in our lab. We were delighted to find that...
treatment of 161 with Pd(OAc)$_2$ and di-tert-butyl (methyl) phosphonium tetrafluoroborate under microwave reaction conditions afforded 162 in an excellent yield of 88%. Removal of the benzyl group was accomplished using catalytic hydrogenation conditions to furnish the key precursor compound 163.

Scheme 2 shows the synthesis of C1 alkoxy/C4 phenyl aporphine analogues from phenol 163. Thus 163 was subjected to Williamson ether synthesis to install several alkyl groups as seen in compounds 164a-h. Subsequent Boc deprotection followed by reductive amination to install an N-methyl functionality afforded our target compounds 165a-h.

Previous SAR studies indicated that an N-substituent larger than a methyl group is detrimental to 5-HT$_{2A}$ receptor affinity.$^{28}$ Nevertheless, in order to see the extent to which small N-alkyl groups can be tolerated when a C4 phenyl ring is present on the nantenine nucleus, N6 alkyl/C4 phenyl analogues were synthesized. Scheme 3 shows the synthesis of N6 alkyl/C4 phenyl
analogs from the key precursor compound 163. Sequential $O$-methylation and Boc deprotection of phenol 163, gave compound 166. Reductive amination of 166 with cyclopropanecarboxaldehyde afforded the $N$-cyclopropylmethyl analogue 165k. Alternatively 166 was converted to the acetamide 167, followed with reduction to furnish the $N$-ethyl analogue 165j. Finally phenol 163 was subjected to Boc deprotection and reductive amination to afford analogue 165i.

Analogs 165a-k were evaluated for affinity across various 5-HT (serotonin) receptors at the Psychoactive Drug Screening Program (PDSP). A detailed experimental procedure is described in section 2.6. Briefly, the compounds were initially screened in a primary radioligand binding assay that measures inhibition of serotonin binding at a compound concentration of 10 $\mu$M. Compounds with inhibition values less than 50 % at a particular receptor, are deemed inactive at that receptor. Compounds that displayed more than 50 % inhibition of serotonin binding in the primary assays, were evaluated for determination of $K_i$ values in secondary assays. Table 3 shows the results of the primary (% inhibition) assay.

As shown in Table 3, surprisingly all compounds displayed a remarkable selectivity profile. All the compounds were inactive at the 5-HT$_{2A}$ receptor and at most other sites. Interestingly, the majority of compounds were active at the 5-HT$_{2B}$ receptor (except compound 165h). 165c and 165f were also active at the 5-HT$_{1B}$ receptor.

Table 4 shows the data for the secondary assay. All the tested compounds displayed moderate to low affinity at the 5-HT$_{2B}$ receptor ($K_i$ values ranging between 96 to 1429 nM).
Table 3: Primary assay data for analogues 165a-k at 5-HT receptors

<table>
<thead>
<tr>
<th>Compound</th>
<th>5-HT&lt;sub&gt;1A&lt;/sub&gt;</th>
<th>5-HT&lt;sub&gt;1B&lt;/sub&gt;</th>
<th>5-HT&lt;sub&gt;1D&lt;/sub&gt;</th>
<th>5-HT&lt;sub&gt;1E&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;3&lt;/sub&gt;</th>
<th>5-HT&lt;sub&gt;5A&lt;/sub&gt;</th>
<th>5-HT&lt;sub&gt;7&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>165a</td>
<td>5.5</td>
<td>22.6</td>
<td>34.5</td>
<td>10.1</td>
<td>33.5</td>
<td>92.2</td>
<td>31.9</td>
<td>8</td>
<td>-12.2</td>
<td>36.1</td>
</tr>
<tr>
<td>165b</td>
<td>-0.9</td>
<td>20.6</td>
<td>29.7</td>
<td>10.3</td>
<td>40.1</td>
<td>83.3</td>
<td>13.5</td>
<td>-2.7</td>
<td>-7.6</td>
<td>26</td>
</tr>
<tr>
<td>165c</td>
<td>27</td>
<td>51.7</td>
<td>22.4</td>
<td>2.1</td>
<td>32.3</td>
<td>84</td>
<td>13.1</td>
<td>0.7</td>
<td>5.2</td>
<td>8.6</td>
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<tr>
<td>165d</td>
<td>18.4</td>
<td>41.4</td>
<td>24</td>
<td>7.3</td>
<td>40.9</td>
<td>76.1</td>
<td>48</td>
<td>11.6</td>
<td>17.6</td>
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<td>13.4</td>
<td>2.4</td>
<td>19.9</td>
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<td>0.8</td>
<td>-6.2</td>
<td>-14.6</td>
</tr>
<tr>
<td>165f</td>
<td>-9.8</td>
<td>60.2</td>
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<td>27.4</td>
<td>42.9</td>
<td>77.1</td>
<td>32.4</td>
<td>7.7</td>
<td>5.2</td>
<td>6.9</td>
</tr>
<tr>
<td>165g</td>
<td>19.2</td>
<td>19.4</td>
<td>22.6</td>
<td>3.3</td>
<td>47.8</td>
<td>72.1</td>
<td>28.8</td>
<td>8.3</td>
<td>-9.8</td>
<td>24.7</td>
</tr>
<tr>
<td>165h</td>
<td>25.9</td>
<td>2.1</td>
<td>9.8</td>
<td>-0.1</td>
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<td>24.4</td>
<td>-6.6</td>
<td>-7.4</td>
<td>-9.3</td>
<td>-22.6</td>
</tr>
<tr>
<td>165i</td>
<td>24.2</td>
<td>-5.2</td>
<td>15.8</td>
<td>11</td>
<td>29.2</td>
<td>50.3</td>
<td>-2.3</td>
<td>4.1</td>
<td>1.8</td>
<td>31.3</td>
</tr>
<tr>
<td>165j</td>
<td>3.3</td>
<td>23.6</td>
<td>27.4</td>
<td>5</td>
<td>12.8</td>
<td>88.4</td>
<td>-1.6</td>
<td>-4.1</td>
<td>-12.1</td>
<td>8.9</td>
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<tr>
<td>165k</td>
<td>13.3</td>
<td>16.2</td>
<td>22.4</td>
<td>3.5</td>
<td>9.6</td>
<td>73.7</td>
<td>9.4</td>
<td>-13.3</td>
<td>-18.2</td>
<td>18.3</td>
</tr>
<tr>
<td>55&lt;sup&gt;b&lt;/sup&gt;</td>
<td>na&lt;sup&gt;c&lt;/sup&gt;</td>
<td>100.3</td>
<td>na&lt;sup&gt;c&lt;/sup&gt;</td>
<td>60</td>
<td>89.3</td>
<td>92.8</td>
<td>103.0</td>
<td>44.6</td>
<td>92.8</td>
<td>96.7</td>
</tr>
</tbody>
</table>

<sup>a</sup> Hot ligands: [<sup>3</sup>H]-8-OH-DPAT (5-HT<sub>1A</sub>), [<sup>3</sup>H]GR125743 (5-HT<sub>1B</sub>), [<sup>3</sup>H]GR125743 (5-HT<sub>1D</sub>), [<sup>3</sup>H]5-HT (5-HT<sub>1E</sub>), [<sup>3</sup>H]ketanserin (5-HT<sub>2A</sub>), [<sup>3</sup>H]LSD (5-HT<sub>2B</sub>), [<sup>3</sup>H]mesulergine (5-HT<sub>2C</sub>), [<sup>3</sup>H]LY278584 (5-HT<sub>3</sub>), [<sup>3</sup>H]LSD(5-HT<sub>5A</sub>), [<sup>3</sup>H]LSD(5-HT<sub>7</sub>);

<sup>b</sup> Corresponding Ki data previously reported in reference 69

<sup>c</sup> na – data not available
Table 4: Secondary assay data for analogues 165a-k

![Chemical structure of compound 165](image)

<table>
<thead>
<tr>
<th>Compound</th>
<th>5-HT\textsubscript{1B}\textsuperscript{b}</th>
<th>5-HT\textsubscript{2B}\textsuperscript{c}</th>
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<tbody>
<tr>
<td>165a</td>
<td>nd\textsuperscript{d}</td>
<td>96 ± 12</td>
</tr>
<tr>
<td>165b</td>
<td>nd</td>
<td>209 ± 27</td>
</tr>
<tr>
<td>165c</td>
<td>1,608 ± 210</td>
<td>307 ± 40</td>
</tr>
<tr>
<td>165d</td>
<td>nd</td>
<td>601 ± 78</td>
</tr>
<tr>
<td>165e</td>
<td>nd</td>
<td>663 ± 86</td>
</tr>
<tr>
<td>165f</td>
<td>2,466 ± 320</td>
<td>299 ± 39</td>
</tr>
<tr>
<td>165g</td>
<td>nd</td>
<td>416 ± 54</td>
</tr>
<tr>
<td>165h</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>165i</td>
<td>nd</td>
<td>715 ± 92</td>
</tr>
<tr>
<td>165j</td>
<td>nd</td>
<td>419 ± 54</td>
</tr>
<tr>
<td>165k</td>
<td>nd</td>
<td>1,429 ± 180</td>
</tr>
<tr>
<td>55</td>
<td>100 ± 3</td>
<td>543 ± 53</td>
</tr>
<tr>
<td>Ergotamine</td>
<td>10</td>
<td>-</td>
</tr>
<tr>
<td>SB206553</td>
<td>-</td>
<td>23</td>
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</table>

\textsuperscript{a} Values represent mean ± SEM for at least three independent experiments
\textsuperscript{b} hot ligand = [\textsuperscript{3}H]GR125743
\textsuperscript{c} hot ligand = [\textsuperscript{3}H]LSD
\textsuperscript{d} nd = not determined (<50 % inhibition in primary assay)
In contrast to our expectations all the C4 phenyl compounds displayed no affinity for the 5-HT$_{2A}$ receptor. This clearly indicates that a phenyl group at the C4 position of nantenine is detrimental for 5-HT$_{2A}$ receptor affinity. This in turn might be due to the inability of the 5-HT$_{2A}$ binding cavity to accommodate the C4 phenyl group or due to a steric clash between a receptor side chain and the C4 phenyl group.

Surprisingly all the compounds displayed affinity for the 5-HT$_{2B}$ receptor with remarkable selectivity. Compound 165a had the highest affinity for the 5-HT$_{2B}$ receptor ($K_i = 96$ nM). Nantenine was found to have poor affinity for the 5-HT$_{2B}$ receptor ($K_i = 534$ nM). Thus when nantenine is compared to compound 165a it is apparent that the C4 phenyl substituent positively impacts 5-HT$_{2B}$ affinity and selectivity.

A clear trend between the length of alkyl group at the C1 position and 5-HT$_{2B}$ receptor affinity was observed. Thus with increasing C1 alkyl chain length, the 5-HT$_{2B}$ receptor affinity was found to decrease as evident from compound 165a to 165e. A similar trend was also observed with respect to the size of the N6 alkyl substituent in compounds 165i to 165j. Thus the larger the N-alkyl group the lower is the 5-HT$_{2B}$ receptor affinity. Both the trends suggest that the binding pocket occupied by the C1 alkyl and N6 alkyl groups are small and do not accommodate larger substituents. The C1 cyclopropylmethyl analogue (165f, $K_i = 299$ nM) has similar affinity compared to the propyl analogue (165c, $K_i = 307$ nM), which indicates that some degree of branching is tolerated. The allyl analogue (165g, $K_i = 416$ nM) had reduced affinity compared to its saturated analogue (165c, $K_i = 307$ nM) suggesting that saturation in this part of the alkyl chain is not tolerated. A phenolic OH group is not well tolerated for 5-HT$_{2B}$ receptor affinity as indicated by 165i ($K_i = 715$ nM).
165a which was the compound with the highest affinity identified in this series was selected for further assays. In order to investigate the selectivity profile, compound 165a was screened across a broad panel of CNS receptors. In addition to the 5-HT receptors noted in Table 4, compound 165a did not display any affinity across the following receptors (< 50% inhibition in primary assays): α1A, α1B, α1D, β1, β2, β3, BZP rat brain site, CB2, D1, D2, D3, D4, D5, DAT, DOR, GABA_A, H1, H2, H3, H4, KOR, M2, M3, M4, M5, MOR, NET, NMDA, SERT, sigma-1, sigma-2. 165a showed affinities for the following receptors other than 5-HT2B: 5-HT6 (627 nM), α2a (719 nM), α2B (3220 nM), α2C (433 nM) M1 (> 10,000 nM) and PBR (2897 nM). In the 5-HT2B functional activity testing, 165a displayed antagonistic activity (IC50 = 1 µM). It is also of relevance that no 5-HT2B agonist activity was found.

5-HT2B receptors are widely distributed in the cardiac muscles and regulate cardiomyocyte development and growth.130 Activation of 5-HT2B receptors in these regions is related to pulmonary hypertension and fatal cardiac failure.131 Moreover the anti-obesity drug fenfluramine and the anti-Parkinson’s disease drug pergolide were withdrawn from the US market because of severe valvular heart diseases induced by agonism of 5-HT2B receptors.132-134 As a result, 5-HT2B agonists are considered as anti-targets in any drug discovery program. Since 165a does not have any agonist activity, it can be considered to be a good lead for developing 5-HT2B antagonists.

5-HT2B antagonists are considered as emerging therapeutics for the treatment of migraine, Irritable Bowel Syndrome (IBS), pulmonary hypertension, liver fibrosis and lung fibrosis.135-138 However, due to the high homology of the 5-HT2B receptor with 5-HT2A and 5-HT2C receptors, it is difficult to obtain selective 5-HT2B antagonists.139 Figure 43 shows some selective 5-HT2B antagonists that are commercially available and used mainly as biological tools.
Although 165a has low affinity for the 5-HT$_{2B}$ receptor in comparison to 168 and 169, 165a to the best of our knowledge is the most potent 5-HT$_{2B}$ aporphinoid antagonist known till date.$^{140}$ Although not as potent as 168 or 169, compound 165a is a highly selective 5-HT$_{2B}$ antagonist and is a promising lead for the development of selective and highly potent 5-HT$_{2B}$ antagonists. Moreover 165a represents a new class of pharmacophore for the development of 5-HT$_{2B}$ antagonists.

Since all the C4 phenyl nantenine compounds were tested as a mixture of diastereomers, it remains to be determined the extent to which the two chiral centers present impact 5-HT$_{2B}$ receptor affinity and activity. For future studies the individual diastereomers of 165a should be evaluated at 5-HT$_{2B}$ receptors. Moreover the steric and electronic tolerance of the phenyl ring needs to be determined. Analogues of 165a having different substitutions on the phenyl ring should be synthesized and evaluated for affinity at the 5-HT$_{2B}$ receptor.
2.4.2 Isochroman Analogues:

**Rationale:** Molecular docking studies indicate that the protonated amine nitrogen (N6) of aporphines is required for binding to the 5-HT$_{2A}$ receptor via interactions with an aspartate residue (Asp 155) in the ligand cavity.\textsuperscript{123} Figure 44 shows a docking pose of nantenine (55) in the binding cavity of the 5-HT$_{2A}$ receptor. As can be seen, the carboxylate side chain of Asp155 forms a salt bridge interaction (shown as a dashed line) with the protonated nitrogen atom of nantenine (55).

![Docking pose of nantenine (55) in the 5-HT$_{2A}$ receptor binding cavity](image)

**Figure 44:** Docking pose of nantenine (55) in the 5-HT$_{2A}$ receptor binding cavity

However, the requirement of this salt bridge interaction has not been rigorously tested via synthesis and evaluation of appropriate analogs. Moreover with regards to the previously reported cytotoxic activity of aporphines, the requirement for an N6 nitrogen atom has not been thoroughly
investigated. As a result, the synthesis and evaluation of oxygen containing analogues (isochroman compounds like 170) as N6 isosteres was undertaken.

![Structures of Nantenine (55) and Nantenine N6 isostere (170)](image)

Figure 46 shows the approach used for the synthesis of compound 170. Thus the isochroman compound 170 was synthesized via an intramolecular C-H activation in compound 171, which in turn was synthesized using an Oxa Pictet-Spengler reaction between the alcohol 172 and aldehyde 173.

![Retrosynthetic analysis for compound 170](image)

The Oxa Pictet-Spengler reaction is the oxygen variant of the classical Pictet-Spengler reaction. Figure 47 shows a proposed mechanism for the Oxa Pictet-Spengler reaction.
between 172 and 173. Thus in presence on an acid catalyst, alcohol 172 attacks the activated carbonyl carbon in 174, to generate intermediate 175. Internal proton transfer followed by loss of water results in formation of oxocarbenium intermediate 177.

Subsequent cyclization followed by re-aromatization leads to the formation of the isochroman compound 171. The use of a Dean-Stark apparatus helps in driving the equilibrium in the forward direction by removing water from the system.

Using this strategy (shown in Figure 47), synthesis of ring A isochroman analogues was accomplished as shown in Scheme 4. The synthesis commences with the commercially available acid 179 (isovanillic acid). Treatment of 179 with benzyl bromide afforded the dibenzylated intermediate 180 which after reduction with Lithium Aluminum Hydride (LAH) afforded the alcohol 181. Treatment of 181 with the easily accessible aldehyde 173 under Oxa Pictet-Spengler reaction conditions furnished the isochroman 182. With 182 in hand, the stage was set to attempt an intramolecular C-H activation reaction which would furnish intermediate 183. Prior synthesis
of the aporphine template using this reaction utilized ligands such as 2’-(diphenylphosphino)-N,N’-dimethyl-(1,1’-biphenyl)-2-amine (PhDavePhos, Table 5, Ligand A), di-tert-butyl (methyl) phosphonium tetrafluoroborate (Table 5, Ligand B), 2-Dicyclohexylphosphino-2’-(N,N-dimethylamino)biphenyl (Table 5, Ligand D) and tricyclohexylphosphine.29

In order to make a direct comparison between the synthesis of aporphine alkaloids and the isochroman scaffold, we screened ligands A-D as shown in Table 5. As seen in Table 5, tricyclohexylphosphine tetrafluoroborate was found as the optimal catalyst with a yield of 93 % for the cyclization of 171 to 170. Other ligands resulted in lower isolated yields (ligands A and B) or gave no reaction (ligand D).
Table 5: Microwave assisted C-H activation on compound 171 with various ligands

<table>
<thead>
<tr>
<th>Entry</th>
<th>Ligand</th>
<th>Temperature (°C)</th>
<th>Yielda (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A</td>
<td>135</td>
<td>40</td>
</tr>
<tr>
<td>2</td>
<td>B</td>
<td>140</td>
<td>22</td>
</tr>
<tr>
<td>3</td>
<td>C</td>
<td>140</td>
<td>93</td>
</tr>
<tr>
<td>4</td>
<td>D</td>
<td>140</td>
<td>No Reaction</td>
</tr>
</tbody>
</table>

a Isolated yield after purification

Accordingly in the presence of Pd(OAc)$_2$ and tricyclohexylphosphine tetrafluoroborate, compound 182 was smoothly transformed to 183 in 90 % yield. Catalytic hydrogenation of 183 afforded phenol 184, which was subsequently derivatized to furnish the target isochroman analogues 170 and 185a-d. Scheme 5 shows the synthesis of ring D isochroman analogues. The commercially available acid 186 was first converted to the ester 187 followed by reduction to furnish alcohol 172. The C-H activation step from 189a-d proceeded to give target isochroman
compounds 185e-h in excellent yields indicating tolerance of a variety of substitution pattern in the lower aromatic ring.

The effect of catalyst loadings and catalyst structure on the C-H activation reaction was also examined. In case of cyclization from 189a to 185e we observed a decrease in isolated yield with lower catalyst loadings. With 5 % Pd(OAc)$_2$/10 % tricyclohexylphosphine tetrafluoroborate catalytic system the isolated yield of 185e was 76 %. This was slightly improved to 82 % when 10 % Pd(OAc)$_2$/20 % tricyclohexylphosphine tetrafluoroborate catalytic system was employed. The best yield observed was 89 % which was obtained 17 % Pd(OAc)$_2$/34 % tricyclohexylphosphine tetrafluoroborate catalytic system. Overall the synthesis of novel

\[
\begin{array}{l}
\text{Scheme 5: Reagents and Conditions: a) MeOH, H$_2$SO$_4$, reflux, 30 min, 99 %; b) NaBH}_4, \text{CaCl}_2, \text{EtOH, 0 °C-rt, 16h, 70 %; c) 188a-d, PTSA, Tolune,reflux (Dean Stark Apparatus). 18h; d) Pd(OAc)$_2$, tricyclohexylphosphine tetrafluoroborate, DMSO, 140 °C, microwaves, 200W, 10 min; e) Dess-Martin Periodinane, DCM, rt, 30 min.}
\end{array}
\]
isochroman heterocycles in excellent yields was achieved via a palladium catalyzed C-H activation reaction.\textsuperscript{143}

Analogues 170 and 185b were screened at a concentration of 10 µM in multi-well format for intrinsic (agonist) and antagonist activity at the human 5-HT\textsubscript{2A} and α\textsubscript{1A} receptors using the Fluorescence Imaging Plate Reader (FLIPR) based functional assay that detects receptor mediated mobilization of internal calcium with a calcium sensitive fluorescent dye (see section 2.6 for a detailed experimental procedure). Table 6 shows the apparent binding affinities of 170 and 185b.

### Table 6: Apparent binding affinities for isochroman compounds 170 and 185b

<table>
<thead>
<tr>
<th>Compound</th>
<th>K\textsubscript{e} ± SEM\textsuperscript{a} (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5-HT\textsubscript{2A}</td>
</tr>
<tr>
<td>170</td>
<td>&gt;3000</td>
</tr>
<tr>
<td>185b</td>
<td>&gt;3000</td>
</tr>
<tr>
<td>(±)-55\textsuperscript{b}</td>
<td>850 ± 6</td>
</tr>
<tr>
<td>Prazosin</td>
<td>-</td>
</tr>
<tr>
<td>Ketanserin\textsuperscript{b,c}</td>
<td>32</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Values represent mean ± SEM for at least three independent experiments

\textsuperscript{b} Corresponding data previously reported in reference 69

\textsuperscript{c} IC\textsubscript{50} determined in the presence of 5-HT EC\textsubscript{80}
As seen in Table 6, isochroman compounds 170 and 185b were found to be completely devoid of affinity for the 5-HT2A and α1A receptors. This suggest that the presence of N6 nitrogen atom is critical for affinity at both these receptors.144

Indra and co-workers had proposed a binding mode of nantenine at the 5-HT2A receptor.70 Their study which was based on a homology model of the rat 5-HT2A receptor, showed the N6 nitrogen atom involved in a strong H-bond interaction with the Asp155 residue of the 5-HT2A receptor. Previous molecular docking studies done in our group also confirmed this H-bond interaction between the protonated N6 nitrogen atom and the Asp155 residue in a homology model of the human 5-HT2A receptor.123 Furthermore Sowdhamini and co-workers docked several 5-HT2A antagonists like ketanserin, haloperidol and clozapine and displayed the presence of an H-bond interaction between Asp155 and the protonated amine functionality of a particular ligand.145 Findings from the above mentioned studies are now corroborated by this study which shows that both 170 and 185b lacking the nitrogen atom are devoid of 5-HT2A receptor affinity.

Table 7: Cytotoxicity data for compound 185e

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Percent growth inhibitor</th>
</tr>
</thead>
<tbody>
<tr>
<td>IGROV (ovarian cancer)</td>
<td>69.25 %</td>
</tr>
<tr>
<td>T-47D (breast cancer)</td>
<td>75.81 %</td>
</tr>
</tbody>
</table>
The cytotoxic evaluations of nantenine (55) at the human colon cancer cell lines HCT-116 and Caco-2 were previously reported. Results from this study indicated that an \(N\)-methyl functionality is preferred for cytotoxicity over other \(N\)-substituted aporphines. In order to study the importance of the \(N6\) nitrogen on the cytotoxic activity of nantenine, all the isochroman heterocycle containing analogues (except 185b) were screened for cytotoxicity at the NCI-60 DTP Human Tumor Cell Line Screen. Detailed experimental procedures and results of the cytotoxic screen are described in section 2.7 and appendix C respectively. All tested compounds were found to have moderate to low cytotoxic activity, thus indicating that the nitrogen atom of nantenine is not absolutely required for its cytotoxic activity. Compound 185e displayed 69.25 % inhibition of cell growth against the IGROV1 (ovarian) cancer cell line as well as 75.81 % inhibition of cell growth against the T-47D (breast) cancer cell line.

**Synthesis of phenanthrene alkaloids via isochroman analogues:**

As an extension to this work, the opportunity to transform the isochroman heterocycles to the phenanthrene alkaloid scaffold was initiated. Compounds containing the phenanthrene nucleus have been shown to possess a wide range of biological activities including anticancer, antimicrobial, anxiolytic and sedative activities. Phenanthrene alkaloids in particular possess anti-inflammatory, antitumour and anti-acetylcholinesterase, anti-\(\alpha\)-glucosidase and antifungal activities. Phenanthrene alkaloids are also known precursors for the synthesis of aporphine alkaloids.

Scheme 6 summarizes previous methods for the synthesis of phenanthrene alkaloids. Route A shows the synthesis of phenanthrene alkaloid 195 from dibenzo[d,e,g]chromanone compound 191. Compound 191 was accessed from 190 via a radical-mediated cyclization using toxic tin reagents. Another approach previously reported for the synthesis of phenanthrene alkaloids is
shown in route B. Thus compound 194 containing the phenanthrene core was obtained via a photocyclization reaction of stilbene 193.\(^{157}\) Alternatively phenanthrene alkaloids can be accessed through an aryne mediated cycloaddition as shown in route C.\(^{158,159}\) These methods have several drawbacks including the use of toxic tin reagents and uncontrolled regioselectivity during the biaryl bond formation process.

Our strategy to synthesize phenanthrene alkaloid scaffold from the isochroman motif, involved cleavage of the isochroman moiety to reveal a phenanthrene ethyl alcohol unit, which could be easily transformed to the phenanthrene alkaloid moiety. Initial attempts to cleave the isochroman ring in 185e using zinc dust and acetyl chloride resulted in a complex mixture of
products. After experimenting with a number of acidic cleavage conditions, it was found that treatment with 33% HBr-AcOH afforded the desired alcohol 200 along with the acetate 199 (Scheme 7). Acetate 199 could be easily hydrolyzed to the alcohol 200. Alcohol 200 was subjected to oxidation using Dess-Martin Periodinane, followed by reductive amination to generate the known phenanthrene alkaloid 201.

**Scheme 7: Reagents and conditions:** a) 33% HBr-AcOH, 10 °C, 30 min; b) 20% NaOH, methanol, rt, 2h, 99%; c) Dess Martin Periodinane, DCM, rt, 30 min; d) N,N-dimethylamine, NaB(OAc)₃H, DCM, 0 °C-rt, 2h, 59% from 200

As shown in Scheme 6, the requisite biaryl motif for the synthesis of phenanthrene alkaloids was obtained via a radical mediated cyclization (63% yield), photocyclization (30-54% yield) or via aryne mediated cycloaddition (15% yield). In comparison to these previous methods, our method involves a high yielding palladium catalyzed biaryl coupling reaction (90%), tolerant of a variety of substitution patterns in both aryl rings. Furthermore this method avoids the use of
toxic tin reagents as well as results in the formation of a single product. Hence this method serves as a good alternative for the synthesis of phenanthrene alkaloids.

Overall a novel method for the synthesis of two biologically relevant scaffolds viz. isochroman and phenanthrenes has been developed. These method involves high yielding Oxa Pictet-Spengler reaction and a microwave assisted intramolecular C-H activation as the key reactions. Moreover the isochroman analogues 170 and 185b confirm results from molecular docking studies that basic a nitrogen atom is required for affinity at the 5-HT$_2$A receptors. These novel oxygen isosteres of aporphine alkaloids, also have moderate cytotoxic profile and hence are important leads for future design of cytotoxic agents.
2.4.3 Ring D Indole-Aporphine Analogues:

**Rationale:** Based on molecular docking studies, the “methylenedioxy unit” of C1 nantenine analogues is involved in a hydrogen bond interaction with a serine (Ser242) residue of the 5-HT$_{2A}$ receptor.$^{123}$ Figure 48 shows the 2D representation of the binding pose of the C1 propyl analogue (133) and C1 pentyl analogue (202). The hydrogen bond interaction between the oxygen of the methylene dioxy unit and hydroxyl group of Ser242 residue is shown in a dashed line.
It is hypothesized that the phenyl methylenedioxy unit of nantenine can be replaced with other heteroaromatic rings with similar electronic characteristics. Amongst the numerous known heteroaromatic rings, the indole unit is considered as a privileged scaffold for drug design.\(^{160,161}\) There were seven indole containing commercial drugs in the top 200 bestselling drugs of 2012 (by US retail sales).\(^{162}\) Moreover, the indole nucleus is found in several marketed CNS drugs such as sumatriptan (Imitrex), rizatriptan (Maxalt), pindolol and oxypertine among others.\(^{163,164}\) Furthermore, the indole nucleus has also been reported in several 5-HT\(_2A\) ligands.\(^{165-168}\) Considering the rich CNS pharmacology of indoles and aporphine alkaloids, it was decided to synthesize novel fused indole-aporphine compounds having the general structure 207.
Previously Bremner and co-workers had reported the synthesis and evaluation of compound 208 as an α₁A antagonist (Kᵢ = 4 nM). The structure of compound 208 bears great
resemblance to the general structure of the proposed fused indole aporphine analogues (207). The only difference between the two structures is the alkyl group attached to the nitrogen atom. In the initial SAR studies conducted on nantenine, it was revealed that the incorporation of an N-propyl functionality results in selective affinity for the \( \alpha_{1A} \) receptor.\(^{28} \) Shortening the alkyl chain to an N-methyl group resulted in combined affinities for the 5-HT\(_{2A} \) and \( \alpha_{1A} \) receptors. Based on these observations it is expected that this change (from N-propyl in 208 to N-methyl group in 207) will enhance the affinity for the 5-HT\(_{2A} \) receptor.

As shown in Figure 51, Bremner and co-workers accomplished the synthesis of 208 via a Fischer indole synthesis reaction on compound 209.\(^{169} \) Compound 209 was synthesized after 8 steps from the commercially available amine 210. In order to shorten the synthetic route and to allow a late stage modification of the nitrogen functionality, ketone 211 was targeted as a precursor for the synthesis of the desired indole-aporphine analogues. (See Scheme 8)
Scheme 8: Reagents and conditions: a) 213, HCOOH, rt, 16h, 67%; b) 10 % NaOH, MeOH, 2h, rt, 99%; c) TFA, TFAA, rt, 4 h, 67%; d) 216a-e, TFA, Toluene, reflux, 3 h

Scheme 8 shows the initial approach that was used to synthesize the fused indole-aporphine analogues via ketone 211. Acyl Pictet-Spengler reaction between carbamate 212 and acetal 213 proceeded smoothly to provide intermediate 214. Initial attempts to transform ester 214 directly to the ketone 211 using PPA\textsuperscript{170} was unsuccessful. As a result ester 214 was first hydrolyzed to the acid 215, which would be more facile to convert to the ketone 211. Next we attempted the cyclization reaction of 215 to 211 using several acid catalysts. Initial experiments with PTSA\textsuperscript{171}, HCl\textsuperscript{172} and PPA\textsuperscript{173} resulted in either no product formation or led to the formation of decomposed products. However treatment with a mixture of TFA (catalytic) and TFAA\textsuperscript{174} smoothly transformed 215 to the desired ketone 211 in 67 % yield. Once 211 was synthesized Fischer indole synthesis conditions were employed to furnish novel fused indole-aporphine derivatives 217a-d.

Having synthesized 217a-d, the next step was to reduce the N-carbamate functionality to an N-methyl group. Unfortunately the initial attempt of reduction using LAH did not produce the
desired product but instead resulted in the formation of phenanthrene derivative 219. Other efforts to avoid the formation of this unexpected product (as shown in Table 8) were also unsuccessful. Thus using less equivalents of LAH (1.2 equiv., entry 2) resulted in the formation of 219 as the sole product. Coop and his co-workers had reported the use of L-selectride as a reagent for carbamate deprotection of opium alkaloids and its derivatives. Unfortunately the use of L-selectride (entry 3) proved unsuccessful as was the case with NaBH₄ (entry 4). Efforts to hydrolyze the carbamate group using NaOH (entry 5) were also not fruitful.

Table 8: Reduction of 217d to 219

<table>
<thead>
<tr>
<th>Entry</th>
<th>Reagents</th>
<th>Conditions</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>LAH (2 equiv.)</td>
<td>THF, 0 ºC - rt-reflux, 6 h</td>
<td>219</td>
</tr>
<tr>
<td>2</td>
<td>LAH (1.2 equiv.)</td>
<td>THF, 0 ºC, 4 h</td>
<td>219</td>
</tr>
<tr>
<td>3</td>
<td>NaBH₄</td>
<td>MeOH, reflux, 16 h</td>
<td>No Reaction</td>
</tr>
<tr>
<td>4</td>
<td>L-Selectride</td>
<td>THF, 0 ºC-rt-reflux, 48 h</td>
<td>No Reaction</td>
</tr>
<tr>
<td>5</td>
<td>20 % NaOH</td>
<td>EtOH, reflux, 32 h</td>
<td>No Reaction</td>
</tr>
</tbody>
</table>
A plausible mechanism for the formation of 219 is proposed in Figure 52. Thus reduction of carbamate 217d would first furnish intermediate 222, which would subsequently be reduced to the iminium 224. At this stage deprotonation of the relatively acidic proton H7 can lead to ring A opening, to furnish imine 225, which on subsequent reduction gets transformed into 219. Phenanthrene alkaloids have been reported to be synthesized from aporphine alkaloids under basic Hofmann’s degradation conditions and involve an aporphinoid intermediate with a quaternary ammonium group. In the case of intermediate 224, the iminium nitrogen has the same formal charge as in a quaternary ammonium group, and hence it is very likely that the phenanthrene 225 is synthesized from 224. Carbamate reductions in aporphine alkaloids have been achieved successfully using LAH. Although mechanistically similar, the formation of phenanthrene type of compounds is not reported in the case of aporphines. It is possible, that the presence of an indole unit increases the acidity of H7 through inductive effects and hence drives the formation of compound 219.
Unable to control the reduction of the carbamate group, an alternative approach to synthesize the desired fused indole-aporphine compounds was initiated shown in Scheme 9. This approach involves the synthesis of ketone 226 already having the N-methyl functionality, thus precluding the necessity of a protection-deprotection sequence.

Scheme 9: Reagents and conditions: a) succinic anhydride, toluene, reflux, 4 h; b) conc. H₂SO₄, MeOH, reflux, 4 h, 70 % over 2 steps; c) POCl₃, toluene, reflux, 6 h; d) NaBH₄, MeOH, 0 °C-rt, 4 h, 52 % over 2 steps; e) 10% NaOH, EtOH, reflux, 30 mins f) TFA, TFAA, 0 °C-rt, 16 h, 33 % over 2 steps; g) 216a-i, TFA, toluene, reflux, 3 h

The new synthesis commenced with the commercially available amine 227, which was first reacted with succinic anhydride followed by esterification to furnish the amide 228. Amide 228 was then reacted under Bischler-Napieralski conditions to produce iminium ion 229, which on reduction with NaBH₄ was converted to the ester 230. Having already optimized the conditions for the intramolecular cyclization, we found that hydrolysis of ester 230 followed by treatment of the intermittent acid with TFA (catalytic) and TFAA afforded the desired ketone 226. Treatment of ketone 226 with commercially available phenylhydrazines 216a-i under Fischer indole synthesis conditions furnished the desired novel fused indole-aporphine compounds 218a-i.
Overall novel fused indole aporphine compounds (218a-i) were synthesized via a short synthetic sequence comprising of 7 steps. Compounds 218a-i are being currently evaluated for affinity at serotonin 5-HT receptors through the PDSP. We expect these compounds to have enhanced affinity for the 5-HT$_{2A}$ receptor. Binding affinity data from these compounds will help understand the importance of the phenyl methylenedioxy unit for 5-HT$_{2A}$ antagonism. Moreover, the extent to which an indole ring will be tolerated on the aporphine core will also be revealed.
2.4.4 C3 Analogues:

![Diagram showing structures of 232 and proposed C3 analogues 233a-b]

**Rationale:** 3-bromo nantenine (232) is 16 times more potent than nantenine at the 5-HT\textsubscript{2A} receptor ($K_c = 48$ nM).\textsuperscript{28} Molecular docking studies indicate that the C3 bromine atom of 232 lies in close proximity to the Phe339/Phe340 residues of the 5-HT\textsubscript{2A} receptor.\textsuperscript{176} Figure 54 shows a docking pose of 232 in the 5-HT\textsubscript{2A} receptor. It is thus hypothesized that analogues (233a-b) with small hydrophobic functional groups at this position should enhance the affinity at the 5-HT\textsubscript{2A} receptor (through hydrophobic interactions with Phe339/Phe340 residues).
Figure 54: Docking pose of 3-bromo nantenine (232) in the 5-HT$_{2A}$ receptor binding cavity

Synthesis of the target C3 analogues is shown in Scheme 10. The synthesis commences with compound 214, which was condensed with nitromethane to afford the nitrostryene derivative 235. LiBH$_4$ mediated reduction of 235 produced primary amine 236, which was coupled with the acid 158 in the presence of CDI to furnish the amide 237. Amide 237 was first treated with PCl$_5$ to generate the intermediate Bischler-Napieralski product, which on reduction followed by Boc protection gave intermediate 238.
Microwave assisted biaryl coupling reaction of 238 was achieved using Pd(OAc)$_2$ and di-tert-butyl (methyl) phosphonium tetrafluoroborate to furnish 239 in 93 % yield. Catalytic hydrogenation of 239 produced phenol 240 in 88 % yield. 240 was then first treated with NBS to install a bromine atom at the C3 position followed by O-methylation to generate the Suzuki coupling precursor compound 241.

Initial attempts to synthesize 242a from 241 under thermal conditions resulted in the formation of an inseparable 9:1 mixture of 242a: 241 (52 % yield, ratio determined by NMR). In order to improve the outcome of this reaction, microwave reaction conditions were explored. The
use of microwave irradiation in chemistry is gaining increasingly popularity due to superior reaction rates, selectivity and better yields than thermal conditions.\textsuperscript{177,178} More particularly the application of microwave irradiations towards Suzuki coupling reaction is now commonplace.\textsuperscript{179-181}

**Table 9**: Optimization efforts for microwave accelerated Suzuki coupling reaction:

<table>
<thead>
<tr>
<th>Entry</th>
<th>Solvent</th>
<th>Metal Catalyst</th>
<th>Base</th>
<th>Conditions</th>
<th>Yields\textsuperscript{b,c}</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Toluene</td>
<td>0.05 % Pd(PPh\textsubscript{3})\textsubscript{4}</td>
<td>Na\textsubscript{2}CO\textsubscript{3}</td>
<td>Thermal, 100 °C, 16 h</td>
<td>52 % (9:1)</td>
</tr>
<tr>
<td>2</td>
<td>Toluene</td>
<td>0.05 % Pd(PPh\textsubscript{3})\textsubscript{4}</td>
<td>Na\textsubscript{2}CO\textsubscript{3}</td>
<td>MW, 120 °C, 20 min</td>
<td>82 % (9:1)</td>
</tr>
<tr>
<td>3</td>
<td>Toluene</td>
<td>0.1 % Pd(PPh\textsubscript{3})\textsubscript{4}</td>
<td>Na\textsubscript{2}CO\textsubscript{3}</td>
<td>MW, 120 °C, 20 min</td>
<td>87 % (9:1)</td>
</tr>
<tr>
<td>4</td>
<td>Toluene</td>
<td>0.2 % Pd(PPh\textsubscript{3})\textsubscript{4}</td>
<td>Na\textsubscript{2}CO\textsubscript{3}</td>
<td>MW, 120 °C, 20 min</td>
<td>81 % (9:1)</td>
</tr>
<tr>
<td>5</td>
<td>Toluene</td>
<td>0.05 % Pd(dppf)Cl\textsubscript{2}</td>
<td>Na\textsubscript{2}CO\textsubscript{3}</td>
<td>MW, 120 °C, 20 min</td>
<td>No Reaction</td>
</tr>
<tr>
<td>6</td>
<td>1,4 dioxane</td>
<td>0.05 % Pd(PPh\textsubscript{3})\textsubscript{4}</td>
<td>Na\textsubscript{2}CO\textsubscript{3}</td>
<td>MW, 110 °C, 20 min</td>
<td>70 % (7:3)</td>
</tr>
<tr>
<td>7</td>
<td>Toluene</td>
<td>0.05 % Pd(PPh\textsubscript{3})\textsubscript{4}</td>
<td>CsF</td>
<td>MW, 110 °C, 30 min</td>
<td>85 % (9:1)</td>
</tr>
<tr>
<td>8</td>
<td>DMF</td>
<td>0.05 % Pd(PPh\textsubscript{3})\textsubscript{4}</td>
<td>K\textsubscript{2}CO\textsubscript{3}</td>
<td>MW, 140 °C, 10 min</td>
<td>No Reaction</td>
</tr>
<tr>
<td>9</td>
<td>Toluene</td>
<td>0.05 % Pd(PPh\textsubscript{3})\textsubscript{4}</td>
<td>Na\textsubscript{2}CO\textsubscript{3}</td>
<td>MW, 120 °C, 20 min</td>
<td>77 % (9:1)</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Entries 1-8: phenyl boronic acid used, entry 9: allyl boronic acid pinacol ester used
\textsuperscript{b}Isolated yields
\textsuperscript{c}Entries 1-8: ratio for 242a:241, entry 9 ratio for 242b:241
Table 9 summarizes our optimization for the microwave assisted Suzuki coupling reaction. Thus treatment of 241 with phenyl boronic acid (entry 1) in the presence of 0.05 % Pd(PPh₃) resulted in the formation of an inseparable 9:1 mixture of 242a and 241. Other attempts to completely transform 241 to 242a also had similar outcomes. Increasing the catalyst loadings to 0.01 % (entry 3) and 0.2 % (entry 4) also gave a 9:1 mixture of 242a and 241. No reaction product was formed with the use of 0.05 % Pd(dppf)Cl₂ (entry 5). Changing the solvent to 1,4 dioxane (entry 6) or DMF (entry 8) resulted in a diminished yield or no reaction respectively. Changing the base to CsF had an outcome similar to the use of Na₂CO₃. Using a different boronic acid coupling partner (entry 9) also gave us a 9:1 mixture of the corresponding product.

Nevertheless the inseparable mixtures (242a or 242b and 241) obtained after this step was subjected to boc deprotection and reductive amination. The resulting mixture of (242a or 242b and 241) were purified using preparative Thin Layer Chromatography to furnish the desired compounds 233a and 233b.

Prior to this work, there were limited reports of Suzuki coupling reactions attempted on the aporphine scaffold. Hedberg and Linnanen have independently reported the synthesis of C10 and C11 aporphine analogues using the Suzuki coupling reaction. Both groups however used the corresponding aryl triflates as the Suzuki coupling precursor. This work is the first Suzuki coupling reaction reported on an aporphinoid aryl bromide substrate. Furthermore, the microwave reaction conditions developed for the Suzuki coupling reaction are highly efficient compared to reaction under thermal conditions. This synthetic manipulation thus sets the stage for the rapid generation of C3 analogues as well as analogues at other positions.

Analogues 233a and 233b are currently being evaluated for affinity at the serotonin receptors through the PDSP. It is expected that these compounds will have enhanced 5-HT₂A
receptor potency by virtue of hydrophobic interactions of the C3 substituents. Based on this information, 5-HT$_{2A}$ antagonists having modifications at more than one position (for example C1 and C3) will be synthesized.

2.6 Conclusions:

Selective 5-HT$_{2A}$ antagonists have therapeutic potential in the mitigation of several neuropsychiatric disorders such as sleep disorders, depression, anxiety, schizophrenia and drug abuse. However, there are very few truly selective 5-HT$_{2A}$ antagonists known at this time (and furthermore no such compound is clinically available). As a result, there is an unmet need to identify and develop new selective 5-HT$_{2A}$ antagonists. The privileged aporphine scaffold present in the naturally available alkaloid nantenine, is an attractive target for developing selective 5-HT$_{2A}$ antagonists. However, in order to understand the structural tolerance of the nantenine scaffold for 5-HT$_{2A}$ antagonism, an exhaustive SAR study was initiated.

Accordingly a library of nantenine analogues having structural modifications at the C3, C4 positions, oxygen containing isosteric analogues and ring D indole analogues was designed and successfully synthesized. The C4 phenyl analogues were synthesized with the aim of enhancing 5-HT$_{2A}$ receptor affinity (via structural similarity to known 5-HT$_{2A}$ pharmacophores). However, SAR studies of these analogues indicate that introduction of phenyl ring at the C4 position of nantenine is detrimental for 5-HT$_{2A}$ receptor affinity. Surprisingly these compounds displayed affinity and selectivity for the 5-HT$_{2B}$ receptor. The isochroman analogues were devoid of any affinity for 5-HT$_{2A}$ receptor, thus indicating that a basic nitrogen atom is required for affinity at the 5-HT$_{2A}$ receptor. This result also corroborated findings from previous molecular docking
studies, suggesting a salt bridge interaction between the basic nitrogen atom and Asp155. All the
isochroman compounds also displayed low to moderate cytotoxicity, thus indicating that the
nitrogen atom might not be critical for cytotoxic effects of nantenine and other aporphines. A
library of C3 analogues and fused indole aporphine analogues are currently being evaluated for 5-
HT$_{2A}$ affinity. These compounds are expected to have enhanced 5-HT$_{2A}$ affinity.

This work has positively impacted the field of medicinal chemistry. Results from current SAR studies have expanded our understanding of the structural tolerance of the aporphine core required for 5-HT$_{2A}$ antagonism. This information will be useful for the design of future aporphinoid 5-HT$_{2A}$ antagonists. Moreover, novel lead molecules such as compound 165a (selective 5-HT$_{2B}$ antagonist) or compound 185e (cytotoxic agent against IGROV1-ovarian cancer cell line and T-47D-breast cancer cell line) were identified.

From a synthetic chemistry perspective this work has significant outcomes. i) a method utilizing palladium catalyzed C-H activation for the synthesis of two bioactive scaffolds-isochroman analogues and phenanthrene alkaloids was developed. ii) novel fused indole aporphine alkaloids were synthesized iii) a microwave accelerated Suzuki coupling reaction has been developed, enabling the rapid generation of C3 aporphine analogues.

To continue our quest for developing novel aporphine 5-HT$_{2A}$ antagonists, information obtained from previous and current SAR studies will be used to guide the future design of analogues. For example, information obtained from the evaluation of fused indole aporphine analogues will be used for the design of other ring D heterocyclic isosteres. Similarly nantenine analogues having a combination of 5-HT$_{2A}$ preferring moieties at the C1 and C3 positions will also be synthesized and evaluated.
Overall a systematic exploitation of the untapped potential of aporphine alkaloids will lead to the development of novel, potent and unique CNS ligands.

2.6 Experimental:

2.6.1 Chemistry

*General methods and instrumentation:*

All glass apparatus were oven-dried prior to use. A CEM Discover microwave reactor was used to carry out microwave-assisted C-H arylation reactions. **HRESIMS** spectra were obtained using an Agilent 6520 Q-TOF instrument. $^1$H NMR and $^{13}$C NMR spectra were recorded using a Bruker DPX-500 spectrometer (operating at 500 MHz for $^1$H; 125 MHz for $^{13}$C) using CDCl$_3$ as solvent. Tetramethylsilane (δ 0.00 ppm) served as an internal standard in $^1$H NMR and $^{13}$C NMR unless stated otherwise. Chemical shift (δ 0.00 ppm) values are reported in parts per million and coupling constants in Hertz (Hz). Splitting patterns are described as singlet (s), doublet (d), triplet (t), and multiplet (m). Melting points were obtained on a Mel-Temp capillary electrothermal melting point apparatus. Reactions were monitored by TLC with Whatman Flexible TLC silica gel G/UV 254 precoated plates (0.25 mm). TLC plates were visualized in UV light (254 nm) and by staining with phosphomolybdate spray reagent, vanillin or iodine. Flash column chromatography was performed with silica gel 60 (EMD Chemicals, 230-400 mesh, 0.04-0.063 mm particle size). Preparative thin layer chromatography was performed with silica gel GF plates (Analtech, catalog # 02003). All chemicals and reagents were obtained from Sigma-Aldrich and Fischer Scientific (USA) in reagent grade and were used without further purification.
A) Synthesis of C4 Phenyl analogues:

1-(benzyloxy)-2-methoxy-4-(2-nitro-1-phenylethyl) benzene (156): Phenylmagnesium bromide (9.5 g, 53 mmol) was added to anhydrous THF (50 mL) under N₂ atmosphere at 0 ºC. To this reaction mixture, a cold solution of compound 155 (5.0 g, 18 mmol) in anhydrous THF (60 mL) was added dropwise over 30 min. The reaction mixture was allowed to stir at 0 ºC for another 1 h, and then at rt for 16 h. HCl (1M solution, 10 mL) was added and the reaction was stirred for 1 h. The THF was evaporated to get a crude residue to which water (60 mL) was added. The crude mixture was then extracted with ethyl acetate (3 x 30 mL). The combined organic layer was dried over Na₂SO₄, and evaporated under vacuum to afford a brown oil which was purified on silica gel, eluting in 5-20 % acetone-petroleum ether to provide 156 as a yellow powder; 32 %; mp 75 ºC-77 ºC; ¹H NMR (500 MHz, CDCl₃): δ 7.40 (d, 2H, J = 7.5 Hz), 7.37-7.29 (m, 6H), 7.22 (d, 2H, J = 7.3 Hz), 6.82 (d, 1H, J = 8.2 Hz), 6.73-6.70 (m, 2H), 5.11 (s, 2H), 4.93 (d, 2H, J = 8.0 Hz), 4.83 (t, 1H, J = 8.0 Hz), 3.83 (s, 3H); ¹³C NMR (125 MHz, CDCl₃): δ 149.9, 147.6, 139.3, 137.0, 132.1, 129.0, 128.5, 127.9, 127.6, 127.5, 127.2, 119.4, 114.0, 111.7, 79.4, 71.0, 56.0, 48.6; HRESIMS: calcd. for C₂₂H₂₁NO₄Na [M+Na]⁺ 386.1369; found 386.1361.

2-(4-(benzyloxy)-3-methoxyphenyl)-2-phenylethan-1-amine (157): Compound 156 (0.5 g, 1.4 mmol) and tin (II) chloride (6.2 g, 28 mmol) were dissolved in ethanol (60 mL). The resulting reaction mixture was refluxed for 16 h. The reaction was cooled to rt and the solvent evaporated. To the crude residue obtained, ethyl acetate (50 mL) was added followed by the addition of 1 M NaOH to adjust to pH 8. The organic layers were extracted with ethyl acetate (2 x 30 mL). The combined organic layer was dried over Na₂SO₄, and evaporated under vacuum to afford 157 as yellow flakes which were used without further purification; 65 %
N-(2-(4-(benzyloxy)-3-methoxyphenyl)-2-phenylethyl)-2-(6-bromobenzo[d][1,3]dioxol-5-yl)acetamide (159): A solution of bromoacid 158 (0.07 g, 0.3 mmol) in anhydrous THF (30 mL) under N\textsubscript{2} atmosphere was cooled to 0 °C. To this solution CDI (0.04 g, 0.3 mmol) was added in three portions over a period of 30 min at 0 °C. The resulting reaction mixture was allowed to stir for 1.5 h at 0 °C, after which the temperature was raised to rt and allowed to stir for another 1 h. The solution was again cooled to 0 °C and allowed to stir for 30 min, after which a solution of 157 (0.09 g, 0.3 mmol) in anhydrous THF (10 mL) was added dropwise. The resulting reaction mixture was allowed to stir for 30 min at 0 °C and then at rt for another 15 h. The precipitate was filtered and washed with ether affording 159 as a white solid; 60 %; mp 99 °C-103 °C; \textsuperscript{1}H NMR (500 MHz, CDCl\textsubscript{3}): δ 7.42 (d, 2H, J = 7.2 Hz), 7.36 (dd, 2H, J = 7.2, 7.2 Hz), 7.31-7.24 (m, 2H), 7.24 (s, 1H), 7.19 (t, 1H, J = 7.2 Hz), 7.14 (d, 2H, J = 7.2 Hz), 6.87 (s, 1H), 6.77 (d, 1H, J = 8.2 Hz), 6.67 (s, 1H), 6.63 (s, 1H) 6.61 (s, 1H), 5.95 (s, 2H), 5.33 (broad s, 1H), 5.10 (s, 2H), 4.03 (t, 1H, J = 8.1 Hz), 3.82-3.50 (m, 5H), 3.49 (s, 2H); \textsuperscript{13}C NMR (125 MHz, CDCl\textsubscript{3}): δ 169.5, 147.0, 141.6, 134.4, 128.6, 128.5, 127.8, 127.3, 127.2, 126.7, 119.8, 113.9, 112.7, 111.8, 110.7, 101.9, 71.0, 55.9, 49.9, 43.8, 43.7; HRESIMS: calcd. for C\textsubscript{31}H\textsubscript{29}BrNO\textsubscript{5} [M+H]+ 576.1209; found 576.1200

\textit{tert}-butyl 7-(benzylloxy)-1-((6-bromobenzo[d][1,3]dioxol-5-yl)methyl)-6-methoxy-4-phenyl-3,4-dihydroisoquinoline-2(1H)-carboxylate (161): Compound 158 (5.0 g, 8.7 mmol) was dissolved in dichloromethane (50 mL) and the resulting solution was cooled to 0 °C and allowed to stir for 20 min. PCl\textsubscript{5} (2.7 g, 13 mmol) was added in 3 portions over a period of 30 min. The resulting reaction mixture was allowed to stir at 0 °C for 30 min and then at rt for the next 16 h. To this reaction mixture 20 mL NaHCO\textsubscript{3} was added and the organic layers were extracted with dichloromethane (2 x 10 mL). The combined organic layers were dried over Na\textsubscript{2}SO\textsubscript{4}, and evaporated under vacuum to get the intermediate imine as a brown oil, which was used without
further purification. Sodium borohydride (1.3 g, 34 mmol) was added to the solution of intermittent imine (4.8 g, 8.6 mmol) in methanol (50 mL) at 0 ºC. The reaction mixture was then brought to rt and allowed to stir for 4 h. After 4 h, methanol was evaporated to get a crude mixture, to which water (30 mL) was added, followed by extraction with ethyl acetate (3 x 30 mL). The combined organic layers were dried over Na₂SO₄, and evaporated under vacuum to get an intermediate amine (29) which was used in the next step without purification. The crude amine (160, 4.8 g, 8.2 mmol), (Boc)₂O (2.84 mL, 2.7 g, 12.3 mmol) and DIPEA (2.83 mL, 2.1 g, 16 mmol) and DMAP (catalytic) were dissolved in dichloromethane (100 mL). The resulting reaction mixture was stirred at rt for 16 h. After 16 h, the reaction mixture was evaporated to dryness and the crude product was purified on silica gel eluting with 30 % ethyl acetate-petroleum ether to afford 161 as a white solid; 85 %; mp 67 ºC-70 ºC; rotamers observed, all signals reported; ¹H NMR (500 MHz, CDCl₃): δ 7.48-7.42 (2H), 7.40-7.29 (m, 7H), 7.23-7.19 (2H), 7.04-6.79 (1H), 6.84, 6.70 (1H), 6.54-6.51 (1H), 6.31, 6.26 (1H), 5.95-5.85 (2H), 4.48-4.44, 4.24-4.20 (1H), 4.16-4.0 (1H), 3.62-3.61 (3H), 6.31, 6.26 (1H), 5.95-5.85 (2H), 4.48-4.44, 4.24-4.20 (1H), 4.16-4.0 (1H), 3.62-3.61 (3H), 3.25-3.07 (2H), 2.96-2.89 (1H), 1.38-1.30, 1.17 (9H); ¹³C NMR (125 MHz, CDCl₃): δ 154.0, 148.4, 147.3, 147.2, 146.8, 142.5, 137.4, 130.6, 129.6, 129.1, 129.0, 128.7, 128.6, 128.0, 127.9, 127.3, 127.2, 127.1, 127.0, 115.3, 112.7, 112.50, 112.48, 112.4, 112.3, 112.1, 111.3, 110.8, 101.6, 101.5, 79.6, 76.8, 71.3, 71.2, 56.0, 55.9, 54.2, 53.5, 45.1, 44.6, 44.0, 42.4, 41.8, 28.2, 28.0, 14.2; HRESIMS: calcd. for C₃₆H₃₆BrNO₆Na [M+Na]+ 680.1624; found 680.1618.

**tert-butyl** 1-(benzyloxy)-2-methoxy-4-phenyl-4,5,6a,7-tetrahydro-6H [1,3]dioxolo[4',5':4,5]benzo[1,2-g]benzo[de]quinoline-6-carboxylate (162): Compound 161 (0.1 g, 0.2 mmol), Pd(OAc)₂ (0.006 g, 0.03 mmol), K₂CO₃ (0.04 g, 0.3 mmol) and di-tert-butyl methyl phosphonium tetrafluoroborate (0.02 g, 0.06 mmol) were dissolved in DMSO (1 mL) in a 10 mL microwave reaction vial. The reaction mixture was then irradiated with microwaves at 140 ºC, 200
psi for 10 min. The resulting crude was purified directly on a silica gel column chromatography using 30% ethyl acetate–petroleum ether to afford 162 as a white solid; 88%; mp 149 °C-152 °C;\n\n$^1$H NMR (500 MHz, CDCl$_3$): $\delta$ 8.04 (s, 1H), 7.38-7.35 (m, 4H), 7.35-7.30 (m, 4H), 7.29-7.22 (m, 2H), 6.76 (s, 1H), 6.22 (s, 1H), 5.99 (d, 1H, $J = 1.0$ Hz), 5.97 (d, 1H, $J = 1.0$ Hz), 4.82 (d, 1H, $J = 10.5$ Hz), 4.74 (broad s, 1H), 4.70 (d, 1H, $J = 10.5$ Hz), 4.40 (broad s, 1H), 4.01 (m, 1H), 3.59 (s, 3H), 3.08 (m, 1H), 2.86 (m, 2H), 1.49 (s, 9H); $^{13}$C NMR (125 MHz, CDCl$_3$): $\delta$ 154.5, 151.9, 146.7, 146.4, 143.6, 141.2, 137.2, 133.9, 131.4, 129.1, 128.8, 128.7, 128.2, 127.9, 127.2, 126.4, 125.4, 111.2, 109.8, 108.3, 100.9, 80.0, 74.5, 55.9, 52.4, 46.1, 45.3, 35.2, 28.6; HRESIMS: calcd. for C$_{36}$H$_{35}$NO$_6$Na [M+Na]$^+$ 600.2362; found 600.2348.

**tert-butyl 1-hydroxy-2-methoxy-4-phenyl-4,5,6a,7-tetrahydro-6H [1,3]dioxolo[4',5':4,5]benzo[1,2-g]benzo[de]quinoline-6-carboxylate** (163): Compound 162 (0.3 g, 0.7 mmol) was dissolved in methanol-THF mixture (50 mL; 1:1), followed by the addition of 10% Pd/C (10 mg). The resulting suspension was stirred for 6 h under hydrogen atmosphere. After 6 h, the reaction mixture was subjected to vacuum filtration over celite. The filtrate was then evaporated under reduced pressure to afford a crude residue, which was purified using silica gel column chromatography in 30% ethyl acetate – petroleum ether, to afford 163 as a white solid; 90%; mp 225 °C-227 °C; $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ 8.04 (s, 1H), 7.37-7.35 (m, 2H), 7.31-7.29 (m, 1H), 7.21 (m, 2H), 6.77 (s, 1H), 6.15 (s, 2H), 5.99 (s, 1H), 5.97 (s, 1H), 4.80 (m, 1H), 4.37 (m, 1H), 4.01 (dd, 1H, $J = 4.0$, 11.5 Hz), 3.63 (s, 3H), 3.08 (t, 1H, $J = 9.0$ Hz), 2.90 (m, 2H), 1.49 (s, 9H); $^{13}$C NMR (125 MHz, CDCl$_3$): $\delta$ 154.5, 146.3, 146.2, 146.5, 141.5 141.2, 131.0, 129.1, 129.0, 128.6, 127.1, 126.6, 109.4, 109.0, 108.4, 100.9, 80.0, 56.2, 52.6, 45.9, 45.3, 35.4, 28.6; HRESIMS: calcd. for C$_{29}$H$_{29}$NO$_6$Na [M+Na]$^+$ 510.1893; found 510.1880.
General procedure for the synthesis of 165a-i and 165k: (Using 165a as a representative)

Methylation: Compound 163 (0.05 g, 0.10 mmol) was dissolved in acetonitrile (20 mL) followed by the addition of methyl iodide (0.1 mL, 0.02 g, 0.23 mmol) and K$_2$CO$_3$ (0.03 g, 0.21 mmol). The resulting reaction mixture was refluxed for 16 h. After 16 h the reaction mixture was cooled to rt and was subjected to vacuum filtration. The filtrate was then evaporated affording a crude product methylated product which was purified on a silica gel column using 20 % ethyl acetate-petroleum ether. Boc deprotection: The methylated product (0.05 g, 0.1 mmol) and ZnBr$_2$ (0.2 g, 1.0 mmol) were dissolved in dichloromethane (20 mL). The resulting reaction mixture was stirred at rt for 16 h. After 16 h, the reaction mixture was vacuum filtered, and the filtrate collected. To this filtrate saturated NaHCO$_3$ solution (10 mL) was added. The organic layer was then extracted with dichloromethane (2 x 10 mL). The combined organic layer was dried over Na$_2$SO$_4$, and evaporated under vacuum to get the secondary amine (0.04 g). Reductive amination: This secondary amine (0.04 g, 0.1 mmol) was dissolved in dichloromethane (10 mL) followed by the addition of 37 % formaldehyde solution in water (0.03 mL, 1.0 mmol) and sodium triacetoxyborohydride (0.1 g, 0.5 mmol). The resulting reaction mixture was stirred at 16 h. After 16 h, saturated NaHCO$_3$ solution (20 mL) was added to the reaction mixture. The organic layers were then extracted with dichloromethane (2 x 20 mL). The combined organic layer was dried over Na$_2$SO$_4$, and evaporated under vacuum to get a crude product, which was purified on a preparative TLC plate using 0.02 % methanol-DCM as the solvent system to give 165a. (Note: Compounds 165b-h were prepared similarly; For 165k After O-methylation and Boc deprotection steps, reductive amination was done using cyclopropanecarboxaldehyde (0.06 g, 0.87 mmol) and sodium triacetoxyborohydride (0.04 g, 0.17 mmol). Compound 165i was prepared from 163 via Boc deprotection and reductive amination steps).
1,2-dimethoxy-6-methyl-4-phenyl-5,6,6a,7-tetrahydro-4H-[1,3]dioxolo[4',5':4,5]benzo[1,2-g]benzo[de]quinoline (165a): white solid; 56%; mp 109 °C-114 °C; \(^1\)H NMR (500 MHz, CDCl\(_3\)): δ 7.96 (s, 1H), 7.32-7.25 (m, 4H), 7.22-7.18 (m, 1H), 6.77 (s, 1H), 6.46 (s, 1H), 5.99 (d, 1H, \(J = 1.5\) Hz), 5.97 (d, 1H, \(J = 1.5\) Hz), 3.99 (d, 1H, \(J = 3.9\) Hz), 3.74 (s, 3H), 3.68 (s, 3H), 3.01 (dd, 1H, \(J = 3.8, 13.6\) Hz), 2.95 (dd, 1H, \(J = 4.1, 16.8\) Hz), 2.84 (dd, 1H, \(J = 4.4, 11.5\) Hz), 2.61 (m, 1H), 2.38 (s, 3H); \(^{13}\)C NMR (125 MHz, CDCl\(_3\)): δ 152.1, 146.9, 146.5, 146.4, 144.8, 131.1, 130.8, 130.6, 128.80, 128.4, 128.0, 128.6, 126.1, 125.6, 125.1, 124.9, 124.3, 123.8, 123.3, 122.8, 121.7, 118.9, 108.9, 108.3, 100.9, 62.6, 60.13, 60.11, 55.8, 44.9, 44.1, 35.4; HRESIMS: calcd. for C\(_{26}\)H\(_{26}\)NO\(_4\) [M+H]\(^+\) 416.1862; found 416.1830

1-ethoxy-2-methoxy-6-methyl-4-phenyl-5,6,6a,7-tetrahydro-4H

[1,3]dioxolo[4',5':4,5]benzo[1,2-g]benzo[de]quinoline (165b): light brown solid; 45%; mp 113 °C-116 °C; \(^1\)H NMR (500 MHz, CDCl\(_3\)): δ 8.03 (s, 1H), 7.32-7.24 (m, 4H), 7.21-7.17 (m, 1H), 6.76 (s, 1H), 6.45 (s, 1H), 5.99 (d, 1H, \(J = 1.4\) Hz), 5.96 (d, 1H, \(J = 1.4\) Hz), 3.99 (s, 1H), 3.97-3.89 (m, 1H), 3.77-3.69 (m, 4H), 3.03-2.94 (m, 4H), 2.61 (t, 1H, \(J = 15.5\) Hz), 2.39 (s, 3H), 1.30 (t, 3H, \(J = 7.1\) Hz); \(^{13}\)C NMR (125 MHz, CDCl\(_3\)): δ 152.2, 146.9, 146.0 (x2), 143.8, 131.0, 130.6, 128.8, 128.3, 128.0, 127.0, 126.1, 125.9, 111.6, 109.2, 108.2, 100.8, 68.6, 62.7, 60.1, 55.8, 44.8, 44.1, 35.3, 15.7; HRESIMS: calcd. for C\(_{27}\)H\(_{28}\)NO\(_4\) [M+H]\(^+\) 430.2018; found 430.2019

2-methoxy-6-methyl-4-phenyl-1-propoxy-5,6,6a,7-tetrahydro-4H-

[1,3]dioxolo[4',5':4,5]benzo[1,2-g]benzo[de]quinoline (165c): white solid; 36%; mp 113 °C-117 °C; \(^1\)H NMR (500 MHz, CDCl\(_3\)): δ 8.01 (s, 1H), 7.32-7.25 (m, 4H), 7.21-7.15 (m, 1H), 6.77 (s, 1H), 6.45 (s, 1H), 5.99 (d, 1H, \(J = 1.3\) Hz), 5.95 (d, 1H, \(J = 1.3\) Hz), 3.98 (d, 1H, \(J = 3.8\) Hz), 3.84-3.79 (m, 1H), 3.72 (s, 3H), 3.62-3.57 (m, 1H), 3.01 (dd, 1H, \(J = 3.8, 8.6\) Hz), 2.94 (d, 2H, \(J = 10.5\) Hz), 2.84 (dd, 1H, \(J = 4.4, 11.5\) Hz), 2.60 (t, 1H, \(J = 13.4\) Hz), 2.40 (s, 3H), 1.76-1.67 (m, 2H), 0.95 (t, 3H, \(J = 7.4\) Hz); \(^{13}\)C NMR (125 MHz, CDCl\(_3\)): δ 152.2, 146.9, 146.3 (x2), 144.0,
130.9, 130.5, 128.8, 128.4, 127.9, 126.9, 126.1, 125.8, 111.8, 109.4, 108.2, 100.8, 74.7, 62.7, 60.1, 55.8, 44.8, 44.2, 35.4, 23.5, 10.5; HRESIMS: calcd. for C_{28}H_{30}NO_{4} [M+H]^+ 444.2175; found 444.2172

1-butoxy-2-methoxy-6-methyl-4-phenyl-5,6,6a,7-tetrahydro-4H-
[1,3]dioxolo[4',5':4,5]benzo[1,2-g]benzo[de]quinoline (165d): light brown solid; 26 %; mp 118 °C -123 °C; H NMR (500 MHz, CDCl\textsubscript{3}): δ 8.00 (s, 1H), 7.31-7.25 (m, 4H), 7.21-7.18 (m, 1H), 6.76 (s, 1H), 6.45 (s, 1H), 5.99 (d, 1H, J = 1.2 Hz), 5.96 (d, 1H, J = 1.2 Hz), 3.98 (d, 1H, J = 3.3 Hz), 3.86-3.82 (m, 1H), 3.72 (s, 3H), 3.66-3.62 (m, 1H), 3.02-2.93 (m, 3H), 2.84 (dd, 1H, J = 4.4, 11.4 Hz), 2.60 (t, 1H, J = 13.4 Hz), 2.38 (s, 3H), 2.84 (dd, 1H, J = 7.1, 11.4 Hz), 2.60 (t, 3H, J = 13.4 Hz), 2.38 (s, 3H), 1.74-1.63 (m, 2H), 1.49-1.36 (m, 2H), 0.9 (t, 3H, J = 7.4 Hz); C NMR (125 MHz, CDCl\textsubscript{3}): δ 152.2, 146.9, 146.3 (x2), 144.0, 130.9, 130.5, 128.8, 128.4, 127.9, 126.9, 126.1, 125.8, 111.7, 109.3, 108.1, 100.8, 72.8, 62.7, 60.2, 55.8, 44.8, 44.2, 35.3, 32.3, 19.2, 13.8; HRESIMS: calcd. for C_{29}H_{32}NO_{4} [M+H]^+ 458.2331; found 458.2329

1-(hexyloxy)-2-methoxy-6-methyl-4-phenyl-5,6,6a,7-tetrahydro-4H-
[1,3]dioxolo[4',5':4,5]benzo[1,2-g]benzo[de]quinoline (165e): brown solid; 40 %; mp 108 °C-110 °C; H NMR (500 MHz, CDCl\textsubscript{3}): δ 7.99 (s, 1H), 7.32-7.27 (m, 4H), 7.21-7.15 (m, 1H), 6.76 (s, 1H), 6.44 (s, 1H), 5.98 (d, 1H, J = 1.5 Hz), 5.96 (d, 1H, J = 1.5 Hz), 3.98 (d, 1H, J = 3.8 Hz), 3.87-3.80 (m, 1H), 3.72 (s, 3H), 3.65-3.60 (m, 1H), 3.01-2.99 (m, 1H), 2.84 (d, 2H, J = 12.4 Hz), 2.84 (dd, 1H, J = 4.3, 11.5 Hz), 2.60 (t, 1H, J = 13.4 Hz), 2.38 (s, 3H), 1.75-1.62 (m, 2H), 1.46-1.32 (m, 2H), 1.31-1.21 (m, 4H), 0.87 (t, 3H, 6.8 Hz); C NMR (125 MHz, CDCl\textsubscript{3}): δ 152.2, 146.9, 146.25 ( □ 2), 144.0, 130.9, 130.5, 128.8, 128.4, 127.9, 126.9, 126.1, 125.8, 111.7, 109.4, 108.1, 100.8, 73.1, 62.7, 60.2, 55.8, 44.8, 44.2, 35.4, 31.7, 30.2, 25.8, 22.6, 14.1; HRESIMS: calcd. for C_{31}H_{36}NO_{4} [M+H]^+ 486.2644; found 456.2648
1-(cyclopropylmethoxy)-2-methoxy-6-methyl-4-phenyl-5,6,6a,7-tetrahydro-4H-[1,3]dioxolo[4',5':4,5]benzo[1,2-g]benzo[de]quinoline (165f): light brown solid; 58 %; mp 114 °C-118 °C; \(^1\)H NMR (500 MHz, CDCl\(_3\)): \(\delta\) 8.13 (s, 1H), 7.32-7.28 (m, 4H), 7.23-7.21 (m, 1H), 6.79 (s, 1H), 6.47 (s, 1H), 6.02 (d, 1H, J = 1.2 Hz), 5.99 (d, 1H, J = 1.2 Hz), 4.02 (d, 1H, J = 3.7 Hz), 3.78-3.73 (m, 4H), 3.45 (dd, 1H, J = 7.6, 9.8 Hz), 3.05-2.96 (m, 3H), 2.87 (dd, 1H, J = 4.3, 11.4 Hz), 2.61 (t, 1H, J = 13.3 Hz), 2.41 (s, 3H), 1.21-1.16 (m, 1H), 0.52-0.49 (m, 2H), 0.21-0.13 (m, 2H); \(^{13}\)C NMR (125 MHz, CDCl\(_3\)): \(\delta\) 152.2, 146.9, 146.2, 128.8, 128.4, 128.0, 127.1, 126.1, 125.7, 111.7, 109.3, 108.2, 100.9, 73.7, 62.6, 60.2, 55.8, 44.8, 44.2, 35.4, 11.0, 3.4, 3.0; HRESIMS: calcd. for C\(_{29}\)H\(_{30}\)NO\(_4\) [M+H]\(^+\) 456.2175; found 456.2172

1-(allyloxy)-2-methoxy-6-methyl-4-phenyl-5,6,6a,7-tetrahydro-4H-[1,3]dioxolo[4',5':4,5]benzo[1,2-g]benzo[de]quinoline (165g): brown solid; 60 %; mp 132 °C-135 °C; \(^1\)H NMR (500 MHz, CDCl\(_3\)): \(\delta\) 8.02 (s, 1H), \(\delta\) 7.33-7.28 (m, 4H), 7.24-7.21 (m, 1H), 6.79 (s, 1H), 6.48 (s, 1H), 6.08-5.98 (m, 3H), 5.31 (dd, 1H, J = 1.2, 17.2 Hz), 5.19 (d, 1H, J = 10.4 Hz), 4.4 (dd, 1H, J = 5.9, 11.9 Hz), 4.25 (dd, 1H, J = 5.7, 11.9 Hz), 4.02 (d, 1H, J = 3.5 Hz), 4.25 (d, 1H, J = 11.5 Hz), 2.64 (t, 1H, J = 13.4 Hz), 2.41 (s, 3H); \(^{13}\)C NMR (125 MHz, CDCl\(_3\)): \(\delta\) 152.2, 146.9, 146.2, 143.6, 130.9, 130.6, 128.8, 128.4, 128.0, 127.1, 126.1, 125.7, 111.7, 109.3, 108.2, 100.9, 73.7, 62.6, 60.2, 55.8, 44.9, 44.2, 35.4; HRESIMS: calcd. for C\(_{28}\)H\(_{28}\)NO\(_4\) [M+H]\(^+\) 442.2018; found 442.2013

1-((4-bromobenzyl)oxy)-2-methoxy-6-methyl-4-phenyl-5,6,6a,7-tetrahydro-4H-[1,3]dioxolo[4',5':4,5]benzo[1,2-g]benzo[de]quinoline (165h): brown solid; 45 %; mp 147 °C-151 °C; \(^1\)H NMR (500 MHz, CDCl\(_3\)): \(\delta\) 7.87 (s, 1H), 7.35 (d, 2H, J = 8.3 Hz), 7.31-7.28 (m, 2H), 7.24-7.19 (m, 3H), 7.10 (d, 2H, J = 8.3 Hz), 6.74 (s, 1H), 6.45 (s, 1H), 5.99 (d, 1H, J = 1.5 Hz), 5.94 (d, 1H, J = 1.5 Hz), 4.81 (d, 1H, J = 10.9 Hz), 4.69 (d, 1H, J = 10.9 Hz), 3.99 (d, 1H, J = 3.8 Hz).
Hz), 3.73 (s, 3H), 2.99-2.90 (m, 3H), 2.83 (m, 1H), 2.46 (m, 1H), 2.31 (s, 3H); \(^{13}\text{C NMR}\) (125 MHz, CDCl\(_3\)): \(\delta\) 152.1, 146.8, 146.4, 146.2, 142.6, 135.9, 131.0, 130.6, 128.8, 128.4, 128.0, 127.6, 126.1, 125.6, 121.9, 111.7, 108.1, 100.9, 73.7, 62.5, 60.1, 55.8, 44.8, 44.2, 35.2; HRESIMS: calcd. for C\(_{32}\)H\(_{29}\)NO\(_4\) [M+H]\(^+\) 572.1280; found 572.1251

2-methoxy-6-methyl-4-phenyl-5,6,6a,7-tetrahydro-4H-[1,3]dioxolo[4',5':4,5]benzo[1,2-g]benzo[de]quinolin-1-ol (165i): brown solid; 35 %; mp 188 °C-191 °C; \(^1\text{H NMR}\) (500 MHz, CDCl\(_3\)): \(\delta\) 7.97 (s, 1H), 7.31-7.29 (m, 4H), 7.22-7.20 (m, 1H), 6.80 (s, 1H), 6.44 (s, 1H), 6.00 (broad s, 2H), 4.01 (d, 1H, \(J = 3.6\) Hz), 3.81 (s, 3H), 3.07-2.96 (m, 2H), 2.88 (dd, 1H, \(J = 4.3, 11.4\) Hz), 2.69-2.63 (m, 1H), 2.41 (s, 3H) \(^{13}\text{C NMR}\) (125 MHz, CDCl\(_3\)): \(\delta\) 147.0, 146.2, 146.0, 145.9, 141.0, 130.6, 128.8, 128.6, 128.0, 126.1, 126.0, 125.7, 119.1, 109.9, 109.1, 108.3, 100.8, 62.7, 60.4, 56.1, 44.7, 44.2, 35.2; HRESIMS: calcd. for C\(_{25}\)H\(_{24}\)NO\(_4\) [M+H]+ 402.1705; found 402.1708

6-(cyclopropylmethyl)-1,2-dimethoxy-4-phenyl-5,6,6a,7-tetrahydro-4H-[1,3]dioxolo[4',5':4,5]benzo[1,2-g]benzo[de]quinoline (165k): light brown oil; 31 %; \(^1\text{H NMR}\) (500 MHz, CDCl\(_3\)): \(\delta\) 7.95 (s, 1H), 7.37 (d, 2H, \(J = 7.2\) Hz), 7.28-7.25 (m, 2H), 7.20-7.17 (m, 1H), 6.77 (s, 1H), 6.48 (s, 1H), 5.99 (d, 1H, \(J = 1.5\) Hz), 5.97 (d, 1H, \(J = 1.5\) Hz), 3.99 (s, 1H), 3.75 (s, 3H), 3.66 (s, 3H), 3.34 (dd, 1H, \(J = 3.5, 13.5\) Hz), 3.29 (dd, 1H, \(J = 1.3, 11.5\) Hz), 3.01 (dd, 1H, \(J = 3.7, 13.8\) Hz), 2.94 (dd, 1H, \(J = 2.2, 9.6\) Hz), 2.79 (dd, 1H, \(J = 5.8, 13.5\) Hz), 2.57 (t, 1H, \(J = 13.6\) Hz), 2.37-2.33 (m, 1H), 1.20 (s, 1H), 0.76-0.68 (m, 1H), 0.45-0.40 (m, 1H), 0.32-0.20 (m, 1H), 0.09-0.04 (m, 1H); \(^{13}\text{C NMR}\) (125 MHz, CDCl\(_3\)): \(\delta\) 149.5 (x2), 144.4, 144.0, 143.9, 143.2, 142.2, 128.9, 126.4, 125.4, 124.3, 123.5, 123.2, 109.2, 106.5, 105.8, 98.4, 57.7, 56.8, 55.8, 53.6, 53.3, 42.5, 32.8, 4.9, 2.5 (x2); HRESIMS: calcd. for C\(_{29}\)H\(_{30}\)NO\(_4\) [M+H]+ 456.2175; found 456.2168
1-(1,2-dimethoxy-4-phenyl-4,5,6a,7-tetrahydro-6H-[1,3]dioxolo[4',5':4,5]benzo[1,2-g]benzo[de]quinolin-6-yl)ethan-1-one (167): A solution of acetic acid (0.02 g, 0.35 mmol) in anhydrous THF (30 mL) under N₂ atmosphere was cooled to 0 ºC. To this solution CDI (0.05 g, 0.32 mmol) was added in three portions over a period of 30 min at 0 ºC. The resulting reaction mixture was allowed to stir for 1.5 h at 0 ºC, after which the temperature was raised to rt and allowed to stir for another 1 h. The solution was again cooled to 0 ºC and allowed to stir for 30 min, followed by the addition of the intermediate secondary amine (0.13 g, 0.32 mmol) in anhydrous THF (10 mL) was added dropwise. The resulting reaction mixture was allowed to stir for 30 min at 0 ºC and then at rt for another 15 h. The product was filtered and washed with ether to afford 167 as a white solid (87%) which was used without further purification.

6-ethyl-1,2-dimethoxy-4-phenyl-5,6,6a,7-tetrahydro-4H-[1,3]dioxolo[4',5':4,5]benzo[1,2-g]benzo[de]quinoline (165j): Lithium aluminum hydride (0.03 g, 0.86 mmol) was carefully transferred to a 50 mL three necked round bottom flask under nitrogen, and anhydrous THF (15 mL) added. The resulting suspension was cooled to 0 ºC. After 15 min, a solution of 167 (0.078 g, 0.17 mmol) in THF (15 mL) was added dropwise to the suspension. The resulting reaction mixture was stirred for another 15 min at 0 ºC, and then at rt for 7 h. After 7 h the reaction mixture was cooled to 0 ºC and water was carefully added. The resulting suspension was filtered over celite and filtrate was evaporated to dryness. Saturated NaHCO₃ solution (20 mL) was added and extracted with ethyl acetate (3 x 10 mL). The combined organic layers were dried over Na₂SO₄, and evaporated under vacuum to get a crude product which was purified on a preparative TLC plate using 0.02 % methanol-DCM as the solvent system, to afford 165j as light brown oil ; 35 %;

\(^1\)H NMR (500 MHz, CDCl₃): δ 7.95 (s, 1H), 7.35 (d, 2H, \(J = 9.3 \) Hz), 7.29-7.25 (m, 2H), 7.21-7.17 (m, 1H), 6.77 (s, 1H), 6.47 (s, 1H), 5.99 (s, 1H), 5.97 (s, 1H), 3.98 (s, 1H), 3.74 (s, 3H), 3.66
(s, 3H), 3.26 (dd, 1H, \( J = 4.1, 16.9 \) Hz), 3.07-2.99 (m, 2H), 2.95-2.90 (m, 1H), 2.82 (dd, 1H, \( J = 5.0, 14.3 \) Hz), 2.59 (m, 1H), 2.50-2.39 (m, 1H), 0.92 (t, 3H, \( J = 8.8 \) Hz); \(^{13}\text{C NMR}\) (125 MHz, CDCl\(_3\)): \( \delta \) 151.91 (x 2), 146.7, 146.4, 146.3, 144.6, 131.3, 128.8, 127.8, 126.8, 126.0, 125.7, 111.7, 108.9, 108.3, 100.8, 60.1, 59.2, 55.8, 55.2, 47.5, 44.8, 35.3, 10.8; \(^{13}\text{C NMR}\): calcd. for C\(_{27}\)H\(_{28}\)NO\(_4\) [M+H]\(^+\) 430.2018; found 430.1993

\[ \text{B) Synthesis of isochroman analogues:} \]

\textbf{benzyl 2-(4-(benzyloxy)-3-methoxyphenyl)acetate (180):} A solution of 179 (5.0 g, 27.47 mmol), K\(_2\)CO\(_3\) (7.58 g, 54.94 mmol) and benzyl bromide (6.5 mL, 54.94 mmol) in anhydrous acetonitrile (150 mL) was refluxed for 4 h. After 4 h, the reaction was cooled to rt and was subjected to vacuum filtration. The filtrate was then evaporated under reduced pressure. The resulting crude was purified on silica gel column chromatography using 10 % ethyl acetate-petroleum ether, to afford 180 as a pale yellow solid; 95 %; \( \text{mp} \) 50 °C -53 °C; \(^1\text{H NMR}\) (500 MHz, CDCl\(_3\)): \( \delta \) 7.42 (d, 2H, \( J = 7.3 \) Hz), \( \delta \) 7.37-7.27 (m, 8H), \( \delta \) 6.81 (d, 2H, \( J = 7.9 \) Hz), \( \delta \) 6.74 (dd, 1H, \( J = 8.2, 1.8 \) Hz), \( \delta \) 5.13 (d, 4H, \( J = 4.8 \) Hz), \( \delta \) 3.84 (s, 3H), \( \delta \) 3.59 (s, 2H); \(^{13}\text{C NMR}\) (125 MHz, CDCl\(_3\)): \( \delta \) 171.5, 149.6, 147.3, 137.1, 135.8, 128.55 (x 2), 128.54, 128.2, 128.1, 127.8, 127.2 (x 2), 126.9, 121.4, 114.0 (x 2), 112.9 (x 2), 71.0, 66.6, 55.9, 40.9; \(^{13}\text{C NMR}\): calcd for C\(_{23}\)H\(_{22}\)O\(_4\)Na[M+Na]\(^+\) 386.1444; found 386.1446

\textbf{2-(4-(benzyloxy)-3-methoxyphenyl)ethanol (181):} Lithium Aluminum Hydride (1.09 g, 28.8 mmol) was added to a three neck round bottom flask under argon, followed by the addition of anhydrous THF (70 mL). The resulting suspension was cooled to 0 °C and stirred for 30 min, followed by drop wise addition of a solution of 180 (2.0 g, 5.76 mmol) in anhydrous THF (50 mL).
The resulting reaction mixture was allowed to stir at 0 °C for another 30 min, and then at rt for 3 h. After 3 h the reaction mixture was cooled to 0 °C, and water (2 mL) was carefully and drop wise added, followed by the addition of 2N NaOH solution (10 mL). The reaction mixture was allowed to stir for 30 min, after which it was subjected to vacuum filtration using celite as the filter aid. The filtrate was evaporated under reduced pressure. Water (30 mL) was added to the crude, which was then extracted dichloromethane (3 x 20 mL). The combined organic layer was dried over Na$_2$SO$_4$, and evaporated under reduced pressure to give 181 as a white solid; 85 %; mp 53 °C - 57 °C; $^1$H NMR (500 MHz, CDCl$_3$): δ 7.43 (d, 2H, $J = 7.5$ Hz), 7.36 (t, 2H, $J = 7.5$ Hz), 7.29 (t, 1H, $J = 7.2$ Hz), 6.82 (d, 1H, $J = 8.1$ Hz), 6.77 (d, 1H, $J = 1.8$ Hz), 6.70 (dd, 1H, $J = 8.1, 1.9$ Hz), 5.13 (s, 2H), 3.88 (s, 3H), 3.83 (q, 2H, $J = 6.2$ Hz), 2.80 (t, 2H, $J = 6.4$ Hz); $^{13}$C NMR (125 MHz, CDCl$_3$): δ 149.7, 146.8, 137.3, 131.5, 128.5 (x 2), 127.7, 127.2 (x 2), 120.9, 114.3, 112.8, 71.1, 63.7, 56.0, 38.7; HRESIMS: calcd for C$_{16}$H$_{18}$O$_3$Na[M+Na]$^+$ 281.1154; found 281.1156

7-(benzyloxy)-1-((6-bromobenzo[d][1,3]dioxol-5-yl)methyl)-6-methoxyisochroman (182): To a three neck round bottom flask attached with a Dean stark apparatus, a solution of 181 (1.2 g, 4.65 mmol), 173 (0.56 g, 2.32 mmol) and PTSA (catalytic) in anhydrous toluene (100 mL) was added. The resulting reaction mixture was for refluxed for 18 h. After 18 h, the reaction mixture was cooled to rt and transferred to a separatory funnel containing water (30 mL). The aqueous layer was extracted with ethyl acetate (2 x 20 ml). The combined organic layer was dried over Na$_2$SO$_4$, and evaporated under reduced pressure. The resulting crude was purified on a silica gel column chromatography using 30 % ethyl acetate-petroleum ether to afford 182 as a white solid; 63 %; mp 71 °C -75 °C; (a mixture of rotamers as evident from NMR data-all signals observed are reported); $^1$H NMR (500 MHz, CDCl$_3$): δ 7.42 (dd, 2H, $J = 7.5, 7.5$ Hz), 7.36 (m, 2H), 7.30 (m, 1H), 7.00 (s, 0.5H), 6.81 (s, 0.5H), 6.72 (m, 1H), 6.62 (m, 1H), 6.53 (m, 0.5H), 5.94 (m, 2H),
5.13 (d, 1H, J = 2.9 Hz), 5.07 (s, 1H), 4.81 (d, 1H, J = 8.3 Hz), 4.07 (m, 1H), 3.88 and 3.86 (s, 3H), 3.72 (m, 1H), 3.14 (dd, 1H, J = 14.4, 2.8 Hz), 2.7 (m, 2H), 2.64 (m, 1H); \textbf{\textsuperscript{13}C NMR} (125 MHz, CDCl\textsubscript{3}): δ 148.41, 148.40, 147.4, 147.10, 146.5, 146.3, 145.9, 137.22, 137.21, 132.5, 131.4, 129.4, 128.6, 128.4, 127.87, 127.84, 127.32, 127.28, 127.0, 126.7, 122.4, 114.8, 112.5, 112.0, 111.9, 111.8, 111.54, 111.50, 108.0, 101.6, 100.8, 75.0, 71.4, 62.9, 62.5, 56.0, 42.6, 42.2, 28.7; \textbf{HRESIMS}: calcd for C\textsubscript{25}H\textsubscript{23}BrO\textsubscript{5} [M+H]\textsuperscript{+} 483.0807; found 483.0802

\textbf{1-(benzylxy)-2-methoxy-4,5,6a,7-tetrahydro-[1,3]dioxolo[4',5':4,5]benzo[1,2g]benzo[de]chromene (183): Compound 182} (100 mg, 0.207 mmol), Pd(OAc)\textsubscript{2} (0.009 g, 0.042 mmol), K\textsubscript{2}CO\textsubscript{3} (0.057 g, 0.414 mmol) and tricyclohexyl phosphine tetrafluoroborate (0.031 g, 0.083 mmol) were dissolved in DMSO (1 mL) in a 10 mL microwave reaction vial. The reaction mixture was then irradiated with microwaves at 140 °C, 200 psi for 10 min. The resulting crude was purified directly on a silica gel column chromatography using 20 % acetone- petroleum ether to afford 183 as a white solid; 90 %; mp 105 °C -108 °C; \textbf{\textsuperscript{1}H NMR} (500 MHz, CDCl\textsubscript{3}): δ 7.99 (s, 1H), 7.36 (m, 2H), 7.30 (m, 3H), 6.72 (s, 1H), 6.63 (s, 1H), 5.96 (d, 1H, J = 1.5 Hz), 5.93 (d, 1H, J = 1.5 Hz), 4.84 (d, 1H, J = 10.5 Hz), 4.71 (d, 1H, J = 10.5 Hz), 4.48 (dd, 1H, J = 13.5, 5.0 Hz), 4.25 (dd, 1H, J =12.0, 6.5 Hz), 3.88 (s, 3H), 3.80 (td, 1H, J = 10.0, 3.5 Hz), 3.13 (m, 1H), 2.83 (dd, 1H, J = 12.5,7.5 Hz), 2.73 (t, 1H, J = 13.5 Hz), 2.61 (dd, 1H, J = 16.0, 3.0 Hz); \textbf{\textsuperscript{13}C NMR} (125 MHz, CDCl\textsubscript{3}): δ 152.5, 146.5, 143.0, 137.1, 129.2, 128.7 (x 2), 128.1 (x 2), 127.9, 127.47, 127.45, 126.8, 125.4, 110.8, 109.4, 108.5, 100.9, 74.7, 73.3, 64.7, 55.9, 36.5, 28.1; \textbf{HRESIMS}: calcd for C\textsubscript{25}H\textsubscript{22}O\textsubscript{5}Na [M+Na]\textsuperscript{+} 425.1365; found 425.1360

\textbf{2-methoxy-4,5,6a,7-tetrahydro-[1,3]dioxolo[4',5':4,5]benzo[1,2g]benzo[de]chromen-1-ol (184): Compound 183} (0.3 g, 0.746 mmol) was dissolved in methanol-THF mixture (50mL, 1:1), followed by the addition of activated palladium over charcoal (10 mg). The resulting suspension
was stirred for 6 h under hydrogen atmosphere. After 6 h the reaction mixture was subjected to vacuum filtration over celite. The filtrate was then evaporated under reduced pressure to afford a crude residue, which was purified using silica gel column chromatography using 20 % ethyl acetate – petroleum ether, to afford 184 as a white solid; 91 %; mp 108 °C -112 °C; \(^1\)H NMR (500 MHz, CDCl\(_3\)): δ 7.96 (s, 1H), 6.75 (s, 1H), 6.57 (s, 1H), 6.12 (s, 1H), 5.97 (dd, 2H, \(J = 7.5, 1.5\) Hz), 4.54 (dd, 1H, \(J = 13.5, 5.4\) Hz), 4.25 (dd, 1H, \(J = 11.4, 6.0\) Hz), 3.92 (s, 3H), 3.81 (td, 1H, \(J = 11.9, 3.6\) Hz), 3.12 (m,1H), 2.87 (dd, 1H, \(J = 13.7, 5.3\) Hz), 2.80 (t, 1H, \(J = 13.5\) Hz), 2.57 (dd,1H, \(J = 16.2, 3.4\) Hz); \(^{13}\)C NMR (125 MHz, CDCl\(_3\)): δ 146.4, 146.2, 146.15, 146.12, 140.7, 128.8, 127.5, 125.6, 122.4, 118.6, 100.8, 73.4, 64.9, 56.2, 36.5, 27.9; HRESIMS: calcd for C\(_{18}\)H\(_{16}\)O\(_5\) [M+H]+ 312.0998; found 312.0995

**General procedure for the synthesis of 170 and 185a-d:** Compound 184 (0.03 g, 0.096 mmol) was dissolved in anhydrous acetonitrile (10 mL) followed by the addition of appropriate alkyl halide (0.1152 mmol) and K\(_2\)CO\(_3\) (0.198 g, 1.44 mmol). The resulting reaction mixture was refluxed for 6 h. After 6 h the reaction mixture was cooled to rt and was subjected to vacuum filtration. The filtrate was then evaporated and the resulting crude was purified using on a silica gel prep TLC plate using 30 % ethyl acetate-petroleum ether to afford the respective compounds.

1,2-dimethoxy-4,5,6a,7-tetrahydro-[1,3]dioxolo[4′,5′:4,5]benzo[1,2-g]benzo[de]chromene (170): White Solid; 95 %; mp 106 °C -109 °C; \(^1\)H NMR (500 MHz, CDCl\(_3\)): δ 7.99 (s, 1H), 6.78 (s,1H), 6.64 (s, 1H), 5.99 (d, 2H, \(J = 4.4\) Hz), 4.52 (dd, 1H, \(J = 13.4, 5.25\) Hz), 4.27 (dd, 1H, \(J = 11.4, 6.5\) Hz), 3.91 (s, 3H), 3.84 (td, 1H, \(J = 11.9, 3.5\) Hz), 3.71 (s, 3H), 3.15 (m, 1H), 2.88 (dd, 1H, \(J = 13.7, 5.4\) Hz), 2.82 (t, 1H, \(J = 13.5\) Hz), 2.63 (dd, 1H, \(J = 16.3, 2.9\) Hz); \(^{13}\)C NMR (125 MHz, CDCl\(_3\)): δ 152.4, 146.6, 146.5, 144.5, 129.3, 127.4, 127.3, 126.1, 125.4, 110.8, 108.85, 108.70,
100.9, 73.3, 64.7, 60.2, 55.9, 36.6, 28.1; HRESIMS: calcd for C_{19}H_{18}O_{5} [M]^+ 326.1154; found 326.1151

2-methoxy-1-propoxy-4,5,6a,7-tetrahydro-[1,3]dioxolo[4',5':4,5]benzo[1,2-g]benzo[de]chromene (185a): White Solid; 86%; mp 110 °C-113 °C; \(^1\)H NMR (500 MHz, CDCl\(_3\)): \(\delta\) 8.03 (s, 1H), 6.77 (s, 1H), 6.63 (s, 1H), 6.01 (s, 1H), 5.98 (s, 1H), 4.51 (dd, 1H, \(J = 13.3, 5.2\) Hz), 4.27 (dd, 1H, \(J = 11.6, 6.5\) Hz), 3.89 (s, 3H), 3.85 (m, 2H), 3.64 (m, 1H), 3.15 (m, 1H), 2.87 (t, 2H, \(J = 13.7\) Hz), 2.63 (dd, 1H, \(J = 16.3, 3.0\) Hz), 1.7 (m, 2H), 0.99 (t, 3H, \(J = 7.4\) Hz); \(^{13}\)C NMR (125 MHz, CDCl\(_3\)): \(\delta\) 152.5, 146.46, 146.43, 143.7, 129.1, 127.4, 127.0, 126.4, 125.6, 110.8, 109.2, 108.5, 100.9, 74.8, 73.4, 64.8, 55.9, 36.6, 28.1, 23.4, 10.5; HRESIMS: calcd for C\(_{21}\)H\(_{22}\)O\(_5\) [M]^+ 354.1467; found 354.1463

1-(allyloxy)-2-methoxy-4,5,6a,7-tetrahydro-[1,3]dioxolo[4',5':4,5]benzo[1,2-g]benzo[de]chromene (185b): White Solid; 87%; mp 120 °C-124 °C; \(^1\)H NMR (500 MHz, CDCl\(_3\)): \(\delta\) 8.02 (s, 1H), 6.77 (s, 1H), 6.63 (s, 1H), 6.07 (m, 1H), 6.00 (s, 1H), 5.98 (s, 1H), 5.31 (dd, 1H, \(J = 17.2, 1.4\) Hz), 5.20 (d, 1H, \(J = 10.3\) Hz), 4.52 (dd, 1H, \(J = 5.35, 5.15\) Hz), 4.41 (dd, 1H, \(J = 12.0, 5.9\) Hz), 4.27 (m, 2H), 3.89 (s, 3H), 3.83 (td, 1H, \(J = 11.9, 3.6\) Hz), 3.14 (m, 1H), 2.88 (dd, 1H, \(J = 13.6, 5.2\) Hz), 2.80 (t, 1H, \(J = 13.5\) Hz), 2.63 (dd, 1H, \(J = 16.3, 3.0\) Hz); \(^{13}\)C NMR (125 MHz, CDCl\(_3\)): \(\delta\) 152.5, 146.53, 146.50, 143.1, 134.1, 129.2, 127.4, 127.3, 126.5, 125.5, 117.6, 110.8, 109.1, 108.6, 100.9, 73.7, 73.3, 64.7, 55.9, 36.6, 28.1; HRESIMS: calcd for C\(_{21}\)H\(_{20}\)O\(_5\)Na [M+Na]^+ 375.1209; found 375.1202

1-(cyclopropylmethoxy)-2-methoxy-4,5,6a,7-tetrahydro [1,3]dioxolo[4',5':4,5]benzo[1,2-g]benzo[de]chromene (185c): White Solid; 95%; mp 95 °C-98 °C; \(^1\)H NMR (500 MHz, CDCl\(_3\)): \(\delta\) 8.13 (s, 1H), 6.76 (s, 1H), 6.62 (s, 1H), 6.00 (s, 1H), 5.98 (s, 1H), 4.52 (dd, 1H, \(J = 12.8, 4.2\) Hz), 4.27 (dd, 1H, \(J = 11.5, 6.75\) Hz), 3.89 (s, 3H), 3.85 (m, 1H), 3.76 (m, 1H), 3.45 (t, 1H, \(J = 8.5\) Hz),
3.15 (m, 1H), 2.87 (dd, 1H, \( J = 13.7, 4.9 \) Hz), 2.80 (t, 1H, \( J = 13.5 \) Hz), 2.62 (d, 1H, \( J = 16.3 \) Hz), 1.21 (m, 1H), 0.53 (d, 2H, \( J = 8.1 \) Hz), 0.20 (m, 2H); \(^{13}\text{C NMR} \) (125 MHz, CDCl\(_3\)): \( \delta \) 152.5, 146.4, 146.4, 143.4, 129.1, 127.3, 127.1, 126.5, 126.7, 110.7, 109.4, 108.5, 100.9, 77.8, 73.4, 64.8, 55.9, 36.6, 28.1, 11.0, 3.4, 3.1; HRESIMS: calcd for C\(_{22}\)H\(_{22}\)O\(_5\)Na [M+Na]+ 389.1365; found 389.1363

2-methoxy-1-(prop-2-yn-1-loyloxy)-4,5,6a,7-tetrahydro-[1,3]dioxolo[4',5':4,5]benzo[1,2-g]benzo[de]chromene (185d): White Solid; 90%; mp 160 °C-164 °C; \(^1\text{H NMR} \) (500 MHz, CDCl\(_3\)): \( \delta \) 8.01 (s, 1H), 6.76 (s, 1H), 6.64 (s, 1H), 6.01 (s, 1H), 5.99 (s, 1H), 4.60 (dd, 1H, \( J = 15.2, 2.4 \) Hz), 4.50 (m, 1H), 4.27 (dd, 1H, \( J = 11.3, 6.5 \) Hz), 3.90 (s, 3H), 3.82 (td, 1H, \( J = 11.9, 3.6 \) Hz), 3.15 (m, 1H), 2.87 (dd, 2H, \( J = 13.6, 5.2 \) Hz), 2.80 (t, 1H, \( J = 13.5 \) Hz), 2.63 (dd, 1H, \( J = 16.4, 2.9 \) Hz), 2.39 (t, 1H, \( J = 2.3 \) Hz); \(^{13}\text{C NMR} \) (125 MHz, CDCl\(_3\)): \( \delta \) 152.4, 146.6 (x 2), 142.0, 129.3, 128.0, 127.5, 126.9, 125.3, 110.7, 109.3, 108.3, 100.9, 79.0, 75.3, 75.0, 64.7, 59.8, 55.9, 36.6, 28.1; HRESIMS: calcd for C\(_{21}\)H\(_{18}\)O\(_5\)Na [M+Na]+ 373.1052; found 373.1046

methyl 2-(3,4-dimethoxyphenyl)acetate (187): A solution of compound 55 (5.00 g, 27.62 mmol) and conc. H\(_2\)SO\(_4\) (catalytic) in anhydrous methanol (50 mL) was refluxed for 30 min. After 30 min, the reaction mixture was cooled to rt and was evaporated under reduced pressure. To the resulting crude water (30 mL) was added, followed by extraction with dichloromethane (3 x 30 mL). The combined organic layers were dried over Na\(_2\)SO\(_4\), and were evaporated under reduced pressure to afford 186 as brown oil. (5.29 g, 99%). Data in accordance with previously reported.

2-(3,4-dimethoxyphenyl)ethanol (172): In a three neck round bottom flask, a solution of CaCl\(_2\) (1.26 g, 11.52 mmol) in ethanol (50 mL) was added under argon atmosphere. The resulting solution was cooled to 0 °C, followed by the addition of NaBH\(_4\) (0.22 g, 5.76 mmol) at the same temperature. The resulting suspension was allowed to stir at 0 °C for 30 min, after which a solution of 187 (1.05 g, 3.84 mmol) in ethanol (50 mL) was added at the same temperature. The resulting reaction
mixture was allowed to stir at 0 °C for another 30 min, and then at rt for 15 h. After 15 h, a mixture of water and dichloromethane (50 mL, 1:1) was added to the reaction mixture which was then sonicated for 1 h. The resulting mixture was then subjected to vacuum filtration over celite. The filtrate was collected and evaporated under reduced pressure. Water (50 mL) was added to the resulting crude, which was then extracted with dichloromethane (3 x 20 mL). The combined organic layer was dried over Na₂SO₄, and evaporated under reduced pressure to afford 172 as a white solid. (0.63g, 70 %); mp 63 °C-67 °C; ¹H NMR (500 MHz, CDCl₃): δ 6.82 (s, 1H), δ 6.78 (d, 1H), δ 6.75 (d, 1H), 3.87 (s, 3H), 3.86 (s, 3H), 3.83 (t, 2H, J = 6.5 Hz), 2.81 (t, 2H, J = 6.5 Hz); ¹³C NMR (125 MHz, CDCl₃): δ 149.0, 147.7, 130.9, 120.9, 112.2, 111.3, 63.7, 55.9, 55.8, 38.7; HRESIMS: calcd for C₁₀H₁₄O₃Na[M+Na]⁺ 205.0841; found 205.0842

General procedure for the synthesis of 189a–d:

To a three neck round bottom flask attached with a Dean stark apparatus, a solution of 172 (2 equiv.) in anhydrous toluene (10 mL), 188a-d (1 equiv.) and PTSA (catalytic) were added. The resulting reaction mixture was refluxed for 18 h. After 18 h, the reaction mixture was cooled to rt and transferred to a separatory funnel containing water (20 mL). The aqueous layer was extracted, with ethyl acetate (3 x 20 mL). The combined organic layer was dried over Na₂SO₄, and evaporated under reduced pressure. The resulting crude was purified on a silica gel column chromatography using 10-30 % ethyl acetate-petroleum ether to afford 189a-d.

1-(2-bromo-4,5-dimethoxybenzyl)-6,7-dimethoxyisochroman (189a): White Solid; 73 %; mp 107 °C-110 °C; ¹H NMR (500 MHz, CDCl₃): δ 7.06 (s, 1H), 6.89 (s, 1H), 6.72 (s, 1H), 6.63 (s, 1H) 5.00 (d, 1H, J = 6.1 Hz), 4.16 (m, 1H), 3.89 (s, 3H), 3.88 (s, 3H), 3.87 (s, 3H), 3.86 (s, 3H), 3.78 (m, 1H), 3.35 (dd, 1H, J = 14.4, 3.3 Hz), 3.06 (dd,1H, J = 14.4, 9.0 Hz); ¹³C NMR (125 MHz, CDCl₃): δ 148.2, 148.0, 147.6, 147.3, 130.2, 129.4, 126.0, 115.2, 114.6, 114.5, 111.3, 108.3, 75.2,
62.8, 56.1, 56.0, 55.9, 55.8, 42.3, 28.6; **HRESIMS**: calcd for C_{20}H_{23}BrO_5Na [M+Na]^+ 445.0621; found 445.0618

**1-(2-bromo-5-methoxybenzyl)-6,7-dimethoxyisochroman (189b)**: White Solid; 72 %; **mp** 92 °C-95 °C; **^1H NMR** (500 MHz, CDCl₃): δ 7.47 (d, 1H, J = 8.8 Hz), 6.94 (d, 1H, J = 3.1 Hz), 6.71 (m, 2H), 6.64 (s, 1H), 5.03 (dd, 1H, J = 9.3, 3.0 Hz), 4.16 (m, 1H), 3.89 (s, 3H), 3.85 (s, 3H), 3.80 (s, 3H), 3.79 (m, 1H), 3.36 (dd, 1H, J = 14.3, 3.3 Hz), 3.08 (dd, 1H, J = 14.3, 9.3 Hz), 2.90 (m, 1H), d 2.71 (td, 1H, J = 16.1, 4.6 Hz); **^13C NMR** (125 MHz, CDCl₃): δ 158.7, 147.7, 147.4, 139.2, 133.1, 129.4, 126.0, 117.8, 115.2, 113.8 (x 2), 108.3, 74.7, 62.8, 55.95, 55.90, 55.4, 43.0, 28.6; **HRESIMS**: calcd for C_{19}H_{21}BrO_4Na [M+Na]^+ 415.0515; found 445.0516

**1-(2-bromo-4-methoxybenzyl)-6,7-dimethoxyisochroman (189c)**: White Solid; 74 %; **mp** 98 °C-103 °C; **^1H NMR** (500 MHz, CDCl₃): δ 7.27 (d, 1H, J = 8.4 Hz), 7.16 (d, 1H, J = 2.5 Hz), 6.85 (dd, 1H, J = 8.4, 2.5 Hz), 6.71 (s, 1H), 6.63 (s, 1H), 4.99 (dd, 1H, J = 9.1, 2.2 Hz), 4.14 (m, 1H), 3.89 (s, 3H), 3.85 (s, 3H), 3.81 (s, 3H), 3.79 (m, 1H), 3.34 (dd, 1H, J = 14.4, 3.3 Hz), 3.06 (m, 1H), 2.88 (m, 1H), 2.70 (dt, 1H, J = 16.0, 4.5 Hz); **^13C NMR** (125 MHz, CDCl₃): δ 158.7, 147.6, 147.3, 132.4, 130.2, 129.6, 126.0, 124.8, 117.8, 113.8, 113.4, 111.4, 108.3, 74.7, 62.5, 55.9, 55.8, 55.5, 41.9, 28.6; **HRESIMS**: calcd for C_{19}H_{21}BrO_4Na [M+Na]^+ 415.0515; found 445.0514

**1-(2-bromobenzyl)-6,7-dimethoxyisochroman (189d)**: Clear Oil; 59 %; **^1H NMR** (500 MHz, CDCl₃): δ 7.60 (d, 1H, J = 8.0 Hz), 7.37 (dd, 1H, J = 1.5, 7.6 Hz), 7.32 (m, 1H), 7.13 (ddd, 1H, J = 7.6, 7.6, 1.5 Hz), 6.70 (s, 1H), 6.64 (s, 1H), 5.05 (dd, 1H, J = 9.3, 3.0 Hz), 4.15 (m, 1H), 3.89 (s, 3H), 3.80 (s, 3H), 3.78 (m, 1H), 3.40 (dd, 1H, J = 14.3, 3.3 Hz), 3.12 (m, 1H), 2.89 (m, 1H), 2.73 (td, 1H, J = 13.3, 7.6 Hz); **^13C NMR** (125 MHz, CDCl₃): δ 147.7, 147.3, 138.3, 132.7, 130.2, 129.5, 128.1, 127.2, 126.2, 126.0, 114.4, 108.3, 74.7, 62.4, 55.9, 55.8, 42.9, 28.6; **HRESIMS**: calcd for C_{18}H_{19}BrO_3Na [M+Na]^+ 385.0410; found 385.0409
General procedure for the synthesis of 185e–h

Compound 189a-d (1 equiv.), Pd(OAc)₂ (0.2 equiv.), K₂CO₃ (2 equiv.) and tricyclohexyl phosphate tetrafluoroborate (0.4 equiv.) were dissolved in DMSO (1 mL) in a 10 mL microwave reaction vial. The reaction mixture was then irradiated with microwaves at 140 °C, 200 psi for 10 min. The resulting crude was purified directly on a silica gel column chromatography using 20 % acetone- petroleum ether to afford 185e–h

1,2,9,10-tetramethoxy-4,5,6a,7-tetrahydrodibenzo[de,g]chromene (185e): White Solid; 89 %; mp 110 °C-112°C; ¹H NMR (500 MHz, CDCl₃): δ 8.12 (s, 1H), 6.77 (s, 1H), 6.62 (s, 1H), 4.54 (dd, 1H, J = 12.8, 6.0 Hz), 4.26 (dd, 1H, J = 11.4, 6.4 Hz), 3.92 (s, 3H), d 3.91 (s, 3H), 3.82 (dd, 1H, J = 11.8, 3.6 Hz), 3.68 (s, 3H), 3.14 (m, 1H), 2.89 (m, 2H), 2.62 (dd, 1H, J = 11.4, 3.2 Hz); ¹³C NMR (125 MHz, CDCl₃): δ 152.4, 148.1, 147.5, 144.3, 127.9, 127.6, 127.4, 126.0, 124.3, 111.5, 111.2, 110.6, 73.5, 64.8, 60.2, 55.9, 55.88, 55.85, 36.0, 28.2; HRESIMS: calcd for C₂₀H₂₂O₅Na [M+Na]⁺ 365.1365; found 365.1360

1,2,9-trimethoxy-4,5,6,7-tetrahydrodibenzo[de,g]chromene (185f): White Solid; 84 %; mp 107 °C-110°C; ¹H NMR (500 MHz, CDCl₃): δ 8.37 (d, 1H, J = 8.8 Hz), 6.88 (dd, 1H, J = 8.8, 2.5 Hz), 6.83 (d, 1H, J = 2.5 Hz), 6.63 (s, 1H), 4.57 (dd, 1H, J = 13.0, 5.4 Hz), 4.29 (dd, 1H, J = 11.4, 6.5 Hz), 3.91 (s, 3H), 3.87 (s, 3H), 3.83 (m, 1H), 3.70 (s, 3H), 3.15 (m, 1H), 2.94 (m, 2H), 2.64 (dd, 1H, J = 16.3, 2.8 Hz); ¹³C NMR (125 MHz, CDCl₃): δ 158.7, 152.4, 144.6, 136.8, 129.6, 127.4, 127.3, 126.0, 124.7, 113.8, 112.3, 110.6, 73.3, 64.8, 60.0, 55.9, 55.2, 37.0, 28.2; HRESIMS: calcd for C₁₉H₂₀O₅ [M+H]⁺ 313.1440; found 313.1434

1,2,10-trimethoxy-4,5,6a,7-tetrahydrodibenzo[de,g]chromene (185g): Brown Oil; 86 %; ¹H NMR (500 MHz, CDCl₃): δ 8.09 (d, 1H, J = 2.1 Hz), 7.20 (d, 1H, J = 8.3 Hz), 6.84 (dd, 1H, J =
8.3, 2.1 Hz), 6.69 (s, 1H), 4.54 (dd, 1H, J = 13.5, 4.95 Hz), 4.29 (dd, 1H, J = 11.4, 6.6 Hz), 3.92 (s, 3H), 3.86 (s, 3H), 3.82 (dd, 1H, J = 11.5, 3.45 Hz), 3.73 (s, 3H), 3.17 (m, 1H), 2.95 (dd, 1H, J = 13.5, 5.1 Hz), 2.83 (t, 1H, J = 13.5 Hz), 2.65 (dd, 1H, J = 16.2, 2.5 Hz); $^{13}$C NMR (125 MHz, CDCl$_3$): $\delta$ 158.7, 152.4, 145.1, 132.8, 129.0, 128.2, 127.5, 127.1, 126.0, 113.8, 112.2, 110.5, 73.6, 64.8, 60.3, 55.9, 55.3, 35.6, 28.2; HRESIMS: calcd for C$_{19}$H$_{20}$O$_5$ [M+H]$^+$ 313.1440; found 365.1434

1,2-dimethoxy-4,5,6a,7-tetrahydrodibenzo[de,g]chromene (185h): Colorless Oil; 79 %; $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ 8.42 (d, 1H, J = 7.9 Hz), 7.28 (m, 3H), 6.68 (s, 1H), 4.57 (dd, 1H, J = 13.5, 5.2 Hz), 4.29 (dd, 1H, J = 11.4, 6.4 Hz), 3.92 (s, 3H), 3.84 (dd, 1H, J = 11.8, 11.8, 3.6 Hz), 3.71 (s, 3H) d 3.18 (m, 1H), 3.00 (dd, 1H, J = 13.7, 5.15 Hz), 2.91 (t, 1H, J = 13.6 Hz), 2.65 (dd, 1H, J = 16.4, 3.2 Hz); $^{13}$C NMR (125 MHz, CDCl$_3$): $\delta$ 151.4, 150.4, 134.0, 128.3, 128.2, 127.7, 127.3, 127.0, 126.9, 124.2, 111.6, 106.7, 73.3, 65.5, 59.8, 56.5, 36.5, 29.9; HRESIMS: calcd for C$_{18}$H$_{16}$O$_3$ [M]$^+$ 280.1096; found 280.1094

2-(3,4,6,7-tetramethoxyphenanthren-1-yl)ethanol (200): 33 % HBr-AcOH (20 mL) was added to a two neck round bottom flask containing compound 185e (0.5 g, 1.46 mmol) at 10 °C. The resulting reaction mixture was allowed to stir at the same temperature for 30 min, after which water (10 mL) was added. The aqueous layers were extracted with dichloromethane (2 x 20 mL). The combined organic layer was dried over Na$_2$SO$_4$, and evaporated to get a crude residue, which was purified on a silica gel column using 30-70 % ethyl acetate hexane, to afford compound 199 (0.22 g) and compound 200 (0.31 g). Compound 199 was then dissolved in anhydrous methanol (10 mL), followed by the addition of 20 % NaOH solution (10 mL). The reaction mixture was stirred at rt for 2h, after which the organic layer was evaporated to get a crude mixture. Water (10 mL) was added to this crude, and the aqueous layers were extracted with dichloromethane (3 x 10 ml). The
combined organic layer was dried over Na₂SO₄, and evaporated under reduced pressure to get compound 200 as a pale yellow solid; 98 %

2-(3,4,6,7-tetramethoxyphenanthren-1-yl)ethyl acetate (199): Brown Oil; ¹H NMR (500 MHz, CDCl₃): δ 9.28 (s, 1H), 7.80 (d, 1H, J = 9.1 Hz), 7.56 (d, 1H, J = 9.1 Hz), 7.21 (s, 1H), 7.20 (s, 1H), 4.41 (t, 2H, J = 7.5 Hz), 4.08 (s, 3H), 4.05 (s, 3H), 4.03 (s, 3H), 3.92 (s, 3H), 3.41 (t, 2H, J = 7.5 Hz), 2.07 (s, 3H); ¹³C NMR (125 MHz, CDCl₃): δ 173.4, 152.6, 151.1, 150.8, 147.5, 132.8, 130.5, 128.2, 127.3, 127.0, 126.6, 122.8, 116.6, 111.2, 110.1, 66.9, 62.3, 58.9, 58.0, 35.4, 32.0, 23.3; HRESIMS: calcd for C₂₂H₂₄O₆Na [M+Na]⁺ 407.1471; found 407.1462

2-(3,4,6,7-tetramethoxyphenanthren-1-yl)ethanol (200): White Solid; mp 134 °C-136 °C; ¹H NMR (500 MHz, CDCl₃): δ 9.21 (s, 1H), 7.75 (d, 1H, J = 9.0 Hz), 7.50 (d, 1H, J = 9.0 Hz), 7.19 (s, 1H), 7.17 (s, 1H), 4.06 (s, 3H), 4.02 (s, 3H), 3.99 (s, 3H), 3.89 (s, 3H), 3.33 (t, 2H, J = 6.6 Hz); ¹³C NMR (125 MHz, CDCl₃): δ 150.1, 148.7, 148.4, 131.4, 128.1, 125.7, 124.8, 124.7, 124.2, 120.6, 114.4, 108.8, 107.7, 63.2, 60.0, 56.5, 55.8, 55.75, 55.72, 31.1; HRESIMS: calcd for C₂₀H₂₂O₅Na [M+Na]⁺ 365.1365; found 365.1368

N,N-dimethyl-2-(3,4,6,7-tetramethoxyphenanthren-1-yl)ethanamine (201): To a solution of compound 200 (0.11 g, 0.3275 mmol) anhydrous dichloromethane (30 mL), Dess martin periodinane (0.152 g, 0.36 mmol) was added. The resulting reaction mixture was allowed to stir at rt for 15 min, after which it was filtered on a silica gel bed. The filtrate was evaporated to get the resulting aldehyde as a brown residue (0.138 g). The crude aldehyde was then dissolved in anhydrous dichloromethane (40 mL) and cooled to 0 °C followed by the addition of 1 N dimethylamine solution in THF (0.82 mL, 0.8116 mmol). The resulting reaction mixture was allowed to stir for 15 min at 0 °C, after which Na(AcO)₃BH (0.1720 g, 0.8116 mmol) was added. The solution was then stirred at 0 °C for 1h and then at rt for another 1h. After 1 h, water (15 mL)
was added, followed by the extraction of the organic layer with dichloromethane (2 x 20 mL). The combined organic layer was dried over Na$_2$SO$_4$, and evaporated to get a crude residue which was purified on a silica gel column using 5% - 20% methanol-dichloromethane, to afford 201 as brown oil; 59%; $^1$H NMR (500 MHz, CDCl$_3$): δ 9.27 (s, 1H), 7.76 (d, 1H, $J = 9.1$ Hz), 7.55 (d, 1H, $J = 9.0$ Hz), 7.20 (d, 1H, $J = 3.6$ Hz), 4.07 (s, 3H), 4.04 (s, 3H), 4.03 (s, 3H), 3.91 (s, 3H), 3.30 (t, 2H, $J = 8.1$ Hz), 2.72 (t, 2H, $J = 8.3$ Hz), 2.44 (s, 3H); $^{13}$C NMR (125 MHz, CDCl$_3$): δ 150.3, 148.8, 148.4, 144.8, 132.9, 128.2, 125.5, 124.8, 124.7, 124.3, 120.6, 113.9, 108.9, 107.7, 60.7, 60.0, 56.7, 56.5, 45.2 (x 2), 32.3, 29.7; HRESIMS: calcd for C$_{22}$H$_{28}$NO$_4$ [M+H]$^+$ 370.2018; found 370.2010

C) Synthesis of fused indole aporphine analogues:

ethyl 1-(3-ethoxy-3-oxopropyl)-6,7-dimethoxy-3,4-dihydroisoquinoline-2(1H)-carboxylate (214): To a solution of compound 212 (5.0 g, 19.7 mmol) in formic acid (50 mL) was added compound 213 (4.15 g, 23.6 mmol). The resulting solution was stirred at room temperature for 16 h. After 16 h formic acid was evaporated followed by the addition of water (40 mL). The resulting mixture was extracted with dichloromethane (3 x 30 mL). The combined organic layer was dried over Na$_2$SO$_4$, and evaporated to get a crude residue which was purified on a silica gel column using 20% - 40% ethylacetate-hexane, to afford 214 as a brown oil; 67%; $^1$H NMR (500 MHz, CDCl$_3$): δ 6.59 (s, 1H), 6.55 (s, 1H), 4.13-4.04 (m, 2H), 3.84 (s, 6H), 3.43 (t, 3H, $J = 2.8$ Hz), 3.06-3.01 (m, 1H), 2.68 (t, 2H, $J = 5.6$ Hz), 2.63-2.58 (m, 1H), 2.42-2.34 (merged, 4H), 2.20-2.13 (m, 2H), 2.06-1.98 (m, 1H), 1.23 (t, 3H, $J = 7.1$ Hz); $^{13}$C NMR (125 MHz, CDCl$_3$): δ 174.3, 147.3, 147.2, 129.1, 127.2, 111.1, 109.9, 62.5, 60.1, 55.9, 55.8, 51.4, 48.5, 42.9, 30.0, 29.8, 25.9, 14.2; HRESIMS: calcd for C$_{18}$H$_{25}$NO$_6$ [M+Na]$^+$ 374.1580; found 374.1575
3-(2-(ethoxycarbonyl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolin-1-yl)propanoic acid (215): Compound 214 (4.5 g, 12.32 mmol) was dissolved in ethanol (100 mL) followed by the addition of 20 % NaOH solution (20 mL). The resulting reaction mixture was allowed to stir under reflux conditions for 2 h. After 2 h the organic layers were evaporated under vacuum, followed by addition of 1 N HCl (25 mL) to adjust the pH to 1. The resulting aqueous solution was extracted with ethyl acetate (3 x 30 mL). The combined organic layer was dried over Na$_2$SO$_4$, and evaporated to dryness to afford 215 as clear oil; 99 %. 215 was used without further purification.

ethyl 5,6-dimethoxy-7-oxo-2,3,7,8,9,9a-hexahydro-1H-benzo[de]quinoline-1-carboxylate (211): To an ice cooled solution of compound 215 (4.0 g, 11.86 mmol) in anhydrous dichloromethane (60 mL), trifluoroacetic anhydride (1.84 mL, 2.74 g, 13.05 mmol) and trifluoroacetic acid (0.14 mL, 0.21 g, 1.86 mmol) were added. The resulting solution was stirred at room temperature for 4 h. After 4 h, the organic layers were evaporated followed by addition of 1N NaOH (20 mL). The resulting mixture was extracted with dichloromethane (3 x 30 mL). The combined organic layers were dried over Na$_2$SO$_4$, and evaporated to get a crude residue which was purified on a silica gel column using 10 % - 40 % ethyl acetate-hexanes to afford 211 as a brown solid; 67 %; mp 93 °C-97 °C; $^1$H NMR (500 MHz, CDCl$_3$): δ 6.88 (s, 1H), 4.80 (dd, 1H, $J = 12.1$, 2.4 Hz), 4.44 (d, 1H, $J = 9.3$ Hz), 4.25-4.19 (m, 2H), 3.88 (d, 6H, $J = 6.9$ Hz), 2.90-2.82 (m, 2H), 2.79-2.68 (m, 2H), 2.67-2.64 (m, 1H), 2.32 (t, 1H, $J = 6.0$ Hz), 1.99-1.90 (m, 1H), 1.93 (t, 3H, $J = 6.9$ Hz); $^{13}$C NMR (125 MHz, CDCl$_3$): δ 196.1, 155.2, 152.1, 147.9, 131.1, 130.1, 126.4, 116.8, 61.7, 61.5, 56.1, 51.1, 38.7, 38.3, 30.3, 27.9, 14.7; HRESIMS: calcd for C$_{17}$H$_{21}$NO$_5$ [M+Na]$^+$ 342.1318; found 342.1314

General procedure for the synthesis of 217a–d
To a solution of 211 (0.1 g, 0.3132 mmol) and appropriate phenyl hydrazine (0.93 mmol) in anhydrous toluene (20 mL), trifluoroacetic acid (0.19 mL, 2.5 mmol) was added. The resulting mixture was heated for 3 h. After 3 h, the mixture was cooled, followed by the addition of saturated NaHCO$_3$ (20 mL). The resulting mixture was extracted with ethyl acetate (3 x 20 mL). The combined organic layer was dried over Na$_2$SO$_4$, and evaporated to dryness. The resulting crude was purified on a silica gel column (5-50 % ethyl acetate-hexanes)

**ethyl 1,2-dimethoxy-4,6a,7,12-tetrahydroisoquinolino[8,1-ab]carbazole-6(5H)-carboxylate (217a):** Brown solid; 93 %; mp 154 °C-157 °C; $^1$H NMR (500 MHz, CDCl$_3$): δ 10.02 (s, 1H), 8.13 (d, 1H, $J = 7.8$ Hz), 8.07 (d, 1H, $J = 8.8$ Hz), 7.74 (d, 1H, $J = 8.7$ Hz), 7.62 (d, 1H, $J = 8.1$ Hz), 7.45 (t, 1H, $J = 7.3$ Hz), 7.29 (t, 1H, $J = 7.7$ Hz), 7.16 (s, 1H), 4.73 (s, 1H), 4.17-4.14 (merged, 5H), 4.04 (s, 3H), 3.60 (d, 2H, $J = 6.0$ Hz), 3.34 (t, 2H, $J = 6.3$ Hz), 1.25 (t, 3H, $J = 6.9$ Hz); $^{13}$C NMR (125 MHz, CDCl$_3$): δ 156.7, 147.6, 142.1, 138.0, 133.9, 132.2, 126.7, 124.7, 122.8, 119.7, 119.5, 118.3, 118.0, 115.2, 114.9, 111.1, 61.1, 60.8, 56.8, 41.6, 34.1, 14.7; HRESIMS: calcd for C$_{23}$H$_{24}$N$_2$O$_4$ [M+Na]$^+$ 415.1634; found 415.1628

**ethyl 9-fluoro-1,2-dimethoxy-4,6a,7,12-tetrahydroisoquinolino[8,1-ab]carbazole-6(5H)-carboxylate (217b):** Light brown solid; 96 %; mp 163 °C-165 °C; $^1$H NMR (500 MHz, CDCl$_3$): δ 10.00 (s, 1H), 7.98 (d, 1H, $J = 8.5$ Hz), 7.75 (td, 2H, $J = 9, 2.5$ Hz), 7.53 (dd, 1H, $J = 9.0, 4.5$ Hz), 7.22-7.16 (m, 2H), 4.74 (s, 1H), 4.17-4.13 (merged, 5H), 4.04 (s, 3H), 3.59 (d, 2H, $J = 6.5$ Hz), 3.34 (t, 2H, $J = 7.0$ Hz), 1.25 (t, 3H, $J = 7.0$ Hz); $^{13}$C NMR (125 MHz, CDCl$_3$): δ 158.6, 156.7, 156.6, 147.7, 135.2, 134.4, 132.38, 132.36, 126.9, 123.3, 123.2, 118.2, 117.9, 117.4, 115.3, 112.9, 112.7, 111.7, 111.6, 105.1, 104.9, 61.22, 61.22, 60.8, 56.8, 41.7, 34.1, 14.7; HRESIMS: calcd for C$_{23}$H$_{23}$FN$_2$O$_4$ [M+Na]$^+$ 433.1540; found 433.1535
ethyl 9-bromo-1,2-dimethoxy-4,6a,7,12-tetrahydroisoquinolino[8,1-ab]carbazole-6(5H)-
carboxylate (217c): Light brown solid; 89 %; mp 167 °C -170 °C; ¹H NMR (500 MHz, CDCl₃):
δ 10.04 (s, 1H), 8.24 (s, 1H), 7.98 (d, 1H, J = 8.5 Hz), 7.75 (d, 1H, J = 9.0 Hz), 7.53-7.48 (m, 2H),
7.18 (s, 1H), 4.74 (s, 1H), 4.17-4.13 (merged, 5H), 4.04 (s, 3H), 3.59 (d, 2H, J = 6.5 Hz), 3.33 (t,
2H, J = 6.5 Hz), 1.25 (t, 3H, J = 7.0 Hz); ¹³C NMR (125 MHz, CDCl₃): δ 156.6, 147.7, 142.2,
136.6, 134.6, 132.4, 127.4, 126.9, 124.6, 122.4, 117.7, 117.5, 117.3, 115.7, 115.4, 112.5, 112.4,
61.24, 60.8, 56.8, 41.7, 34.1, 14.7; HRESIMS: calcd for C₂₃H₂₃ClN₂O₄ [M+Na]⁺ 449.1244; found
449.1238

ethyl 1,2,9-trimethoxy-4,6a,7,12-tetrahydroisoquinolino[8,1-ab]carbazole-6(5H)-
carboxylate (217d): Light brown solid; 85 %; mp 146 °C-150 °C; ¹H NMR (500 MHz, CDCl₃):
δ 9.91 (s, 1H), 8.01 (d, 1H, J = 9.0 Hz), 7.70 (d, 1H, J = 8.5 Hz), 7.58 (s, 1H), 7.51 (d, 1H, J = 9.0
Hz), 7.15 (s, 1H), 7.10 (dd, 1H, J = 8.5, 2 Hz), 4.74 (s, 1H), 4.17-4.13 (merged, 5H), 4.03 (s, 3H),
3.96 (s, 3H), 3.60 (d, 2H, J = 6.0 Hz), 3.33 (t, 2H, J = 6.5 Hz), 1.25 (t, 3H, J = 7.0 Hz); ¹³C NMR
(125 MHz, CDCl₃): δ 156.6, 154.0, 147.6, 134.6, 133.1, 132.2, 126.7, 123.2, 118.2, 118.0, 117.5,
115.0, 114.7, 114.5, 111.8, 101.9, 61.1, 60.8, 56.8, 56.0, 41.6, 34.1, 14.7; HRESIMS: calcd for
C₂₄H₂₆N₂O₅ [M+Na]⁺ 445.1740; found 445.1735

methyl 4-((3,4-dimethoxyphenethyl)(methyl)amino)-4-oxobutanoate (228): 227 (10.0 g, 51.28
mmol) and succinic anhydride (5.28 g, 52.82 mmol) were dissolved in anhydrous toluene (100
mL). The resulting solution was refluxed for 3 h. The reaction mixture was cooled to rt and organic
layer was evaporated. The resulting crude was dissolved in dichloromethane (100 mL) and washed
with water (3 x 30 mL). The combined organic layer was dried over Na₂SO₄, and evaporated to
dryness to afford the intermediate acid as a brown oil which was used without further purification
(16g crude). The crude was dissolved in anhydrous methanol (100 mL) followed by the addition
of conc. H$_2$SO$_4$ (0.5 mL). The resulting reaction mixture was refluxed for 30 min. After cooling the reaction to rt, methanol was evaporated under vacuum. The resulting crude was dissolved in dichloromethane (100 mL) which was washed first with water (2 x 30 mL) and then saturated NaHCO$_3$ (2 x 30 mL). The combined organic layer was dried over Na$_2$SO$_4$, and evaporated to dryness. The resulting crude was purified on a silica gel column (1-5 % MeOH-CH$_2$Cl$_2$) to afford 228 as light brown oil; 70 %; (a mixture of rotamers as evident from NMR data-all signals observed are reported); $^1$H NMR (500 MHz, CDCl$_3$): δ 6.81-6.70 (m, 3H), 3.84 (s, 3H), 3.82 (s, 3H), 3.65 (d, 3H, $J$ = 14.8 Hz), 3.53 (q, 2H, $J$ = 7.5 Hz), 2.90 (s, 3H), 2.78 (dt, 2H, $J$ = 23.0, 6.9 Hz), 2.60 (dt, 2H, $J$ = 26.7, 5.7 Hz), 2.51 (t, 1H, $J$ = 6.5 Hz), 2.39 (t, 1H, $J$ = 6.5 Hz); $^{13}$C NMR (125 MHz, CDCl$_3$) δ 173.4, 173.4, 171.0, 170.8, 149.1, 148.9, 147.8, 147.5, 131.7, 130.8, 120.8, 120.7, 112.1, 111.6, 111.4, 55.88, 55.82, 51.58, 51.52, 51.4, 50.1, 35.7, 34.1, 33.5, 33.2, 29.1, 29.0, 28.3, 27.5; HRESIMS: calcd for C$_{16}$H$_{24}$NO$_5$ [M+H]$^+$ 310.1654; found 310.1682

methyl 3-(6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinolin-1-yl)propanoate (230): Compound 228 (11.0 g, 35.59 mmol) was dissolved in anhydrous toluene (100 mL) followed by the addition of POCl$_3$ (16.52 mL, 27.27 g, 177.95 mmol). The reaction mixture was refluxed for 6 h after which it was cooled to rt. The reaction mixture was evaporated under vacuum to get a light brown viscous oil (229) which was used without further purification. Crude 229 was then dissolved in anhydrous methanol (100 mL) and cooled to 0 ºC. To this solution, NaBH$_4$ (9.78 g, 257 mmol) was added in several portions while maintaining the temperature 0 ºC. The resulting reaction mixture was allowed to stir for 4 h. After 4 h, methanol was evaporated under vacuum and saturated NaHCO$_3$ (100 mL) was added. The aqueous layer was then extracted with dichloromethane (5 x 30 mL). The combined organic layer was dried over Na$_2$SO$_4$, and evaporated to dryness. The resulting crude was purified on a silica gel column (1-5 % MeOH-CH$_2$Cl$_2$) to
afford 230 as a light brown oil: 52 % from 228; ¹H NMR (500 MHz, CDCl₃): δ 6.59 (s, 1H), 6.55 (s, 1H), 3.82 (s, 3H), 3.84 (s, 6H), 3.62 (s, 3H), 3.43 (s, 1H), 3.06-3.00 (m, 1H), 2.69-2.67 (m, 2H), 2.63-2.58 (m, 1H), 2.45-2.38 (merged, m, 4H), 2.22-2.13 (m, 2H); 2.07-1.97 (m, 1H); ¹³C NMR (125 MHz, CDCl₃): δ 174.7, 147.3, 147.2, 128.9, 127.2, 111.2, 109.8, 62.4, 55.9, 55.8, 51.3, 48.5, 42.9, 29.7, 25.9; HRESIMS: calcd for C₁₆H₂₄NO₄ [M+H]+ 294.1705; found 294.1703

sodium 3-(6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinolin-1-yl)propanoate (231): Compound 230 (5.5 g, 17.91 mmol) was dissolved in ethanol (70 mL) followed by addition of 10 % aq. NaOH (20 mL). The resulting solution was refluxed for 30 min. The reaction mixture was cooled to rt and evaporated to dryness. The crude mixture was then azeotroped with toluene (3 x 100 mL), evaporated to dryness and used without further purification.

5,6-dimethoxy-1-methyl-1,2,3,8,9,9a-hexahydro-7H-benzo[de]quinolin-7-one (226): Crude 231 (7.3 g) was added to a three neck round bottom flask under argon atmosphere and was cooled to 0 ºC. To this flask, trifluoroacetic acid (0.77 ml, 1.15 g, 10.12 mmol) was carefully added, followed by the addition of trifluoroacetic anhydride (14.8 mL, 21.25 g, 101.2 mmol) at 0 ºC. The resulting reaction mixture was then allowed to stir at rt for 16 h. After 16 h the reaction mixture was evaporated under vacuum followed by careful addition of 10 % aq. NaOH (30 mL) to adjust pH to 8. The aqueous layer was then extracted with dichloromethane (4 x 30 mL). The combined organic layers were dried over Na₂SO₄, and evaporated to dryness. The resulting crude was then purified on a silica gel column (1-5 % MeOH-CH₂Cl₂) to afford 236 as dark brown oil; 33 %; ¹H NMR (500 MHz, CDCl₃): δ 6.84 (s, 1H), 3.86 (merged d, 6H, J = 5.3 Hz), 3.17–3.05 (m, 3H), 2.81-2.75 (m, 1H), 2.72-2.69 (m, 1H), 2.59-2.51 (m, 2H), 2.50 (s, 3H), 2.44-2.41 (m, 1H), 1.76-1.67 (m, 1H); ¹³C NMR (125 MHz, CDCl₃): δ 197.0, 152.1, 147.8, 132.8, 129.2, 125.6, 118.0,
HRESIMS: calcd for C₁₅H₂₀NO₃ [M+H]⁺ 262.1443; found 262.1442

**General procedure for the synthesis of 218a–i**

To a solution of 226 (0.1 g, 0.3132 mmol) and appropriate phenyl hydrazine (0.93 mmol) in anhydrous toluene (20 mL), trifluoroacetic acid (0.19 mL, 0.28 g, 2.5 mmol) was added. The resulting mixture was refluxed for 3 h. After 3 h, the mixture was cooled, followed by the addition of saturated NaHCO₃ (20 mL). The resulting mixture was extracted with dichloromethane (3 x 20 ml). The combined organic layer was dried over Na₂SO₄, and evaporated to dryness. The resulting crude was purified on a silica gel column (1-10 % MeOH-CH₂Cl₂)

1,2-dimethoxy-6-methyl-4,5,6a,7,12-hexahydroisoquinolino[8,1-ab]carbazole (218a): Light brown solid; 45 %; mp 113 °C-117 °C; ¹H NMR (500 MHz, CDCl₃): δ 9.34 (s, 1H), 7.59 (d, 1H, J = 8.0 Hz), 7.42 (d, 1H, J = 8.5 Hz), 7.19 (t, 1H, J = 7.0 Hz), 7.13 (t, 1H, J = 7.0 Hz), 6.54 (s, 1H), 3.90 (s, 3H), 3.89 (s, 3H), 3.44 (dd 1H, J = 12.0, 6.0 Hz), 3.40-3.35 (m, 1H), 3.21-3.14 (m, 1H), 3.10 (dd, 1H, J = 11.5, 6.0 Hz), 2.68-2.63 (m, 2H), 2.60 (s, 3H), 2.55 (dd, 1H, J = 12.0, 3.0 Hz); ¹³C NMR (125 MHz, CDCl₃): δ 151.1, 142.2, 136.4, 130.8, 130.7, 126.2, 125.6, 122.2, 121.1, 119.5, 118.4, 111.2, 110.7, 110.6, 63.0, 60.9, 55.7, 54.0, 44.1, 29.3, 25.3; HRESIMS: calcd for C₂₁H₂₃N₂O₂ [M+H]⁺ 335.1759; found 335.1758

9-fluoro-1,2-dimethoxy-6-methyl-4,5,6a,7,12-hexahydroisoquinolino[8,1-ab]carbazole (218b): Light brown solid; 30 %; mp 116 °C-119 °C; ¹H NMR (500 MHz, CDCl₃): δ 9.34 (s, 1H), 7.32 (dd, 1H, J = 8.5, 4.0 Hz), 7.21 (dd, 1H, J = 9.5, 2.5 Hz), 6.93 (td, 1H, J = 9.0, 2.5 Hz), 6.55 (s, 1H), 3.91 (s, 3H), 3.88 (s, 3H), 3.39-3.33 (m, 2H), 3.21-3.1 (m, 2H), 2.66 (d, 2H, J = 17.5 Hz), 2.60 (s, 3H), 2.56 (dd, 1H, J = 12.0, 3.5 Hz); ¹³C NMR (125 MHz, CDCl₃): δ 158.8, 156.9, 151.1,
142.3, 132.8, 132.5, 130.8, 126.5, 126.4, 120.7, 111.8, 111.7, 111.0, 110.5, 110.3, 103.3, 103.1, 62.8, 60.9, 55.7, 53.9, 43.9, 29.2, 25.2; **HRESIMS**: calcd for C$_{21}$H$_{22}$FN$_2$O$_2$ [M+H]$^+$ 353.1665; found 353.1664

9-chloro-1,2-dimethoxy-6-methyl-4,5,6,6a,7,12-hexahydroisoquinolino[8,1-ab]carbazole (218c): Light brown solid; 56%; mp 126 °C-130 °C; $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ 9.36 (s, 1H), 7.54 (s, 1H), 7.32 (d, 1H, $J = 8.6$ Hz), 7.13 (dd, 1H, $J = 8.6$, 2.0 Hz), 6.56 (s, 1H), 3.91 (s, 3H), 3.89 (s, 3H), 3.39-3.35 (m, 2H), 3.20-3.08 (m, 2H), 2.68-2.62 (m, 2H), 2.62 (s, 3H), 2.54 (dd, 1H, $J = 11.6$, 3.4 Hz); $^{13}$C NMR (125 MHz, CDCl$_3$): $\delta$ 151.1, 142.3, 134.6, 132.2, 130.9, 127.3, 125.6, 125.2, 122.3, 120.6, 117.9, 112.1, 111.1, 110.3, 62.8, 60.9, 55.7, 54.0, 44.0, 29.3, 25.1; **HRESIMS**: calcd for C$_{21}$H$_{22}$ClN$_2$O$_2$ [M+H]$^+$ 369.1370; found 369.1371

9-bromo-1,2-dimethoxy-6-methyl-4,5,6,6a,7,12-hexahydroisoquinolino[8,1-ab]carbazole (218d): Light brown solid; 30%; mp 134 °C-137 °C; $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ 9.38 (s, 1H), 7.70 (s, 1H), 7.28-7.23 (merged, 2H), 6.54 (s, 1H), 3.90 (s, 3H), 3.87 (s, 3H), 3.38-3.32 (m, 2H), 3.19-3.07 (m, 2H), 2.66-2.61 (m, 2H), 2.57 (s, 3H), 2.53 (dd, 1H, $J = 11.6$, 3.6 Hz); $^{13}$C NMR (125 MHz, CDCl$_3$): $\delta$ 151.1, 142.3, 134.9, 132.0, 130.8, 127.9, 125.5, 124.8, 120.9, 120.5, 112.7, 112.6, 111.1, 110.1, 62.8, 60.9, 55.7, 53.9, 44.0, 29.2, 25.1; **HRESIMS**: calcd for C$_{21}$H$_{22}$BrN$_2$O$_2$ [M+H]$^+$ 413.0864; found 413.0859

1,2,9-trimethoxy-6-methyl-4,5,6,6a,7,12-hexahydroisoquinolino[8,1-ab]carbazole (218e): Light brown solid; 60%; mp 118 °C-121 °C; $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ 9.25 (s, 1H), 7.31 (d, 1H, $J = 8.7$ Hz), 7.01 (s, 1H), 6.86 (d, 1H, $J = 8.7$ Hz), 6.53 (s, 1H), 3.90-3.88 (merged, 9H), 3.41-3.35 (m, 2H), 3.21-3.10 (m, 2H), 2.66 (d, 2H, $J = 15.6$ Hz), 2.61 (s, 3H), 2.59-2.55 (m, 1H); $^{13}$C NMR (125 MHz, CDCl$_3$): $\delta$ 154.1, 151.1, 142.0, 131.5, 131.4, 130.6, 126.3, 125.3, 121.1,
112.7, 112.0, 110.5, 110.4, 99.7, 62.9, 60.8, 55.8, 55.7, 53.9, 44.0, 29.2, 25.2; **HRESIMS**: calcd for C$_{22}$H$_{24}$N$_2$O$_3$ [M+H]$^+$ 365.1865; found 365.1866

1,2-dimethoxy-6-methyl-9-(trifluoromethoxy)-4,5,6,6a,7,12-hexahydroisoquinolinolo[8,1-ab]carbazole (218f): Light brown solid; 57 %; **mp** 123 °C-126 °C; **$^1$H NMR** (500 MHz, CDCl$_3$): δ 9.43 (s, 1H), 7.42 (s, 1H), 7.38 (d, 1H, $J = 8.7$ Hz), 7.05 (d, 1H, $J = 8.8$ Hz), 6.57 (s, 1H), 3.91 (s, 3H), 3.89 (s, 3H), 3.40-3.34 (m, 2H), 3.20-3.12 (m, 2H), 3.11-3.08 (m, 2H), 2.68 (t, 2H, $J = 3.4$ Hz), 2.67-2.62 (m, 1H), 2.66 (s, 3H), 2.54 (dd, 1H, $J = 11.6$, 3.4 Hz); **$^{13}$C NMR** (125 MHz, CDCl$_3$): δ 152.2, 142.5, 141.9, 134.5, 135.4, 131.2, 128.2, 125.2, 121.4, 120.4, 119.4, 119.3, 115.7, 113.5, 111.5, 110.4, 60.9, 60.8, 60.5, 55.8, 51.7, 40.8, 25.6, 21.7; **HRESIMS**: calcd for C$_{22}$H$_{22}$F$_3$N$_2$O$_3$ [M+H]$^+$ 406.1633; found 406.1642

1,2-dimethoxy-6,9-dimethyl-4,5,6,6a,7,12-hexahydroisoquinolinolo[8,1-ab]carbazole (218g): Light brown solid; 76 %; **mp** 137 °C-141 °C; **$^1$H NMR** (500 MHz, CDCl$_3$): δ 9.24 (s, 1H), 7.38 (s, 1H), 7.31 (d, 1H, $J = 10.5$ Hz), 7.02 (d, 1H, $J = 9.0$ Hz), 6.53 (s, 1H), 3.89 (d, 6H, $J = 5.0$ Hz), 3.44-3.35 (m, 2H), 3.21-3.09 (m, 2H), 2.65 (d, 2H, $J = 16.5$ Hz), 2.60 (s, 3H), 2.57-2.55 (m, 1H), 2.46 (s, 3H); **$^{13}$C NMR** (125 MHz, CDCl$_3$): δ 151.1, 142.1, 134.7, 130.8, 130.7, 128.7, 126.4, 125.5, 123.9, 121.3, 118.0, 110.9, 110.5, 110.2, 63.0, 60.9, 55.7, 54.0, 44.0, 29.3, 25.2, 21.5; **HRESIMS**: calcd for C$_{22}$H$_{25}$N$_2$O$_2$ [M+H]$^+$ 349.1916; found 349.1916

11-chloro-1,2-dimethoxy-6-methyl-4,5,6,6a,7,12-hexahydroisoquinolinolo[8,1-ab]carbazole (218h): Light brown solid; 48 %; **mp** 124°C-128 °C; **$^1$H NMR** (500 MHz, CDCl$_3$): δ 9.51 (s, 1H), 7.47 (d, 1H, $J = 7.9$ Hz), 7.18 (d, 1H, $J = 7.0$ Hz), 7.06 (t, 1H, $J = 7.8$ Hz), 6.57 (s, 1H), 3.94 (s, 3H), 3.89 (s, 3H), 3.42 (dd, 1H, $J = 14.0$, 4.3 Hz), 3.30-3.20 (broad, 2H), 2.76-2.66 (merged, 6H); **$^{13}$C NMR** (125 MHz, CDCl$_3$): δ 151.1, 142.2, 136.4, 130.8, 130.7, 126.2, 125.6, 122.2, 121.1,
119.5, 118.4, 111.2, 110.7, 110.6, 63.0, 60.9, 55.7, 54.0, 44.1, 29.3, 25.3; **HRESIMS**: calcd for C\textsubscript{21}H\textsubscript{22}ClN\textsubscript{2}O\textsubscript{2} [M+H]\textsuperscript{+} 367.1370; found 367.1370

11-bromo-1,2-dimethoxy-6-methyl-4,5,6a,7,12-hexahydroisoquinolino[8,1-ab]carbazole (218j): Light brown solid; 37 %; **mp** 131°C-135 °C; **\textsuperscript{1}H NMR** (500 MHz, CDCl\textsubscript{3}): \(\delta\) 9.50 (s, 1H), 7.52 (d, 1H, \(J = 7.5\) Hz), 7.33 (d, 1H, \(J = 7.5\) Hz), 7.00 (t, 1H, \(J = 7.5\) Hz), 6.57 (s, 1H), 3.94 (s, 3H), 3.87 (s, 3H), 3.42-3.36 (m, 2H), 3.21-3.09 (m, 2H), 2.67 (d, 2H, \(J = 15.5\) Hz); 2.59 (s, 3H), 2.55 (dd, 1H, \(J = 12, 4\) Hz) **\textsuperscript{13}C NMR** (125 MHz, CDCl\textsubscript{3}): \(\delta\) 151.2, 142.4, 135.0, 131.4, 130.8, 127.3, 125.5, 124.3, 120.6, 120.5, 117.5, 111.6, 111.2, 104.7, 62.8, 60.9, 55.8, 54.0, 44.0, 29.3, 25.5; **HRESIMS**: calcd for C\textsubscript{21}H\textsubscript{22}BrN\textsubscript{2}O\textsubscript{2} [M+H]\textsuperscript{+} 413.0864; found 413.0858

**D) Synthesis of C3 analogues:**

(E)-2-(benzyloxy)-1-methoxy-4-(2-nitrovinyl)benzene (235): Compound 234 (15 g, 61.98 mmol) was dissolved in acetic acid (100 mL) followed by the addition of ammonium acetate (2.04 g, 61.98 mmol) and nitromethane (16.5 mL, 18.91 g, 309.9 mmol). The resulting reaction mixture was heated under reflux conditions for a 6 h. After 6 h the reaction mixture was allowed to cool at rt and allowed to stand overnight at rt. The crushed out product was then subject to vacuum filtration to afford compound 235 as yellow crystals; 94 %; **mp**121 °C-123 °C; **\textsuperscript{1}H NMR** (500 MHz, CDCl\textsubscript{3}): \(\delta\) 7.90 (dd, 1H, \(J = 13.5, 1.4\) Hz), 7.45–7.38 (m, 5H), 7.34-7.31 (m, 1H), 7.19–7.15 (m, 1H), 7.03 (s, 1H), 6.92 (dd, 1H, \(J = 8.3, 0.5\) Hz), 5.17 (s, 2H), 3.94 (s, 3H); **\textsuperscript{13}C NMR** (125 MHz, CDCl\textsubscript{3}): \(\delta\) 153.4, 148.5, 139.3, 136.2, 135.0, 128.7, 128.2, 127.3, 124.9, 122.5, 112.9, 111.6, 71.0, 56.1; **HRESIMS**: calcd for C\textsubscript{16}H\textsubscript{16}NO\textsubscript{4} [M+H]\textsuperscript{+} 286.1079; found 286.1088
2-(3-(benzyloxy)-4-methoxyphenyl)ethan-1-amine (236): LiBH$_4$ (4.8 g, 224.5 mmol) was carefully added to a three necked round bottom flask under nitrogen atmosphere followed by the careful addition of anhydrous THF (150 mL). To this suspension, TMSCl (57.18 mL, 48.95 g, 449.1 mmol) was carefully added over a period of 15 minutes. The resulting reaction mixture was allowed to stir at rt for 15 additional minutes after which compound 235 (16.0 g, 56.14 mmol) was added in several portions over a period of 30 min. The resulting reaction mixture was heated under reflux conditions for 6 h. After 6 h the reaction mixture was first cooled to rt and then at 0 ºC, followed by the careful addition of methanol (100 mL) over a period of 30 min. After the reaction mixture was quenched, the organic layers were evaporated under vacuum. The resulting crude was then cooled to 0 ºC, followed by the careful addition of 20 % KOH (30 mL) to adjust the pH to 8.

The resulting crude was then extracted with dichloromethane (4 x 30 ml). The combined organic layer was dried over Na$_2$SO$_4$ and evaporated to dryness under vacuum to afford 236 as a light brown oil: 69 %. Compound 98 was used without further purification.

N-(3-(benzyloxy)-4-methoxyphenethyl)-2-(6-bromobenzo[d][1,3]dioxol-5-yl)acetamide (237): A solution of bromoacid 158 (5.55 g, 21.38 mmol) in anhydrous THF (100 mL) under N$_2$ atmosphere was cooled to 0 ºC. To this solution CDI (3.14 g, 19.43 mmol) was added in three portions over a period of 30 min at 0 ºC. The resulting reaction mixture was allowed to stir for 1.5 h at 0 ºC, after which the temperature was raised to rt and allowed to stir for another 1 h. The solution was again cooled to 0 ºC and allowed to stir for 30 min, after which a solution of 236 (5 g, 19.43 mmol) in 50 mL anhydrous THF was added over a period of 5 min. The resulting reaction mixture was allowed to stir for 30 min at 0 ºC and then at rt for another 15 h. After 15 h, the reaction mixture was filtered to collect pale yellow solid, which was washed with diethyl ether to afford a white solid as the first batch of product 237. The combined filtrate was evaporated to give
a crude mixture to which saturated NaHCO₃ (40 mL) was added. The resulting mixture was extracted with dichloromethane (2 x 30 ml). The combined organic layer was dried over Na₂SO₄ and evaporated to dryness under vacuum. The resulting crude was washed with diethyl ether followed by vacuum filtration to afford the second batch of product 237; white solid; 78 %; mp 161 °C -163 °C; ¹H NMR (500 MHz, CDCl₃): δ 7.45 (d, 2H, J = 7.7 Hz), 7.39 (t, 2H, J = 7.4 Hz), 7.33 (d, 1H, J = 7.5 Hz), 7.00 (s, 1H), 6.79 (d, 1H, J = 8.1 Hz), 6.74 (s, 1H), 6.69 (s, 1H), 6.66 (d, 1H, J = 8.2 Hz), 5.98 (s, 2H), 5.38 (s, 1H), 5.12 (s, 2H), 3.88 (s, 3H), 3.55 (s, 2H), 3.44 (q, 2H, J = 6.5 Hz), 2.68 (t, 2H, J = 6.8 Hz); ¹³C NMR (125 MHz, CDCl₃): δ 169.5, 148.4, 148.2, 147.8, 147.7, 137.0, 130.9, 128.5, 127.8, 127.5, 127.3, 121.4, 115.2, 114.6, 112.8, 112.0, 110.9, 101.9, 71.0, 56.0, 43.8, 40.5, 34.8; HRESIMS: calcd for C₂₅H₂₅BrNO₄ [M+H]⁺ 498.0916; found 498.0916

tert-butyl 6-(benzyloxy)-1-(((6-bromobenzo[d][1,3]dioxol-5-yl)methyl)-7-methoxy-3,4-dihydroisoquinoline-2(1H)-carboxylate (238): Compound 237 (7.5 g, 15.06 mmol) was dissolved in anhydrous dichloromethane (100 mL) and the resulting solution was cooled to 0 °C and allowed to stir for 20 min. PCl₅ (4.7 g, 22.5 mmol) was added in 3 portions over a period of 30 min. The resulting reaction mixture was allowed to stir at 0 °C for 30 min and then at rt for the next 18 h. To this reaction mixture saturated NaHCO₃ (60 mL) was added and the organic layers were extracted with dichloromethane (3 x 30 mL). The combined organic layer was dried over Na₂SO₄, and evaporated under vacuum to get the intermediate imine as a pale yellow, which was used without further purification. Sodium borohydride (1.58 g, 41.66 mmol) was added to the solution of this imine (5.0 g, 10.41 mmol) in anhydrous methanol (100 mL) at 0 °C. The reaction mixture was then brought to rt and allowed to stir for 6 h. After 6 h, methanol was evaporated to get a crude mixture, to which water (50 mL) was added, followed by extraction with ethyl acetate
(3 × 30 mL). The combined organic layer was dried over Na₂SO₄, and evaporated under vacuum to get an intermediate amine, which was used in the next step without purification. The crude amine (4.8 g, 9.95 mmol), (Boc)₂O (3.42 mL, 3.25 g, 14.9 mmol) and DIPEA (3.46 mL, 2.57 g, 19.9 mmol) and DMAP (catalytic) were dissolved in anhydrous dichloromethane (100 mL). The resulting reaction mixture was stirred at rt for 16 h. After 16 h, the reaction mixture was evaporated to dryness and the crude was purified on silica gel eluting with 30 % ethyl acetate-petroleum ether to afford 238 as a white solid; 70 % (over 3 steps); mp 131 °C-133 °C; ¹H NMR (500 MHz, CDCl₃): δ 7.47 (d, 2H, J = 7.4 Hz), 7.39 (t, 2H, J = 7.5 Hz), 7.33 (t, 1H, J = 7.0 Hz), 7.06-7.01 (1H), 6.82 & 6.64-6.62 (1H), 6.67 (s, 1H), 6.60 (s, 1H), 5.97-5.90 (dd, 2H, J = 24.0, 6.2 Hz), 5.36-5.30 (m 1H), 5.15-5.14 (s, 2H), 4.37-4.33 & 4.00-3.98 (1H), 3.89 & 3.80 (3H), 3.27-3.18 (m, 2H), 3.00-2.75 (m, 2H), 2.61 (d, 1H, J = 16.0 Hz), 1.39-1.22 (9H); ¹³C NMR (125 MHz, CDCl₃): δ 169.5, 148.4, 148.2, 147.8, 147.7, 137.0, 130.9, 128.5, 127.8, 127.5, 127.3, 121.4, 115.2, 114.6, 112.8, 112.0, 110.9, 101.9, 71.0, 56.0, 43.8, 40.5, 34.8; HRESIMS: calcd for C₃₀H₃₂BrNO₆ [M+Na]⁺ 604.1311; found 604.1301

tert-butyl 2-(benzylxy)-1-methoxy-4,5,6a,7-tetrahydro-6H-[1,3]dioxolo[4',5':4,5]benzo[1,2-g]benzo[de]quinoline-6-carboxylate (239): Compound 238 (0.2 g, 0.34 mmol), Pd(OAc)₂ (0.015 g, 0.068 mmol), K₂CO₃ (0.093 g, 0.68 mmol) and di-tert-butyl methyl phosphonium tetrafluoroborate (0.069 g, 0.028 mmol) were dissolved in DMSO (1 mL) in a 10 mL microwave reaction vial. The reaction mixture was then irradiated with microwaves at 140 °C, 200 psi for 15 min. The resulting crude was purified directly on a silica gel column chromatography using 30 % ethyl acetate-petroleum ether to afford 239 as a white solid; 93 %; mp 169 °C-173 °C; ¹H NMR (500 MHz, CDCl₃): δ 8.04 (s, 1H), 7.50 (d, 2H, J = 7.6 Hz), 7.42 (t, 2H, J = 7.4 Hz), 7.36 (t, 1H, J = 7.5 Hz), 6.77 (s, 1H), 6.71 (s, 1H), 6.00 (s, 2H), 5.16 (q, 2H, J = 11.8 Hz), 4.65 (d, 1H, J =
11.3 Hz), 4.41 (d, 1H, J = 7.8 Hz), 3.74 (s, 3H), 2.95-2.74 (m, 4H), 2.62 (d, 2H, J = 14.8 Hz), 1.51 (s, 9H); $^{13}$C NMR (125 MHz, CDCl$_3$): δ 151.1, 146.6, 146.5, 145.4, 137.1, 131.5, 129.7, 128.6, 127.9, 127.3, 126.4, 125.2, 112.8, 109.0, 108.3, 100.9, 79.8, 70.9, 60.1, 51.7, 30.3, 28.5; HRESIMS: calcd for C$_{30}$H$_{31}$NO$_6$ [M+Na]$^+$ 524.2049; found 524.2045

tert-butyl 2-hydroxy-1-methoxy-4,5,6a,7-tetrahydro-6H-[1,3]dioxolo[4',5':4,5]benzo[1,2-g]benzo[de]quinoline-6-carboxylate (240): Compound 239 (4.0 g, 7.98 mmol) was dissolved in a 1:1 mixture of anhydrous methanol-THF (150 mL) followed by the addition of 10 % Pd/C (15 mg). The resulting suspension was stirred for 6 h under hydrogen atmosphere. After 6 h, the reaction mixture was subjected to vacuum filtration over celite. The filtrate was then evaporated under reduced pressure to afford a crude residue, which was purified using silica gel column chromatography in 30 % ethyl acetate – petroleum ether to afford 240 as a white solid; 88 %; mp 218 °C-221 °C; $^1$H NMR (500 MHz, CDCl$_3$): δ 7.87 (s, 1H), 6.76 (s, 1H), 6.70 (s, 1H), 5.99 (s, 2H), 5.88 (s, 1H), 4.59 (d, 1H, J = 10.3 Hz), 4.38 (br, 1H), 3.58 (s, 3H), 2.92-2.87 (m, 2H), 2.82-2.70 (m, 2H), 2.61 (d, 1H, J = 15.8 Hz), 1.49 (s, 9H); $^{13}$C NMR (125 MHz, CDCl$_3$): δ 154.9, 148.0, 146.8, 146.8, 142.5, 131.5, 131.0, 126.5, 125.4, 124.8, 124.7, 113.4, 108.6, 107.8, 101.0, 79.9, 60.2, 51.7, 28.5; HRESIMS: calcd for C$_{23}$H$_{25}$NO$_6$ [M+Na]$^+$ 434.1580; found 434.1574

tert-butyl 3-bromo-1,2-dimethoxy-4,5,6a,7-tetrahydro-6H-[1,3]dioxolo[4',5':4,5]benzo[1,2-g]benzo[de]quinoline-6-carboxylate (241): Compound 240 (2.80 g, 6.81 mmol) was dissolved in anhydrous dichloromethane (100 mL) followed by the addition of freshly re-crystalized NBS (1.44 g, 8.1 mmol). The resulting reaction mixture was stirred for 30 min under nitrogen atmosphere at rt. After 30 min water (20 mL) was added to the reaction mixture followed by extraction with dichloromethane (3 x 30 ml). The combined organic layer was dried over Na$_2$SO$_4$ and evaporated to dryness under vacuum to afford a crude, which was purified using silica gel
column chromatography in 30 % ethyl acetate – petroleum ether to afford the corresponding 3-bromo derivative; yellow solid; 68 %. This solid (2.26 g, 4.61 mmol) was then dissolved in anhydrous DMF (60 mL) followed by the addition of K₂CO₃ (0.95 g, 6.91 mmol) and methyl iodide (0.34 mL, 0.79g, 5.07 mmol). The resulting reaction mixture was allowed to stir at rt for 20 h. After 20 h, the reaction mixture was first subject to vacuum filtration, followed by evaporating the filtrate under vacuum. 1N HCl (20 ml) was then added to the resulting crude, followed by extraction with dichloromethane (3 x 30 ml). The combined organic layer was dried over Na₂SO₄ and evaporated to dryness under vacuum to afford a crude residue which was purified using silica gel column chromatography in 30 % ethyl acetate – petroleum ether to afford 241 as a light brown solid; 88 %; mp 164 °C-166 °C; ¹H NMR (500 MHz, CDCl₃): δ 7.93 (s, 1H), 6.75 (s, 1H), 5.98 (s, 2H), 4.66 (br, 1H), 4.40 (d, 1H, J = 9.2 Hz), 3.92 (s, 3H), 3.73 (s, 3H), 3.00 (d, 1H, J = 16.0 Hz), 2.88 (t, 1H, J = 13.0 Hz), 2.84–2.75 (m, 2H), 2.69–2.62 (m, 1H), 1.48 (s, 9H); ¹³C NMR (125 MHz, CDCl₃): δ 154.2, 150.0, 149.6, 146.8, 146.6, 131.4, 130.9, 130.1, 127.3, 124.5, 118.1, 108.8, 108.4, 101.0, 80.0, 60.7, 60.3, 51.8, 30.6, 28.5; HRESIMS: calcd for C₂₄H₂₆BrNO₆ [M+Na]⁺ 526.0842; found 526.0812

**tert-butyl 1,2-dimethoxy-3-phenyl-4,5,6a,7-tetrahydro-6H-[1,3]dioxolo[4',5':4,5]benzo[1,2-g]benzo[de]quinoline-6-carboxylate (242a):** Compound 241 (0.1 g, 1.98 mmol), Pd(PPh₃)₄ (0.014 g, 0.12 mmol), Phenyl boronic acid (0.026 g, 0.21 mmol) and 1M Na₂CO₃ (0.76 ml) were dissolved in anhydrous toluene (1 mL) in a 10 mL microwave reaction vial. The reaction mixture was then irradiated with microwaves at 120 °C, 200 psi for 20 min. The reaction mixture was then evaporated under vacuum followed by the addition of saturated NaHCO₃ (5 mL) to the crude. The aqueous layer was then extracted with dichloromethane (2 x 10 ml). The combined organic layer
was dried over Na$_2$SO$_4$ and evaporated under vacuum to afford a 9:1 inseparable mixture of 242a and 241 which was used in the next step without further purification.

1,2-dimethoxy-6-methyl-3-phenyl-5,6,6a,7-tetrahydro-4H-[1,3]dioxolo[4',5':4,5]benzo[1,2-g]benzo[de]quinolone (233a): A mixture of 242a and 241 (0.085 g) was dissolved in anhydrous dichloromethane (10 mL) followed by addition of zinc bromide (0.15 g, 0.67 mmol). The resulting reaction mixture was stirred at rt for 16 h. After 16 h, saturated NaHCO$_3$ (10 mL) was added to the reaction mixture followed by extraction with dichloromethane (2 x 20 ml). The combined organic layer was dried over Na$_2$SO$_4$ and evaporated under vacuum to afford the corresponding crude deprotected intermediate (0.06 g). The resulting crude was dissolved in anhydrous dichloromethane (10 mL) followed by the addition of 37 % formaldehyde solution in water (0.15 mL, 1.49 mmol) and sodium triacetoxyborohydride (0.1 g, 0.350 mmol) and stirred for 16 h. After 16 h, saturated NaHCO$_3$ solution (20 mL) was added to the reaction mixture. The aqueous layers were then extracted with dichloromethane (2 x 20 mL). The combined organic layer was dried over Na$_2$SO$_4$, and evaporated under vacuum to get a crude product, which was purified on a preparative TLC plate using 0.02 % methanol-DCM as the solvent system to give 233a as a light brown solid; 67 %; mp 153 °C-157 °C; $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ 7.87 (s, 1H), 7.43 (t, 2H, $J = 7.7$ Hz), 7.37 (t, 1H, $J = 1.2$ Hz), 7.27 – 7.22 (merged, 2H), 6.77 (s, 1H), 5.98 (s, 2H), 3.75 (s, 3H), 3.66 (s, 3H), 3.00 (dd, 2H, $J = 14.3$, 3.7 Hz), 2.90 (dd, 1H, $J = 11.1$, 5.3 Hz), 2.82 – 2.75 (m, 1H), 2.58 (t, 1H, $J = 14.3$ Hz), 2.50 (s, 3H), 2.35 (td, 1H, $J = 11.7$, 3.7 Hz), 2.26 (dd, 1H, $J = 16.7$, 3.4 Hz); $^{13}$C NMR (125 MHz, CDCl$_3$): $\delta$ 150.0, 148.4, 146.4, 146.3, 137.0, 134.7, 130.9, 130.7, 129.7, 129.2, 128.3, 128.1, 127.6, 127.0, 126.8, 125.3, 108.8, 108.1, 100.8, 63.2, 60.9, 60.2, 53.2, 44.0, 34.9, 28.3; HRESIMS: calcd for C$_{26}$H$_{26}$NO$_4$ [M+H]$^+$ 416.1862; found 416.1857
2.6.2 Biological Evaluations:

A) Primary and secondary radioligand binding assays:

Both the assays were done at the PDSP facility. In the primary binding assays, compounds were usually tested at single concentrations (10 µM) in quadruplicate in 96-well plates. Compounds that showed a minimum of 50% inhibition at 10 µM were tagged for secondary radioligand binding assays to determine equilibrium binding affinity at specific targets. In the secondary binding assays, selected compounds were usually tested at 11 concentrations (0.1, 0.3, 1, 3, 10, 30, 100, 300 nM, 1, 3, 10 µM) and in triplicate (3 sets of 96-well plates). Both primary and secondary radioligand binding assays were carried out in a final of volume of 125 µl per well in appropriate binding buffer. The hot ligand concentration was usually at a concentration close to the $K_d$ (unless otherwise indicated). Total binding and nonspecific binding were determined in the absence and presence of 10 µM appropriate reference compound, respectively. In brief, plates were usually incubated at room temperature and in the dark for 90 min (unless otherwise indicated). Reactions were stopped by vacuum filtration onto 0.3% polyethyleneimine (PEI) soaked 96-well filter mats using a 96-well Filtermate harvester, followed by three washes with cold wash buffer. Scintillation cocktail was then melted onto the microwave-dried filters on a hot plate and radioactivity was counted in a Microbeta counter. For detailed experimental details please refer to the PDSP website http://pdsp.med.unc.edu/ and click on ‘Binding Assay’ or ‘Functional Assay’ on the menu bar.

B) FLIPR Calcium Mobilization Assay:

Our collaborator Dr. Hernan Navarro has stably expressed the human 5-HT$_{2A}$ receptor in CHO-K1 cells (ATCC). This clonal cell line was used to develop a robust FLIPR-based functional assay
for receptor activation. The calcium 4 dye assays (Molecular Devices, Sunnyvale, CA) were run according to manufacturer’s specifications. Briefly, wells of black clear bottom 96-well tissue culture treated plates were seeded with 20,000 cells the afternoon before assay. The day of assay, the cells were incubated with the calcium indicator dye for 1 hour at 37 °C. For antagonist assay the test compound was pre-incubated with the cells during the last 15 min of the dye incubation. The plate was then placed into FlexStation pre-warmed to 37 °C. Basal or unstimulated fluorescence intensity was recorded for 13 seconds followed by the addition of the test compound (intrinsic activity) or 5-HT (agonist and K<sub>e</sub> assays). Fluorescence intensity was recorded for an additional 47 seconds. The effect of test compound was determined by subtracting the minimum from the maximum fluorescence recorded for each well during the 47-seconds recording period. Thus, each well served as its own control. All samples were run in duplicate. For the agonist screening assays, the compounds were added directly to the cells and their effect on basal fluorescence was recorded. For the antagonist screening assays, the cells were pre-incubated with the test compounds for 15 minutes at 37 °C before the FlexStation added 5-HT at its EC<sub>80</sub> (~20 nM). Any compound that inhibits the effects of 5-HT by at least 50%, its apparent affinity (K<sub>e</sub>) were determined (see below). On-plate controls for screening assays: It is important to monitor assay performance because cell responsiveness can change over 90 time. For this reason, a 6-point log-unit 5-HT curve, 1 mM ketanserin (selective for 5-HT<sub>2A</sub> receptor), and 1 mM SB 206553 (selective for 5-HT<sub>2B/2C</sub> receptors) were run on each assay plate. If the EC<sub>50</sub> value for 5-HT was more than two SD different from the historical average that assay plate had to be rerun. If a consistent shift in the 5-HT EC<sub>50</sub> was observed, a new batch of 5-HT<sub>2A</sub>–expressing cells were used. Antagonists had their apparent affinity or K<sub>e</sub> values determined by running a 8-point half log 5-HT concentration response curve in the presence and absence of a single concentration of
antagonist. The EC$_{50}$ values were calculated for 5-HT (A) and 5-HT + test compound (A'), and these were used to calculate the test compound K$_e$ using the formula: K$_e$ = [L]/(DR-1), where [L] equals to concentration of test compound in the assay and DR equals the dose ratio or A'/A. The concentration of antagonist was chosen such that it caused at least a 2-fold increase (shift to right) in the 5-HT curve. If an antagonist was found to produce an insurmountable inhibition of 5-HT, it was assumed to be acting in a noncompetitive manner. In these cases, the compound had its IC$_{50}$ determined by running a concentration response curve in presence of the 5-HT EC$_{80}$. Assay performance for compound characterization was monitored as described above for the screening assays. In the case of the K$_e$ experiments, the 5-HT curve also served to monitor assay performance.

C) Cytotoxic Evaluations (NCI-60 DTP Human Tumor Cell Line Screen):

The human tumor cell lines of the cancer screening panel were grown in RPMI 1640 medium containing 5% fetal bovine serum and 2 mM L-glutamine. For a typical screening experiment, cells were inoculated into 96 well microtiter plates in 100 μL at plating densities ranging from 5,000 to 40,000 cells/well depending on the doubling time of individual cell lines. After cell inoculation, the microtiter plates were incubated at 37 °C, 5% CO$_2$, 95% air and 100% relative humidity for 24 h prior to addition of experimental drugs. After 24 h, two plates of each cell line were fixed in situ with TCA, to represent a measurement of the cell population for each cell line at the time of drug addition (Tz). Experimental drugs were solubilized in dimethyl sulfoxide at 400-fold the desired final maximum test concentration and stored frozen prior to use. At the time of drug addition, an aliquot of frozen concentrate was thawed and diluted to twice the desired final maximum test concentration with complete medium containing 50 μg/ml gentamicin. Additional four, 10-fold or ½ log serial dilutions were made to provide a total of five drug concentrations plus
control. Aliquots of 100 μl of these different drug dilutions were added to the appropriate microtiter wells already containing 100 μl of medium, resulting in the required final drug concentrations.

Following drug addition, the plates were incubated for an additional 48 h at 37 °C, 5 % CO₂, 95 % air, and 100 % relative humidity. For adherent cells, the assay was terminated by the addition of cold TCA. Cells were fixed in situ by the gentle addition of 50 μl of cold 50 % (w/v) TCA (final concentration, 10 % TCA) and incubated for 60 minutes at 4 °C. The supernatant was discarded, and the plates were washed five times with tap water and air dried. Sulforhodamine B (SRB) solution (100 μl) at 0.4 % (w/v) in 1 % acetic acid was added to each well, and plates were incubated for 10 minutes at room temperature. After staining, unbound dye was removed by washing five times with 1 % acetic acid and the plates were air dried. Bound stain was subsequently solubilized with 10 mM trizma base, and the absorbance was read on an automated plate reader at a wavelength of 515 nm. For suspension cells, the methodology was the same except that the assay was terminated by fixing settled cells at the bottom of the wells by gently adding 50 μl of 80 % TCA (final concentration, 16 % TCA). Using the seven absorbance measurements [time zero, (Tz), control growth, (C), and test growth in the presence of drug at the five concentration levels (Ti)], the percentage growth was calculated at each of the drug concentrations levels. Percentage growth inhibition was calculated as:

\[
\frac{(\text{Tz} - \text{Tz})}{(\text{C} - \text{Tz})} \times 100 \text{ for concentrations for which Ti} \geq \text{Tz}
\]

\[
\frac{(\text{Tz} - \text{Tz})}{\text{Tz}} \times 100 \text{ for concentrations for which Ti} < \text{Tz}.
\]

Three dose response parameters were calculated for each experimental agent. Growth inhibition of 50 % (GI₅₀) was calculated from \((\text{Tz} - \text{Tz})/(\text{C} - \text{Tz})\) x 100 = 50, which is the drug concentration
resulting in a 50% reduction in the net protein increase (as measured by SRB staining) in control cells during the drug incubation. The drug concentration resulting in total growth inhibition (TGI) was calculated from $T_i = T_z$. The $LC_{50}$ (concentration of drug resulting in a 50% reduction in the measured protein at the end of the drug treatment as compared to that at the beginning) indicating a net loss of cells following treatment was calculated from $[(T_i - T_z)/T_z] \times 100 = -50$. Values were calculated for each of these three parameters if the level of activity was reached; however, if the effect was not reached or was exceeded, the value for that parameter was expressed as greater or less than the maximum or minimum concentration tested.
CHAPTER III: SYNTHESIS & EVALUATION OF TRIS-(PHENYLALKYL) AMINES
3.1 Introduction:

As a part of the preliminary investigations on the nantenine scaffold, the requirement of a rigid aporphine core of nantenine (55) for 5-HT$_{2A}$ antagonism was studied. To this effect compounds 147-150 were synthesized previously and evaluated for 5-HT$_{2A}$ receptor affinity.$^{123}$ Compounds 147, 148 and 150, which have an intact tetrahydroisoquinoline ring (but truncated aporphine ring) were completely inactive at the 5-HT$_{2A}$ receptor. The flexible compound 149 was found to have poor affinity for the 5-HT$_{2A}$ receptor compared to its parent compound nantenine (55).

![Figure 55: Structures of flexible compounds 147-150 and Nantenine (55)](image)

147: R = H $K_e > 10,000$ nM  
148: R = Me $K_e > 10,000$ nM  
150 $K_e > 10,000$ nM  
149 $K_e = 5180$ nM  
55 $K_e = 830$ nM

Although this study established that an intact aporphine core is required for 5-HT$_{2A}$ antagonism, we were also interested in the structure of compound 149. Other compounds having structural similarity to compound 149 have been reported in literature.$^{184,185}$ However, no precedence existed for such compounds to have affinity at the 5-HT$_{2A}$ receptor. Although compound 149 has poor affinity at the 5-HT$_{2A}$ receptor, it was considered that compound 149 could be used as a starting point for developing 5-HT$_{2A}$ receptor ligands.
The \(N\)-phenylalkyl moiety represents an important pharmacophoric element for CNS drug design. Ligands containing the \(N\)-phenylalkyl moiety have been studied at the histamine H\(_3\) (compound 243\(^{186}\)), NMDA (compound 244\(^{187}\)), dopamine D\(_3\) (compound 245\(^{188}\)) and sigma \(\sigma_1\) receptors (compound 246\(^{189,190}\)). Moreover, there are several \(N\)-phenylalkyl containing compounds that are reported as ligands at the 5-HT\(_{1A}\) (compound 247\(^{191}\)) and 5-HT\(_{2A}\) receptors (compound 248\(^{192,193}\)). Some examples are shown in Figure 56.

![Figure 56: Structures of 5-HT\(_{2A}\) ligands (243-248) containing the \(N\)-phenylalkyl pharmacophore](image)

### 3.2 Rationale:

Compound 149 has poor affinity for the 5-HT\(_{2A}\) receptor. It was foreseen that the \(N\)-methyl group of compound 149 could be replaced with other \(N\)-phenylalkyl groups. We considered that this approach would enhance the affinity towards the 5-HT\(_{2A}\) receptor primarily due to two reasons i) the flexibility of ligands might allow the ligands multiple possibilities for interaction of the receptor with the \(N\)-phenylalkyl group ii) The \(N\)-phenylalkyl group is a known 5-HT receptor
pharmacophore. Additionally, it was expected that this approach would lead to more diverse series of analogs and a much shorter synthetic route to the compounds, precluding laborious synthesis of the aporphine template.

3.3 Preliminary Screening:

In order to find the optimum length of the linker between the phenylalkyl group and the nitrogen atom, compounds 249a-j were synthesized. Our initial design featured linker length of either 1, 2 or 3 carbons between the phenylalkyl group and the nitrogen atom, as well as phenyl groups having methoxy group substituents. These compounds were designed with the aim to increase 5-HT\textsubscript{2A} receptor affinity. Contrary to the expected result, it was observed that all compounds displayed high affinity for the 5-HT\textsubscript{2B} receptor and a range of selectivity (from 2 to almost 90 fold) vs. the 5-HT\textsubscript{2A} and 5-HT\textsubscript{2C} subtypes. Most of the analogs had 5-HT\textsubscript{2B} affinities that were similar or superior to the standard ligand used – SB206553, which had 5-HT\textsubscript{2B} affinity of 21 nM.
Table 10: Binding affinities of 249a-j at 5-HT2 receptors

![Structure diagram]

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
<th>n</th>
<th>m</th>
<th>Kᵢ (nM)</th>
<th>Selectivity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5-HT₂A</td>
</tr>
<tr>
<td>249a</td>
<td>H</td>
<td>0</td>
<td>1</td>
<td>3531 ± 460</td>
<td>59 ± 8.8</td>
</tr>
<tr>
<td>249b</td>
<td>H</td>
<td>1</td>
<td>1</td>
<td>1472 ± 190</td>
<td>17 ± 2.5</td>
</tr>
<tr>
<td>249c</td>
<td>2-MeO</td>
<td>2</td>
<td>1</td>
<td>165 ± 25</td>
<td>26 ± 2.3</td>
</tr>
<tr>
<td>249d</td>
<td>3-MeO</td>
<td>2</td>
<td>1</td>
<td>140 ± 15</td>
<td>5.8 ± 0.6</td>
</tr>
<tr>
<td>249e</td>
<td>4-MeO</td>
<td>2</td>
<td>1</td>
<td>200 ± 22</td>
<td>4.6 ± 0.5</td>
</tr>
<tr>
<td>249g</td>
<td>2,5-DiMeO</td>
<td>2</td>
<td>1</td>
<td>919 ± 120</td>
<td>36 ± 4.6</td>
</tr>
<tr>
<td>249h</td>
<td>2,4,5-triMeO</td>
<td>2</td>
<td>1</td>
<td>146 ± 19</td>
<td>4.1 ± 0.5</td>
</tr>
<tr>
<td>249i</td>
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<td>59 ± 7.6</td>
</tr>
<tr>
<td>249j</td>
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<td>2</td>
<td>0</td>
<td>2234 ± 290</td>
<td>231 ± 25</td>
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</table>

Clozapine          |      |    |    | 15      |             |             |         |               |
SB206553           |      |    |    | 21      |             |             |         |               |
Ritanserin         |      |    |    | 1.8     |             |             |         |               |

¹ Radioligands are [³H]Ketanserin, [³H]LSD and [³H]mesulergine for 5-HT₂A, 5-HT₂B and 5-HT₂C respectively, b na not active defined as : % inhibition at 10 µM < 50 % in primary assay

3.4 Synthesis & evaluation of ring C halogenated analogues:

Results from the preliminary screen indicated that the tris-(phenylalkyl)amine analogues displayed higher affinity for the 5-HT₂B receptor. Nevertheless, more information was needed to understand the structural tolerance of the tris-(phenylalkyl)amine scaffold for affinity at 5-HT receptors. Compound 249c, which is a non-competitive antagonist at the 5-HT₂B receptor was
selected as a lead candidate. The focus of this study was to understand the steric and electronic tolerance of ring C. Accordingly a series of ring C halogenated analogues (of compound 249c) were synthesized and evaluated.

![Figure 57: Structure of 249c]

Scheme 11 shows the synthesis of ring C halogenated tris-(phenylalkyl) amine derivatives. The synthesis commenced through a CDI mediated coupling of commercially available amine 210 and acid 250 to furnish the amide 251. Reduction of amide 251 using borane-THF resulted in the formation of secondary amine 252. Amine 252 was then coupled with commercially available acids 253a-l to afford amides 254a-l. The final step of the synthesis utilized a borane-THF mediated reduction of amides 254a-l, to generate the target compounds 255a-l.
Scheme 11: Reagents & Conditions: a) 250, CDI, THF, 0 °C - rt, 16 h, 80 %; b) BH₃-THF, BF₃·Et₂O, THF, rt-reflux, 4h, 73 %; c) 253a-l, EDCI, HOBr, DIPEA, DMF, 0 °C - rt, 6h; d) BH₃-THF, BF₃·Et₂O, THF, rt-reflux, 4h
Table 11: Binding affinities of 255a-l at 5-HT<sub>2</sub> receptors

![Chemical structure](image)

<table>
<thead>
<tr>
<th>Compound</th>
<th>K&lt;sub&gt;i&lt;/sub&gt; (nM)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Selectivity</th>
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<tr>
<td></td>
<td>5-HT&lt;sub&gt;2A&lt;/sub&gt;</td>
<td>5-HT&lt;sub&gt;2B&lt;/sub&gt;</td>
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<tr>
<td>249c</td>
<td>H H H</td>
<td>165 ± 25</td>
</tr>
<tr>
<td>255a</td>
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<td>65</td>
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<tr>
<td>255b</td>
<td>Cl H H</td>
<td>43</td>
</tr>
<tr>
<td>255c</td>
<td>Br H H</td>
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</tr>
<tr>
<td>255d</td>
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<td>75</td>
</tr>
<tr>
<td>255e</td>
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<tr>
<td>255f</td>
<td>H Cl H</td>
<td>125</td>
</tr>
<tr>
<td>255g</td>
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<td>103</td>
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<tr>
<td>255h</td>
<td>H I H</td>
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</tr>
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<tr>
<td>255j</td>
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</tr>
<tr>
<td>255k</td>
<td>H H Br</td>
<td>221</td>
</tr>
<tr>
<td>255l</td>
<td>H H I</td>
<td>288</td>
</tr>
</tbody>
</table>

Clozapine | 15 |
SB206553 | 21 |
Ritanserin | 1.8 |

<sup>a</sup> Radioligands are [³H]Ketanserin, [³H]LSD and [³H]mesulergine for 5-HT<sub>2A</sub>, 5-HT<sub>2B</sub> and 5-HT<sub>2C</sub> respectively

All the synthesized compounds were evaluated for affinity at 5-HT<sub>2</sub> receptors through the Psychoactive Drug Screening Program (PDSP) of the National Institute of Health (NIH). Here, the submitted compounds were first screened in a primary radioligand binding assay (in quadruplicate) at a concentration of 10 µM at the three human 5-HT<sub>2</sub> receptor sites. Compounds which displayed a minimum of 50 % inhibition for a particular receptor in this preliminary assay were then...
evaluated in secondary radioligand binding assays (11 concentrations; each in triplicate) to determine $K_i$ values. These $K_i$ values are compiled in Table 11.

As seen in Table 11, all synthesized compounds displayed affinities for the 5-HT$_{2B}$ receptor in the range of 1 to 60 nM. The most potent compound identified in this series was 255a with a $K_i$ value of 1.7 nM at the 5-HT$_{2B}$ receptor and had a 38 and 46 fold selectivity over the 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors respectively.

In the 2′ substituted series (255a-d), introduction of a fluorine atom at the 2′ position (255a, $K_i = 1.7$ nM) resulted in a 15 fold increase in affinity at the 5-HT$_{2B}$ receptor (compared with compound 249c). The affinity of 255a at the 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors as compared to 249c was also found to increase by 2.5 and 5 folds respectively. Moreover the selectivity of 255a at both these receptors was better as compared to 249c (38 vs 6 fold over 5-HT$_{2A}$ and 46 vs 15 fold over 5-HT$_{2C}$). Similarly 255b (2′-chloro, $K_i = 3.1$ nM) and 255c (2′-bromo, $K_i = 3.4$ nM) were also found to be 8 times more potent than their parent compound 255c ($K_i = 26$ nM), whereas 255d (2′-iodo, $K_i = 13$ nM) was found to be 2 times potent than 249c at the 5-HT$_{2B}$ receptor. 255a, which is 12 times more potent than the standard 5-HT$_{2B}$ ligand (SB206553), was the most potent compound identified in this series.

Introduction of a halogen atom at the 3′ position in ring C (255e-h) resulted in a moderate increase in affinity at the 5-HT$_{2B}$ receptor than the parent compound 249c. The 3′ fluoro (255e, $K_i = 13$ nM) and 3′ iodo (255h, $K_i = 14$ nM) had a 2 fold increase in affinity at the 5-HT$_{2B}$ receptor. Increased affinity was also observed for 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors. Interestingly all the 3′ substituted compounds (255e-h) had similar affinities indicative of the tolerance of halogen atoms at this position.
Compound 255i which has a fluorine atom in the 4' position of ring C displayed slightly improved affinity ($K_i = 14 \text{ nM}$) at the 5-HT$_{2B}$ receptor than compound 249c. This change also led to an increase in selectivity over the 5-HT$_{2A}$ receptor (13 fold vs 6 fold), but a decrease in selectivity over the 5-HT$_{2C}$ receptor (9 fold vs 15 fold). Contrarily with the 4'-chloro (255j, $K_i = 54 \text{ nM}$), 4'-bromo (255k, $K_i = 45 \text{ nM}$) and 4'-iodo (255l, $K_i = 51 \text{ nM}$) compounds slight drop in 5-HT$_{2B}$ receptor affinity as well as decrease in selectivity over 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors was observed, although no clear trend was identified. Only fluorination at this position resulted in increased affinity for all 5-HT$_{2A}$, 5-HT$_{2B}$ and 5-HT$_{2C}$ receptors.

A small trend was observed in the 2' series (255a-d), where affinity at the 5-HT$_{2B}$ receptor was found to decrease with increasing size of the halogen atom. This trend was also seen with respect to the selectivity of the respective compounds over 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors.

Except the fluoro compounds (255a, 255e and 255i), affinity at the 5-HT$_{2B}$ receptor decreased in the following order: 2' substituted compounds > 3' substituted compounds > 4' substituted compounds. Thus 255b ($K_i = 3.1 \text{ nM}$) was 5 times potent than 255f ($K_i = 17 \text{ nM}$), which in turn was 3 times more potent than 255j ($K_i = 54 \text{ nM}$). This trend was also observed for the affinities of the same compounds at the 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors.

3.5 Discussion:

The 5-HT$_{2B}$ receptor is involved in the regulation of CNS, gastric and intestinal motility and cardiovascular functions. 5-HT$_{2B}$ antagonists have been explored as potential pharmacotherapeutics for migraine, irritable bowel syndrome, pulmonary hypertension and heart failure.$^{196-201}$ 5-HT$_{2B}$ receptor agonists display antidepressant activity and 5-HT$_{2B}$ receptor activation is required for antidepressant actions of selective serotonin reuptake inhibitors.
However, 5-HT\textsubscript{2B} agonism is known to be associated with the development of valvular heart disease (VHD) and as such is regarded as an anti-target in most drug discovery programs. \textsuperscript{203-205} Despite the promise of 5-HT\textsubscript{2B} antagonists as useful therapeutics, there are no 5-HT\textsubscript{2B} antagonists that are clinically approved for the clinical indications mentioned previously. This is partly because many known ligands are not truly 5-HT\textsubscript{2B} selective (5-HT\textsubscript{2B} ligands often also have affinity for the related 5-HT\textsubscript{2A} and 5-HT\textsubscript{2C} receptors) and even when selective there are issues related to ADME properties of the compounds that prohibit clinical translational studies.

Figure 58 shows some selective 5-HT\textsubscript{2B} antagonists that are commercially available; these compounds are predominantly used as biological tools. \textsuperscript{206-208} The identification of new 5-HT\textsubscript{2B} preferring scaffolds is critical in the pursuit of novel chemical entities that may be developed as useful 5-HT\textsubscript{2B} antagonist therapeutics.

In a previous study we identified a novel tris-(phenylalkyl)amine scaffold as a 5-HT\textsubscript{2B} antagonist. These compounds were designed with the aim of replacing the $N$-methyl group of
nantenine with an N-phenylalkyl moiety as well as increasing the molecular flexibility of nantenine. Compound 249c having a propyl linker was selected as our lead compound.

In order to understand the functional group tolerance of the phenyl ring (of the N-phenylpropyl moiety) we decided to synthesize a series of 2’, 3’ and 4’ substituted halogenated analogues. This compounds should reveal the electronic tolerance of the phenyl ring towards 5-HT2B receptor affinity.

Based on the biological evaluations, it seems clear that ring C is tolerant towards halogen substitution in any position. In most cases, halogen substitution also resulted in increased affinity (compared to the non-halogenated parent compound 249c), with some compounds being more potent than the positive control SB-200646.

Introduction of a halogen atom can have several beneficial effects on the affinities of a ligand. There are several plausible ways in which halogen atom can interact with a protein or other relevant target. Classically halogens are considered to be hydrophobic moieties and lewis bases in accordance to their electronegativities. However the halogen atom can also act as a lewis acid (via σ-hole) and interact with an electron donating residue of a protein. This interaction termed as halogen bonding is described in detail in chapter 4. Bromine and chlorine atoms may be involved in bonding interactions with backbone amino acids that contain carbonyl oxygen atoms. Additionally, halogen atoms are also involved in bond formation with side chain groups such as hydroxyls in serine, thereonine, and tyrosine, carboxylate groups in aspartate and glutamate, sulfurs in cysteine and methionine, nitrogens in histidine and the π surfaces of phenyl alanine, tyrosine, histidine and tryptophan.

In the case of tris-(phenylalkyl)amines it is not clear which of the above mentioned interactions are responsible for their enhanced affinity. Molecular docking studies should throw
further light on their possible modes of interactions with the 5-HT\textsubscript{2B} receptor and help in future ligand design.

### 3.6 Conclusions:

A series of ligands bearing a tris-phenylalkyl)amine scaffold with high affinity and selectivity for the 5-HT\textsubscript{2B} receptor were synthesized. This ligands featured a halogen substituent in ring C. In general halogen substitution was well tolerated in ring C and resulted in increased affinity and selectively for the 5-HT\textsubscript{2B} receptor (compared to their non-halogenated parent compound). Compound \textbf{255a} having a 2’ fluoro group in ring C, was identified as the most potent compound in this series. \textbf{255a} (Ki = 1.7 nM) is 12 times more potent than the standard 5-HT\textsubscript{2B} ligand SB206553. At this time the role of halogen substitution in the increased affinity remains to be determined. The ease of synthesis of this scaffold makes it particularly attractive for further structure-activity work to optimize 5-HT\textsubscript{2B} affinity, selectivity and antagonist activity in the quest for 5-HT\textsubscript{2B} antagonist drugs.

The synthetic feasibility of this newly identified template (4 high-yielding synthetic steps from commercially available materials) makes this scaffold attractive for the synthesis of larger libraries of analogs and promise for optimization of 5-HT\textsubscript{2B} affinity and selectivity. Additional exploration of the scaffold should provide new tool compounds that will be useful for the study of 5-HT\textsubscript{2B} receptor functions. Future \textit{in vitro} as well as \textit{in vivo} pharmacological characterization accompanied with molecular docking studies would provide a platform for such studies.
3.7 Experimental:

3.7.1 Chemistry

*General methods and instrumentation:*

All glass apparatus were oven-dried prior to use. A CEM Discover microwave reactor was used to carry out microwave-assisted C-H arylation reactions. **HRESIMS** spectra were obtained using an Agilent 6520 Q-TOF instrument. $^1$H NMR and $^{13}$C NMR spectra were recorded using a Bruker DPX-500 spectrometer (operating at 500 MHz for $^1$H; 125 MHz for $^{13}$C) using CDCl$_3$ as solvent. Tetramethylsilane ($\delta$ 0.00 ppm) served as an internal standard in $^1$H NMR and $^{13}$C NMR unless stated otherwise. Chemical shift ($\delta$ 0.00 ppm) values are reported in parts per million and coupling constants in Hertz (Hz). Splitting patterns are described as singlet (s), doublet (d), triplet (t), and multiplet (m). Melting points were obtained on a Mel-Temp capillary electrothermal melting point apparatus. Reactions were monitored by TLC with Whatman Flexible TLC silica gel G/UV 254 precoated plates (0.25 mm). TLC plates were visualized in UV light (254 nm) and by staining with phosphomolybdic acid spray reagent, vanillin or iodine. Flash column chromatography was performed with silica gel 60 (EMD Chemicals, 230-400 mesh, 0.04-0.063 mm particle size). Preparative thin layer chromatography was performed with silica gel GF plates (Analtech, catalog # 02003). All chemicals and reagents were obtained from Sigma-Aldrich and Fischer Scientific (USA) in reagent grade and were used without further purification.
2-(benzo[d][1,3]dioxol-5-yl)-N-(3,4-dimethoxyphenethyl)acetamide (251): A solution of acid 250 (4.96 g, 27.62 mmol) in anhydrous THF (50 mL) under N₂ atmosphere was cooled to 0 °C. To this solution CDI (5.30 g, 32.7 mmol) was added in three portions over a period of 30 min at 0 °C. The resulting reaction mixture was allowed to stir for 1.5 h at 0 °C, after which the temperature was raised to rt and allowed to stir for another 1 h. The solution was again cooled to 0 °C and allowed to stir for 30 min, after which a solution of 251 (5.00 g, 27.62 mmol) in THF (10 mL) was added dropwise. The resulting reaction mixture was allowed to stir for 30 min at 0 °C and then at rt for another 15 h. After 15 h, the crushed out solid was filtered and washed with diethyl ether to furnish 251; white solid; 80 %; mp 99 °C-103 °C. Data in accordance with previously reported.

2-(benzo[d][1,3]dioxol-5-yl)-N-(3,4-dimethoxyphenethyl)ethan-1-amine (252): To a solution of amide 251 (7.00 g, 20.43 mmol) in anhydrous THF (100 mL) under N₂, BF₃-Et₂O (5.04 mL, 5.80 g, 40.86 mmol) was added. The resulting reaction mixture was heated under reflux for 30 min, after which a 1M solution of BH₃-THF (46.54 mL, 4.39 g, 51.05 mmol) was carefully added dropwise. The resulting reaction mixture was heated under reflux for another 3.5 h. After 3.5 h the reaction mixture was allowed to cooled to 0 °C, followed by the careful dropwise addition of 3N HCl (30 mL). The resulting solution was evaporated under vacuum, followed by addition of 3N NaOH (45 mL) to adjust pH to 8-9. The resulting aqueous solution was extracted using dichloromethane (3 x 30 mL). The combined organic layer was dried over Na₂SO₄ and evaporated to dryness under vacuum to afford amine 252 as a light brown oil; 73 %. Compound 252 was used without further purification.

General procedure for the synthesis of 254a-l: HoBT (1.2 equiv.) was added to an ice cooled solution of EDCI (1.2 equiv.) in anhydrous DMF (10 mL) under N₂. The resulting solution was allowed to stir for 10 mins at 0 °C, followed by the addition of the appropriate acid (1.2 equiv.)
and DIPEA (2.5 equiv.). The resulting solution was stirred for another 15 min at 0 °C, after which a solution of amine 252 (1.0 equiv.) in anhydrous DMF (5 mL) was added. The resulting reaction mixture was allowed to stir first at 0 °C for 30 mins, then at rt for 5 h. The reaction mixture was then evaporated to dryness under vacuum. Saturated NaHCO₃ solution (10 mL) was then added to the resulting crude followed by extraction of the aqueous layer using dichloromethane (3 x 15 mL). The combined organic layer was dried over Na₂SO₄ and evaporated to dryness under vacuum to afford the corresponding amides which were purified using flash column chromatography (30 – 50 % ethyl acetate –petroleum ether).

N-(2-(benzo[d][1,3]dioxol-5-yl)ethyl)-N-(3,4-dimethoxyphenethyl)-3-(2-fluorophenyl)propanamide (254a): clear oil; 75 %; rotamers observed, all signals reported; 

\[^{1}H \text{ NMR} \text{ (500 MHz, CDCl₃): } \delta 7.20-7.15 (m, 2H), 7.06-6.97 (m, 2H), 6.78 (m, 1H), 6.76-6.70 (1.5H), 6.69-6.68 (m, 1H), 6.61 (d, 1H, } J = 7.7 \text{ Hz), 6.56 (dd, 1H, } J = 7.7 \text{ Hz, 1.9 Hz), 6.52-6.50 (0.5 H), 5.90 (merged d, 2H, } J = 1.6 \text{ Hz), 3.86-3.81 (6H), 3.48 (q, 2H, } J = 9.3 \text{ Hz), 3.28 (q, 2H, } J = 7.0 \text{ Hz), 2.96-2.90 (m, 4H), 2.80-2.73 (m, 2H), 2.64 (q, 2H, } J = 7.4 \text{ Hz), 2.43 (t, 1H, } J = 8.0 \text{ Hz), 2.40 (t, 1H, } J = 7.6 \text{ Hz) ; } ^{13}C \text{ NMR} \text{ (125 MHz, CDCl₃): } \delta 171.75, 171.70, 162.1, 160.2, 149.0, 148.9, 147.8, 147.6, 147.5, 146.3, 146.0, 133.1, 131.8, 131.7, 131.07, 131.02, 130.9, 130.5, 128.1, 128.0, 127.9, 127.8, 124.0, 121.6, 120.7, 120.6, 115.3, 115.2, 115.1, 115.0, 112.0, 111.8, 111.4, 111.2, 109.2, 109.0, 108.4, 108.2, 100.9, 100.8, 55.88, 55.86, 50.2, 50.1, 48.4, 35.0, 34.9, 33.8, 33.6, 33.2, 25.3, 25.2; \text{ HRESIMS: calcd for C}_{28}H_{31}FNO_{5} \left[M+H\right]^+ 480.2186; \text{ found 480.2181}

N-(2-(benzo[d][1,3]dioxol-5-yl)ethyl)-3-(2-chlorophenyl)-N-(3,4-dimethoxyphenethyl)propanamide (254b): clear oil; 82 %; rotamers observed, all signals reported; 

\[^{1}H \text{ NMR} \text{ (500 MHz, CDCl₃): } \delta 7.34-7.31 (m, 1H), 7.23-7.20 (m, 1H), 7.18-7.14 (m, 2H), 6.77 (dd, 1H, } J = 13.1, 8.05 \text{ Hz), 6.74-6.73 (m, 1H), 6.72-6.68 (1.5H), 6.62-6.60 (m, 1H), 6.55 (dd,
1H, J = 8.5, 1.9 Hz), 6.51-6.49 (0.5H), 5.90 (merged d, 2H, J = 2.1 Hz), 3.86-3.81 (6H), δ 3.48 (q, 2H, J = 7.7 Hz), 3.28-3.25 (m, 2H), 3.05-3.00 (m, 2H), 2.80-2.74 (m, 2H), 2.62 (q, 2H, J = 7.0 Hz), 2.47-2.41 (m, 2H); 13C NMR (125 MHz, CDCl3): δ 171.7, 171.6, 149.0, 148.9, 147.8, 147.6, 147.5, 146.3, 146.0, 138.8, 133.8, 133.7, 133.1, 131.8, 131.7, 130.98, 130.91, 130.5, 129.47, 129.42, 127.7, 126.9, 121.6, 120.7, 120.6, 112.0, 111.8, 111.3, 111.2, 109.2, 109.0, 108.4, 108.2, 100.9, 100.8, 55.9, 55.8, 50.2, 50.1, 48.52, 48.50, 35.1, 34.9, 33.8, 33.6, 32.8, 29.7, 29.6;

HRESIMS: calcd for C28H31ClNO5 [M+H]+ 496.1891; found 496.1886

N-(2-(benzo[d][1,3]dioxol-5-yl)ethyl)-3-(2-bromophenyl)-N-(3,4-dimethoxyphenethyl)propanamide (254c): clear oil; 89%; rotamers observed, all signals reported; 1H NMR (500 MHz, CDCl3): δ 7.53-7.50 (m, 1H), 7.26-7.21 (m, 2H), 7.08-7.04 (m, 1H), 6.77 (dd, 1H, J = 13.0, 8.0 Hz), 6.74-6.71 (1.5H), 6.69 (d, 1H, J = 8.0 Hz), 6.61 (dt, 1H, J = 8.0, 2.0 Hz), 6.56 (dd, 1H, J = 8.0, 2.0 Hz), 6.52-6.50 (0.5H), 5.91 (merged d, 2H, J = 2.4 Hz), 3.86-3.82 (6H), 3.48 (q, 2H, J = 7.0 Hz), 3.28-3.25 (m, 2H), 3.06-3.00 (m, 2H), 2.80-2.74 (m, 2H), 2.63 (q, 2H, J = 7.0 Hz), 2.48-2.42 (m, 2H); 13C NMR (125 MHz, CDCl3): δ 171.6, 171.6, 149.0, 148.9, 147.8 147.6, 147.5, 146.3, 146.0, 140.5, 133.1, 132.79, 132.74, 131.8, 131.7, 130.99, 130.93, 130.5, 127.9, 127.62, 127.61, 124.29, 124.23, 121.68, 121.65, 120.7, 120.6, 112.0, 111.8, 111.3, 111.2, 109.2, 109.0, 108.4, 108.2, 100.9, 100.8, 55.91, 55.90, 50.28, 50.20, 48.55, 48.53, 35.1, 34.9, 33.8, 33.6, 32.9, 32.2, 32.1; HRESIMS: calcd for C28H31BrNO5 [M+H]+ 540.1385; found 526.1386

N-(2-(benzo[d][1,3]dioxol-5-yl)ethyl)-N-(3,4-dimethoxyphenethyl)-3-(2-iodophenyl)propanamide (254d): clear oil; 76%; rotamers observed, all signals reported; 1H NMR (500 MHz, CDCl3): δ 7.80-7.78 (m, 1H), 7.28-7.20 (m, 2H), 6.91-6.87 (m, 1H), 6.78 (dd, 1H, J = 13.3, 8.0 Hz), 6.74-6.71 (1.5H), 6.69 (d, 1H, J = 7.9 Hz), 6.62 (dd, 1H, J = 7.9, 1.5 Hz),
6.57 (dd, 1H, 7.3, 1.8 Hz), 6.54-6.53 (0.5H), 5.91 (merged d, 2H, 7.3 Hz), 3.87-3.82 (6H), 3.48 (q, 2H, 7.3 Hz), 3.28-3.25 (m, 2H), 3.05-3.00 (m, 2H), 2.80-2.74 (m, 2H), 2.63 (q, 2H, 7.3 Hz), 2.43 (quin, 2H, 7.3 Hz); $^{13}$C NMR (125 MHz, CDCl$_3$): δ 171.5, 171.4, 149.0, 148.9, 147.8, 147.6, 147.5, 146.3, 146.0, 143.9, 143.8, 139.59, 139.50, 139.4, 133.1, 131.8, 131.7, 130.5, 130.0, 128.1, 121.7, 121.6, 120.8, 120.6, 112.0, 111.9, 111.3, 111.2, 109.2, 109.1, 108.4, 108.2, 100.9, 100.8, 100.1, 55.96, 55.92, 55.90, 50.3, 50.2, 48.6, 48.5, 36.58, 36.53, 35.1, 34.9, 33.8, 32.6, 32.2; HRESIMS: calcd for C$_{28}$H$_{31}$NO$_5$ [M+H]$^+$ 588.1247; found 588.1241

$N$-(2-(benzo[d][1,3]dioxol-5-yl)ethyl)-$N$-((3,4-dimethoxyphenethyl)-3-(3-fluorophenyl)propanamide (254e): clear oil; 90 %; rotamers observed, all signals reported; $^1$H NMR (500 MHz, CDCl$_3$): δ 7.21-7.19 (m, 1H), 6.93-6.77 (m, 4H), 6.74-6.71 (m, 2H), 6.69-6.68 (0.5H), 6.62 (td, 1H, 7.3 Hz), 6.54 (dd, 1H, 7.3 Hz), 6.51-6.49 (0.5H), 5.92 (merged d, 2H, 7.3 Hz), 3.87-3.82 (6H), 3.50 (q, 2H, 7.3 Hz), 3.27 (m, 2H), 2.89-2.75 (m, 4H), 2.64 (q, 2H, 7.3 Hz), 2.35 (t, 1H, 7.3 Hz), 2.30 (t, 1H, 7.3 Hz); $^{13}$C NMR (125 MHz, CDCl$_3$): δ 171.6, 171.5, 163.87, 163.85, 161.92, 161.90, 149.0, 148.9, 147.9, 147.6, 147.5, 146.4, 146.0, 144.09, 144.06, 144.03, 144.00, 133.1, 131.81, 131.81, 130.6, 129.89, 129.87, 129.82, 129.82, 124.06, 124.05, 121.7, 121.6, 120.7, 120.6, 115.3, 115.2, 115.13, 115.01, 113.04, 113.01, 112.88, 112.84, 112.0, 111.9, 111.4, 111.2, 110.0, 100.8, 100.8, 100.5, 100.3, 101.0, 100.0, 55.9, 55.8, 50.1, 50.0, 48.44, 48.42, 34.9, 34.7, 34.5, 34.4, 33.7, 33.6, 31.0; HRESIMS: calcd for C$_{28}$H$_{31}$FNO$_5$ [M+H]$^+$ 480.2186; found 480.2185

$N$-(2-(benzo[d][1,3]dioxol-5-yl)ethyl)-3-(3-chlorophenyl)-$N$-((3,4-dimethoxyphenethyl)propanamide (254f): clear oil; 77 %; rotamers observed, all signals reported; $^1$H NMR (500 MHz, CDCl$_3$): δ 7.15-7.13 (m, 2H), 7.11 (d, 1H, 7.3 Hz), 7.01 (dd, 1H, 7.3 Hz), 6.79 (d, 1H, 7.3 Hz), 6.74-6.70 (m, 2H), 6.69-6.68 (0.5H), 6.61 (dt, 1H,
$J = 7.85, 1.75 \text{ Hz}, 6.54 (d, 1H, $J = 3.8 \text{ Hz}), 6.51-6.49 (0.5H), 5.91 (s, 2H), 3.86-3.82 (6H), 3.49 (q, 2H, $J = 7.6 \text{ Hz}), 3.27 (q, 2H, $J = 4.9 \text{ Hz}), \delta 2.87-2.75 (m, 4H), \delta 2.66-2.61 (m, 2H), 2.34 (t, 1H, $J = 7.9 \text{ Hz}), 2.29 (t, 1H, $J = 8.0 \text{ Hz})$; $^{13}$C NMR (125 MHz, CDCl$_3$): $\delta 171.56, 171.50, 149.0, 148.9, 147.9, 147.6, 147.4, 146.0, 143.53, 143.51, 134.14, 134.10, 133.0, 131.8, 131.7, 130.6, 129.7, 128.5, 128.4, 126.6, 126.28, 126.24, 121.7, 121.6, 120.7, 120.6, 112.0, 111.9, 111.4, 111.2, 109.1, 109.0, 108.5, 108.2, 101.0, 100.8, 55.9, 55.8, 50.08, 50.03, 48.4, 34.9, 34.7, 34.5, 34.4, 33.7, 33.5, 31.0; HRESIMS: calcd for C$_{28}$H$_{31}$ClNO$_5$ [M+H]$^+$ 496.1891; found 496.1889

$N$-(2-(benzo[d][1,3]dioxol-5-yl)ethyl)-3-(3-bromophenyl)-N-(3,4-dimethoxyphenethyl)propanamide (254g): clear oil; 84%; rotamers observed, all signals reported; $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ 7.33-7.29 (m, 1H), 7.26 (d, 1H, $J = 5.7 \text{ Hz}), 7.15-7.11 (m, 1H), 7.05 (dd, 1H, $J = 16.5, 7.6 \text{ Hz}), 6.79 (d, 1H, $J = 8.1 \text{ Hz}), 6.74-6.70 (m, 2H), 6.69-6.68 (0.5H), 6.61 (d, 1H, $J = 4.6 \text{ Hz}), 6.54 (d, 1H, $J = 4.7 \text{ Hz}), 6.51-6.49 (0.5H), 5.91 (s, 2H), 3.86-3.82 (6H), 3.49 (q, 2H, $J = 7.7 \text{ Hz}), 3.27 (q, 2H, $J = 6.9 \text{ Hz}), 2.86-2.75 (m, 4H), 2.66-2.61 (m, 2H), 2.34 (t, 1H, $J = 7.9 \text{ Hz}), 2.29 (t, 1H, $J = 8.0 \text{ Hz}); ^{13}$C NMR (125 MHz, CDCl$_3$): $\delta$ 171.57, 171.51, 149.1, 148.9, 147.93, 147.92, 147.6, 146.5, 146.0, 143.85, 143.83, 133.0, 131.8, 131.7, 131.4, 131.3, 130.6, 130.0, 129.24, 129.20, 127.1, 122.48, 122.44, 121.7, 121.6, 120.7, 120.6, 112.0, 111.9, 111.4, 111.2, 109.2, 109.0, 108.5, 108.3, 101.0, 100.8, 55.95, 55.93, 55.91, 50.10, 50.06, 48.4, 34.9, 34.7, 34.5, 34.4, 33.8, 33.6, 31.0; HRESIMS: calcd for C$_{28}$H$_{31}$BrNO$_5$ [M+H]$^+$ 540.1385; found 526.1381

$N$-(2-(benzo[d][1,3]dioxol-5-yl)ethyl)-N-(3,4-dimethoxyphenethyl)-3-(3-iodophenyl)propanamide (254h): clear oil; 71%; rotamers observed, all signals reported; $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ 7.53-7.48 (m, 2H), 7.11 (dd, 1H, $J = 17.0, 7.6 \text{ Hz}), 7.01-6.98 (m, 1H), 6.79 (d, 1H, $J = 6.1 \text{ Hz}), 6.74-6.70 (1H), 6.69-6.68 (0.5H), 6.61 (d, 1H, $J = 8.0 \text{ Hz}), 6.54 (d, 1H,
$J = 3.4 \text{ Hz}$, 6.51-6.49 (0.5H), 5.91 (s, 2H), 3.86-3.82 (6H), 3.49 (q, 2H, $J = 7.4 \text{ Hz}$), 3.26 (q, 2H, $J = 6.7 \text{ Hz}$), 2.94-2.75 (m, 4H), 2.66-2.60 (m, 2H), 2.33 (t, 2H, $J = 7.9 \text{ Hz}$), 2.28 (t, 2H, $J = 8.0 \text{ Hz}$); $^{13}$C NMR (125 MHz, CDCl$_3$): $\delta$ 171.5, 171.4, 149.0, 148.9, 147.88, 147.86, 147.6, 147.5, 146.3, 146.0, 143.93, 143.91, 137.3, 137.2, 135.17, 135.13, 133.0, 131.78, 131.76, 130.5, 130.1, 127.7, 127.6, 121.6, 120.7, 120.6, 112.0, 111.9, 111.4, 111.2, 109.1, 109.0, 108.5, 108.2, 100.9, 100.8, 55.94, 55.93, 55.90, 55.8, 50.06, 50.03, 48.4, 34.9, 34.7, 34.5, 33.7, 33.4, 33.6, 30.88, 30.87; HRESIMS: calcd for C$_{28}$H$_{31}$INO$_5$ [M+H]$^+$ 588.1247; found 588.1241

$^{N}$-(2-(benzo[d][1,3]dioxol-5-yl)ethyl)-$^N$-(3,4-dimethoxyphenethyl)-3-(4-fluorophenyl)propanamide (254i): clear oil; 84 %; rotamers observed, all signals reported; $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ 7.08 (ddd, 2H, $J = 15.9$, 8.5, 5.5 Hz), 6.97-6.93 (m, 2H), 6.78 (t, 1H, $J = 8.1 \text{ Hz}$), 6.74-6.70 (m, 2H), 6.68 (0.5H), 6.61 (t, 1H, $J = 8.2 \text{ Hz}$), 6.54 (t, 1H, $J = 1.8 \text{ Hz}$), 6.51-6.40 (0.5H), 5.91 (merged d, 2H, $J = 3.7 \text{ Hz}$), 3.86-3.81 (6H), 3.49 (q, 2H, $J = 8.9 \text{ Hz}$), 3.26 (q, 2H, $J = 6.9 \text{ Hz}$), 2.87-2.74 (m, 4H), 2.66-2.61 (m, 2H), 2.36 (t, 1H, $J = 7.9 \text{ Hz}$), 2.29 (t, 1H, $J = 7.9 \text{ Hz}$); $^{13}$C NMR (125 MHz, CDCl$_3$): $\delta$ 171.8, 171.7, 162.36, 162.34, 160.42, 160.40, 149.1, 148.9, 147.92, 147.90, 147.6, 147.5, 146.3, 146.0, 137.09, 137.03, 133.1, 133.8, 131.8, 130.6, 129.8, 129.79, 129.76, 129.73, 121.69, 121.66, 120.7, 120.6, 115.26, 115.22, 115.09, 115.06, 112.0, 111.9, 111.4, 111.2, 109.2, 109.0, 108.5, 108.3, 100.8, 100.0, 55.92, 55.90, 55.0, 48.43, 48.42, 35.0, 34.9, 34.8, 34.7, 33.7, 33.6, 30.5; HRESIMS: calcd for C$_{28}$H$_{31}$FNO$_5$ [M+H]$^+$ 480.2186; found 480.2184

$^{N}$-(2-(benzo[d][1,3]dioxol-5-yl)ethyl)-3-(4-chlorophenyl)-$^{N}$-(3,4-dimethoxyphenethyl)propanamide (254j): clear oil; 79 %; rotamers observed, all signals reported; $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ 7.23-7.20 (m, 2H), 7.04 (dd, 2H, $J = 17.0$, 8.3 Hz), $\delta$ 6.79-6.69 (3.5H), 6.60 (t, 1H, $J = 4.9 \text{ Hz}$), 6.58 (s,1H), 6.50-6.48 (0.5H), 5.89 (d, 2H, $J = 3.0 \text{ Hz}$),

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N-(2-(benzo[d][1,3]dioxol-5-yl)ethyl)-3-(bromophenyl)-N-(3,4-
dimethoxyphenethyl)propanamide (254k): clear oil; 76 %; rotamers observed, all signals reported; \(^1H\) NMR (500 MHz, CDCl\(_3\)): \(\delta\) 7.39-7.36 (m, 2H), 7.00 (dd, 2H, \(J = 8.1, 1.5\) Hz), 6.79 (t, 1H, \(J = 1.7\) Hz), 6.77-6.70 (m, 2H), 6.68 (0.5H), 6.60 (t, 1H, \(J = 7.5\) Hz), 6.54-6.53 (m, 1H), 5.91 (2H), 3.86-3.81 (6H), 3.48 (q, 2H, \(J = 7.3\) Hz), 3.26 (t, 2H, \(J = 2.2\) Hz), 2.85-2.74 (m, 4H), 2.63 (q, 2H, \(J = 7.4\) Hz), 2.31 (t, 1H, \(J = 1.6\) Hz), 2.28 (t, 1H, \(J = 1.5\) Hz); \(^13C\) NMR (125 MHz, CDCl\(_3\)): \(\delta\) 171.58, 171.51, 149.0, 148.9, 147.89, 147.87, 147.6, 147.5, 146.3, 146.0, 140.45, 140.42, 133.0, 131.7, 131.43, 131.41, 130.6, 130.19, 130.17, 121.68, 121.63, 120.7, 120.6, 119.79, 119.75, 112.0, 111.9, 111.4, 111.2, 109.1, 109.0, 108.4, 108.2, 100.9, 100.8, 55.9, 55.8, 50.0, \(\delta\) 48.36, \(\delta\) 48.35, \(\delta\) 34.9, \(\delta\) 34.7, \(\delta\) 34.5, \(\delta\) 34.4, \(\delta\) 33.7, \(\delta\) 33.5, \(\delta\) 30.66, \(\delta\) 30.64; HRESIMS: calcd for C\(_{28}\)H\(_{31}\)BrNO\(_5\) [M+H]\(^+\) 540.1385; found 526.1383

N-(2-(benzo[d][1,3]dioxol-5-yl)ethyl)-N-(3,4-dimethoxyphenethyl)-3-(4-
iodophenyl)propanamide (254l): clear oil; 81 %; rotamers observed, all signals reported; \(^1H\) NMR (500 MHz, CDCl\(_3\)): \(\delta\) 7.59-7.57 (m, 2H), 6.96 (dd, 2H, \(J = 11.9, 8.3\) Hz), 6.87 (t, 1H, \(J = 8.1\) Hz), 6.79-6.69 (m, 2H), 6.68 (0.5H), 6.60 (td, 1H, \(J = 7.8, 1.6\) Hz), 6.53 (dd, 1H, \(J = 4.0, 1.5\) Hz), 6.50-6.48 (0.5H), 5.91 (merged d, 2H, \(J = 3.5\) Hz), 3.86-3.81 (6H), 3.48 (q, 2H, \(J = 8.5\) Hz), 3.26 (q, 2H, \(J = 4.0\) Hz), 2.83-2.74 (m, 4H), 2.63 (q, 2H, \(J = 4.0\) Hz), 2.33 (t, 1H, \(J = 8.0\) Hz), 2.27
(t, 1H, J = 8.0 Hz); $^{13}$C NMR (125 MHz, CDCl$_3$): δ 171.6, 171.5, 149.0, 148.9, 147.92, 147.90, 147.6, 147.5, 146.4, 146.0, 141.14, 137.46, 137.43, 133.0, 131.7, 130.6, 130.55, 130.53, 121.7, 121.6, 120.7, 120.6, 112.0, 111.9, 111.4, 111.2, 109.2, 109.0, 108.5, 108.3, 101.0, 100.8, 91.1, 91.0, 55.93, 55.91, 50.0, 48.4, 34.9, 34.7, 34.5, 33.7, 33.5, 33.4, 30.78, 30.76; HRESIMS: calcd for C$_{28}$H$_{31}$INO$_5$ [M+H]$^+$ 588.1247; found 588.1243

**General procedure for the synthesis of 255a-l:** To a solution of appropriate amide 254a-l (1.0 equiv.) in anhydrous THF (10 ml) under N$_2$, BF$_3$-Et$_2$O (2 equiv.) was added. The resulting reaction mixture was heated under reflux for 30 min after which a 1M solution of BH$_3$-THF (2.5 equiv.) was carefully added dropwise. The resulting reaction mixture was heated under reflux for another 3.5 h. After 3.5 h the reaction mixture was allowed to cooled to 0 °C, followed by the careful dropwise addition of 3N HCl (15 mL). The resulting solution was evaporated under vacuum, followed by addition of 3N NaOH (20 mL) to adjust pH to 8-9. The resulting aqueous solution was extracted using dichloromethane (3 x 30 ml). The combined organic layer was dried over Na$_2$SO$_4$ and evaporated to dryness under vacuum to afford the corresponding amine 255a-l. The crude amines were purified on a silica gel column using 2 – 5 % MeOH – DCM.

**N-(2-(benzo[d][1,3]dioxol-5-yl)ethyl)-N-(3,4-dimethoxyphenethyl)-3-(2-fluorophenyl)propan-1-amine (255a):** light brown oil; 67 %; $^1$H NMR (500 MHz, CDCl$_3$): δ 7.16 (t, 2H, J = 8.3 Hz), 7.04 (t, 1H, J = 8.2 Hz), 6.98 (d, 1H, J = 10.5 Hz), 6.78 (d, 1H, J = 10.8 Hz), 6.72-6.70 (m, 3H), 6.66 (s, 1H), 6.60 (d, 1H, J = 8.2 Hz), 5.89 (s, 2H), 3.85 (merged s, 6H), 2.73-2.58 (m, 12H), 1.82-1.74 (m, 2H); $^{13}$C NMR (500 MHz, CDCl$_3$): δ 162.4, 159.9, 148.8, 147.5, 147.3, 145.7, 134.5, 133.3, 130.6, 130.6, 129.2, 129.0, 127.5, 127.4, 123.94, 123.91, 121.4, 120.6, 115.0, 112.1, 111.3, 109.2, 108.1, 100.7, 56.2, 55.9, 55.8, 53.4, 33.5, 33.4, 27.7, 26.9, 25.6; HRESIMS: calcd for C$_{28}$H$_{33}$FNO$_4$ [M+H]$^+$ 466.2393; found 466.2394
**N-(2-(benzo[d][1,3]dioxol-5-yl)ethyl)-3-(2-chlorophenyl)-N-(3,4-dimethoxyphenethyl)propan-1-amine (255b):** light brown oil; 83 %; $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ 7.33 (d, 1H, $J = 9.4$ Hz), 7.17 (d, 2H, $J = 5.1$ Hz), 7.14-7.10 (m, 1H), 6.79 (d, 1H, $J = 10.8$ Hz), 6.73-6.71 (m, 3H), 6.67 (s, 1H), 6.61 (d, 1H, $J = 8.1$ Hz), 5.90 (s, 2H), 3.85 (merged s, 6H), 2.74-2.61 (m, 12H), 1.79 (quin, 2H, $J = 9.7$ Hz); $^{13}$C NMR (500 MHz, CDCl$_3$): $\delta$ 148.8, 147.5, 147.3, 145.7, 139.8, 134.4, 133.8, 133.2, 130.3, 129.4, 127.2, 126.7, 121.4, 120.5, 112.0, 111.2, 109.1, 108.1, 100.7, 56.126, 56.120, 55.9, 55.8, 53.3, 33.4, 33.2, 31.5, 27.1; HRESIMS: calcd for C$_{28}$H$_{33}$ClNO$_4$ [M+H]$^+$ 482.2098; found 482.2100

**N-(2-(benzo[d][1,3]dioxol-5-yl)ethyl)-3-(2-bromophenyl)-N-(3,4-dimethoxyphenethyl)propan-1-amine (255c):** light brown oil; 81 %; $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ 7.51 (d, 1H, $J = 9.0$ Hz), 7.24-7.15 (m, 2H), 7.03 (t, 1H, $J = 9.3$ Hz), 6.78 (d, 1H, $J = 10.8$ Hz), 6.72-6.70 (m, 3H), 6.67 (s, 1H), 6.61 (d, 1H, $J = 8.0$ Hz), 5.89 (s, 2H), 3.85 (merged s, 6H), 2.75-2.61 (m, 12H), 1.78 (quin, 2H, $J = 9.5$ Hz); $^{13}$C NMR (500 MHz, CDCl$_3$): $\delta$ 148.8, 147.5, 147.3, 145.7, 141.6, 134.4, 133.3, 132.8, 130.3, 127.5, 127.4, 124.4, 121.5, 120.6, 112.1, 111.2, 109.2, 108.1, 100.7, 56.19, 56.18, 55.9, 55.8, 53.3, 34.1, 33.5, 31.3, 27.4; HRESIMS: calcd for C$_{28}$H$_{33}$BrNO$_4$ [M+H]$^+$ 526.1593; found 526.1595

**N-(2-(benzo[d][1,3]dioxol-5-yl)ethyl)-N-(3,4-dimethoxyphenethyl)-3-(2-iodophenyl)propan-1-amine (255d):** light brown oil; 77 %; $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ 7.82 (d, 1H, $J = 9.8$ Hz), 7.29 (d, 2H, $J = 9.0$ Hz), 7.21-7.16 (m, 1H), 6.91 (t, 1H, $J = 9.0$ Hz), 6.80 (d, 1H, $J = 10.8$ Hz), 6.74-6.71 (m, 3H), 6.67 (s, 1H), 6.65-6.60 (m, 2H), 5.92 (s, 2H), 3.87-3.85 (merged d, 6H, $J = 6.9$ Hz), 3.12-2.57 (broad s, 12H), 2.17-1.78 (broad s, 2H); $^{13}$C NMR (500 MHz, CDCl$_3$): $\delta$ 148.8, 147.5, 147.4, 145.8, 144.6, 137.4, 134.9, 130.1, 127.7 (x 2), 121.5, 120.5, 112.0, 111.3, 109.1,
108.2, 100.8, 94.4, 55.95, 55.94, 55.92, 55.91, 52.9, 33.2, 33.1, 33.0, 28.4; **HRESIMS**: calcd for C\textsubscript{28}H\textsubscript{33}INO\textsubscript{4} [M+H]\textsuperscript{+} 574.1474; found 574.1450

\textbf{N-(2-(benzo[d][1,3]dioxol-5-yl)ethyl)-N-(3,4-dimethoxyphenethyl)-3-(3-fluorophenyl)propan-1-amine (255e)}: light brown oil; 69 \%; **\textsuperscript{1}H NMR** (500 MHz, CDCl\textsubscript{3}): δ 7.24-7.19 (m, 1H), 6.92 (d, 1H, \textit{J} = 7.5 Hz), 6.88-6.84 (m, 2H), 6.79 (d, 1H, \textit{J} = 8.0 Hz), 6.72-6.69 (m, 3H), 6.66 (s, 1H), 6.60 (d, 1H, \textit{J} = 6.0 Hz), 5.90 (s, 2H), 3.85 (merged s, 6H), 2.74-2.63 (m, 8H), 2.63-2.54 (m, 4H), 1.76 (quin, 2H, \textit{J} = 7.50 Hz); **\textsuperscript{13}C NMR** (125 MHz, CDCl\textsubscript{3}): δ 163.8, 161.9, 148.8, 147.5, 147.3, 145.7, 145.0, 144.9, 134.4, 133.2, 129.68, 129.62, 124.04, 124.02, 121.4, 120.5, 115.2, 115.1, 112.6, 112.4, 112.0, 111.2, 109.1, 108.1, 100.7, 56.18, 56.15, 55.9, 55.8, 53.2, 33.5, 33.39, 33.35, 33.3, 28.7; **HRESIMS**: calcd for C\textsubscript{28}H\textsubscript{33}INO\textsubscript{4} [M+H]\textsuperscript{+} 466.2393; found 466.2394

\textbf{N-(2-(benzo[d][1,3]dioxol-5-yl)ethyl)-3-(3-chlorophenyl)-N-(3,4-dimethoxyphenethyl)propan-1-amine (255f)}: light brown oil; 75 \%; **\textsuperscript{1}H NMR** (500 MHz, CDCl\textsubscript{3}): δ 7.32-7.30 (m, 2H), 7.14 (t, 1H, \textit{J} = 7.7 Hz), 7.07 (d, 1H, \textit{J} = 7.6 Hz), 6.79 (d, 1H, \textit{J} = 7.8 Hz), 6.73-6.70 (m, 3H), 6.66 (s, 1H), 6.61 (d, 1H, \textit{J} = 6.4 Hz), 5.91 (s, 2H), 3.86 (merged s,
6H), 2.74-2.68 (m, 8H), 2.57-2.54 (m, 4H), 1.77 (t, 2H, J = 8.4 Hz); ¹³C NMR (125 MHz, CDCl₃): δ 148.8, 147.5, 147.3, 145.7, 144.5, 134.2, 133.0, 131.4, 129.8, 128.9, 127.0, 122.4, 121.4, 120.5, 112.0, 111.2, 109.1, 108.2, 100.8, 56.0, 55.99, 55.94, 55.8, 53.0, 33.2, 33.18, 33.13, 28.5; HRESIMS: calcd for C₂₈H₃₃BrNO₄ [M+H]+ 526.1593; found 526.1596

N-(2-(benzo[d][1,3]dioxol-5-yl)ethyl)-N-(3,4-dimethoxyphenethyl)-3-(3-iodophenyl)propan-1-amine (255h): light brown oil; 78%; ¹H NMR (500 MHz, CDCl₃): δ 7.53-7.50 (m, 2H), 7.10 (d, 1H, J = 7.6 Hz), 7.00 (t, 1H, J = 7.7 Hz), 6.79 (d, 1H, J = 7.9 Hz), 6.73-6.70 (m, 3H), 6.66 (s, 1H), 6.61 (d, 1H, J = 6.3 Hz), 5.91 (s, 2H), 3.86 (merged s, 6H), 2.73-2.64 (m, 8H), 2.57-2.51 (m, 4H), 1.76 (t, 2H, 7.1 Hz); ¹³C NMR (125 MHz, CDCl₃): δ 148.8, 147.5, 147.3, 145.7, 144.6, 137.45, 137.45, 134.8, 130.08, 130.08, 127.6, 121.4, 120.5, 112.0, 111.2, 109.1, 108.2, 100.7, 94.4, 56.02, 56.00, 55.9, 55.8, 53.0, 33.2, 33.1, 33.0, 28.6; HRESIMS: calcd for C₂₈H₃₃INO₄ [M+H]+ 574.1474; found 574.1455

N-(2-(benzo[d][1,3]dioxol-5-yl)ethyl)-N-(3,4-dimethoxyphenethyl)-3-(4-fluorophenyl)propan-1-amine (255i): light brown oil; 73%; ¹H NMR (500 MHz, CDCl₃): δ 7.11-7.07 (m, 2H), 6.95 (t, 2H, J = 9.7 Hz), 6.79 (d, 1H, J = 10.0 Hz), 6.73-6.69 (m, 3H), 6.66 (s, 1H), 6.60 (d, 1H, J = 8.0 Hz), 5.90 (s, 2H), 3.85 (merged s, 6H), 2.73-2.65 (m, 8H), 2.58-2.54 (m, 4H), 1.75 (quin, 2H, J = 10.0 Hz); ¹³C NMR (125 MHz, CDCl₃): δ 162.1, 160.2, 148.8, 147.5, 147.3, 145.7, 137.88, 137.86, 134.4, 133.2, 129.69, 129.63, 121.4, 120.5, 115.0, 114.9, 112.0, 111.2, 109.1, 108.1, 100.7, 56.17, 56.13, 55.9, 55.8, 53.2, 33.4, 33.3, 32.7, 29.1; HRESIMS: calcd for C₂₈H₃₃FNO₄ [M+H]+ 466.2393; found 466.2396

N-(2-(benzo[d][1,3]dioxol-5-yl)ethyl)-3-(4-chlorophenyl)-N-(3,4-dimethoxyphenethyl)propan-1-amine (255j): light brown oil; 81%; ¹H NMR (500 MHz, CDCl₃): δ 7.24 (t, 2H, J = 5.5 Hz), 7.07 (d, 2H, J = 8.3 Hz), 6.79 (d, 1H, J = 7.8 Hz), 6.73-6.71
(m, 3H), 6.69 (s, 1H), 6.60 (d, 1H, J = 6.4 Hz), 5.91 (s, 2H), 3.85 (merged s, 6H), 2.74-2.68 (m, 8H), 2.58-2.55 (m, 4H), 1.76 (t, 2H, J = 6.8 Hz); \textbf{\textsuperscript{13}C NMR} (125 MHz, CDCl\textsubscript{3}): δ 148.8, 147.5, 147.4, 145.8, 140.5, 134.1, 132.9, 131.5, 129.7 (x 2), 128.4 (x 2), 121.47, 120.5, 112.0, 111.2, 109.1, 108.2, 100.8, 56.0, 55.99, 55.94, 55.8, 53.1, 33.19, 33.13, 32.8, 28.6; \textbf{HRESIMS}: calcd for C\textsubscript{28}H\textsubscript{33}ClNO\textsubscript{4} [M+H]\textsuperscript{+} 482.2098; found 482.2101

\textbf{\textit{N-(2-(benzo[d][1,3]dioxol-5-yl)ethyl)-3-(4-bromophenyl)-N-(3,4-dimethoxyphenethyl)propan-1-amine}} (255k): light brown oil; 80 %; \textbf{\textsuperscript{1}H NMR} (500 MHz, CDCl\textsubscript{3}): δ 7.38 (d, 2H, J = 10.4 Hz), 7.01 (d, 2H, J = 10.3 Hz), 6.79 (d, 1H, J = 9.8 Hz), 6.73-6.69 (m, 3H), 6.65 (s, 1H), 6.60 (d, 1H, J = 7.9 Hz), 5.90 (s, 2H), 3.85 (merged s, 6H), 2.74-2.62 (m, 8H), 2.56-2.52 (m, 4H), 1.73 (quin, 2H, J = 9.6 Hz); \textbf{\textsuperscript{13}C NMR} (125 MHz, CDCl\textsubscript{3}): δ 148.8, 147.5, 147.3, 145.7, 141.2, 134.4, 133.2, 131.3 (x 2), 130.1 (x 2), 121.4, 120.5, 119.4, 112.1, 111.2, 109.2, 108.1, 100.8, 56.16, 56.12, 55.9, 55.8, 53.2, 33.4, 33.3, 33.0, 28.9; \textbf{HRESIMS}: calcd for C\textsubscript{28}H\textsubscript{33}BrNO\textsubscript{4} [M+H]\textsuperscript{+} 526.1593; found 526.1594

\textbf{\textit{N-(2-(benzo[d][1,3]dioxol-5-yl)ethyl)-N-(3,4-dimethoxyphenethyl)-3-(4-iodophenyl)propan-1-amine}} (255l): light brown oil; 75 %; \textbf{\textsuperscript{1}H NMR} (500 MHz, CDCl\textsubscript{3}): δ 7.24-7.13 (m, 3H), 7.01 (d, 2H, J = 9.0 Hz), 6.79 (d, 1H, J = 10.0 Hz), 6.72-6.70 (m, 3H), 6.66 (s, 1H), 6.60 (d, 1H, J = 7.8 Hz), 5.89 (s, 2H), 3.85 (merged s, 6H), 2.73-2.64 (m, 8H), 2.57-2.53 (m, 4H), 1.75 (quin, 2H, J = 9.6 Hz); \textbf{\textsuperscript{13}C NMR} (125 MHz, CDCl\textsubscript{3}): δ 148.8, 147.5, 147.3, 145.7, 144.4, 134.4, 134.0, 133.2, 129.5, 128.5, 126.6, 125.9, 121.4, 120.5, 112.1, 111.3, 109.2, 108.1, 100.8, 56.13, 56.11, 55.9, 55.8, 53.1, 33.4, 33.3, 33.2, 28.7; \textbf{HRESIMS}: calcd for C\textsubscript{28}H\textsubscript{33}INO\textsubscript{4} [M+H]\textsuperscript{+} 574.1474; found 574.1460
3.7.2 Biological Evaluations:

A) Primary and secondary radioligand binding assays:

Both the assays were done at the PDSP facility. In the primary binding assays, compounds were usually tested at single concentrations (10 µM) in quadruplicate in 96-well plates. Compounds that showed a minimum of 50% inhibition at 10 µM were tagged for secondary radioligand binding assays to determine equilibrium binding affinity at specific targets. In the secondary binding assays, selected compounds were usually tested at 11 concentrations (0.1, 0.3, 1, 3, 10, 30, 100, 300 nM, 1, 3, 10 µM) and in triplicate (3 sets of 96-well plates). Both primary and secondary radioligand binding assays were carried out in a final of volume of 125 µl per well in appropriate binding buffer. The hot ligand concentration was usually at a concentration close to the $K_d$ (unless otherwise indicated). Total binding and nonspecific binding were determined in the absence and presence of 10 µM appropriate reference compound, respectively. In brief, plates were usually incubated at room temperature and in the dark for 90 min (unless otherwise indicated). Reactions were stopped by vacuum filtration onto 0.3% polyethyleneimine (PEI) soaked 96-well filter mats using a 96-well Filtermate harvester, followed by three washes with cold wash buffer. Scintillation cocktail was then melted onto the microwave-dried filters on a hot plate and radioactivity was counted in a Microbeta counter. For detailed experimental details please refer to the PDSP website http://pdsp.med.unc.edu/ and click on ‘Binding Assay’ or ‘Functional Assay’ on the menu bar.
CHAPTER IV: MOLECULAR DOCKING STUDIES
4.1 Introduction

The central goal of our research is to design and synthesize aporphine alkaloid analogs as selective and potent 5-HT\textsubscript{2A} antagonists. In order to do so, an exhaustive SAR study to identify structural tolerance of the aporphine template, required for 5-HT\textsubscript{2A} antagonism has been conducted (reported previously as well as described in the previous chapter). The analogs in these studies were designed to investigate the role of substitution at the C1, C2, C3 and C4 positions, to study the importance of the nitrogen atom and to study the tolerance of an indole ring on the aporphine scaffold.\textsuperscript{69,122,123,140,144,176,213} These SAR studies have resulted in the identification of several high affinity 5-HT\textsubscript{2A} ligands.

In order to understand the possible binding modes of nantenine analogs at the human 5-HT\textsubscript{2A} receptor as well as for the retrospective evaluation of high affinity ligands, molecular docking studies were undertaken.

Since the crystal structure of the 5-HT\textsubscript{2A} receptor is not available, we relied on a homology model of the 5-HT\textsubscript{2A} receptor. The use of homology modelling has been reported to become common in recent times.\textsuperscript{214} Our group had previously reported the construction and optimization of a homology model of the 5-HT\textsubscript{2A} receptor using the human \(\beta_2\)-adrenoceptor (PDB code: 2RH1) as the template, using ICM Pro software.\textsuperscript{123,215} Results of the molecular docking studies performed using this model are described.

4.2 Results and Discussion:
4.2.1 Molecular docking studies of C3 halogenated analogues:

In order to understand the structural tolerance for antagonistic activity and selectivity of the aporphine scaffold at the 5-HT$_{2A}$ receptor, a series of ring A nantenine analogs were synthesized and evaluated. As a part of this study, a series of C3 halogenated nantenine analogs (259-262 and 232) were synthesized and evaluated, to test the effect of halogenation at the C3 position on 5-HT$_{2A}$ receptor affinity. Table 12 displays the corresponding binding affinity data for these compounds.

![Figure 59: Structures of compound 258, nantenine (55) and C3 halogenated aporphinoid 5-HT$_{2A}$ antagonists 259-262 and 232](image)

Table 12 displays the corresponding binding affinity data for these compounds.
Table 12: \(K_e\) values of C3 analogues at the 5-HT\(_{2A}\) receptor.

As shown in Table 12, incorporation of a halogen atom to the C3 position of compound 258 (i.e. compounds 259-261) resulted in doubling of the 5-HT\(_{2A}\) antagonist potency irrespective of the halogen substituent. A greater enhancement in potency was observed when a halogen atom was added to the C3 position of compound 55 (i.e. compounds 262 and 232). Thus incorporation of chlorine at the C3 position (compound 262) resulted in a 13 fold increase in 5-HT\(_{2A}\) receptor affinity compared to compound 55. The 3-bromo compound (232) was 17 times more potent than
the parent compound nantenine (55) and was the most potent compound identified in this series. When compound 262 is compared with 259 it is apparent that methylation of the C2 phenol group in 259 results in improved 5-HT_{2A} potency. The same trend was observed with compound 232 and 260. In general halogenation at the C3 position for 5-HT_{2A} antagonism is well tolerated when either a C2 hydroxyl or C2 methoxyl group is present.

To rationalize the improved affinity of the C3 halogenated compounds at the 5-HT_{2A} receptor, these compounds were docked into a homology model of the 5-HT_{2A} receptor. The homology model was constructed as previously described with ICM Pro using a human β₂-adrenoceptor template. Docking was performed using ICM Pro software as previously described.

**Figure 60:** Compounds 259 (green), 260 (green) and 261 (violet) docked in the 5-HT_{2A} receptor
Figure 60 shows the binding pose of compounds 259, 260 and 261. As it can be seen, all the three compounds have a similar binding pose, where the halogen atom seems interact with Phe339/Phe340 residues of the 5-HT$_{2A}$ receptor. The enhanced affinities of these compound (compared to 1) might be due to this interaction.

Figure 61: 2D representations of the binding pose of compound 259, 260 and 261 in the 5-HT$_{2A}$ receptor
A two-dimensional representation (prepared using Maestro) of the binding interactions for compounds 259, 260 and 261 is shown in Figure 61. Apart from the important Asp155 interaction with the protonated nitrogen atom in the ligand, several hydrophobic residues as well as the hydrophilic residues such as Asn343, Ser239, Ser242, Ser159, and Asn363 are important for the binding of these compounds.

A direct comparison between the binding poses of compounds 258 and 260 can be seen in Figure 62. As seen in Figure 62, compound 258 which lacks a halogen atom, has a completely different binding orientation than the halogenated compound 260.

**Figure 62:** Docking poses of compound 258 (purple) and 260 (black) in the 5-HT$_{2A}$ receptor

Overall, the docking studies suggest that the presence of a C3 halogen atom allows for a different orientation of the halogenated analogs in the receptor as compared to non-halogenated analogs

Figure 63 shows an overlay of binding poses for compounds 260, 262 and 232. These compounds allow for some qualitative comparison of the effect of different halogen atoms at C3
(i.e., 262 versus 232) as well as the effect of the presence of a methoxyl or hydroxyl group at C2 (i.e. 260 versus 232).

Figure 63: Compounds 260 (green), 262 (cyan) and 232 (blue) docked in the 5-HT₂A receptor

Halogen atoms have been in used in several cases of hit-to-lead as well as lead-to-drug conversions.²¹⁶-²¹⁸ Halogenation is one of the common method to increase the metabolic stability and lipophilicity of pharmacologically active compounds.²¹⁹ Moreover, incorporation of halogen atoms is known to enhance Blood Brain Barrier (BBB) permeability and hence is widely employed in drugs that target CNS.²²⁰
At a molecular level, halogen atoms are involved in non-covalent interactions which are now commonly referred as halogen bonding.\textsuperscript{221} Halogen bonding refers to an interaction of the type R-X····Y-R', where the halogen atom X acts as a Lewis acid and Y can be any electron donor moiety.\textsuperscript{209} This halogen bonding often results due to the σ-hole, which is a positively charged region on the back side of X, along the R-X bond axis that is caused by an anisotropy of electron density on the halogen atom.\textsuperscript{222,223} Figure 64 shows a schematic display of halogen bonding and the σ-hole.

![Figure 64: Schematic representation of halogen binding. (Most positive surface potential (including the σ-hole) is colored in red, while most negative surface potential is colored in cyan. (J. Med. Chem., 2013, 56, 1363-1388)](image)

Thus any potential Lewis base in the binding pocket could interact with the ligand halogen atom. Such potential donors can be present either in the backbone groups (e.g. carbonyl groups)\textsuperscript{210,211} or in the side chain groups (e.g. hydroxyl or carboxylate groups).\textsuperscript{224,225} Other side chain interactions involve hydroxyls in serine, threonine and tyrosine, sulfurs in cysteine and methionine, and the π surfaces of phenylalanine, tyrosine, histidine and tryptophan.\textsuperscript{209}

Xu and co-workers demonstrated the application of halogen bonding in drug design through the synthesis and evaluation of Phosphodiesterase Type 5 (PDE5) inhibitors. The halogenated analogues 8a-d were found to be more potent than their non-halogenated parent. Figure 65 shows the docking pose of 263a-d, where the tyrosine residue forms a halogen bond.\textsuperscript{226}
Electrostatic potential (ESP) studies done on halogenated aromatic systems suggests that the size of the σ-hole (or the positive charge) increases with increasing size of the halogen. In other words, the strength of the halogen bond interaction increases with increasing size of the halogen atom.\textsuperscript{210,227} However, it should be noted that due to its high electronegativity, the fluorine atom is not capable of forming a σ-hole.\textsuperscript{228}

![Diagram of compound 263a-d](image)

\textbf{Figure 65}: Phosphodiesterase Type 5 (PDE5) inhibitors 263a-d docked in the PDE5. (\textit{J. Med. Chem.}, \textbf{2011}, 54, 5607)

Results from our studies show that the C3 halogen atoms (in compound 259-262 and 232) lie in close proximity to the phenyl ring of the Phe339 and Phe340 residues. The proximity of the halogen atoms in the ligands with Phe339/Phe340 suggests that this interaction is a significant one and may in part be responsible for the higher affinity observed in the C3-halogenated compounds. The difference in affinities between compounds 262 and 232 can be rationalized through the larger size of bromine atom (and hence a potentially larger σ-hole) which results in stronger interaction. However since compounds 259-261 have similar affinities, the role of other hydrophobic and polar residues in the receptor pocket cannot be ruled out to explain this difference.
4.2.2 Molecular docking studies of C1 nantenine analogues:

Prior SAR studies of nantenine have been largely directed towards the C1 and C2 positions of the aporphine scaffold of nantenine. These studies have shown that substituents at the C1 position of nantenine may be manipulated to improve 5-HT$_2$A affinity and selectivity.$^{122,213}$ SAR results indicate that n-alkyl substituents at the C1 position are particularly well tolerated. Among several derivatives synthesized and evaluated, the C1 allyl analogue 138 is one of the most potent compounds identified ($K_e = 70$ nM). Compared to nantenine ($55$, $K_e = 830$ nM), compound 138 has 12 times higher 5-HT$_2$A receptor affinity. Moreover, unlike nantenine, compound 138 is devoid of affinity for the $\alpha_{1A}$ adrenergic receptor. This improvement in 5-HT$_2$A receptor affinity and selectivity can be attributed to the electron rich nature of the allyl group. However other effects such as steric and hydrophobic effects cannot be ruled out. In order to probe whether the improvement in affinity was due to the electronic nature of the allyl group, a series C1 isosteric analogues, having diverse electronic, steric and hydrophobic characteristics were synthesized and evaluated at the 5-HT$_2$A receptor.$^{229}$

![Figure 66: Structures of C1 allyl analogue 138 and C1 allyl isosteres 264-266](image)

Since the benzyl group contains an allylic substructure, compound 264, 265 and 266 having a substituted benzyl functionality were synthesized as a part of this study. Compound 264 having a $p$-chloro benzyl moiety did not have any appreciable 5-HT$_2$A receptor affinity ($K_e = 6046$ nM).
Replacing the chloro group with a CF$_3$ group (compound 265), resulted in slight improvement in affinity ($K_e = 2458$ nM). Switching to the $p$-bromo benzyl group (compound 266), however resulted in a dramatic increase in 5-HT$_2A$ receptor affinity ($K_e = 9.2$ nM). Compound 266 is 7 times more potent than the allyl analogue 138 and 92 times more potent than nantenine (55). In fact compound 266 is the most potent 5-HT$_2A$ aporphinoid antagonist reported till date.

![Figure 67: Compounds 264 (cyan), 265 (brown) and 266 (purple) docked in the 5-HT$_2A$ receptor](image)

In order to rationalize the dramatic difference in the affinities of compound 264, 265 and 266, they were docked into the homology model of 5-HT$_2A$ receptor.
Figure 67 shows an overlay of the binding pose of 264, 265 and 265. As seen, all the three benzyl substituted compounds have strikingly similarly binding orientation. The ICM docking scores for compounds 264, 265 and 265 are -81.77 kcal/mole, -85.06 kcal/mole and -77.34 kcal/mole respectively. Unfortunately these ICM docking scores do not correlate with the order/rank of affinities. In fact docking scores are not always useful in predicting the rank of affinities; they are more useful for predicting activity vs non-activity.\(^ {230}\)

While comparing the activities of compound 264 and 266, compound 266 has higher affinity possibly due to the higher hydrophobicity of the larger bromine atom. Figure 68 shows an overlay of binding pose of compounds 264 and 266, with the halogen atoms depicted using space filling model. As seen in Figure 68, the larger bromine atom seems to be buried deeply in the binding pocket compared to the chlorine atom.

In the case of affinities of compound 265 and 266, the larger CF\(_3\) group (in 265) would be expected to be buried deeply in the binding pocket. However, this postulate is contradicted by the relatively large difference in binding affinities of 265 and 266. One plausible reason could be that the sp\(^2\) C-CF\(_3\) bond length in compound 265 (1.49 Å) is significantly lower than the sp\(^2\) C-Br bond length in compound 266 (1.88 Å), thus positioning compound 266 deeper in the binding pocket than 265. It might also be possible that the electron withdrawing CF\(_3\) group in 265, might have an unfavorable interaction with a particular side chain residue. Figure 69 shows an overlay of binding pose of compounds 265 and 266, with the halogen atoms depicted using space filling model.

Finally it could be argued that the larger and more hydrophobic CF\(_3\) group (in compound 265) could lead to a favorable binding interaction with the receptor and hence has better affinity when compared to compound 264.
Figure 68: Compounds 264 (cyan) and 266 (purple) docked in the 5-HT$_2A$ receptor (halogen atoms are depicted using space filling model).
**Figure 69:** Compounds 265 (brown) and 266 (purple) docked in the 5-HT$_2A$ receptor (halogen atoms are depicted using space filling model).

Figures A-89, A-90 and A-91 (see appendix-B) show the 2D view of the docking poses of compounds 264, 265 and 266 respectively.

Overall the current 5-HT$_2A$ receptor model does not predict accurately the binding behavior of compounds 264, 265 and 266. It is quite plausible that combination of effects (steric and electronegativity) might be responsible for this dramatic difference in the observed binding affinities. A better refined 5-HT$_2A$ receptor homology model and larger library of analogues might help understand the binding behavior of these compounds.
4.2.3 Molecular docking studies of C2 analogues:

As described earlier, SAR studies of nantenine have largely focused on the synthesis of C1 and C2 analogues. Previous SAR data indicates that only small alkyl groups are tolerated at the C2 position. Replacement with larger alkyl groups was found to be detrimental for affinity at the 5-HT$_{2A}$ receptor. However only a small group of C2 analogues were synthesized in this study. Furthermore, the synthesized compounds were tested as racemates. In order to investigate the tolerance of the C2 position towards other alkyl substitution patterns a library of C2 analogues was synthesized. The synthesis of these analogues was achieved using a semi-synthetic route starting from the commercially available aporphine alkaloid boldine (56). (See scheme 3.1, in appendix)

Based on the SAR data compound 268 and 269 were identified as potent 5-HT$_{2A}$ ligands. Compound 268 which has a benzyl group at the C-2 position displayed a $K_i$ value of 22 nM, while the C-2 p-bromo phenylethyl substituted compound 269 has a $K_i$ value of 20 nM. The tetra-alkylated aporphine alkaloid glaucine (267) displays moderate affinity for the 5-HT$_{2A}$ receptor. Compared to glaucine (267), analogues 268 and 269 display 48 fold higher potency.

![Figure 70: Structures of boldine (56), glaucine (267) and C-2 analogues 268 and 269](image-url)
In order to rationalize their enhanced affinity, compound 268 and 269 were docked into a homology model of the 5-HT$_{2A}$ receptor.

Figure 71 shows the binding pose of compound 268 in the 5-HT$_{2A}$ receptor. As shown, the protonated nitrogen atom of compound 268 forms a hydrogen bond interaction with the Asp155 residue. Apart from this interaction the C2 benzyl group seems to interact with the L228, L229 and K350 residues.

Figure 71: Binding pose of compound 268 in the 5-HT$_{2A}$ receptor

The binding pose of compound 269 in the binding cavity of 5-HT$_{2A}$ receptor is shown in Figure 72. Compound 269 has a similar binding orientation to compound 268. However in the case of compound 269, the $p$-bromo phenyl ring seems to be oriented towards the K350 residue.
Figure 72: Binding pose of compound 269 docked in the 5-HT$_{2A}$ receptor

An overlay of the binding poses of compound 267, 268 and 269 is shown in Figure 73. As it can be seen, all the compounds have a similar orientation. However the enhanced affinity of compound 268 and 269 can be attributed to the interaction of the C2 functionality with Leu228, Leu229 and Lys350 residues. The low affinity of glaucine (267) can be attributed to the lack of these interactions.
**Figure 73**: Compounds 267 (green) and 268 (red) and 269 (cyan) docked in the 5-HT$_{2A}$ receptor

Figures A-92, A-93 and A-94 (see appendix-B) show the 2D binding pose of compounds 268, 269 and 267 respectively. Figure A-95 (see appendix-B) displays compound 269 and its binding pocket.
4.3 Conclusions:

Molecular docking studies were used to rationalize the enhanced affinities of selected high affinity compounds. In the case of C3 halogenated compounds, interaction between the halogen atoms and Phe223/Phe224 residues might be responsible for the higher affinity of these compounds. Furthermore based on this information we have designed a series a C3 analogs having hydrophobic groups at this position (described in the previous chapter). In the case of the C2 $p$-bromo phenylethyl analogue, molecular docking studies helped to identify a key interaction between the C2 benzyl substituent and Lys350 residue.

Overall molecular docking studies were successfully employed to understand the binding modes of nantenine analogues and to provide retrospective insight into the high affinity of ligands. This information has expanded our existing knowledge about the interaction of nantenine analogues with the $5$-HT$_{2A}$ receptor, and thus helps us design better analogues.

Future efforts will focus on refining the existing 5-HT$_{2A}$ homology model as well as construction of new model using new templates (for example 5-HT$_{2B}$ receptor).

4.4 Experimental:

A) Ligand Preparation:

All the compounds were drawn as 2D structures with ChemDraw Ultra version 9.0, with a formal positive charge centered on the nitrogen, and then energy minimized through Chem3D Ultra version 9.0/MOPAC, Job Type: Minimum RMS Gradient of 0.010 kcal/mol and RMS distance of 0.1 Å, and saved as MDL MolFiles (*.mol).
B) ICM docking:

A homology model of the 5-HT$_{2A}$ receptor (*.pdb file) was first converted to an ICM file, followed by identification of the binding site. The binding site was reviewed and adjusted: ICM made a box around the ligand binding site based on the information entered in the receptor setup section. The position of the box encompassed the residues expected to be involved in ligand binding. Then the receptor maps were made: Energy maps of the environment within the docking box were constructed. Flexibility of the receptor residues was set to 4.0. The ligand was then converted to 3D using ICM ligand editor. Interactive docking was used to dock nantenine and the other analogs. The thoroughness level was set to the maximum value of 10. Once docked, all the receptor–ligand complexes were energy minimized and redocked, and visualized in 3-D using ICM Pro v 3.6. ICM calculated a score for the complexes. Poses depicted in this chapter are for the highest ranked complexes.

C) 2D Visualization using Maestro:

2D diagrams of selected docking poses were plotted using the ligand interaction tool of Maestro.
APPENDIX A: NMR SPECTRA OF FINAL ANALOGUES
Figure A-1: $^1$H NMR (500 MHz, CDCl$_3$) of compound 165a

Figure A-2: $^{13}$C NMR (500 MHz, CDCl$_3$) of compound 165a
Figure A-3: $^1$H NMR (500 MHz, CDCl$_3$) of compound 165b

Figure A-4: $^{13}$C NMR (500 MHz, CDCl$_3$) of compound 165b
Figure A-5: $^1$H NMR (500 MHz, CDCl$_3$) of compound 165c

Figure A-6: $^{13}$C NMR (500 MHz, CDCl$_3$) of compound 165c
Figure A-7: $^1$H NMR (500 MHz, CDCl$_3$) of compound 165d

Figure A-8: $^{13}$C NMR (500 MHz, CDCl$_3$) of compound 165d
Figure A-9: $^1$H NMR (500 MHz, CDCl$_3$) of compound 165e

Figure A-10: $^{13}$C NMR (500 MHz, CDCl$_3$) of compound 165e
Figure A-11: $^1$H NMR (500 MHz, CDCl$_3$) of compound 165f

Figure A-12: $^{13}$C NMR (500 MHz, CDCl$_3$) of compound 165f
Figure A-13: $^1$H NMR (500 MHz, CDCl$_3$) of compound 165g

Figure A-14: $^{13}$C NMR (500 MHz, CDCl$_3$) of compound 165g
Figure A-15: $^1$H NMR (500 MHz, CDCl$_3$) of compound 165h

Figure A-16: $^{13}$C NMR (500 MHz, CDCl$_3$) of compound 165h
Figure A-17: $^1$H NMR (500 MHz, CDCl$_3$) of compound 165i

Figure A-18: $^{13}$C NMR (500 MHz, CDCl$_3$) of compound 165i
Figure A-19: $^1$H NMR (500 MHz, CDCl$_3$) of compound 165k

Figure A-20: $^{13}$C NMR (500 MHz, CDCl$_3$) of compound 165k
Figure A-21: $^1H$ NMR (500 MHz, CDCl$_3$) of compound 165j

Figure A-22: $^{13}C$ NMR (500 MHz, CDCl$_3$) of compound 165j
Figure A-23: $^1$H NMR (500 MHz, CDCl$_3$) of compound 170

Figure A-24: $^{13}$C NMR (500 MHz, CDCl$_3$) of compound 170
Figure A-25: $^1$H NMR (500 MHz, CDCl$_3$) of compound 185a

Figure A-26: $^{13}$C NMR (500 MHz, CDCl$_3$) of compound 185a
Figure A-27: $^1$H NMR (500 MHz, CDCl$_3$) of compound 185b

Figure A-28: $^{13}$C NMR (500 MHz, CDCl$_3$) of compound 185b
Figure A-29: $^1$H NMR (500 MHz, CDCl$_3$) of compound 185c

Figure A-30: $^{13}$C NMR (500 MHz, CDCl$_3$) of compound 185c
Figure A-31: $^1$H NMR (500 MHz, CDCl$_3$) of compound 185d

Figure A-32: $^{13}$C NMR (500 MHz, CDCl$_3$) of compound 185d
Figure A-33: $^1$H NMR (500 MHz, CDCl$_3$) of compound 185e

Figure A-34: $^{13}$C NMR (500 MHz, CDCl$_3$) of compound 185e
Figure A-35: $^1$H NMR (500 MHz, CDCl$_3$) of compound 185f

Figure A-36: $^{13}$C NMR (500 MHz, CDCl$_3$) of compound 185f
**Figure A-37**: $^1$H NMR (500 MHz, CDCl$_3$) of compound 185g

**Figure A-38**: $^{13}$C NMR (500 MHz, CDCl$_3$) of compound 185g
Figure A-39: $^1$H NMR (500 MHz, CDCl$_3$) of compound 185h

Figure A-40: $^{13}$C NMR (500 MHz, CDCl$_3$) of compound 185h
Figure A-41: $^1$H NMR (500 MHz, CDCl$_3$) of compound 201

Figure A-42: $^{13}$C NMR (500 MHz, CDCl$_3$) of compound 201
Figure A-43: $^1$H NMR (500 MHz, CDCl$_3$) of compound 226

Figure A-44: $^{13}$C NMR (500 MHz, CDCl$_3$) of compound 226
Figure A-45: $^1$H NMR (500 MHz, CDCl$_3$) of compound 218a

Figure A-46: $^{13}$C NMR (500 MHz, CDCl$_3$) of compound 218a
Figure A-47: $^1$H NMR (500 MHz, CDCl$_3$) of compound 218b

Figure A-48: $^{13}$C NMR (500 MHz, CDCl$_3$) of compound 218b
Figure A-49: $^1$H NMR (500 MHz, CDCl$_3$) of compound 218c

Figure A-50: $^{13}$C NMR (500 MHz, CDCl$_3$) of compound 218c
Figure A-51: $^1$H NMR (500 MHz, CDCl$_3$) of compound 218d

Figure A-52: $^{13}$C NMR (500 MHz, CDCl$_3$) of compound 218d
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Figure A-54: $^{13}$C NMR (500 MHz, CDCl$_3$) of compound 218e
Figure A-55: $^1$H NMR (500 MHz, CDCl$_3$) of compound 218f

Figure A-56: $^{13}$C NMR (500 MHz, DMSO) of compound 218f
Figure A-57: $^1$H NMR (500 MHz, CDCl$_3$) of compound 218g

Figure A-58: $^{13}$C NMR (500 MHz, CDCl$_3$) of compound 218g
Figure A-59: $^1$H NMR (500 MHz, CDCl$_3$) of compound 218h

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Figure A-63: $^1$H NMR (500 MHz, CDCl$_3$) of compound 233a

Figure A-64: $^{13}$C NMR (500 MHz, CDCl$_3$) of compound 233a
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Figure A-66: $^{13}$C NMR (500 MHz, CDCl$_3$) of compound 255a
Figure A-67: $^1$H NMR (500 MHz, CDCl$_3$) of compound 255b

Figure A-68: $^{13}$C NMR (500 MHz, CDCl$_3$) of compound 255b
Figure A-69: $^1$H NMR (500 MHz, CDCl$_3$) of compound 255c

Figure A-70: $^{13}$C NMR (500 MHz, CDCl$_3$) of compound 255c
Figure A-71: $^1$H NMR (500 MHz, CDCl$_3$) of compound 255d

Figure A-72: $^{13}$C NMR (500 MHz, CDCl$_3$) of compound 255d
Figure A-73: $^1$H NMR (500 MHz, CDCl$_3$) of compound 255e

Figure A-74: $^{13}$C NMR (500 MHz, CDCl$_3$) of compound 255e
Figure A-75: $^1$H NMR (500 MHz, CDCl$_3$) of compound 255f

Figure A-76: $^{13}$C NMR (500 MHz, CDCl$_3$) of compound 255f
Figure A-77: $^1$H NMR (500 MHz, CDCl$_3$) of compound 255g

Figure A-78: $^{13}$C NMR (500 MHz, CDCl$_3$) of compound 255g
Figure A-79: $^1$H NMR (500 MHz, CDCl$_3$) of compound $255h$

Figure A-80: $^{13}$C NMR (500 MHz, CDCl$_3$) of compound $255h$
Figure A-81: $^1$H NMR (500 MHz, CDCl$_3$) of compound 255i

Figure A-82: $^{13}$C NMR (500 MHz, CDCl$_3$) of compound 255i
Figure A-83: $^1$H NMR (500 MHz, CDCl$_3$) of compound 255j

Figure A-84: $^{13}$C NMR (500 MHz, CDCl$_3$) of compound 255j
Figure A-85: $^1$H NMR (500 MHz, CDCl$_3$) of compound 255k

Figure A-86: $^{13}$C NMR (500 MHz, CDCl$_3$) of compound 255k
Figure A-87: $^1$H NMR (500 MHz, CDCl$_3$) of compound 255l

Figure A-88: $^{13}$C NMR (500 MHz, CDCl$_3$) of compound 255l
APPENDIX B: SUPPORTING FIGURES FOR MOLECULAR DOCKING STUDIES
Figure A-89: 2D representation of the binding pose of compound 264 in the 5-HT$_2$A receptor
Figure A-90: 2D representation of the binding pose of compound 265 in the 5-HT$_{2A}$ receptor
Figure A-91: 2D representation of the binding pose of compound 266 in the 5-HT$_{2A}$ receptor
**Figure A-92**: 2D representation of the binding pose of compound **268** in the 5-HT$_2$A receptor.
Figure A-93: 2D representation of the binding pose of compound 269 in the 5-HT$_{2A}$ receptor
Figure A-94: 2D representation of the binding pose of compound 267 in the 5-HT$_{2A}$ receptor
Figure A-95: Binding pocket of compound 269 in the 5-HT$_{2A}$ receptor (white = neutral, green = hydrophobic, red = hydrogen bonding acceptor potential, blue = hydrogen bonding donor potential)
Scheme A-1: General scheme showing the synthesis of C-2 analogues from boldine
APPENDIX C: CYTOTOXIC EVALUATION RESULTS FOR ISOCHROMAN ANALOGUES
Figure A-96: Cytotoxic evaluation results of compound 184
**Figure A-97**: Cytotoxic evaluation results of compound 170
Figure A-98: Cytotoxic evaluation results of compound 185a
Figure A-99: Cytotoxic evaluation results of compound 185c
### Figure A-100: Cytotoxic evaluation results of compound 185d

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Figure A-101: Cytotoxic evaluation results of compound 185e
Figure A-102: Cytotoxic evaluation results of compound 185g
Figure A-103: Cytotoxic evaluation results of compound 185f
Figure A-104: Cytotoxic evaluation results of compound 185h

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(2) Shamma, M.; Slusarchyk, W. A. Chem. Rev. 1964, 64, 59.


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