ORTHOSTERIC AND ALLOSTERIC PROBES FOR
CANNABINOID RECEPTORS

A dissertation presented

by

Ritesh B. Tichkule

to

The Department of Chemistry and Chemical Biology

In partial fulfillment of the requirements for the degree of
Doctor of Philosophy
In the field of
Chemistry
Northeastern University
Boston, Massachusetts
December, 2012
ORTHOSTERIC AND ALLOSTERIC PROBES FOR CANNABINOID RECEPTORS

by

Ritesh B. Tichkule

ABSTRACT OF DISSERTATION

Submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Chemistry in the Graduate School of Northeastern University December, 2012
Abstract

Plant-derived and endogenous cannabinoids (endocannabinoids) produce their cellular effects by interacting with cannabinoid receptors (CBs), which are members of the G-protein coupled receptor (GPCR) superfamily. Two subtypes CBRs have been designated as CB\textsubscript{1} and CB\textsubscript{2} which are activated by various CB ligands. These CB ligands have therapeutic potential in numerous medical conditions including obesity, osteoporosis, pain, cancer and inflammation.\textsuperscript{1} Cannabinoid ligands bind at orthosteric as well as allosteric binding sites of the receptors. Ligands for both orthosteric and allosteric sites have been synthesized and discussed here.

Cannabinoid agonists Marinol (Dronabinol, Δ\textsuperscript{9}-THC) and Cesamet [Nabilone (Eli Lilly)] are marketed to control nausea and emesis in chemotherapy. But the major problem with CB agonist is CNS side effects due to penetration through the blood brain barrier. There is potential value for the development of selective CB agonists with minimal or no blood brain barrier penetration. With this aim to limit CNS side effects, novel biaryl ligands and their salts are synthesized and promising results are presented. This work led to the discovery of AM9405 which showed high affinity and efficacy at both CB receptors.

Recently, rimonabant (Acomplia\textsuperscript{TM}, SR141716A), a CB\textsubscript{1} antagonist was approved in Europe for the treatment of obesity. However, in human trials rimonabant exhibited nausea and psychiatric disorders as major side effects, causing the withdrawal of this drug from the market. This highlighted the need to develop safer alternatives. A CB\textsubscript{1} antagonist with a different pharmacological mechanism may represent such a safer alternative. A promising approach is the development of negative allosteric modulators of CB\textsubscript{1} receptor which would inhibit the action of endocannabinoids. The primary goal of this project is to develop novel high-affinity, potent and efficacious allosteric modulators of the CB\textsubscript{1} receptor based on the structure of the current lead
molecule PSNCBAM-1 (negative allosteric modulator) which will hopefully be devoid of side effects. Detailed SAR around PSNCBAM-1 template is presented. Series of analogs with modifications focused on stereoelectronic effects on aryl ring are synthesized and evaluated for allosteric modulation of CB₁. Further modifications involve bioisosteric replacement of urea group and investigation of its mode of binding with the receptor in the context of H-bonding. Final modifications are focused on optimal conformation of PSNCBAM-1. All synthesized analogs were profiled for functional activity in both cAMP and β-arrestin assays.
In memory of my Father

Bhanudas K. Tichkule
(1941-2011)

And

For my Mother

Usha B. Tichkule
Acknowledgements

I am very thankful to my research advisor, Dr. Alexandros Makriyannis, for giving me this opportunity to do research in his laboratory and for his guidance to solve medicinal chemistry problems. His inputs to interpret various biological activity data and focused future direction made my research work on orthosteric cannabinoid project (Chapter 2) possible.

Also, I am very grateful to my research co-advisor, Dr. Ganesh Thakur, for teaching me basic scientific skills not only limited to synthetic chemistry, medicinal chemistry, biological assay interpretation, but also for helping me to evolve as a good medicinal scientist. His scientific training on daily basis made my research work on allosteric modulators (Chapter 3) possible. I am greatly indebted to his tremendous support and faith while doing my graduate work. I am also very thankful to NIDA/NIH for providing grant to our lab (PI: Dr. G.A. Thakur; DA027113), without which this work would not have been possible.

I would like to thank my committee members, Dr. Robert Hanson and Dr. Graham Jones, for their valuable time to review my dissertation and provide valuable inputs. Their teaching in medicinal and organic chemistry courses and encouragement to master fundamental concepts in the classes helped me to develop as a good scientist.

I would also like to thank my lab group members Dr. Subramanian Kumara Vadivel, Dr. Kiran Vemuri, Dr. Spyros Nikas, Dr. Vidhyanand Shukla, Dr. JodiAnne Wood, Dr. Anna Bowman, Dr. Rick Duclos, Marsha D’Souza and Kyle Whitten for their help in lab research work. I would like to thank Dr. Jessica Garcia for correcting my thesis. Also, I
would like to give thanks to administrative staff; Dr. David Janero, Shawntelle Dillon, Sarah Strassburger and Brett Greene.

Special thanks go to my lovely wife Shama Tichkule for her constant support, understanding and love during one of the most challenging phases of my life. Her constant motivation during my graduate work made it possible. I also want to thank my mother Usha B. Tichkule, my late father Bhanudasji K. Tichkule, sisters Varshatai Chole & Rajani Chaudhari, brother Rajesh Tichkule, sister-in-law Rupali Tichkule, Shirya, Mehul, Vidhi, Ridhi and Shaurya who always encouraged me to continue my research work and supported me to bring my work to its logical end. I am grateful to all above mentioned persons forever.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Abstract</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dedication</td>
<td>5</td>
</tr>
<tr>
<td>Acknowledgement</td>
<td>6</td>
</tr>
<tr>
<td>Table of Contents</td>
<td>8</td>
</tr>
<tr>
<td>List of Tables</td>
<td>12</td>
</tr>
<tr>
<td>List of Figures</td>
<td>13</td>
</tr>
<tr>
<td>List of Synthetic schemes</td>
<td>15</td>
</tr>
<tr>
<td>List of Abbreviations</td>
<td>18</td>
</tr>
</tbody>
</table>

## Chapter One: Introduction to Cannabinoids

1.1 History of Cannabinoids     | 21 |
1.2 Endocannabinoid System      | 22 |
1.3 Cannabinoid Receptors       | 23 |
   1.3.1 CB₁ Receptors           | 23 |
   1.3.2 CB₂ Receptors           | 24 |
1.4 Signaling of CB receptors   | 25 |
1.5 Endocannabinoids            | 26 |
1.6 Hydrolyzing Enzymes         | 27 |
1.7 Synthetic Cannabinoid Ligands | 27 |
1.8 Orthosteric Cannabinoid Ligands | 28 |
<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.9</td>
<td>Classical Cannabinoids (CCs)</td>
<td>28</td>
</tr>
<tr>
<td>1.10</td>
<td>Non classical Cannabinoids (NCCs)</td>
<td>29</td>
</tr>
<tr>
<td>1.11</td>
<td>Hybrid Cannabinoids (HCs)</td>
<td>30</td>
</tr>
<tr>
<td>1.12</td>
<td>Aminoalkylindoles (AAIs)</td>
<td>31</td>
</tr>
<tr>
<td>1.13</td>
<td>Diarylpyrazoles</td>
<td>32</td>
</tr>
<tr>
<td>1.14</td>
<td>CB₁ Receptor Neutral Antagonists</td>
<td>33</td>
</tr>
<tr>
<td>1.15</td>
<td>AllostERIC Cannabinoid Ligands</td>
<td>35</td>
</tr>
<tr>
<td>1.16</td>
<td>Therapeutic Potential of Cannabinoids</td>
<td>35</td>
</tr>
<tr>
<td>1.17</td>
<td>Cannabinoid Based Drugs on the Market</td>
<td>36</td>
</tr>
<tr>
<td>1.18</td>
<td>Peripheral Targeting Agents to Treat GI Diseases and Nociception</td>
<td>37</td>
</tr>
<tr>
<td>1.19</td>
<td>Functionally Selective Ligands</td>
<td>38</td>
</tr>
<tr>
<td>1.20</td>
<td>Summary and Objective</td>
<td>39</td>
</tr>
</tbody>
</table>

**Chapter Two: OrthostERIC Cannabinoid Modulators**

<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>Biaryl cannabinoids</td>
<td>42</td>
</tr>
<tr>
<td>2.2</td>
<td>Chemistry</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td>2.2.1 Synthesis of Biaryl Analogs</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td>2.2.2 Synthesis of Biaryl Analogs with Side Chain Modification</td>
<td>49</td>
</tr>
<tr>
<td></td>
<td>2.2.3 Synthesis of Benzimidazolium Analogs</td>
<td>52</td>
</tr>
<tr>
<td></td>
<td>2.2.4 Scale-Up Route for the Synthesis of Quaternary Ammonium Analogs</td>
<td>54</td>
</tr>
<tr>
<td></td>
<td>2.2.5 Benzimidazolium Analogs with 1-Adamantyl Chain</td>
<td>55</td>
</tr>
</tbody>
</table>
3.4.1.1 Site I variations 152

3.4.1.2 Site II variations 157

3.4.1.3 Site III Variations 158

3.5 Conclusions 159

3.6 Experimental 161

Appendix 216

References 225
## List of Tables

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table 2.1</td>
<td>Affinities of Biaryl Analogs towards Cannabinoid Receptors</td>
<td>56</td>
</tr>
<tr>
<td>Table 2.2</td>
<td>cAMP of AM9405</td>
<td>65</td>
</tr>
<tr>
<td>Table 2.3</td>
<td>BBB Penetration Study of AM9405</td>
<td>71</td>
</tr>
<tr>
<td>Table 3.1</td>
<td>EC$_{50}$ values of novel CB$_1$ negative allosteric modulators in cAMP Hunter assay and CNR1 PathHunter assay ($\beta$-arrestin).</td>
<td>145</td>
</tr>
</tbody>
</table>
# List of Figures

| Figure 1.1 | Key cannabinoids from *Cannabis sativa L* | 22 |
| Figure 1.2 | Schematic representation of the CB₁ and CB₂ receptors | 24 |
| Figure 1.3 | Endogenous CB Ligands | 27 |
| Figure 1.4 | Classical Cannabinoids | 29 |
| Figure 1.5 | Representative Non-classical Cannabinoids | 30 |
| Figure 1.6 | Representative Hybrid Cannabinoids | 31 |
| Figure 1.7 | Aminoalkylindoles (AAI) Cannabinoids | 32 |
| Figure 1.8 | Biarylpyrazoles Cannabinoids as CB₁ Antagonist/Inverse Agonists | 33 |
| Figure 1.9 | CB₁ Neutral Antagonist | 35 |
| Figure 2.1 | Biaryl Cannabinoids from Merck Frosst Laboratory | 42 |
| Figure 2.2 | Role of Hydroxyl Group in Biaryl Cannabinoid Modulators | 43 |
| Figure 2.3 | Proposed H-Bonding in Biaryl Cannabinoids | 44 |
| Figure 2.4 | Colonic Propulsion Study | 66 |
| Figure 2.5 | Whole Gut Transit Study | 67 |
| Figure 2.6 | Stress-induced Bowel Movement (IBS-like) Study | 68 |
| Figure 2.7 | Castor Oil Induced Diarrhea Studies | 69 |
| Figure 2.8 | Effect on Visceral Abdominal Pain Induced by Mustard Oil study | 70 |
| Figure 2.9 | Hypothermia Studies | 72 |
| Figure 2.10 | Visceral Abdominal Pain Study | 73 |
| Figure 3.1 | Allosteric modulators of CB₁ receptor | 123 |
| Figure 3.2 | Proposed SAR plan around PSNCBAM-1 scaffold | 124 |
| Figure 3.3 | Crystal Structure of 24a and 24b | 144 |
### List of Synthetic Schemes

<table>
<thead>
<tr>
<th>Scheme</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scheme 2.1</td>
<td>Synthesis of Biaryl Analogs</td>
<td>45</td>
</tr>
<tr>
<td>Scheme 2.2</td>
<td>Direct Arylation of $N$-Methylimidazole with Aryl Bromide</td>
<td>46</td>
</tr>
<tr>
<td>Scheme 2.3</td>
<td>Synthesis of Biaryl Analogs via Suzuki Coupling</td>
<td>47</td>
</tr>
<tr>
<td>Scheme 2.4</td>
<td>Synthesis of Fused Benzohetercyclic Biaryl Analogs</td>
<td>49</td>
</tr>
<tr>
<td>Scheme 2.5</td>
<td>Synthesis of Biaryl Analogs with Side Chain Modification</td>
<td>50</td>
</tr>
<tr>
<td>Scheme 2.6</td>
<td>Synthetic Challenge for Biaryl Analogs with Side Chain Modification 2a</td>
<td>51</td>
</tr>
<tr>
<td>Scheme 2.7</td>
<td>Synthetic Challenge for Biaryl Analogs with Side Chain Modification 2b</td>
<td>51</td>
</tr>
<tr>
<td>Scheme 2.8</td>
<td>Synthesis of Biaryl Analogs with Side Chain Modification</td>
<td>52</td>
</tr>
<tr>
<td>Scheme 2.9</td>
<td>Synthesis of Benzimidazolium Analogs</td>
<td>53</td>
</tr>
<tr>
<td>Scheme 2.10</td>
<td>Scale-up route for the synthesis of 50a (AM9404)</td>
<td>54</td>
</tr>
<tr>
<td>Scheme 2.11</td>
<td>Quaternary Ammonium Analogs with 1-Adamanthyl Chain</td>
<td>55</td>
</tr>
<tr>
<td>Scheme 3.1</td>
<td>Site I Modification of PSNCBAM-1</td>
<td>126</td>
</tr>
<tr>
<td>Scheme 3.2</td>
<td>Site I Modification: Synthesis of Urea and Carbamate from Isocyanate Route</td>
<td>127</td>
</tr>
<tr>
<td>Scheme 3.3</td>
<td>Challenges in Synthesizing 15 from Electron Deficient and Sterically Hindered Aniline 14</td>
<td>128</td>
</tr>
<tr>
<td>Scheme 3.4</td>
<td>Successful synthesis of ureas from isocyanate 16</td>
<td>128</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
<td></td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
<td></td>
</tr>
<tr>
<td>2-AG</td>
<td>2-Arachidonoylglycerol</td>
<td></td>
</tr>
<tr>
<td>AAI</td>
<td>Aminoalkylindole</td>
<td></td>
</tr>
<tr>
<td>ACN</td>
<td>Acetonitrile</td>
<td></td>
</tr>
<tr>
<td>AEA</td>
<td>N-Arachidonylethanolamine (Anandamide)</td>
<td></td>
</tr>
<tr>
<td>AIDS</td>
<td>Acquired Immuno-Deficiency Syndrome</td>
<td></td>
</tr>
<tr>
<td>BBB</td>
<td>Blood-brain barrier</td>
<td></td>
</tr>
<tr>
<td>cAMP</td>
<td>Cyclic Adenosine 5’-Monophosphate</td>
<td></td>
</tr>
<tr>
<td>CB₁</td>
<td>First Cannabinoid Receptor</td>
<td></td>
</tr>
<tr>
<td>CB₂</td>
<td>Second Cannabinoid Receptor</td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>Classical Cannabinoid</td>
<td></td>
</tr>
<tr>
<td>CNS</td>
<td>Central Nervous System</td>
<td></td>
</tr>
<tr>
<td>DCM</td>
<td>Dichloromethane</td>
<td></td>
</tr>
<tr>
<td>DME</td>
<td>1,2-Dimethoxyethane</td>
<td></td>
</tr>
<tr>
<td>DMSO</td>
<td>Dimethylsulfoxide</td>
<td></td>
</tr>
<tr>
<td>DMF</td>
<td>Dimethylformamide</td>
<td></td>
</tr>
<tr>
<td>FAAH</td>
<td>Fatty Acid Amide Hydrolase</td>
<td></td>
</tr>
<tr>
<td>Acronym</td>
<td>Full Form</td>
<td></td>
</tr>
<tr>
<td>---------</td>
<td>-----------</td>
<td></td>
</tr>
<tr>
<td>GDP</td>
<td>Gaunosine 5’-Diphosphate</td>
<td></td>
</tr>
<tr>
<td>GTP</td>
<td>Gaunosine 5’Triphosphate</td>
<td></td>
</tr>
<tr>
<td>GPCR</td>
<td>G Protein-Coupled Receptor</td>
<td></td>
</tr>
<tr>
<td>hCB&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Human CB&lt;sub&gt;2&lt;/sub&gt; receptor</td>
<td></td>
</tr>
<tr>
<td>HRMS</td>
<td>High Resoluation Mass Spectroscopy</td>
<td></td>
</tr>
<tr>
<td>IC</td>
<td>Inhibition Concentration</td>
<td></td>
</tr>
<tr>
<td>&lt;i&gt;Ki&lt;/i&gt;</td>
<td>Affinity Constant</td>
<td></td>
</tr>
<tr>
<td>LAH</td>
<td>Lithium Aluminum Hydride</td>
<td></td>
</tr>
<tr>
<td>MGL</td>
<td>Monoacelglycerol Lipase</td>
<td></td>
</tr>
<tr>
<td>NCC</td>
<td>Non-Classical Cannabinoid</td>
<td></td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear Magnetic Resonance</td>
<td></td>
</tr>
<tr>
<td>RXN</td>
<td>Reaction</td>
<td></td>
</tr>
<tr>
<td>SAH</td>
<td>Southern Aliphatic Hydroxyl</td>
<td></td>
</tr>
<tr>
<td>SAR</td>
<td>Structure Activity Relationship</td>
<td></td>
</tr>
<tr>
<td>THC</td>
<td>Tetrahydrocannabinol</td>
<td></td>
</tr>
<tr>
<td>THF</td>
<td>Tetrahydrofuran</td>
<td></td>
</tr>
<tr>
<td>TLC</td>
<td>Thin Layer Chromatography</td>
<td></td>
</tr>
</tbody>
</table>
Chapter One:

Introduction to Cannabinoids
1.1 History of Cannabinoids

Marijuana (*Cannabis sativa L*) has been in use for over 5000 years as a therapeutic and recreational drug.\(^3\) Beneficial effects of cannabis include amelioration of vomiting, stimulation of hunger in chemotherapy and AIDS patients, analgesic effects, reduction of muscle spasticity, among many others documented in literature.\(^4\)

In the search of psychoactive constituents of the plant *Cannabis sativa L* more than 400 natural constituents have been identified.\(^5\) These include terpenoids, cannabinoids and flavonoids. The proportion of different constituents depends upon the origin of the plant, time of harvest and its genetics. Amongst all the constituents, cannabinoids are pharmacologically the most important class.\(^6\) The active and pharmacologically interesting constituents include \((-\Delta^9\text{-tetrahydrocannabinol (}\Delta^9\text{-THC)}\), cannabinol (CBD), cannabigerol (CBG), \(\Delta^9\text{-tetrahydrocannabinvarin, (}\Delta^9\text{-THCV})\) and cannabinidivarin (CBDV) as shown in Figure 1.1. Although naturally occurring cannabidiol and cannabigerols are not active at cannabinoid receptors, they have other physiological effects. Cannabigerols exhibit antibacterial activity and cannabidiol has potential antipsychotic activity.\(^7\) Amongst all the cannabinoids, \(\Delta^9\text{-THC}\) is the primary and most psychologically active constituent.\(^6\)

During the 1970s medicinal use of marijuana was prohibited due to undesired psychotropic effect. Mechoulam's research group led to the discovery and synthesis of \(\Delta^9\text{-THC}\). This discovery ensured that cannabinoid research progressed with the structural understanding of \(\Delta^9\text{-THC}\).\(^8\) Most of the pharmacology and toxicology research on \(\Delta^9\text{-THC}\) was conducted during 1970s and 1980s at National Institute of Health (NIH). Studies were mainly based on pharmacokinetics of delivery of smoked marijuana and concentration of \(\Delta^9\text{-THC}\) and its primary metabolite, 11-hydroxy-tetrahydrocannabinol (11-OH-THC).
Dronabinol (Marinol®), synthetic Δ⁹-THC, is an FDA approved medication to treat nausea and emesis caused by cancer chemotherapeutic agents as well as to stimulate appetite in AIDS patients suffering from cachexia.⁹ Stereochemistry of the active natural Δ⁹-THC enantiomer is (-)-(6aR, 10aR),¹⁰ whereas, the synthetic enantiomer is (+)-(6aS, 10aS). Also, a change in the position of double bond gives Δ⁸-tetrahydrocannabinol (Δ⁸-THC). Both the isomers are same in biological activity, but Δ⁸-THC is chemically more stable.

![Cannabinoids](image)

**Figure 1.1:** Key cannabinoids from *Cannabis sativa L*

### 1.2 Endocannabinoid System:

The endocannabinoid system consists of the two well characterized cannabinoid receptors (CB₁ and CB₂), endogenous ligands (anandamide and 2-arachidonylglycerol), enzymes including fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MGL) and proteins which are involved in biosynthesis, transport and degradation.¹¹
1.3 Cannabinoid Receptors:

Discovery of Δ⁹-THC and its medicinal applications advanced the progress towards understanding of the biological effects on a molecular basis. In 1992, discovery of the first endogenous ligand of cannabinoid receptors, anandamide, provided conclusive support to the hypothesis that an "endogenous cannabinoid regulatory system" exists in mammalian nervous tissue. Cloning of two cannabinoid receptors CB₁ and CB₂ opened new avenues in the development of cannabinoid field. CB₁ is located predominantly in the central nervous system, whereas CB₂ is expressed in peripheral tissues. Both receptors are members of the G-protein-coupled receptor (GPCR) super family which consist of seven hydrophobic transmembrane domains. Cannabinoid receptors are negatively coupled GPCRs and in transfected cells mainly inhibit adenylyl cyclase along with activation of mitogen-activated protein kinase (MAPK). Interestingly, CB₁, but not CB₂, receptors also inhibit voltage-mediated calcium channels and activate potassium channels. Although both CB₁ and CB₂ receptors are Gᵢₒ-coupled GPCRs, CB₁ but not CB₂ receptors under certain conditions can also be coupled to Gₛ proteins.

1.3.1 CB₁ Receptors:

CB₁ and CB₂ cannabinoid receptors share 44% overall homology and 68% homology in their transmembrane domain. The CB₁ receptor exhibits 97.3% amino-acid sequence identity across human and rat cannabinoid receptors whereas human CB₂ receptor has 81% sequence identity with rat and 82% with mouse. The CB₁ receptor is the most abundantly expressed GPCR in the brain and is responsible for the CNS side effects of Δ⁹-THC. Along with high density of CB₁ in hippocampus, cerebellum and other brain areas, it is also found in various
peripheral tissues including the gastrointestinal (GI) tract, testis, pancreas, liver, eye, lungs, heart and adipose tissue.\textsuperscript{23,17} Because of its wide distribution, CB\textsubscript{1} receptor modulates many functions of the body. In brain, it controls memory, hunger and sensory functions whereas in peripheral tissues it modulates cardiovascular health, metabolism and energy balance.\textsuperscript{16}

1.3.2 CB\textsubscript{2} Receptors:

CB\textsubscript{2} receptors are predominantly found in cells and tissues of the immune system including the thymus, tonsils, B lymphocytes, T lymphocytes, macrophages, monocytes and natural killer (NK) cells.\textsuperscript{24} Recent studies have demonstrated that CB\textsubscript{2} is also expressed within the CNS and that this expression occurs during various states of inflammation.\textsuperscript{25,26} This expression of CB\textsubscript{2} has been localized primarily in microglia, the resident macrophages of the CNS.\textsuperscript{27} CB\textsubscript{2} receptors are up-regulated during inflammation in the body.\textsuperscript{28} The exact role of CB\textsubscript{2} in the brain is not known, however recent data suggests CB\textsubscript{2} receptors play an important role in addiction.\textsuperscript{29}

\textbf{Figure 1.2}: Schematic Representation of the CB\textsubscript{1} and CB\textsubscript{2} Receptors (Source: Elementos, 60, 2005, pp.3-9)
In Figure 1.2, CB₁ and CB₂ receptors have seven transmembrane segments. Common amino acids to the both receptors are shown in black circles whereas different amino acids to the both receptors are shown in circles. These seven transmembrane domains have e₁, e₂, e₃ extracellular loops and i₁, i₂, i₃ intracellular loops.

1.4 Signaling of CB Receptors:

Endocannabinoids are amides and esters of long chain polyunsaturated fatty acids and act as mediators in brain and peripheral tissues. Anandamide, also known as N-arachidonoylethanolamine (AEA) and 2-arachidonoylglycerol (2-AG) are the two most studied endocannabinoids. AEA is synthesized on demand by receptor-activated cleavage of lipid precursors. It is found that AEA is not stored in cellular compartment. Biological activity of AEA at CB receptor is diminished by its removal from extracellular space through cellular uptake by high-affinity transporter (AMT).³⁰ Existence of AMT is still a matter of debate as recent studies show that AEA might cross plasma membrane by passive diffusion. Once entered into cell, AEA is a substrate for FAAH which cleaves the amide bond to give arachidonic acid and ethanolamine. AEA and 2-AG are not stored in cellular compartment but is produced ‘on demand’ by receptor mediated cleavage of lipid precursors. 2-AG biosynthetic pathway involves the hydrolysis of phospholipids by DAGL. 2-AG can migrate across the plasma membrane through 2-AG membrane transporter that might be the same as AMT transporter. Once accumulated in the cell, 2-AG can be degraded by FAAH or more efficiently by MGL. AEA and 2-AG both can bind to CB₁ and CB₂ receptors; however, AEA is a weaker agonist than 2-AG at CB₁ and partial agonist at CB₂. Hence, 2-AG is considered a true endogenous ligand for both subtypes of CB receptors.³⁰ Interestingly, only AEA has high affinity towards TRPV1 receptor and hence considered to be true endovanilloid.³⁰ All members of ECS are present in plasma
membrane, except FAAH, which is present in intracellular membrane, and MGL, which is present in cytosol. 30

1.5 Endocannabinoids:

Endocannabinoids are hydrophobic lipid messengers which bind to CB₁ and CB₂ receptors. Due to this hydrophobicity these molecules cannot move in aqueous environment and upon activation bind to nearby cannabinoid receptors. In the central nervous system (CNS), these lipid moieties act as synaptic modulators. All other messengers or neurotransmitters such as acetylcholine and dopamine are pre-synthesized and stored, whereas endocannabinoids are produced ‘on demand’. AEA also called anandamide (Figure 1.3), was the first endocannabinoid to be identified and was isolated from porcine brain. 31 Soon after the discovery of AEA, another endocannabinoid, 2-AG (Figure 1.3), an ester of arachidonic acid and glycerol was isolated from canine gut. 32 AEA is highly lipophilic molecule and is sensitive to hydrolysis and oxidation. 19 It is synthesized within the lipid bilayer in a 2-step pathway by coupling N-acylethanolamines (NAEs) through Ca²⁺-dependent N-acyltransferase enzyme and then hydrolyzation of the amine intermediate with N-acylphosphatidylethanolamine-hydrolyzing phospholipase D (NAPE-PLD) enzyme. 33 It acts as a partial agonist at both CB₁ and CB₂ receptors with moderate affinity at CB₁ (Kᵢ = 60 nM) and low affinity at CB₂ (Kᵢ = 1900 nM). On the other hand, 2-AG is a full agonist and has low affinity at both CB₁ (Kᵢ = 470 nM) and CB₂ (Kᵢ = 1400 nM) receptors. Concentration of 2-AG in brain is ~200 fold higher than AEA. 2-AG is synthesized from phospholipid through phospholipase C and diacylglycerol lipase. 34 Both AEA and 2-AG are transported across the cell membrane before being degraded by fatty acid amide hydrolase (FAAH). Also, 2-AG can be degraded by enzyme MGL, a serine hydrolase. 35
1.6 Hydrolyzing Enzymes:

Endogenous hydrolyzing enzymes include FAAH and MGL. FAAH mainly hydrolyzes AEA; however, in some cells it also hydrolyzes 2-AG. With advancement in the field of endocannabinoid hydrolyzing enzymes, a variety of FAAH inhibitors are reported. They can be divided mainly in two groups: the first group includes inhibitors that mimic FAAH substrates and have an arachidonoyl-, oleoyl- or palmitoyl-carbon chain similar to the fatty acid chains of anandamide, oleamide and palmitoylethanolamide. The second group involves carbamates, oxazolopyridins, 2-thioxoimidazolidin-4-ones, imidazolidine-2,4-diones and the non-steroidal anti-inflammatory drugs, compounds structurally not similar to the endocannabinoids. On the other hand, fewer MGL inhibitors have been reported.

1.7 Synthetic Cannabinoid Ligands:

Synthetic cannabimimetic ligands based on the structure, role and binding site are divided into different classes. Firstly, based on the site where they bind to cannabinoid receptors, there are orthosteric and allosteric ligands. The allosteric modulators are discussed in Chapter 3.

**Figure 1.3:** Endogenous CB Ligands

![Anandamide (AEA) and 2-Arachidonoylglycerol (2-AG)](image)
1.8 Orthosteric Ligands:

Based on original classification, cannabinergic ligands are classified as classical cannabinoids (CCs), non-classical cannabinoids (NCCs), hybrid cannabinoids (HCs), aminoalkylindoles (AAIs) and biarylpyrazoles. In the last decade numerous other cannabinergic ligands have been discovered and developed. The key ligands from these classes are categorized as shown below.

1.9 Classical Cannabinoids (CCs):

Classical cannabinoids are ABC tricyclic THC type compounds bearing a benzopyran moiety. ABC ring system starts from phenyl ring A, pyran ring B and terpene ring C. This class includes phytocannabinoids, the most explored group of synthetic cannabinoids. The structure activity relationship (SAR) studies of the classical template have identified four pharmacophoric groups which are: a phenolic hydroxyl, a lipophilic alkyl side chain, a northern aliphatic hydroxy and southern aliphatic hydroxyl. Some of the classical cannabinoids are presented in Figure 1.4.

The most explored pharmacophoric group in this template is the C-3 side chain. It has been shown that replacing the pentyl chain with dimethyl heptyl (DMH) has enhanced the binding affinity by 40-fold at both CB receptors. Functionalizing the DMH chain with different functionalities such as bromo, cyano, azide etc. enhances the affinity and potency at CB receptors. Also, replacing the dimethyl group with 3-5 carbon rings is well tolerated. This site also shows good tolerance for various carbocyclic rings (bornyl, isobornyl, 1-adamantyl, 2-adamantyl etc.) and aromatic rings. Interesting, replacing the long alkyl chain with bulky adamantyl group led to potent molecule AM411 (Figure 1.4) with slow desensitization of the
receptor. Functionalization in the northern site by replacing the methyl group with keto, OH or 11-hydroxy enhances the affinity and potency.\textsuperscript{41}

Figure 1.4: Classical Cannabinoids

1.10 Non classical Cannabinoids (NCCs):

NCCs do not have the B ring of ABC ring system. They contain the phenyl A ring, the side chain and the terpenic C ring. Along with that the southern hydroxyl group is another important pharmacophoric component of this class. CP55,940 (Figure 1.5) is very potent and key NCC. Its tritiated analog was the key to the discovery of \textit{CB}_1 receptor\textsuperscript{42} and is used routinely as a
radioligand in cannabinoid binding assays. CP-55,244 is a highly potent CB₁ agonist with $K_i$ value of 0.21 nM and has an analgesic effect.⁴³ HU-308 was synthesized and characterized by Mechoulam and his research group at the Hebrew University of Jerusalem. HU-308 is a highly selective CB₂ agonist with over 5000-fold selectivity and analgesic effect.⁴⁴,⁴⁵

![Chemical structures of CP-55,244, CP-55940, and HU-308]

**Figure 1.5:** Representative Non-classical Cannabinoids.

### 1.11 Hybrid Cannabinoids (HCs):

Hybrid cannabinoids are synthesized based on the SAR profile of classical and non-classical cannabinoids. These are tricyclic cannabinoids with southern aliphatic hydroxyl (SAH) functional group. From the SAR in hybrid cannabinoids it was found that 6-β-(R)-stereochemistry is the desired stereochemistry in the SAH part and it enhances the binding affinity as compared to the inactive 6α-isomer.⁴⁶ AM919 and AM938 are two hybrid cannabinoids that exhibits high binding affinities (Figure 1.6).⁴⁷
Figure 1.6: Representative Hybrid Cannabinoids

1.12 Aminoalkylindoles (AAIs):

Before being recognized as cannabinoid agonists, the AAI class of compounds was used as analgesic and non-steroidal anti-inflammatory drugs (NSAIDs). Later, binding studies with the key AAI ligand WIN 55,212-2 demonstrated that the aminoalkylindoles activate the CBRs similarly to CP-55,940. Some of the ligands are presented in Figure 1.7. WIN 55,212-2 is a cannabinoid receptor agonist with potent analgesic and anti-inflammatory properties. AM1241 is potent aminoalkylindole-based agonist with 80-fold selectivity for CB2 over CB1 receptor. It also acts as an analgesic agent and is effective to treat hyperalgesia and allodynia through activation of CB2 receptor by the release of endogenous opioid peptides and direct activation of TRPV1 channel. AM630 is a potent and 165-fold selective CB2 antagonist. AM630 was the first analog synthesized with substitution at 6-position of indole ring, which was found to be important for both affinity and efficacy at both CB1 and CB2. This led to the development of several indole based derivatives. JWH-015 which came from AM630, is naphthoylindole based CB2 selective agonist which exhibits immunomodulatory effects and may be useful in the treatment of pain and inflammation. JWH-030 is a cannabinoid receptor agonist with analgesic
effects and is a partial agonist with $K_i$ of 87 nM for CB$_1$ and possesses about half of the potency of THC.$^{58}$ It also has series of CNS effects that are problematic.

![Chemical structures](image.png)

**Figure 1.7:** Aminoalkylindoles (AAIs) Cannabinoids

### 1.13 Diarylpyrazoles:

This class of ligands includes cannabinoid receptors antagonists/inverse agonists. Rimonabant (SR141716A), AM251 and SR-144528 block effects of agonist activation of CB$_1$ receptor. These compounds have high affinity towards CB$_1$ receptors over CB$_2$ receptors. These compounds do not have the ability to activate cannabinoid receptors when administered alone,
however, in some cases they induce receptor response in opposite direction from those induced by agonist response. It has been shown that rimonabant can produce inverse cannabimimetic effects in CB₁-independent manner.⁵⁹ Although Rimonabant showed beneficial weight control and metabolic effects in obese patients, its CNS related side effects halted its use as an anti-obesity drug.⁶⁰,⁶⁰b Sanofi developed Ibipinabant (SLV-319), a highly CB₁ selective antagonist which was withdrawn during phase 2 clinical trials.⁶¹ SLV-319 (Figure 1.8) showed clinical activity and efficacy in the treatment of obesity, however, it was dropped because of undesired side effects. SLV-319 (Ibipinabant) is currently used only for laboratory research, mainly for the purpose of structure-activity relationship studies.⁶²,⁶²b

![Figure 1.8: Biarylpyrazoles Cannabinoids as CB₁ Antagonists and/or Inverse Agonists.](image)

### 1.14 CB₁ Receptor Neutral Antagonists:

Current drug discovery research has focused on the development of CB₁ antagonists with intrinsic molecular pharmacology distinct from the inverse agonists. Neutral antagonists might have fewer psychobehavioral side-effects which prevented the development of first generation of CB₁ inverse agonists such as rimonabant and taranabant.⁶⁰a VCHSR (Figure 1.9) is a biarylpyrazole based analog of rimonabant with a modest CB₁ affinity (Kᵢ < 85 nM). It lacks
rimonabant’s ability to form hydrogen bond with CB1 receptor which was thought to contribute toward inverse agonism.\textsuperscript{63} VCHSR, in animal studies, did not alter Ca\textsuperscript{2+} current proving neutral antagonism mode of action. It also showed efficacy in weight reduction and food intake.\textsuperscript{64} BRP0432 is an alkynyl thiophene analog classified as a neutral antagonist. It binds with 150-fold selectively to hCB1 with high affinity of 14 nM, but it does not show any functional potency of its own.\textsuperscript{65} It also suppressed feeding in obese rats.\textsuperscript{66} Although the exact chemical structure of AM4113 is not reported, it is reported in literature as pyrazole-based rimonabant analog with high CB1 affinity (K\textsubscript{i} = 0.9 nM) and more than 100-fold selectivity over CB2.\textsuperscript{67,68} AM4113 behaves as a neutral antagonist with respect to CB1 in cAMP functional assays. AM6545 is also a rimonabant analog with high affinity for CB1 (K\textsubscript{i} = 3.3 nM, mCB1) with over 100-fold selectivity for CB1 vs CB2.\textsuperscript{69,70} It did not hit any other receptors when screened against a panel of over 60 noncannabinergic targets. Unlike rimonabant, AM6545 exhibited neither basal GTP\gammaS binding to CB1 nor the constitutive cAMP activity demonstrating that AM6545 acts as a neutral antagonist and not as an inverse agonist.\textsuperscript{69,70} It has limited CNS penetrance making it periphero-neutral antagonist.\textsuperscript{60a} URB447 was characterized \textit{in vitro} as a neutral antagonist with low CB1 potency (IC\textsubscript{50} = 313 nM). With limited brain permeability after systemic administration, URB447 inhibited peripheral CB1-mediated analgesia without antagonizing central CB1 dependent responses, such as hypothermia and catalepsy.\textsuperscript{71}
Figure 1.9: CB<sub>1</sub> Neutral Antagonist

1.15 Allosteric Ligands:

Traditionally it was assumed that all GPCR signaling caused by agonists occurred through binding to the site where endogenous ligands bind. This site is defined as the orthosteric binding site. It is now recognized that GPCRs may contain allosteric binding sites for both endogenous and synthetic ligands. Because allosteric binding sites are topographically distinct from orthosteric binding sites, structurally distinct allosteric and orthosteric ligands can be developed. Allosteric ligands can modulate activity by inducing conformational changes in the receptor and modulate the function of the receptors orthosteric site indirectly instead of modulating receptor response with orthosteric ligands directly. This aspect is discussed further in Chapter 3.

1.16 Therapeutic Potential of Cannabinoids:

CB<sub>1</sub> and CB<sub>2</sub> receptors are distributed in various tissues and their psychotropic and peripheral effects are well known. It was shown that cannabinoid agonists, antagonists and other
cannabinoid modulators have vast therapeutic potential and are useful in the treatment of multiple sclerosis, neurodegenerative disorders (Parkinson’s disease, Huntington’s disease, Alzheimer’s disease), asthma, schizophrenia, glioma, rheumatoid arthritis, management of cancer chemotherapy side effects, glaucoma, AIDS wasting syndrome, obesity, metabolic syndrome-related disorder and seizure disorders. CB receptor agonists can be useful in the treatment of analgesia, muscle relaxation, anti-inflammation, improvement of mood, stimulation of appetite and neuroprotection. Current clinical research is focused in the area of chronic pain and neurological disorders.\textsuperscript{74,75}

1.17 Cannabinoid Based Drugs on the Market:

Structure activity studies done on $\Delta^9$-THC led to the discovery of three important FDA approved commercial drugs.\textsuperscript{76} The first marketed drug was nabilone (Cesamet\textsuperscript{®}) from Lilly for the treatment of chemotherapy-induced nausea and vomiting (CINV) caused by cytotoxic chemotherapeutic agents. Also, it was shown that nabilone could be useful in the treatment of pain associated with fibromyalgia.\textsuperscript{77} Recently, it was shown that nabilone is useful for treatment-resistant nightmares in post-traumatic stress disorders.\textsuperscript{78} Dronabinol (Marinol\textsuperscript{®}) was the second synthetic $\Delta^9$-THC based drug from Solvay pharmaceuticals (currently Abbott) which was approved for the treatment of CINV and cachexia (weight loss and wasting) as an appetite stimulant in AIDS patients. However, due to poor side effect profile and poor delivery of the active constituents, use of Marinol\textsuperscript{®} is limited. Currently, dronabinol is being evaluated for the treatment of marijuana addiction. Sativex\textsuperscript{®} was the third $\Delta^9$-THC based medication, developed by GW pharmaceuticals and used for the treatment of neuropathic pain in multiple sclerosis (MS) in Canada. Sativex was also approved for pain relief treatment for patients with advanced cancer who experience pain even after treatment with opioid therapy. Sativex is an oromucosal
spray, with cannabis derived active components Δ⁹-THC and non-psychoactive cannabidiol in equal amounts. Recent studies show that the efficacy of the drug is maintained in the treatment of pain in MS patients even after long-term (for several months) use and is being evaluated in late stage clinical testing in Europe and US.

1.18 Peripheral Targeting Agents to Treat GI Diseases and Nociception:

The importance of the cannabinoid system in the treatment of gastrointestinal diseases is well recognized. Cannabinoid receptors (CB₁ and CB₂), endocannabinoids and enzymes responsible for the synthesis of endocannabinoids and their inactivation are the components of cannabinoid system and all have been detected in the GI system. Relaxation of lower osophageal sphincter and inhibition of gastric acid secretion, intestinal motility and fluid secretion are important functions based on modulation of the cannabinoid system. Recently, it was found that during inflammation in both animal models and humans, the endocannabinoid system in small intestine is overstimulated. Izzo's group showed that endocannabinoids and CB₁ receptors are upregulated during intestinal inflammation and cannabinoids can have a protecting effect during intestinal inflammation mainly through CB₁ activation. Traditional use of marijuana for gastrointestinal disorders, such as diarrhea and abdominal cramps, was limited by undesired psychoactive problems. Activation of CB₁ receptor controls numerous actions in gut. More importantly, CB₁ activation leads to the inhibition of acetylcholine release, which ultimately results in the inhibition of contractility in vitro or an attenuation of motility in vivo.

The endocannabinoid system was found to be very important for the peripheral regulation of nociception. Neurotransmitters are involved in pain management through the impulses carried by nociceptive fibers (pain sensing) in the central nervous system (CNS). In addition to this CNS
mechanism, pain transmissions can occur at the terminals of afferent nerves outside of the CNS and one example of peripheral regulation is the release of endogenous opioids from activated immune cells during inflammation to inhibit pain initiation by interacting with opioid receptors.\textsuperscript{83} Researchers at University of California, Irvine reported a potent non-brain penetrant inhibitor of the anandamide-degrading enzyme (FAAH) to control pain initiation devoid of unwanted central effects.\textsuperscript{84}

1.19 Functionally Selective Ligands:

GPCRs may adopt different structural conformations which involve different functional pathways. Some conformations represent the inactive form of the receptor while others represent the active form of receptor in the presence of ligand agonist. In many cases, receptors adopt an active conformation in the presence of ligand and some receptors stay in an inactive conformation in the absence of ligand. However, in some cases receptor shows some form of active conformation even in the absence of ligand, a condition referred to ligand-independent constitutive activity. Agonists bind to the receptor and bring it to the more active conformation as compared to the inactive conformation. In case of inverse agonists, the ligand binds to the receptor and reverses constitutively active conformation. A neutral antagonists show no preference to any conformation of receptor and binds to the receptor in whichever conformation is available.

The effect of active receptor conformation on downstream effectors could be different, including receptor signaling. Preference for one pathway over the other pathway in receptor signaling is called biased agonism. The strength of the signal effect occurs when the ligand binds to the receptor leads to a conformation that prefers a particular pathway over the other. Partial
agonists may activate the receptor partially. The selective preference of ligand binding to a particular receptor conformation causes selective activation of GPCR signaling pathways, leading to functional selectivity. Functionally selective receptor conformations could also influence patterns of desensitization and internalization of the activated receptors.

Allosteric modulators also may preferentially bind to the receptor in its active conformation and generate functionally selective responses. Very few allosteric modulators have been shown to produce functionally selective responses as compared to functional responses resulting from protein-protein interactions. In general an allosteric ligand enhances affinity of the orthosteric ligand while it decreases efficacy of the orthosteric ligand. This result suggests that an allosteric ligand favorably binds to the receptor conformation which prefers to have a high affinity orthosteric ligand but functionally less active. Allosteric modulators may alter receptor conformation to favor one functional pathway over the other suggesting that functionally selective CB allosteric modulators may exist.

1.20 Summary and Objective:

With only three cannabinoid based drugs in the market with limitations such as undesirable CNS side effects, there is a need to develop new classes of cannabinoid drugs without any CNS based side effects. In case of THC, because of its high lipophilicity, it has undesired pharmacokinetic properties. This led to the design and development of new class of cannabinergic ligands which will have only desirable physiological effects without any CNS side effect.

First in the biaryl cannabinoid ligands, the importance of the hydroxyl groups in the A ring was explored. Tolerance of heteroatom in C ring preferably at the ortho position was
explored. The H-bond mediated ‘Intramolecular lock’ concept of hydroxy group to the H-bond acceptor heteroatom was explored.

For the SAR of the PSNCBAM-1 scaffold starting from the left side urea group was explored. First optimization is carried on aniline group with substitutions influencing electronics and steric properties of the aryl ring. After optimizing the aniline group, bioisosteric replacement of urea group explored. A requirement of hydrogen in urea group was explored. Various linear analogs of PSNCBAM-1 were synthesized to probe the conformation of ligand that required for CB₁ activity.
Chapter Two:

Orthosteric Cannabinoid Modulators
2.1 Biaryl Cannabinoids:

The first report of biaryl cannabinoids was published by researchers at Merck Frosst Laboratory by Gaureu, et al.\textsuperscript{85} It was shown that the tricyclic moiety in \textbf{1} is not essential for high cannabinoid receptor affinity as demonstrated by the high affinity of the terpenyl phenol analogs \textbf{2}. Interestingly, researchers also found that replacement of terpenyl moiety of \textbf{2} by a planar biaryl phenol as in \textbf{3} resulted in well-tolerated high affinity analogs at both CB\textsubscript{1} and CB\textsubscript{2} receptors with improved selectivity for CB\textsubscript{2}.

\begin{center}
\textbf{Figure 2.1:} Biaryl Cannabinoids from Merck Frosst Laboratory
\end{center}

The Gaureu group evaluated biaryl phenol analogs for this effectiveness as a hydrogen bond donor by converting \textbf{3} into dimethyl ether analog \textbf{5}. This resulted in significant loss of affinity at hCB\textsubscript{2} (200-fold) with a $K_i$ of 433 nM. Interestingly, for hCB\textsubscript{1} receptor, this analog showed no measurable affinity. This selectivity for hCB\textsubscript{2} receptor was lost when one of the hydroxyl groups was replaced by a hydrogen atom \textbf{4} resulting improved hCB\textsubscript{1} binding affinity.
**Figure 2.2:** Role of Hydroxyl Group in Biaryl Cannabinoid Modulators

To further explore the role of hydroxyl groups in biaryl analogs, we synthesized heteroatom containing biaryl analogs with intramolecular hydrogen bonding capability, as shown in Figure 2.3. The concept of involving H-bond formation between the heteroatoms in ring C and the phenolic hydroxyls in ring A which we designated as ‘Intramolecular Lock’ was explored. ‘Intramolecular Lock’ embedded ligands as shown in compound 6 would possess desired polarity compared to masked hydroxyl functional groups which would have undesired higher lipophilicity compared to compounds 4 and 5. With the help of intramolecular hydrogen bonding of phenolic hydroxyl group with heteroatom in C ring of compound 6, phenolic hydroxyl groups were designed as hydrogen bond acceptors. Additionally, we planned to evaluate the effect of steric hindrance on phenolic hydroxyl groups caused by ortho-substitution on C ring in compound 6. Presence of ortho substitution on aryl C ring would change torsional angle between ring A and ring C. Analogs containing this conformational change induced by ortho substitution on ring C were planned to be synthesized and evaluated. Several heterocyclic rings incorporating dimethylheptyl resorcinols were synthesized. The resulting library was evaluated for their affinities for the cannabinoid receptors.
Figure 2.3: Proposed H-Bonding in Biaryl Cannabinoids

2.2 Chemistry:

2.2.1 Synthesis of Biaryl Analogs

In the biaryl cannabinoid system, the dihydroxy group of phenyl A ring plays a key role in affinity at both CB1 and CB2 receptors.\textsuperscript{85} Analog 17 (AM 9408) was synthesized following the synthetic route reported by Moore et al, as shown in scheme 2.1.\textsuperscript{86} Benzonitrile 10 was refluxed with n-hexylmagnesium bromide 11 in the presence of cuprous bromide under inert reaction conditions, followed by acid-mediated hydrolysis of the resulting imine to produce ketone 12 in high yield (94%). The ketone was converted into a gem-dimethyl group with titanium tetrachloride and dimethyl zinc at -40 °C to give 13 in 90% yield. Regioselective ortho-bromination was done in the presence of 18-crown-6 using bromine to give key aryl bromo intermediate 14 in high yield (97%) under reported conditions.\textsuperscript{87} Suzuki-Miyuara coupling
conditions developed in the Makriyannis group between aryl bromide 14 and phenylboronic acid 15 in the presence of catalytic Pd(PPh₃)₄ and Ba(OH)₂, gave the desired biaryl analog 16 in good yield (84%).⁸⁸ Demethylation of ether 16 was achieved with excess BBr₃ to give desired biaryl resorcinol product 17 in good yield (89%). Controlled mono-demethylation was achieved by treating 16 with two equivalents of BBr₃ at 0 °C for 3 h to give desired product 18 in moderate yield (50%).

**Scheme 2.1: Synthesis of Biaryl Analogs**

![Scheme 2.1: Synthesis of Biaryl Analogs]

**Reagents and Conditions:** (a) cat. CuBr, 15% H₂SO₄, THF, reflux, 20 min, RT, 16 h, 94%; (b) TiCl₄, Me₂Zn, DCM, -40°C, 2 h, 90%; (c) Br₂, 18-crown-6, DCM, 0 °C - RT, 30 min, 97%; (d) Pd(PPh₃)₄, Ba(OH)₂, DCE, 85 °C, 16h, 84%; (e) BBr₃, DCM, 0 °C - RT, 16h, 89%; (f) BBr₃, DCM, 0 °C - RT, 16h, 50%.

Synthesis of biaryl resorcinol analog 20 was attempted following Scheme 2.2.⁸⁹ Since a variety of commercially cheap hetero-aromatic substrates could be used in this approach, this was the most economically attractive approach to introduced heteroatoms at the ortho position of C ring containing biaryl analogs. Aryl bromide 14 was treated with N-methyl imidazole in the
presence of copper (I) iodide and Pd(OAc)$_2$, however no reaction was observed. Our second approach was utilizing Stille coupling to obtain the desired product under standard conditions also was not successful (Scheme 2.2).$^{90}$

**Scheme 2.2:** Direct Arylation of $N$-Methylimidazole with Aryl Bromide

$$\text{Scheme 2.2: Direct Arylation of } N\text{-Methylimidazole with Aryl Bromide}$$

$$\begin{align*}
\text{Reagents and Conditions: (a) CuI, Pd(OAc)$_2$, DMA, 140 °C, 16 h, no rxn; (b) n-ButylLithium, tributyltin chloride, Pd(PPh$_3$)$_4$, THF, -78 °C, 2h & 60 °C, 16 h, no rxn.}
\end{align*}$$

An alternate approach was used to access biaryl analogs 24 using a Suzuki reaction. As instability of 2-pyridylboronic acid or other heteroaromatic boronic acid at 2-position is very well known$^{91}$ the aryl bromide 14 was converted into aryl boronic acid by lithium halogen exchange, followed by quenching with trimethylborate to give the desired product 21 in good yield (81%) as shown in Scheme 2.3. The resulting boronic acid was coupled with 2-bromopyridine 22a in the presence of catalytic Pd(PPh$_3$)$_4$ to give desired pyridyl biaryl analog 23a in 63% yield. Demethylation of the ether was achieved by treating with BBr$_3$ to give the desired biaryl resorcinol product 24a in good yield (69%). A series of analogs were synthesized using this protocol as shown in Scheme 2.3.
Scheme 2.3: Synthesis of Biaryl Analogs via Suzuki Coupling

Reagents and Conditions: (a) n-BuLi, B(OCH₃)₃, THF, -78 °C, 3 h, 81%; (b) Pd(PPh₃)₄, Na₂CO₃, DCE, 85 °C, overnight, 54-67%; (c) BBr₃, DCM, 0 °C - RT, 16 h, 65-79%.

Benzimidazole or other fused benzoheterocycles resorcinol analogs could be accessed by condensation of 1,2-diaminobenzene 26a with benzaldehyde 25, followed by intramolecular cyclization under oxidative conditions as reported in the literature. Benzaldehyde 25 was synthesized by lithium halogen exchange using n-BuLi, followed by quenching with anhydrous dimethylformamide in good yields (92%) as shown in Scheme 2.4. Benzaldehyde 25 was also synthesized by ortholithiation of 13 and subsequent quenching with DMF. Ortho lithiation was carried out directly on the dimethyl resorcinol ether 13 since the methoxy is an ortho-directing group and preference goes to the desirable, least hindered site, i.e., para position to dimethyl.
heptyl group. However, ortho lithiation required longer times (~2 hours at 0 °C), the yield was very low and was often not reproducible. Therefore, a route involving aryl bromide as starting material was chosen to synthesize aldehyde 25 (Scheme 2.4). Benzaldehyde 25 was condensed and cyclized with 1,2-diaminobenzene 26a in the presence of hydrogen peroxide and catalytic cerium ammonium nitrate to give benzimidazole 27a in good yield (71%). Demethylation of the ether under standard tribromoborane conditions gave benzimidazole resorcinol 28a in good yield (78%).
Scheme 2.4: Synthesis of Fused Benzohetercyclic Biaryl Analogs

Reagents and Conditions: (a) n-BuLi, DMF, THF, -78 °C, 3 h, 92%; (b) CAN, H$_2$O$_2$, acetonitrile, RT, overnight, 60-82%; (c) BBr$_3$, DCM, 0 °C - RT, 16 h, 45-91%.

2.2.2 Synthesis of Biaryl Analogs with Side Chain Modification

We decided to probe replacement of dimethylheptyl group with a thiophenyl containing group in biaryl scaffold in order to improve the ligands physicochemical properties. Thiophenol 30 was coupled with bromobenzene 29 in the presence of catalytic copper iodide and benzotriazole under basic conditions to give the diphenyl sulfide 31 in good yield (84%).$^{93}$ Vilsmeier formylation was performed on 31 utilizing phosphorous oxychloride and dimethylformamide to give benzaldehyde 32 in good yield (81%). Benzaldehyde was condensed
with 1,2-diaminobenzene and cyclized under oxidative conditions to give the desired benzimidazole product 33 in good yield (80%). Subsequent demethylation of the ether gave benzimidazole resorcinol 34 in good yield (69%). Benzimidazole phenyl sulfide 33 was oxidized to sulfoxide 35 under standard conditions using sodium periodate in 60% yield, and to sulfone 37 using mCPBA in 80% yield. Subsequent demethylation of ethers 35 and 37 under standard conditions gave 36 and 38 in 65% and 72% yields respectively.

**Scheme 2.5:** Synthesis of Biaryl Analogs with Side Chain Modification

Reagents and Conditions: a) CuI, benzotriazole, t-BuOK, 80 °C, overnight, 84%; (b) POCl₃, DMF, 0 °C - RT, 16 h, 81%; (c) benzene-1,2-diamine, p-benzoquinone, ethanol, reflux, 80%; (d) BBr₃, DCM, 0 °C - RT, 16 h, 69%; (e) sodium periodate, methanol, RT, 3 h, 60%; (f) BBr₃, DCM, 0 °C - RT, 16 h, 64%; (g) mCPBA, DCM, RT, 6 h, 80%; (h) BBr₃, DCM, 0 °C - RT, 16 h, 71%.

Recently, Moore II’s group published the synthesis of novel tri-aryl CB₂ selective cannabinoid ligands. In previous studies they had shown that in a THC template introduction of aryl ring to replace the terpene ring was tolerated with regard to cannabinoid affinity. An observed 600-fold selectivity for CB₂ against CB₁ in one of the analogs. This led to the
replacement of the dimethylheptyl chain with phenyl-OH at the C1’ position of the biaryl template. Retrosynthetic analysis of phenylmethanol side chain analogs required access to benzaldehyde with carboxylic acid functionality in place. Attempts of lithium halogen exchange on 39 and 42 followed by quenching with DMF to get the desired benzaldehyde in the presence of carboxylic acid or carboxylic acid methyl ester as shown in Schemes 2.6 and 2.7 resulted in multiple unknown byproducts, possibly due to high energy di-anion intermediate formation this led us to propose new synthetic route.

**Scheme 2.6: Synthetic Challenge for Biaryl Analogs with Side Chain Modification 2a**

![Scheme 2.6](image)

**Reagents and Conditions:** (a) n-BuLi, DMF, THF, -78 °C, 3 h, no rxn.

**Scheme 2.7: Synthesis Challenge for Biaryl Analogs with Side Chain Modification 2b**

![Scheme 2.7](image)

**Reagents and Conditions:** (a) n-BuLi, DMF, THF, -78 °C, 3 h, no rxn.

Bromobenzaldehyde 44 was used as a starting material (Scheme 2.8). It was reacted with phenyl magnesium bromide to give bromodiphenylmethanol intermediate 46 in good yield (83%). Lithium halogen exchange was performed followed by quenching with
dimethylformamide to give the desired benzaldehyde 47 in 70% yield. The resulting benzaldehyde was condensed and cyclized with 1,2-diaminobenzene under oxidative condition and subsequent demethylation of ether gave desired resorcinol analog 41 in good yield (69%).

**Scheme 2.8: Synthesis of Biaryl Analogs with Side Chain Modification**

**Reagents and Conditions:** (a) THF, 0 °C, 3h, 83%; (b) n-BuLi, DMF, THF, -78 °C, 3 h, 70%; (c) FeCl₃, acetonitrile, RT, overnight, 69%; (d) BBr₃, DCM, 0 °C - RT, 16 h, 55-69%.

**2.2.3 Synthesis of Benzimidazolium Analogs**

Benzimidazolium analogs were planned in order to improve physicochemical properties mainly aqueous solubility of biaryl analogs. Various benzimidazole analogs 27f-27l were synthesized as described earlier in Scheme 2.4. Benzimidazole 27 was treated with methyl iodide in the presence of potassium carbonate in DMF to give N,N-dimethylbenzaimidazolium iodide 49, which precipitated after addition of water at room temperature. Demethylation of ether 49 was done by treating with boron tribromide at room temperature to give desired resorcinol 50 in moderate yield.
**Scheme 2.9** Synthesis of Benzimidazolium Analogs

Reagents and Conditions: (a) CAN, H₂O₂, acetonitrile, RT, overnight, 56-90%; (b) Iodomethane, K₂CO₃, DMF, overnight, 53-80%; (c) BBr₃, DCM, 0 °C - RT, 16 h, 27-84%.
2.2.4 Scale-Up Route for the Synthesis of Benzimidazolium Analogs

Compound 50a (AM9404) was needed in large quantities for further in vivo experiments. During the synthesis of compound 28a, dual addition of aldehyde 52 to 1,2-diaminobenzene 26 under oxidative condition using CAN and H₂O₂ was observed as a byproduct. Because of the formation of this byproduct, this reaction became unattractive for scale-up reactions. After screening a small set of reaction conditions, it was found that treating 1,3-dihydroxybenzaldehyde 52 with 1,2-diaminobenzene 26 in the presence of sodium metabisulfite as an oxidant cleanly gave desired benzimidazole with no formation of bis-addition aldehyde byproduct (Scheme 2.10). Benzimidazole was then N-methylated and demethylation of the ether was done as shown in the previous scheme to give the final desired product 50a.

Scheme 2.10: Scale-up route for the synthesis of 50a (AM9404)

Reagents and Conditions: (a) POCl₃, DMF, DCM, 0 °C, 3 h, 62%; (b) Sodium metabisulfite, DMA, 60°C, overnight, 71%; (c) Iodomethane, K₂CO₃, DMF, overnight, 82%; (d) BBr₃, DCM, 0 °C - RT, 16 h, 72%.
2.2.5 Benzimidazolium Analogs with 1-Adamantyl Chain

The Makriyannis lab has shown that replacing the dimethylheptyl chain in THC, and the biaryl scaffold with a 1-adamantyl group alters selectivity towards either CB₁ or CB₂ receptors. Interestingly, 1-adamantyl in the THC template favors CB₁ whereas 1-adamantyl in the biaryl template favors CB₂. It was decided to introduce the 1-adamantyl group in the benzimidazolium analogs to probe its influence on binding and selectivity towards cannabinoid receptors. Ortho lithiation was performed on dimethyl resorcinol ether 53 followed by quenching with DMF to give aldehyde 54 in 76% yields. Benzaldehyde 54 was condensed and cyclized with 1,2-diaminobenzene in the presence of hydrogen peroxide and catalytic cerium ammonium nitrate to give benzimidazole 55 in good yield (74%). N-methylation of 55 with iodomethane and K₂CO₃ yielded 56. Finally, demethylation of the ether was performed by treating with hydroiodic acid at 60 °C to give desired benzaimidazole resorcinol 57 in moderate yields (62%).

Scheme 2.11: Quaternary Ammonium Analogs with 1-Adamantyl Chain

Reagents and Conditions: (a) n-BuLi, DMF, THF, -78 °C, 3 h, 76%; (b) CAN, H₂O₂, acetonitrile, RT, overnight, 73%; (c) Iodomethane, K₂CO₃, DMF, overnight, 78%; (d) HI, 60 °C, 16 h, 63%. 

55
### Table 2.1 Affinities of Biaryl Analogs towards Cannabinoid Receptors

<table>
<thead>
<tr>
<th>Compound Structure</th>
<th>AM No.</th>
<th>rCB (_1) (nM)</th>
<th>mCB (_2) (nM)</th>
<th>hCB (_2) (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>TYPE A</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><img src="image1" alt="Compound 16" /></td>
<td><strong>16</strong> AM9407</td>
<td>&gt;1,000</td>
<td>&gt;1,000</td>
<td>--</td>
</tr>
<tr>
<td><img src="image2" alt="Compound 17" /></td>
<td><strong>17</strong> AM9408</td>
<td>74±7</td>
<td>145±14</td>
<td>137±13</td>
</tr>
<tr>
<td><img src="image3" alt="Compound 18" /></td>
<td><strong>18</strong> AM9409</td>
<td>600±60</td>
<td>780±80</td>
<td>--</td>
</tr>
<tr>
<td><img src="image4" alt="Compound 24a" /></td>
<td><strong>24a</strong> AM9410</td>
<td>&gt;1,000</td>
<td>710±70</td>
<td>--</td>
</tr>
<tr>
<td><img src="image5" alt="Compound 24b" /></td>
<td><strong>24b</strong> AM9411</td>
<td>71±7</td>
<td>124±12</td>
<td>56±6</td>
</tr>
<tr>
<td><img src="image6" alt="Compound 24c" /></td>
<td><strong>24c</strong> AM9412</td>
<td>&gt;1,000</td>
<td>&gt;1,000</td>
<td>--</td>
</tr>
<tr>
<td>Structure</td>
<td>Name</td>
<td>AM9413</td>
<td>AM9414</td>
<td>AM9415</td>
</tr>
<tr>
<td>-----------</td>
<td>--------</td>
<td>--------</td>
<td>--------</td>
<td>--------</td>
</tr>
<tr>
<td><img src="image1.png" alt="Structure" /></td>
<td><strong>24d</strong></td>
<td>&gt;1,000</td>
<td>--</td>
<td>&gt;1,000</td>
</tr>
<tr>
<td><img src="image2.png" alt="Structure" /></td>
<td><strong>24e</strong></td>
<td>&gt;1,000</td>
<td>--</td>
<td>&gt;1,000</td>
</tr>
<tr>
<td><img src="image3.png" alt="Structure" /></td>
<td><strong>28e</strong></td>
<td>&gt;1,000</td>
<td>&gt;1,000</td>
<td>--</td>
</tr>
<tr>
<td><img src="image4.png" alt="Structure" /></td>
<td><strong>28f</strong></td>
<td>&gt;1,000</td>
<td>--</td>
<td>&gt;1,000</td>
</tr>
<tr>
<td><img src="image5.png" alt="Structure" /></td>
<td><strong>28a</strong></td>
<td>&gt;1,000</td>
<td>&gt;1,000</td>
<td>&gt;1,000</td>
</tr>
<tr>
<td><img src="image6.png" alt="Structure" /></td>
<td><strong>28b</strong></td>
<td>95±10</td>
<td>227±22</td>
<td>297±30</td>
</tr>
<tr>
<td><img src="image7.png" alt="Structure" /></td>
<td><strong>28h</strong></td>
<td>75±8</td>
<td>953±90</td>
<td>210±21</td>
</tr>
<tr>
<td></td>
<td>Structure</td>
<td>Compound</td>
<td>IC50 (μM)</td>
<td>IC50 (μM)</td>
</tr>
<tr>
<td>-----</td>
<td>-----------</td>
<td>----------</td>
<td>-----------</td>
<td>-----------</td>
</tr>
<tr>
<td>27a</td>
<td><img src="image" alt="Structure" /></td>
<td>AM9401</td>
<td>&gt;1,000</td>
<td>&gt;1,000</td>
</tr>
<tr>
<td>27d</td>
<td><img src="image" alt="Structure" /></td>
<td>AM9402</td>
<td>&gt;1,000</td>
<td>&gt;1,000</td>
</tr>
<tr>
<td>28d</td>
<td><img src="image" alt="Structure" /></td>
<td>AM9429</td>
<td>&gt;1,000</td>
<td>&gt;1,000</td>
</tr>
<tr>
<td>28g</td>
<td><img src="image" alt="Structure" /></td>
<td>AM9430</td>
<td>&gt;1,000</td>
<td>&gt;1,000</td>
</tr>
<tr>
<td>49a</td>
<td><img src="image" alt="Structure" /></td>
<td>AM9403</td>
<td>&gt;1,000</td>
<td>&gt;1,000</td>
</tr>
<tr>
<td>50a</td>
<td><img src="image" alt="Structure" /></td>
<td>AM9404</td>
<td>5.68±0.6</td>
<td>76.6±8</td>
</tr>
</tbody>
</table>

**TYPE C**
<table>
<thead>
<tr>
<th>Compound</th>
<th>Formula</th>
<th>Type</th>
<th>Activity (Ki)</th>
</tr>
</thead>
<tbody>
<tr>
<td>50b AM9405</td>
<td><img src="image" alt="Structure" /></td>
<td></td>
<td>23.3±2</td>
</tr>
<tr>
<td>50c AM9406</td>
<td><img src="image" alt="Structure" /></td>
<td></td>
<td>21.2±2</td>
</tr>
<tr>
<td>50j AM9426</td>
<td><img src="image" alt="Structure" /></td>
<td></td>
<td>&gt;1,000</td>
</tr>
<tr>
<td>50h AM9421</td>
<td><img src="image" alt="Structure" /></td>
<td>TYPE D</td>
<td>&gt;1,000</td>
</tr>
<tr>
<td>50i AM9422</td>
<td><img src="image" alt="Structure" /></td>
<td></td>
<td>770±75</td>
</tr>
<tr>
<td>Compound</td>
<td>AM9423</td>
<td>AM9424</td>
<td>AM9425</td>
</tr>
<tr>
<td>------------</td>
<td>--------</td>
<td>--------</td>
<td>--------</td>
</tr>
<tr>
<td>50e</td>
<td>24.7±2</td>
<td>169±16</td>
<td>108±10</td>
</tr>
<tr>
<td>50f</td>
<td>120±12</td>
<td>334±33</td>
<td>325±32</td>
</tr>
<tr>
<td>50g</td>
<td>25±2</td>
<td>157±15</td>
<td>55.4±5</td>
</tr>
<tr>
<td>50d</td>
<td>&gt;1,000</td>
<td>&gt;1,000</td>
<td>--</td>
</tr>
<tr>
<td>TYPE E</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>41</td>
<td>&gt;1,000</td>
<td>&gt;1,000</td>
<td>--</td>
</tr>
<tr>
<td>41a</td>
<td>74.2±7</td>
<td>&gt;1,000</td>
<td>&gt;1,000</td>
</tr>
</tbody>
</table>
Binding affinities for CB₁ and CB₂ were determined using rat brain (CB₁) or membranes from HEK293 cells expressing mouse or human CB₂ and [³H]CP-55,940 as the radioligand. Ki values for these compounds were obtained from one experiment (8 point) run in triplicate when experiments using the two point data (in triplicate) showed Ki values below 1000 nM.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Ki (nM)</th>
<th>S.D. 1</th>
<th>S.D. 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>AM10407</td>
<td>&gt;1,000</td>
<td>&gt;1,000</td>
<td>--</td>
</tr>
<tr>
<td>AM10408</td>
<td>&gt;1,000</td>
<td>&gt;1,000</td>
<td>--</td>
</tr>
<tr>
<td>AM10409</td>
<td>&gt;1,000</td>
<td>&gt;1,000</td>
<td>--</td>
</tr>
<tr>
<td>AM10410</td>
<td>&gt;1,000</td>
<td>&gt;1,000</td>
<td>--</td>
</tr>
<tr>
<td>AM10405</td>
<td>&gt;1,000</td>
<td>590±60</td>
<td>420±42</td>
</tr>
<tr>
<td>AM10406</td>
<td>&gt;1,000</td>
<td>30±3</td>
<td>193±20</td>
</tr>
</tbody>
</table>
2.4 Structure-Activity Relationships (SAR)

The ability of compounds AM9401 – AM9430; AM10405-10410 to displace radiolabeled CP-55,940 from purified rat forebrain synaptosomes and HEK293 cells expressing either mCB₂ or hCB₂ were determined, as described in the experimental Section.

In this study we chose to investigate the effects of modifying ortho-substitutions on rings A and C ring in the biaryl template with regard to their affinity and selectivity for CB₁ and CB₂ receptors. First, we explored the biphenyl analogs in which either both ortho-substituents on ring A are methoxy groups 16 (AM9407), or only one ortho group is present as a methoxy while the other is –OH 18 (AM9409) or both ortho groups are OH 17 (AM9408). AM9407 exhibited no significant affinities for either receptor subtypes, whereas, AM9409 containing one –OH group exhibited moderate affinity Ki (rCB₁) = 600nM and Ki (mCB₂) = 780nM. The affinity for both receptors was further increased when both phenolic –OH groups were present on ring A 17 (AM9408). The compound 17 exhibited preference for rCB₁ (Ki = 74.3nM) over mCB₂ (Ki = 145nM) and hCB₂ (137nM).

Analogs 24a (AM9410) and 24c (AM9412) containing 2-pyridyl ring and 2-pyrimidinyl rings, respectively, were designed so that their C-rings are capable of engaging one or both of the –OH groups in intra-molecular H-bonding, the compound exhibited no affinity (>1000 nM) at either receptor. These data support the significance of A ring –OH groups for suitable receptor interactions. A similarly reduced affinity was observed in analog 24d (AM9413) in which B-ring was 2-imidazolyl. An interesting finding was observed with analog 24b (AM9411) in which an ortho-methyl group present on 2-pyridyl B ring, which exhibited significantly improved affinity for all receptor subtypes (Ki = 71nM for rCB₁; Ki = 124nM for mCB₂; and Ki = 56 nM for
hCB<sub>2</sub>). 24<sub>e</sub> (AM9414) which contains a mono N-methylated 2-imidazolyl ring, did not exhibit the same trend for increased affinity. These initial findings underscored the significance of having two phenolic hydroxyl groups for favorable interactions with both receptor subtypes and highlighted the importance of torsion angle between two phenyl rings.

We further explored the introduction of fused-heterocyclic rings such as benzothiazole 28<sub>e</sub> (AM9415), benzimidazole 28<sub>a</sub> (AM9417) and benzoazole 28<sub>d</sub> (AM9429) as ring C in the biaryl template. All these analogs and their dimethoxy/monomethoxy derivatives 27<sub>a</sub> (AM9401), 28<sub>f</sub> (AM9416), 27<sub>d</sub> (AM9402) and 28<sub>g</sub> (AM9430) exhibited diminished affinity (>1000 nM) for both CB receptor subtypes. Interestingly, the ring A mono N-methylated derivatives, 28<sub>b</sub> (AM9418) and 28<sub>h</sub> (AM9419) exhibited significantly improved affinities for both receptor subtypes.

Encouraged by these findings, we had further developed analogs in which both nitrogen atoms in the benzimidazole rings are methylated. We anticipated that if both methyl groups are tolerated, the resulting permanently charged compounds could serve as peripherally restricted cannabinoids which should be devoid of CNS effects. Analog 50<sub>a</sub> (AM9404) with a chloride counter ion exhibited highest affinity of all analogs synthesized in this series, with a Ki of 5.7nM (rCB<sub>1</sub>), 76.6 nM (mCB<sub>2</sub>) and 53.8 nM (hCB<sub>2</sub>). Other salts bromide 50<sub>b</sub> (AM9505) and iodide 50<sub>c</sub> (AM9406) exhibited nearly the same affinity as AM9404 for both receptor subtypes. Of all the three salts, AM9405 (bromide salt) had slightly better affinities for all CB receptor subtypes, Ki of 23.3 nM (rCB<sub>1</sub>) and 27.9 nM (hCB<sub>2</sub>). Dimethylated 2-imidazolyl compound, AM9426, however, did not exhibit significant affinity for CB receptors.
In an effort to further optimize AM9404, our lead molecule, we incorporated different substituents at the 4’ and 5’-position of benzimidazole ring. Analogs 50f (AM9424) and 50d (AM9420) containing fluoro- and carboxylate groups respectively at the 4’-position, exhibited reduced or no affinities for the CB receptor subtypes. Conversely, 50g (AM9425) with a 4’-chloro group, retained affinity at CB1 (Ki = 25.1) but had reduced affinity at mCB2 (Ki = 157.6nM) and hCB2 (55.4nM) compared to AM9404. Analog 50e (AM9423) with a 4’-CH3 group exhibited a similar affinity as AM9425 but with further reduced hCB2 affinity (Ki = 108nM). Substitution at 5’-position with chloro- 50i (AM9422) or –CF3 50h (AM9421) completely abolished for either receptors.

To explore the compatibility of N,N-dimethylated quaternary benzimidazole ring with other C-3 side chains that were previously shown to impart CB1 or CB2 selectivity, we designed and synthesized analogs 41 (AM9427), 41a (AM9428) and 33-36, 38, 56 and 57 (AM10405-AM10410). Compounds AM9427 and AM9428 containing a C1’-phenyl substitution and –OH group exhibited diminished affinity for both the CB1 and CB2 receptors with similar results for compounds containing a thio ether 33 (AM10407), 34 (AM10408), a phenyl sulfone 36 (AM10409) or a phenyl sufoxide 38 (AM10410). Analog 57 (AM10406), containing 1-adamantyl side chain at C-3 position, exhibited high affinity for mCB2 (Ki = 30nM) and over 50-fold selectivity over rCB1 (Ki = >1000nM). It had reduced affinity at hCB2 (Ki = 100nM) thus exhibiting species difference.
2.5 Functional Assay:

Cannabinoid receptors are members of the G-protein coupled receptors and are negatively coupled to G-proteins. By measuring cyclic adenosine monophosphate (cAMP) levels, the ligand’s functional properties can be determined. Ligands that show decreases in cAMP are agonists and ligands that show increases in cAMP are antagonists. AM9405 was evaluated in hCB₁ and hCB₂ functional assays such as cAMP and β-arrestin (Table 2.2). AM9405 acts as an agonist with 88% inhibition at 1 µM for hCB₁ and 92% inhibition at 1 µM for hCB₂. AM9405 also showed 55% inhibition at 10 nM making it potent agonist. Interestingly, AM9405 was completely inactive in β-arrestin assay suggesting that it may be a biased agonist since cAMP and β-arrestin may be involved in two separate GPCR signaling pathways.

Table 2.2 cAMP of AM9405

<table>
<thead>
<tr>
<th>AM no.</th>
<th>Structure</th>
<th>Functional Data</th>
<th>cAMP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>hCB₁ (Ki)</td>
<td>hCB₂ (Ki)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10nM</td>
<td>1uM</td>
</tr>
<tr>
<td>AM9405</td>
<td><img src="image" alt="Structure" /></td>
<td>-25%</td>
<td>88%</td>
</tr>
</tbody>
</table>
2.6 *In vivo* Studies:

Based on high affinity of AM9405, we decided to advance it for *in vivo* studies in Dr. Carol Paronis Lab at Northeastern University as well as Dr. Martin Storr’s Lab at University of Calgary. The results described here were generated by above investigators and we summarized it in the following sections.

2.6.1 Colonic Propulsion Assay: To further characterize the action of AM9405 on GI transit, we examined its effects on colonic propulsion. The reference compound WIN55212, as non-selective CB₁/CB₂ agonist, slowed colonic propulsion and this effect was almost completely blocked by the CB₁ antagonist AM251. At a similar dose, i.e. 1.0 mg/Kg, AM9405 inhibited colonic propulsion in a manner that was completely blocked by AM251 (Figure 2.4). WIN55,212-2 was slightly more potent than AM9405 in this assay.

![Colonic Propulsion Study](image)

**Figure 2.4:** Colonic Propulsion Study; The effect of vehicle (veh; 4% DMSO, 2% Tween 80 in physiological saline), 1.0 mg/kg AM251 administered 15 minutes before the vehicle, WIN 55212-2 (WIN; 0.1mg/kg) or AM9405 (0.1 mg/kg) on colonic propulsion in mice. Drug solutions were administered intraperitoneally.
2.6.2 Whole Gut Transit Assay: Experiments were carried out to examine the effects of AM9405 on whole gut transit. We used WIN55,212-2, an established CB₁/CB₂ agonist, as a reference compound. WIN55,212-2 did not have any effect on gut transit at a dose of 0.1mg/Kg (Figure 2.5). Previous studies published by Cluny, et al., WIN⁹⁵ was shown to have moderate effects of gut transit at a higher dose of 1.0mg/Kg. We did not look into the high concentration effects of WIN in this experiment. AM9405 slowed whole gut transit at a dose of 0.1mg/Kg. In mice treated with AM251 (a CB₁ antagonist) 15 minutes prior to AM9405, a significant decrease in transit time was observed.

Figure 2.5: Whole Gut Transit Study; The effect of vehicle (veh; 4% DMSO, 2% Tween 80 in physiological saline), 1.0mg/kg AM251 administered 15 minutes before the vehicle, WIN 55212-2 (WIN; 0.1mg/kg) or AM9405 (0.1 mg/kg) on whole gut transit in mice.
2.6.3 Stress-induced Bowel Movement (IBS-like) Assay: The effect of novel peripherally restricted CB₁/CB₂ agonist AM9405 was further determined on stress-induced bowel movements. These animals were exposed to a new environment, i.e. separate cages, which induces stress (as measured by increase in defecation; number of pellets produced per hour). AM9405 and WIN55,212-2 normalized the stress-induced bowel movement response at a dose of 1.0mg/kg (Figure 2.6). However, both cannabinoids did not reduce the number of pellets produced in non-stressed animals.

![Stress-induced Bowel Movement (IBS-like) Study](image)

**Figure 2.6:** Stress-induced Bowel Movement (IBS-like) Study; The effect of vehicle (veh; 4% DMSO, 2% Tween 80 in physiological saline), WIN 55212-2 (WIN; 1.0mg/kg) or AM9405 (1.0 mg/kg) on stress-induced bowel movement. Drug solutions were administered intraperitoneally.
2.6.4 Castor Oil Induced Diarrhea Studies: To further assess the effect of AM9405 on the GI tract, we carried out experiments using castor oil induced diarrhea. Mice treated with castor oil exhibited diarrhea (Figure 2.7). AM9405 at a dose of 1.0mg/kg significantly delayed diarrhea and it was slightly more potent than WIN55,212-2 (1.0mg/kg).

![Castor oil graph](image)

**Figure 2.7:** Castor Oil Induced Diarrhea Studies; The effect of vehicle (veh; 4% DMSO, 2% Tween 80 in physiological saline), WIN 55212-2 (WIN; 1.0mg/kg) or AM9405 (1.0 mg/kg) on castor oil induced diarrhea. Drug solutions were administered intraperitoneally.

2.6.5 Effect on Visceral Abdominal Pain Induced by Mustard Oil: The effect of AM9405 on mustard oil induced visceral abdominal pain was measuring total number of behaviors (Figure 2.8). AM9405 at 0.1mg/kg and 0.3mg/kg significantly reduced visceral abdominal pain induced by mustard oil (1% in 70% EtOH). It was not effective at a very low dose of 0.01mg/kg. CB₁
agonist AM251 completely reversed the effects produced by AM9405. Pretreatment with AM630, a CB$_2$ selective antagonist, together with AM251, did not show any difference with respect to pretreatment with AM251 alone. This test shows that AM9405 reduces abdominal pain through a CB$_1$ mechanism.

**Figure 2.8:** Effect on Visceral Abdominal Pain Induced by Mustard Oil study; The effect of AM9405 (1.0 mg/kg) or AM251 (1.0 mg/kg) and or AM630 (1.0 mg/kg) administered 15 minutes before AM9405 (1.0 mg/kg) on oil of mustard (OM; 1% in 70%EtOH; given intracolonic) induced visceral abdominal pain. AM251, AM630 and AM9405 drug solutions were administered intraperitoneally.
2.6.6 Evidence for the peripheral activity of AM 9405.

1) Preliminary Distribution in Brain/Plasma and Drug Oral Bioavailability: AM9405 was administered both iv (0.25 mg/kg) and orally (2.0 mg/kg) to determine oral bioavailability and brain permeability (Table 2.3). Compound AM9405 possessed only 4-5% oral bioavailability. Given either by iv or orally, this compound was not detected in brain, thus confirming its restricted access to BBB.

Table 2.3: BBB Penetration Study of AM9405

<table>
<thead>
<tr>
<th>Compound</th>
<th>IV plasma (ng/μL)</th>
<th>IV brain (ug/g)</th>
<th>Oral Plasma (ng/μL)</th>
<th>Oral brain (ug/g)</th>
<th>Predicted Oral Bioavailability</th>
<th>Brain:Plasma Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>AM-9405 0.25 mg/kg IV 2 mg/kg oral</td>
<td>0.029 ± 0.001</td>
<td>Not detected, but 0.038 at 2 mg/kg, n=1</td>
<td>0.009 ± 0.01 (30 min) 0.011 ± 0.013 (60 min)</td>
<td>Not detected (30 min) Not detected (60 min)</td>
<td>4-5%</td>
<td>IV = NA Oral (30 min) = NA Oral (60 min) = NA</td>
</tr>
</tbody>
</table>
2) **Hypothermia Studies:** Body temperature was measured in isolated rats over a 6 h period following drug injection. AM9405 did not decrease body temperature when given subcutaneously at a dose of 3.0mg/kg and 10.0mg/kg (Figure 2.9).

**Figure 2.9:** Hypothermia Studies; The effect of vehicle (20% ethanol, 20% emulphor, and 60% saline) and AM9405 (3.0 and 10.0 mg/kg) on body temperature in rats.
3) We further investigated the role of peripheral vs. central CB$_1$ receptors in AM9405’s effect on oil of mustard (OM) induced visceral abdominal pain. A dose of 0.3mg/kg of AM9405 significantly reduces pain induced by OM and this effect was not antagonized by intracerebroventricular (icv) administration of AM251 (Figure 2.10). On the other hand, WIN55,212-2, an established centrally acting CB$_1$/CB$_2$ agonist produced moderate antinociceptive effects (0.3mg/kg) compared to AM9405 and these effects were completely reversed by icv administration of AM251.

![Figure 2.10: Visceral Abdominal Pain Study; The effect of AM9405 (0.3mg/kg; ip), WIN (0.3mg/kg; ip) or AM251 (1.0 mg/kg; icv) administered 15 minutes before AM9405 (1.0 mg/kg) or WIN (0.3mg/kg; ip) on oil of mustard (OM; 1% in 70%EtOH; given intracolonic) induced visceral abdominal pain.](image-url)
2.7 Conclusions:

In summary, early SAR studies showed that both hydroxyls in the ring A within biaryl template are required for affinities for both CB receptors. The presence of a methyl group at the ortho position in the C ring improves binding affinities for both CB receptors. This observation was consistent for the phenyl, pyridine and benzimidazole groups. However, small five membered heterocycles in the C ring did not lead to favorable affinities.

The presence of ‘ortho methyl’ groups in the ring C led to enhanced CB receptor affinities. We discovered a potent quaternary ammonium lead analog AM9405, it contains a dimethylated permanently charged imidazole ring C which is expected to be out of plane of the phenolic ring because of its methyl substituents. Substitutions on the phenyl ring of benzimidazole did not improve affinities for both CB₁ and CB₂. AM9405 was evaluated in vivo studies and observed that it did not significantly enter into the brain and has peripheral activity in a GI track experiment. AM9405 has potential for treating GI track disorders, while exhibiting no CNS cannabinoid side-effects. AM9405 needs to be evaluated further to explore its potential as a therapeutic agent.

2.8 Experimental:

1-(3,5-Dimethoxyphenyl)heptan-1-one (12): To a solution of 1,3-dimethoxybenzene (5 g, 36.2 mmol) in THF (100 mL) was added hexylmagnesium bromide (2M in diethyl ether) (23.52 mL, 47 mmol) followed by copper(I) bromide (0.104 g, 0.724 mmol) under inert atmosphere and the
reaction mixture was refluxed for 15 min. The reaction solution was cooled to room temperature and 8 mL of water was added slowly followed by 45 mL of 15% H$_2$SO$_4$ and stirred for overnight at the same temperature. Reaction was diluted with 100 mL diethyl ether and the organic layer was separated, washed with 50 mL water, brine, dried (MgSO$_4$) and concentrated under reduced pressure. The crude product was purified by silica gel chromatography eluting with 10-50% ethyl acetate : hexanes to afford the desired product 12 (8.5 g, 34.0 mmol, 94% yield) as a white solid.

$^1$H NMR (500 MHz, CDCl$_3$) $\delta$: 7.10 (d, $J = 2.0$ Hz, 2H), 6.64 (t, $J = 2.0$ Hz, 1H), 3.84 (s, 6H), 2.92 (t, $J = 7.0$ Hz, 2H), 1.72 (quintet, $J = 7.5$ Hz, 2H), 1.42-1.28 (m, 6H), 0.89 (t, $J = 7.0$ Hz, 3H). HRMS (ESI) calculated for C$_{15}$H$_{22}$O$_3$: calculated 251.1647; found 251.1639.

![Image](image.png)

**1,3-Dimethoxy-5-(2-methyloct-2-yl)benzene (13):** To a stirring solution of TiCl$_4$ (1M in DCM) (120 mL, 120 mmol) was added a solution of dimethylzinc (1.2M in toluene) (100 mL, 120 mmol) using addition funnel at -40 °C and stirred for 30 min under argon blanket. To the mixture was added a cooled solution of 1-(3,5-dimethoxyphenyl)heptan-1-one (5 g, 19.97 mmol) in DCM (80 mL) using cannula over the period of 5 min while maintaining temperature at -40 °C and stirred for 2 hrs. The reaction mixture was poured in ice cold water under stirring condition to obtained colorless solution from dark red reaction solution. The organic layer was separated, washed with 50 mL water, brine, dried (MgSO$_4$) and concentrated under reduced pressure. The crude product was purified by silica gel chromatography eluting with 5-40% ethyl acetate : hexanes to afford the desired product 13 (4.73 g, 17.89 mmol, 90% yield) as a colorless oil. (Note: The product has little impurity which was difficult to separate and was carried to the next
step without further purification.) HRMS (ESI) calculated for C_{17}H_{28}O_2: calculated 265.2168; found 265.2166.

2-Bromo-1,3-dimethoxy-5-(2-methyloctan-2-yl)benzene (14): To a solution of 1,3-dimethoxy-5-(2-methyloctan-2-yl)benzene (2.02 g, 7.64 mmol) in DCM (75 mL) was added 18-crown-6 (0.202 g, 0.764 mmol) followed by bromine (0.394 mL, 7.64 mmol) at 0 °C. The reaction mixture was stirred for 30 min at room temperature and quenched by adding 50 mL aqueous sodium bisulfite solution. The organic layer was separated, washed with 2x50 mL water and brine, dried (MgSO_4) and concentrated under reduced pressure. The crude product was purified by silica gel chromatography eluting with 5-50% ethyl acetate : hexanes to afford the desired product 14 (2.55 g, 7.43 mmol, 97 % yield) as a white solid. ^1H NMR (500 MHz, CDCl_3) δ: 6.54 (s, 2H), 3.90 (s, 6H), 1.61-1.49 (m, 2H), 1.29 (s, 6H), 1.27-1.11 (m, 6H), 1.10-1.01 (m, 2H), 0.85 (t, J = 7.0 Hz, 3H).

2,6-Dimethoxy-4-(2-methyloctan-2-yl)-1,1'-biphenyl (16): To a solution of 2-bromo-1,3-dimethoxy-5-(2-methyloctan-2-yl)benzene (150 mg, 0.437 mmol) in 1,2-Dichloroethane (3 mL) was added phenylboronic acid (64 mg, 0.525 mmol), barium hydroxide (150 mg, 0.875 mmol) and Pd(Ph_3P)_4 (15.16 mg, 0.013 mmol) and heated to 110 °C for 6 hrs. The reaction mixture was
diluted with 25 mL ethyl acetate and 25 mL water. The organic layer was separated, washed with 2x20 mL water and brine, dried (MgSO₄) and concentrated under reduced pressure. The crude product was purified by silica gel chromatography eluting with 5-50% ethyl acetate : hexanes to afford the desired product 16 (125 mg, 0.367 mmol, 84 % yield) as a white solid. ¹H NMR (500 MHz, CD₃OD) δ: 7.41-7.34 (m, 4H), 7.29 (tt, J = 7.0 Hz, J = 1.5 Hz, 1H), 6.61 (s, 2H), 3.72 (s, 6H), 1.66-1.60 (m, 2H), 1.33 (s, 6H), 1.28-1.21 (m, 6H), 1.18-1.12 (m, 2H), 0.86 (t, J = 7.0 Hz, 3H). HRMS (ESI) calculated for C₂₃H₃₂O₂: calculated 341.2481; found 341.2486.

4-(2-Methyloctan-2-yl)-[1,1'-biphenyl]-2,6-diol (17): To a solution of 2,6-dimethoxy-4-(2-methyloctan-2-yl)-1,1'-biphenyl (100 mg, 0.294 mmol) in DCM (3 mL) was added BBr₃ (0.278 mL, 2.94 mmol) at 0 °C and stirred for overnight while warming to room temperature. The reaction mixture was diluted with 25 mL DCM and 25 mL water. The organic layer was separated, washed with 25 mL sat. NaHCO₃ solution, 25 mL water and brine, dried (MgSO₄) and concentrated under reduced pressure. The crude product was purified by silica gel chromatography eluting with 10-50% ethyl acetate : hexanes to afford the desired product 17 (82 mg, 0.262 mmol, 89 % yield) as a white solid. ¹H NMR (500 MHz, CD₃OD) δ: 7.46-7.39 (m, 4H), 7.30 (tt, J = 7.0 Hz, J = 1.5 Hz, 1H), 6.49 (s, 2H), 1.62-1.55 (m, 2H), 1.30-1.20 (m, 12H, especially, s, 1.27, 6H), 1.18-1.10 (m, 2H), 0.87 (t, J = 7.0 Hz, 3H). HRMS (ESI) calculated for C₂₁H₂₈O₂: calculated 313.2168; found 313.2162.
6-Methoxy-4-(2-methyloctan-2-y1)-[1,1'-biphenyl]-2-ol (18): To a solution of 2,6-dimethoxy-4-(2-methyloctan-2-y1)-1,1'-biphenyl (25 mg, 0.073 mmol) in DCM (3 mL) was added BBr₃ (0.014 mL, 0.147 mmol) at 0 °C and stirred for overnight while warming to room temperature. Reaction was diluted with 25 mL DCM and 25 mL water. The organic layer was separated, washed with 25 mL sat. NaHCO₃ solution, 25 mL water and brine, dried (MgSO₄) and concentrated under reduced pressure. The crude product was purified by silica gel chromatography eluting with 10-50% ethyl acetate : hexanes to afford the desired product 18 (12 mg, 0.037 mmol, 50.1 % yield) as a white solid. ¹H NMR (500 MHz, CDCl₃) δ: 7.50-7.46 (m, 2H), 7.41-7.36 (m, 3H), 6.64 (d, J = 2.0 Hz, 1H), 6.52 (d, J = 1.5 Hz, 1H), 4.95 (s, 1H, OH), 3.73 (s, 3H), 1.63-1.57 (m, 2H), 1.30 (s, 6H), 1.28-1.20 (m, 6H), 1.18-1.10 (m, 2H), 0.86 (t, J = 7.0 Hz, 3H). HRMS (ESI) calculated for C₂₂H₃₁O₂: calculated 327.2324; found 327.2315.

2-(2,6-Dimethoxy-4-(2-methyloctan-2-y1)phenyl)-1-methyl-1H-imidazole (20) as per scheme 2.2: To a solution of 2-bromo-1,3-dimethoxy-5-(2-methyloctan-2-y1)benzene (50 mg, 0.146 mmol), 1,10-phenanthroline (1.312 mg, 7.28 µmol), 1-methyl-1H-imidazole (17.94 mg, 0.218 mmol) and K₃PO₄ (50.7 mg, 0.291 mmol) in DMF (1 mL) was added copper(I) iodide (1.387
mg, 7.28 µmol) and heated to 140 °C for 24 hrs. Only un-reacted starting material was observed and the reaction mixture was discarded.

To a solution of 1-methyl-1H-imidazole (80 mg, 0.974 mmol) in THF (1 mL) was added n-butyllithium (0.260 mL, 0.650 mmol) and stirred for 30 min at -78 °C. After tributyltin chloride (0.176 mL, 0.650 mmol) was added to the solution, reaction was stirred for overnight at room temperature. To this reaction mixture was added 2-bromo-1,3-dimethoxy-5-(2-methylctan-2-yl)benzene (50 mg, 0.146 mmol) and Pd(Ph₃P)₄ (751 mg, 0.650 mmol) and stirred for overnight at 60 °C. Multiple byproducts were observed and the reaction mixture was discarded.

(2,6-Dimethoxy-4-(2-methylctan-2-yl)phenyl)boronic acid (21): To a solution of 2-bromo-1,3-dimethoxy-5-(2-methylctan-2-yl)benzene (600 mg, 1.748 mmol) in THF (5 mL) was added n-butyllithium (1.202 mL, 1.923 mmol) at -78 °C and stirred for 30 min. To the reaction was added trimethyl borate (0.391 mL, 3.50 mmol) and stirred for 3 hrs. The reaction mixture was diluted with 25 mL ethyl acetate and 25 mL aqueous ammonium chloride solution. The organic layer was separated, washed with 2x50 mL water and brine, dried (MgSO₄) and concentrated under reduced pressure. The crude product was purified by silica gel chromatography eluting with 10-50% ethyl acetate : hexanes to afford the desired product 21 (435 mg, 1.411 mmol, 81% yield) as a colorless oil. ¹H NMR (500 MHz, CDCl₃) δ: 6.58 (s, 2H), 3.92 (s, 6H), 1.62-1.56 (m, 2H), 1.30 (s, 6H), 1.26-1.16 (m, 6H), 1.10-1.02 (m, 2H), 0.85 (t, J = 7.0 Hz, 3H). HRMS (ESI)
2-(2,6-Dimethoxy-4-(2-methyloctan-2-yl)phenyl)pyridine (23a): To a solution of (2,6-dimethoxy-4-(2-methyloctan-2-yl)phenyl)boronic acid (50 mg, 0.162 mmol) in 1,2-Dichloroethane (3 mL) was added 2-bromopyridine (30.8 mg, 0.195 mmol) and sodium carbonate (34.4 mg, 0.324 mmol) and argon was bubbled through the reaction solution for 1 min. To the reaction mixture Pd(Ph₃P)₄ (5.62 mg, 4.87 µmol) was added and heated to 110 °C for 6 hrs. The reaction mixture was diluted with 25 mL ethyl acetate and 25 mL water. The organic layer was separated, washed with 2x20 mL water and brine, dried (MgSO₄) and concentrated under reduced pressure. The crude product was purified by silica gel chromatography eluting with 10-50% ethyl acetate : hexanes to afford the desired product 23a (35 mg, 0.102 mmol, 63% yield) as a white solid. ¹H NMR (500 MHz, CDCl₃) δ: 8.93 (d, J = 5.0 Hz, 1H), 8.31 (dt, J = 8.0 Hz, J = 1.5 Hz, 1H), 7.98 (d, J = 8.0 Hz, 1H), 7.70 (dt, J = 7.5 Hz, J = 1.0 Hz, 1H), 6.61 (s, 2H), 3.80 (s, 6H), 1.64-1.58 (m, 2H), 1.31 (s, 6H), 1.28-1.18 (m, 6H), 1.14-1.06 (m, 2H), 0.84 (t, J = 7.0 Hz, 3H). HRMS (ESI) calculated for C₂₂H₃₂N₂O₂: calculated 342.2433; found 342.2430.

5-(2-Methyloctan-2-yl)-2-(pyridin-2-yl)benzene-1,3-diol (24a): To a solution of 2-(2,6-dimethoxy-4-(2-methyloctan-2-yl)phenyl)pyridine (25 mg, 0.073 mmol) in DCM (3 mL) was
added BBr$_3$ (0.069 mL, 0.732 mmol) at 0 °C and stirred for overnight while warming it to room temperature. The reaction mixture was diluted with 25 mL DCM and 25 mL water. The organic layer was separated, washed with 25 mL sat. NaHCO$_3$ solution, 25 mL water and brine, dried (MgSO$_4$) and concentrated under reduced pressure. The crude product was purified by silica gel chromatography eluting with 10-50% ethyl acetate : hexanes to afford the desired product **24a** (16 mg, 0.051 mmol, 69% yield) as a white solid. $^1$H NMR (500 MHz, CDCl$_3$) $\delta$: 8.41 (d, $J = 8.5$ Hz, 1H), 8.33 (d, $J = 5.0$ Hz, 1H), 7.99 (t, $J = 8.0$ Hz, 1H), 7.36 (t, $J = 6.0$ Hz, 1H), 6.54 (s, 2H), 1.50-1.45 (m, 2H), 1.31 (s, 6H), 1.28-1.18 (m, 6H), 1.14-1.06 (m, 2H), 0.84 (t, $J = 7.0$ Hz, 3H). HRMS (ESI) calculated for C$_{20}$H$_{28}$NO$_2$: calculated 314.2120; found 314.2110.

![Structure](image_url)

**2,2',6-Trimethyl-4-(2-methyloctan-2-yl)-1,1'-biphenyl (23b):** The synthesis was carried out as described for **23a** by using (2,6-dimethoxy-4-(2-methyloctan-2-yl)phenyl)boronic acid (50 mg, 0.162 mmol), 2-bromo-3-methylpyridine (33.5 mg, 0.195 mmol), sodium carbonate (34.4 mg, 0.324 mmol) and Pd(Ph$_3$P)$_4$ (5.62 mg, 4.87 µmol) to give **23b** (35 mg, 0.098 mmol, 60% yield) as a white solid. (Note: product has little impurity which was difficult to separate and was carried to next step without further purification). HRMS (ESI) calculated for C$_{23}$H$_{34}$NO$_2$: calculated 356.2590; found 356.2583.
5-(2-Methyloctan-2-yl)-2-(3-methylpyridin-2-yl)benzene-1,3-diol (24b): The synthesis was carried out as described for 24a by using 2-(2,6-dimethoxy-4-(2-methyloctan-2-yl)phenyl)-3-methylpyridine (25 mg, 0.070 mmol) and BBr₃ (0.066 mL, 0.703 mmol) to give 24b (18 mg, 0.055 mmol, 78% yield) as a white solid. ¹H NMR (500 MHz, CD₃OD) δ: 8.59 (dd, J = 6.0 Hz, J = 1.0 Hz, 1H), 8.49 (dd, J = 6.0 Hz, J = 1.0 Hz, 1H), 7.89 (dd, J = 8.0 Hz, J = 5.0 Hz, 1H), 6.54 (s, 2H), 2.41 (s, 3H), 1.64-1.56 (m, 2H), 1.28 (s, 6H), 1.26-1.18 (m, 6H), 1.15-1.08 (m, 2H), 0.87 (t, J = 7.0 Hz, 3H).

2-(2,6-Dimethoxy-4-(2-methyloctan-2-yl)phenyl)pyrimidine (23c): The synthesis was carried out as described for 23a by using (2,6-dimethoxy-4-(2-methyloctan-2-yl)phenyl)boronic acid (50 mg, 0.162 mmol), 2-bromopyrimidine (30.9 mg, 0.195 mmol), sodium carbonate (34.4 mg, 0.324 mmol) and Pd(Ph₃P)₄ (5.62 mg, 4.87 µmol) to give 23c (30 mg, 0.088 mmol, 54% yield) as a white solid. ¹H NMR (500 MHz, CDCl₃) δ: 9.13 (d, J = 5.5 Hz, 2H), 7.52 (t, J = 5.5 Hz, 1H), 6.61 (s, 2H), 3.76 (s, 6H), 1.66-1.58 (m, 2H), 1.33 (s, 6H), 1.29-1.18 (m, 6H), 1.15-1.06 (m, 2H), 0.86 (t, J = 7.0 Hz, 3H). HRMS (ESI) calculated for C₂₁H₃₁N₂O₂: calculated 343.2386; found 343.2381.
5-(2-Methyloctan-2-yl)-2-(pyrimidin-2-yl)benzene-1,3-diol (24c): The synthesis was carried out as described for 24a using 2-(2,6-dimethoxy-4-(2-methyloctan-2-yl)phenyl)pyrimidine (25 mg, 0.073 mmol) and BBr₃ (0.069 mL, 0.730 mmol) to give 24c (15 mg, 0.048 mmol, 65% yield) as a white solid. ¹H NMR (500 MHz, CD₃OD) δ: 8.88 (d, J = 5.5 Hz, 1H), 7.41 (t, J = 5.0 Hz, 1H), 6.43 (s, 2H), 1.62-1.56 (m, 2H), 1.26 (s, 6H), 1.25-1.18 (m, 6H), 1.14-1.06 (m, 2H), 0.85 (t, J = 7.0 Hz, 3H). HRMS (ESI) calculated for C₁₉H₂₇N₂O₂: calculated 315.2073; found 315.2069.

2-(2,6-Dimethoxy-4-(2-methyloctan-2-yl)phenyl)-1H-imidazole (23d): The synthesis was carried out as described for 23a by using (2,6-dimethoxy-4-(2-methyloctan-2-yl)phenyl)boronic acid (50 mg, 0.162 mmol), 2-bromo-1H-imidazole (23.84 mg, 0.162 mmol), sodium carbonate (34.4 mg, 0.324 mmol) and Pd(Ph₃P)₄ (5.62 mg, 4.87 µmol) to give 23d (29 mg, 0.088 mmol, 54.1% yield) as a white solid. (Note: product has little impurity which was difficult to separate. Crude product was carried to next step without further purification.) HRMS (ESI) calculated for C₂₀H₃₀N₂O₂: calculated 330.2307; found 330.2305.
2-(1H-Imidazol-2-yl)-5-(2-methyloctan-2-yl)benzene-1,3-diol (24d): The synthesis was carried out as described for 24a using 2-(2,6-dimethoxy-4-(2-methyloctan-2-yl)phenyl)-1H-imidazole (20 mg, 0.061 mmol) and BBr₃ (0.057 mL, 0.605 mmol) by using 24d (13 mg, 0.043 mmol, 71% yield) as a white solid. ¹H NMR (500 MHz, CD₃OD) δ: 7.41 (s, 2H), 6.61 (s, 2H), 1.64-1.56 (m, 2H), 1.27 (s, 6H), 1.26-1.17 (m, 6H), 1.14-1.04 (m, 2H), 0.86 (t, J = 7.0 Hz, 3H). HRMS (ESI) calculated for C₁₈H₂₇N₂O₂: calculated 303.2073; found 303.2067.

2-(2,6-Dimethoxy-4-(2-methyloctan-2-yl)phenyl)-1-methyl-1H-imidazole (20): The synthesis was carried out as described for 23a by using (2,6-dimethoxy-4-(2-methyloctan-2-yl)phenyl)boronic acid (40 mg, 0.130 mmol), 2-bromo-1-methyl-1H-imidazole (25.07 mg, 0.156 mmol), sodium carbonate (27.5 mg, 0.260 mmol) and Pd(Ph₃P)₄ (4.50 mg, 3.89 µmol) to give 23e (30 mg, 0.087 mmol, 67% yield) as a white solid. ¹H NMR (500 MHz, CDCl₃) δ: 7.55 (d, J = 2.0 Hz, 1H), 7.17 (d, J = 2.0 Hz, 1H), 6.59 (s, 2H), 3.80 (s, 6H), 3.64 (s, 3H), 1.64-1.58 (m, 2H), 1.32 (s, 6H), 1.29-1.20 (m, 6H), 1.15-1.06 (m, 2H), 0.86 (t, J = 7.0 Hz, 3H). HRMS (ESI) calculated for C₂₁H₃₃N₂O₂: calculated 345.2542; found 345.2538.
2-(1-Methyl-1H-imidazol-2-yl)-5-(2-methyloctan-2-yl)benzene-1,3-diol (24e): The synthesis was carried out as described for 24a using 2-(2,6-dimethoxy-4-(2-methyloctan-2-yl)phenyl)-1-methyl-1H-imidazole (22 mg, 0.064 mmol) and BBr₃ (0.060 mL, 0.639 mmol) to give 24e (16 mg, 0.051 mmol, 79% yield) as a white solid. ^1H NMR (500 MHz, CD₃OD) δ: 7.64 (d, J = 2.0 Hz, 1H), 7.59 (d, J = 2.0 Hz, 1H), 6.55 (s, 2H), 3.78 (s, 3H), 1.66-1.60 (m, 2H), 1.36-1.28 (m, 10H especially s, 1.29, 6H), 1.27-1.22 (m, 2H), 1.18-1.08 (m, 2H), 0.89 (t, J = 7.0 Hz, 3H). HRMS (ESI) calculated for C₁₉H₂₉N₂O₂: calculated 317.2229; found 317.2230.

2,6-Dimethoxy-4-(2-methyloctan-2-yl)benzaldehyde (25): To a solution of 2-bromo-1,3-dimethoxy-5-(2-methyloctan-2-yl)benzene (1.76 g, 5.13 mmol) in THF (20 mL) was added n-butyllithium (3.52 mL, 5.64 mmol) at -78 °C and stirred for 30 min. To the reaction was added DMF (1.985 mL, 25.6 mmol) and stirred for 3 hrs. The reaction mixture was diluted with 25 mL ethyl acetate and 25 mL aqueous ammonium chloride solution. The organic layer was separated, washed with 2x50 mL water and brine, dried (MgSO₄) and concentrated under reduced pressure. The crude product was purified by silica gel chromatography eluting with 10-50% ethyl acetate:
hexanes to afford the desired product 25 (1.38 g, 4.72 mmol, 92 % yield) as a colorless oil. HRMS (ESI) calculated for C_{18}H_{29}O_{3}: calculated 293.2117; found 293.2111.

2-(2,6-Dimethoxy-4-(2-methyloctan-2-yl)phenyl)-1H-benzo[d]imidazole (27a): A mixture of 2,6-dimethoxy-4-(2-methyloctan-2-yl)benzaldehyde (55.8 mg, 0.191 mmol), benzene-1,2-diamine (22.70 mg, 0.210 mmol), H_{2}O_{2} (30% concentration) (0.078 mL, 0.763 mmol) and ceric ammonium nitrate (10.46 mg, 0.019 mmol) in acetonitrile (3mL) was stirred for 6 hrs at room temperature. The reaction mixture was diluted with 20 mL of ethyl acetate, 20 mL water and 20 mL of 10% Na_{2}SO_{3}. The organic layer was separated, washed with brine, dried over Na_{2}SO_{4} and concentrated under reduced pressure. Purification by flash column chromatography (10-30% ethyl acetate : hexanes) gave desired product 27 (52 mg, 0.137 mmol, 71% yield) as a white solid. \(^1\)H NMR (500 MHz, CDCl\(_3\)) \(\delta\): 7.55 (dd, \(J = 6.0\) Hz, \(J = 3.5\) Hz, 2H), 7.52 (dd, \(J = 6.0\) Hz, \(J = 3.5\) Hz, 2H), 6.73 (s, 2H), 3.79 (s, 6H), 1.72-1.66 (m, 2H), 1.32 (m, 6H), 1.36 (s, 6H), 1.32-1.20 (m, 6H), 1.18-1.08 (m, 2H), 0.88 (t, \(J = 7.0\) Hz, 3H). HRMS (ESI) calculated for C_{24}H_{33}N_{2}O_{2}: calculated 381.2542; found 381.2541.

2-(1H-Benzo[d]imidazol-2-yl)-5-(2-methyloctan-2-yl)benzene-1,3-diol (28a): The synthesis was carried out as described for 24a using 2-(2,6-dimethoxy-4-(2-methyloctan-2-yl)phenyl)-1H-
benzo[d]imidazole (25 mg, 0.066 mmol) and BBr₃ (0.062 mL, 0.657 mmol) to give 28 (18 mg, 0.051 mmol, 78% yield) as a white solid. ¹H NMR (500 MHz, CDCl₃ + 2 drops CD₃OD) δ: 7.62 (dd, J = 6.0 Hz, J = 3.0 Hz, 2H), 7.31 (dd, J = 6.0 Hz, J = 3.0 Hz, 2H), 6.74 (s, 1H), 6.33 (s, 1H), 1.54-1.49 (m, 2H), 1.21 (s, 6H), 1.20-1.11 (m, 6H), 1.04-0.96 (m, 2H), 0.77 (t, J = 7.0 Hz, 3H). HRMS (ESI) calculated for C₂₂H₂₉N₂O₂: calculated 353.2229; found 353.2233.

2-(2,6-Dimethoxy-4-(2-methyloctan-2-yl)phenyl)-1-methyl-1H-benzo[d]imidazole (27b): The synthesis was carried out as described for 27a using 2,6-dimethoxy-4-(2-methyloctan-2-yl)benzaldehyde (50 mg, 0.171 mmol), N-1-methylbenzene-1,2-diamine (22.98 mg, 0.188 mmol), H₂O₂ (30% concentration) (0.070 mL, 0.684 mmol) and ceric ammonium nitrate (9.37 mg, 0.017 mmol) to give 27b (52 mg, 0.132 mmol, 77% yield) as a white solid. ¹H NMR (500 MHz, CDCl₃) δ: 7.82 (d, J = 7.0 Hz, 1H), 7.38 (d, J = 7.0 Hz, 1H), 7.32-7.22 (m, 2H), 6.60 (s, 2H), 3.74 (s, 6H), 3.58 (s, 3H), 1.66-1.60 (m, 2H), 1.34 (s, 6H), 1.30-1.19 (m, 6H), 1.15-1.06 (m, 2H), 0.87 (t, J = 7.0 Hz, 3H). HRMS (ESI) calculated for C₂₅H₃₅N₂O₂: calculated 395.2699; found 395.2700.
2-(1-Methyl-1H-benzo[d]imidazol-2-yl)-5-(2-methyloctan-2-yl)benzene-1,3-diol (28b): The synthesis was carried out as described for 28a using 2-(2,6-dimethoxy-4-(2-methyloctan-2-yl)phenyl)-1-methyl-1H-benzo[d]imidazole (26 mg, 0.066 mmol) and BBr₃ (0.062 mL, 0.659 mmol) at 0 °C and stirred for overnight warming it to room temperature. The reaction mixture was diluted with 25 mL DCM and 25 mL water. The organic layer was separated, washed with 25 mL sat. NaHCO₃ solution, 25 mL water and brine, dried (MgSO₄) and concentrated under reduced pressure. The crude product was purified by silica gel chromatography eluting with 10-50% ethyl acetate : hexanes to afford the desired 28b (22 mg, 0.060 mmol, 91% yield) as a white solid. ¹H NMR (500 MHz, CD₃OD) δ: 7.90-7.86 (m, 1H), 7.79-7.75 (m, 1H), 7.62 (dt, J = 4.5 Hz, J = 2.0 Hz, 2H), 6.59 (s, 2H), 3.92 (s, 3H), 1.66-1.60 (m, 2H), 1.30 (s, 6H), 1.29-1.21 (m, 6H), 1.17-1.08 (m, 2H), 0.88 (t, J = 7.0 Hz, 3H). HRMS (ESI) calculated for C₂₃H₃₁N₂O₂: calculated 367.2386; found 367.2388.

3-Methoxy-2-(1-methyl-1H-benzo[d]imidazol-2-yl)-5-(2-methyloctan-2-yl)phenol (28h): The synthesis was carried out as described for 18 using 2-(2,6-dimethoxy-4-(2-methyloctan-2-yl)phenyl)-1-methyl-1H-benzo[d]imidazole (26 mg, 0.066 mmol) and BBr₃ (0.031 mL, 0.032
mmol) to give 28h (11 mg, 0.030 mmol, 45% yield) as a white solid. $^1$H NMR (500 MHz, CDCl$_3$) $\delta$: 7.91 (d, $J = 7.5$ Hz, 1H), 7.57-7.51 (m, 2H), 7.51-7.47 (m, 1H), 6.72 (s, 1H), 6.36 (s, 1H), 3.79 (s, 3H), 3.71 (s, 3H), 1.48-1.40 (m, 2H), 1.28-1.12 (m, 6H), 1.04 (s, 6H), 1.02-0.92 (m, 2H), 0.86 (t, $J = 7.0$ Hz, 3H).

2-(2,6-Dimethoxy-4-(2-methyloctan-2-yl)phenyl)benzo[d]oxazole (27c): The synthesis was carried out as described for 27a using 2,6-dimethoxy-4-(2-methyloctan-2-yl)benzaldehyde (50 mg, 0.171 mmol), 2-aminophenol (20.53 mg, 0.188 mmol), H$_2$O$_2$ (30% concentration) (0.070 mL, 0.684 mmol) and ceric ammonium nitrate (9.37 mg, 0.017 mmol) to give 27c (50 mg, 0.131 mmol, 77% yield) as a white solid. $^1$H NMR (500 MHz, CDCl$_3$) $\delta$: 7.97-7.93 (m, 1H), 7.68-7.64 (m, 1H), 7.48-7.44 (m, 2H), 6.64 (s, 2H), 3.89 (s, 6H), 1.68-1.60 (m, 2H), 1.34 (s, 6H), 1.30-1.18 (m, 6H), 1.15-1.06 (m, 2H), 0.87 (t, $J = 7.0$ Hz, 3H). HRMS (ESI) calculated for C$_{24}$H$_{32}$NO$_3$: calculated 382.2382; found 382.2379.

2-(Benzo[d]oxazol-2-yl)-3-methoxy-5-(2-methyloctan-2-yl)phenol (28g): The synthesis was carried out as described for 28a using 2-(2,6-dimethoxy-4-(2-methyloctan-2-
yl)phenyl)benzo[d]oxazole (15 mg, 0.039 mmol) in DCM (3 mL) and BBr₃ (7.43 µl, 0.079 mmol) to give 28g (8 mg, 0.022 mmol, 55% yield) as a white solid. ¹H NMR (500 MHz, CDCl₃) δ: 7.71 (dd, J = 7.0 Hz, J = 1.5 Hz, 1H), 7.65 (dd, J = 7.0 Hz, J = 1.5 Hz, 1H), 7.38 (m, 2H), 6.75 (d, J = 1.5 Hz, 1H), 6.51 (d, J = 1.5 Hz, 1H), 4.02 (s, 3H), 1.65-1.55 (m, 2H), 1.31 (s, 6H), 1.28-1.18 (m, 6H), 1.16-1.04 (m, 2H), 0.84 (t, J = 7.0 Hz, 3H).

2-(Benzo[d]oxazol-2-yl)-5-(2-methyloctan-2-yl)benzene-1,3-diol (28c): The synthesis was carried out as described for 28a using 2-(2,6-dimethoxy-4-(2-methyloctan-2-yl)phenyl)benzo[d]oxazole (22 mg, 0.058 mmol) in DCM (3 mL) and BBr₃ (0.055 mL, 0.577 mmol) to give 28c (14 mg, 0.040 mmol, 68% yield) as a white solid. ¹H NMR (500 MHz, CDCl₃) δ: 7.75 (dd, J = 8.0 Hz, J = 1.5 Hz, 1H), 7.64 (dd, J = 8.0 Hz, J = 1.5 Hz, 1H), 7.44 (dt, J = 7.5 Hz, J = 1.5 Hz, 1H), 7.41 (dt, J = 7.5 Hz, J = 1.5 Hz, 1H), 6.64 (s, 2H), 1.62-1.56 (m, 2H), 1.28 (s, 6H), 1.27-1.17 (m, 6H), 1.14-1.04 (m, 2H), 0.84 (t, J = 7.0 Hz, 3H). HRMS (ESI) calculated for C₂₂H₂₈NO₃: calculated 354.2069; found 354.2073.

6-Chloro-2-(2,6-dimethoxy-4-(2-methyloctan-2-yl)phenyl)benzo[d]oxazole (27d): A mixture of 2,6-dimethoxy-4-(2-methyloctan-2-yl)benzaldehyde (25 mg, 0.085 mmol), 2-amino-5-
chlorophenol (13.50 mg, 0.094 mmol), H$_2$O$_2$ (30% concentration) (0.035 mL, 0.342 mmol) and ceric ammonium nitrate (4.69 mg, 8.55 µmol) in acetonitrile (3mL) was stirred for 6h at room temperature. The reaction mixture was diluted with 20 mL of ethyl acetate, 20 mL water and 20 mL of 10% Na$_2$SO$_3$. The organic layer was separated, washed with brine, dried over Na$_2$SO$_4$ and concentrated under reduced pressure. Purification by flash column chromatography (10-30% ethyl acetate : hexanes) gave desired product 27d (29 mg, 0.070 mmol, 82% yield) as a white solid. $^1$H NMR (500 MHz, CDCl$_3$) $\delta$: 7.78 (d, $J = 2.0$ Hz, 1H), 7.48 (d, $J = 3.5$ Hz, 1H), 7.31 (dd, $J = 4.0$ Hz, $J = 2.0$ Hz, 1H), 6.60 (s, 2H), 3.81 (s, 6H), 1.66-1.59 (m, 2H), 1.33 (s, 6H), 1.28-1.17 (m, 6H), 1.13-1.04 (m, 2H), 0.84 (t, $J = 7.0$ Hz, 3H). HRMS (ESI) calculated for C$_{24}$H$_{31}$ClNO$_3$ calculated 416.1992; found 416.1991.

![Chemical Structure](image)

**2-(6-Chlorobenzo[dl]oxazol-2-yl)-5-(2-methyloctan-2-yl)benzene-1,3-diol (28f):** The synthesis was carried out as described for 28a using 2-(2,6-dimethoxy-4-(2-methyloctan-2-yl)phenyl)-1H-benzo[d]imidazole (25 mg, 0.066 mmol) and BB$_3$ (0.007 mL, 0.131 mmol) to give 28f (12 mg, 0.033 mmol, 50% yield) as a white solid. $^1$H NMR (500 MHz, CD$_3$OD) $\delta$: 7.57 (dd, $J = 6.0$ Hz, $J = 3.5$ Hz, 2H), 7.52 (dd, $J = 6.0$ Hz, $J = 3.5$ Hz, 2H), 6.73 (s, 2H), 3.79 (s, 3H), 1.72-1.66 (m, 2H), 1.36 (s, 6H), 1.32-1.20 (m, 6H), 1.18-1.08 (m, 2H), 0.88 (t, $J = 7.0$ Hz, 3H).
2-(2,6-Dimethoxy-4-(2-methyloctan-2-yl)phenyl)benzo[d]thiazole (27e): The synthesis was carried out as described for 27a by using 2,6-dimethoxy-4-(2-methyloctan-2-yl)benzaldehyde (50 mg, 0.171 mmol), 2-aminobenzenethiol (23.55 mg, 0.188 mmol), H$_2$O$_2$ (30% concentration) (0.070 mL, 0.684 mmol) and ceric ammonium nitrate (9.37 mg, 0.017 mmol) to give 27e (45 mg, 0.113 mmol, 66% yield) as a white solid. $^1$H NMR (500 MHz, CDCl$_3$) $\delta$: 8.40 (d, $J = 8.0$ Hz, 1H), 7.92 (d, $J = 7.5$ Hz, 1H), 7.66 (t, $J = 7.5$ Hz, 1H), 7.55 (t, $J = 8.0$ Hz, 1H), 6.72 (s, 2H), 4.10 (s, 6H), 1.68-1.62 (m, 2H), 1.35 (s, 6H), 1.28-1.18 (m, 6H), 1.14-1.06 (m, 2H), 0.86 (t, $J = 7.0$ Hz, 3H). HRMS (ESI) calculated for C$_{24}$H$_{32}$NO$_2$S: calculated 398.2154; found 398.2167.

2-(Benzo[d]thiazol-2-yl)-5-(2-methyloctan-2-yl)benzene-1,3-diol (28e): The synthesis was carried out as described for 28a using 2-(2,6-dimethoxy-4-(2-methyloctan-2-yl)phenyl)benzo[d]thiazole (25 mg, 0.063 mmol) and BBr$_3$ (0.059 mL, 0.629 mmol) to give 28e (16 mg, 0.043 mmol, 69% yield) as a white solid. $^1$H NMR (500 MHz, CDCl$_3$) $\delta$: 8.0 (d, $J = 8.5$ Hz, 1H), 7.93 (d, $J = 7.5$ Hz, 1H), 7.51 (dt, $J = 7.5$ Hz, $J = 1.0$ Hz, 1H), 7.40 (dt, $J = 8.5$ Hz, $J = 1.0$ Hz, 1H), 6.60 (s, 2H), 1.60-1.55 (m, 2H), 1.27 (s, 6H), 1.25-1.16 (m, 6H), 1.12-1.04 (m, 2H),
0.84 (t, $J = 7.0$ Hz, 3H). HRMS (ESI) calculated for $C_{22}H_{28}NO_2S$: calculated 370.1841; found 370.1843.

(3,5-Dimethoxyphenyl)(phenyl)sulfane (31): To a solution of copper(I) iodide (4.39 mg, 0.023 mmol) and benzotriazole (5.49 mg, 0.046 mmol) in DMSO (5 mL) was added 1-bromo-3,5-dimethoxybenzene (1 g, 4.61 mmol) and stirred for 10 min. To the solution was added benzenethiol (0.508 g, 4.61 mmol) followed by potassium tert-butoxide (0.517 g, 4.61 mmol) and heated to 80 °C for overnight. The reaction mixture was diluted with 50 mL ethyl acetate and 50 mL water. The organic layer was separated, washed with 2x50 mL water and brine, dried ($MgSO_4$) and concentrated under reduced pressure. The crude product was purified by silica gel chromatography eluting with 10-50% ethyl acetate : hexanes to afford the desired product 31 (0.95 g, 3.86 mmol, 84% yield). $^1H$ NMR (500 MHz, CDCl$_3$) $\delta$: 7.39 (d, $J = 1.0$ Hz, 1H), 7.37 (br s, 1H), 7.28 (t, $J = 8.0$ Hz, 2H), 7.22 (t, $J = 7.5$ Hz, 1H), 6.45 (d, $J = 2.5$ Hz, 2H), 6.31 (t, $J = 2.0$ Hz, 1H), 3.68 (s, 6H). HRMS (ESI) calculated for $C_{14}H_{15}O_2S$: calculated 247.0793; found 247.0786.

2,6-Dimethoxy-4-(phenylthio)benzaldehyde (32): To a solution of (3,5-dimethoxyphenyl) (phenyl)sulfane (500 mg, 2.030 mmol) in anhydrous DMF (393 µl, 5.07 mmol) at 0 °C under argon atmosphere was added slowly POCl$_3$ (208 µl, 2.233 mmol). The reaction mixture was
stirred for 16h while warming to room temperature. The reaction mixture was poured onto ice cold water and pH was neutralized by adding 10% NaOH. The reaction mixture was stirred for 10 min and extracted with ethyl acetate (3x20 mL). Combined organic extract was washed with brine, dried over MgSO₄ and concentrated under reduced pressure. The crude mixture was purified by flash column chromatography (10-30% ethyl acetate : hexanes) to afford desired product \(32\) (450 mg, 1.640 mmol, 81% yield). \(^1\)H NMR (500 MHz, CDCl₃) \(\delta\): 10.38 (s, 1H), 7.57-7.53 (m, 2H), 7.46-7.42 (m, 3H), 6.29 (s, 2H), 3.75 (s, 6H). HRMS (ESI) calculated for C₁₅H₁₅O₃S: calculated 275.0742; found 275.0730.

\[\text{2-(2,6-Dimethoxy-4-(phenylthio)phenyl)-1-methyl-1H-benzo[d]imidazole (33):}\]

A mixture of 2,6-dimethoxy-4-(phenylthio)benzaldehyde (200 mg, 0.729 mmol), N-1-methylbenzene-1,2-diamine (89 mg, 0.729 mmol), H₂O₂ (0.022 mL, 0.729 mmol) and ceric ammonium nitrate (4.00 mg, 7.29 µmol) in acetonitrile (10 mL) was stirred for 6 hrs at room temperature. The reaction mixture was diluted with 50 mL of ethyl acetate, 50 mL water and 20 mL of 10% Na₂SO₃. The organic layer was separated, washed with brine, dried over Na₂SO₄ and concentrated under reduced pressure. Purification by flash column chromatography (10-30% ethyl acetate: hexanes) gave desired product \(33\) (220 mg, 0.584 mmol, 80% yield) as a white solid. \(^1\)H NMR (500 MHz, CDCl₃) \(\delta\): 7.83 (d, \(J = 8.0\) Hz, 1H), 7.47 (d, \(J = 7.0\) Hz, 2H), 7.41-7.35 (m, 3H), 7.35-7.30 (m, 1H), 7.30-7.27 (m, 2H), 6.57 (d, \(J = 1.0\) Hz, 2H), 3.64 (s, 6H), 3.57 (s, 3H). HRMS (ESI) calculated for C₂₂H₂₁N₂O₂S: calculated 377.1324; found 377.1326.
2-(1-Methyl-1H-benzo[d]imidazol-2-yl)-5-(phenylthio)benzene-1,3-diol (34): The synthesis was carried out as described for 28a using 2-(2,6-dimethoxy-4-(phenylthio)phenyl)-1-methyl-1H-benzo[d]imidazole (25 mg, 0.066 mmol) and BBr₃ (0.063 mL, 0.664 mmol) to give 34 (16 mg, 0.046 mmol, 69% yield) as a white solid. ¹H NMR (500 MHz, CD₃OD) δ: 7.92-7.88 (m, 1H), 7.79-7.76 (m, 1H), 7.67-7.63 (m, 2H), 7.61-7.57 (m, 2H), 7.51-7.46 (m, 3H), 6.32 (s, 2H), 3.92 (s, 3H). HRMS (ESI) calculated for C₂₀H₁₇N₂O₂S: calculated 349.1011; found 349.1003.

2-(2,6-Dimethoxy-4-(phenylsulfinyl)phenyl)-1-methyl-1H-benzo[d]imidazole (35): To a solution of 2-(2,6-dimethoxy-4-(phenylthio)phenyl)-1-methyl-1H-benzo[d]imidazole (35 mg, 0.093 mmol) in methanol (3 mL) was added sodium periodate (29.8 mg, 0.139 mmol) followed by water (1 mL) and stirred for 3h at room temperature. The reaction mixture was diluted with 25 mL ethyl acetate and 25 mL water. The organic layer was separated, washed with 2x50 mL water and brine, dried (MgSO₄) and concentrated under reduced pressure. The crude product was purified by silica gel chromatography eluting with 10-50% ethyl acetate: hexanes to afford the desired product 35 (22 mg, 0.056 mmol, 60% yield). ¹H NMR (500 MHz, CDCl₃) δ: 8.03-7.98 (m, 1H), 7.76-7.70 (m, 2H), 7.64-7.57 (m, 3H), 7.55 (t, J = 3.0 Hz, 3H), 7.03 (s, 1H), 6.95 (s,
1H), 3.84 (s, 6H), 3.78 (s, 3H). HRMS (ESI) calculated for C$_{22}$H$_{21}$N$_2$O$_3$: calculated 393.1273; found 393.1269.

2-(1-methyl-1H-benzo[d]imidazol-2-yl)-5-(phenylsulfanyl)benzene-1,3-diol (36): The synthesis was carried out as described for 28a using 2-(2,6-dimethoxy-4-(phenylsulfanyl)phenyl)-1-methyl-1H-benzo[d]imidazole (15 mg, 0.038 mmol) and BBr$_3$ (36.1 µl, 0.382 mmol to give 36 (9 mg, 0.025 mmol, 64% yield) as a white solid. $^1$H NMR (500 MHz, CD$_3$OD) δ: 7.95-7.91 (m, 1H), 7.81-7.77 (m, 1H), 7.69-7.66 (m, 2H), 7.66-7.61 (m, 2H), 7.59-7.54 (m, 3H), 6.0 (s, 2H), 3.91 (s, 3H).

2-(2,6-Dimethoxy-4-(phenylsulfonyl)phenyl)-1-methyl-1H-benzo[d]imidazole (37): To a solution of 2-(2,6-dimethoxy-4-(phenylthio)phenyl)-1-methyl-1H-benzo[d]imidazole (30 mg, 0.080 mmol) in DCM (3 mL) was added mCPBA (13.75 mg, 0.080 mmol) and stirred for 6h at room temperature. The reaction mixture was diluted with 25 mL ethyl acetate and 25 mL water. The organic layer was separated, washed with 2x50 mL water and brine, dried (MgSO$_4$) and concentrated under reduced pressure. The crude product was purified by silica gel chromatography eluting with 10-50% ethyl acetate : hexanes to afford the desired product 37 (26
mg, 0.064 mmol, 80% yield). \(^1\)H NMR (500 MHz, CDCl\(_3\)) \(\delta\): 8.05-8.01 (m, 1H), 7.99 (d, \(J = 8.5\) Hz, 2H), 7.67 (t, \(J = 8.0\) Hz, 1H), 7.63-7.57 (m, 5H), 7.26 (s, 2H), 3.88 (s, 6H), 3.79 (s, 3H). HRMS (ESI) calculated for C\(_{22}H_{21}N_2O_4S\): calculated 409.1222; found 409.1219.

![Image 1](image1.png)

### 2-(1-Methyl-1H-benzo[d]imidazol-2-yl)-5-(phenylsulfonyl)benzene-1,3-diol (38)

The synthesis was carried out as described for 28a using 2-(2,6-dimethoxy-4-(phenylsulfonyl)phenyl)-1-methyl-1H-benzo[d]imidazole (15 mg, 0.037 mmol) and BBr\(_3\) (34.7 \(\mu\)L, 0.367 mmol) to give 38 (10 mg, 0.026 mmol, 71% yield) as a white solid. \(^1\)H NMR (500 MHz, CD\(_3\)OD) \(\delta\): 8.01 (s, 1H), 7.99 (d, \(J = 1.5\) Hz, 1H), 7.96-7.92 (m, 1H), 7.84-7.78 (m, 1H), 7.73 (t, \(J = 7.5\) Hz, 1H), 7.70-7.62 (m, 4H), 7.09 (s, 2H), 3.92 (s, 3H). HRMS (ESI) calculated for C\(_{20}H_{17}N_2O_4S\): calculated 381.0909; found 381.0901.

![Image 2](image2.png)

### (4-Bromo-3,5-dimethoxyphenyl)(phenyl)methanol (46)

To a solution of 4-bromo-3,5-dimethoxybenzaldehyde (2 g, 8.16 mmol) in THF (15 mL) was added phenylmagnesium bromide (3M in diethyl ether) (2.72 mL, 8.16 mmol) at 0 °C and stirred for 3 hrs. The reaction mixture was diluted with 50 mL ethyl acetate and 50 mL aqueous ammonium chloride solution. The organic layer was separated, washed with 2x50 mL water and brine, dried (MgSO\(_4\)) and
concentrated under reduced pressure. The crude product was purified by silica gel chromatography eluting with 10-50% ethyl acetate: hexanes to afford the desired product 46 (2.2 g, 6.81 mmol, 83% yield) as a colorless oil. \( ^1 \text{H NMR (500 MHz, CDCl}_3 \text{)} \delta: 7.37-7.35 \text{ (m, 4H), 7.32-7.29 \text{ (m, 1H), 6.63 (s, 2H), 5.81 (d, } J = 3.5 \text{ Hz, 1H), 3.87 (s, 6H), 2.25 (d, } J = 3.5 \text{ Hz, 1H). HRMS (ESI) calculated for C}_{15}\text{H}_{15}\text{BrO}_3: calculated 305.0177; found 305.0171.\)

\[
\text{4-(Hydroxy(phenyl)methyl)-2,6-dimethoxybenzaldehyde (47): To a solution of (4-bromo-3,5-dimethoxyphenyl)(phenyl)methanol (1 g, 3.09 mmol) in THF (6 mL) was added } n\text{-butyllithium (1.6 M in hexanes) (2.127 mL, 3.40 mmol) at -78 °C and stirred for 30 min. To the reaction mixture was added DMF (1.198 mL, 15.47 mmol) and stirred for 3 h. The reaction mixture was diluted with 50 mL ethyl acetate and 50 mL aqueous ammonium chloride solution. The organic layer was separated, washed with 2x50 mL water and brine, dried (MgSO}_4 \text{) and concentrated under reduced pressure. The crude product was purified by silica gel chromatography eluting with 10-50% ethyl acetate: hexanes to afford the desired product 47 (0.590 g, 2.167 mmol, 70% yield) as a colorless oil. \( ^1 \text{H NMR (500 MHz, CDCl}_3 \text{)} \delta: 10.44 \text{ (s, 1H), 7.37 (d, } J = 4.0 \text{ Hz, 3H), 7.36-7.29 \text{ (m, 2H), 6.63 (s, 2H), 5.79 (s, 1H), 3.86 (s, 6H). HRMS (ESI) calculated for C}_{16}\text{H}_{17}\text{O}_4: calculated 273.1127; found 273.1122.}\)
\]
(3,5-Dimethoxy-4-(1-methyl-1H-benzo[d]imidazol-2-yl)phenyl)(phenyl)methanol (48): A mixture of 4-(hydroxy(phenyl)methyl)-2,6-dimethoxybenzaldehyde (50 mg, 0.184 mmol), N1-methylbenzene-1,2-diamine (24.68 mg, 0.202 mmol), and FeCl₃ (33 mg, 0.202 mmol) in acetonitrile (3mL) was stirred for 6h at room temperature. The reaction mixture was diluted with 20 mL of ethyl acetate, 20 mL water and 10% 20 mL of Na₂SO₃. The organic layer was separated, washed with brine, dried over Na₂SO₄ and concentrated under reduced pressure. Purification by flash column chromatography (10-30% ethyl acetate: hexanes) gave desired product 48 (48 mg, 0.128 mmol, 69% yield) as a white solid. ¹H NMR (500 MHz, CD₃OD) δ: 8.06-8.02 (m, 1H), 7.60-7.56 (m, 3H), 7.43 (dd, J = 9.0 Hz, J = 1.5 Hz, 2H), 7.38 (t, J = 7.5 Hz, 2H), 7.33 (td, J = 7.0 Hz, J = 2.0 Hz, 1H), 6.86 (s, 1H), 6.67 (s, 1H), 5.85 (s, 1H), 3.79 (s, 1H), 3.77 (s, 6H). HRMS (ESI) calculated for C₂₃H₂₃N₂O₃: calculated 375.1709; found 375.1703.

5-(Hydroxy(phenyl)methyl)-2-(1-methyl-1H-benzo[d]imidazol-2-yl)benzene-1,3-diol (41): The synthesis was carried out as described for 28a using (3,5-dimethoxy-4-(1-methyl-1H-benzo[d]imidazol-2-yl)phenyl)(phenyl)methanol (25 mg, 0.067 mmol) and BBr₃ (0.063 mL, 0.668 mmol) to give 41 (16 mg, 0.046 mmol, 69% yield) as a white solid. ¹H NMR (500 MHz, CDCl₃ + 2 drops CD₃OD) δ: 7.77-7.74 (m, 2H), 7.65-7.61 (m, 2H), 7.43 (s, 1H), 7.41 (s, 1H),
7.36 (t, \( J = 7.0 \, \text{Hz}, \, 2\text{H} \)), 7.29 (t, \( J = 7.0 \, \text{Hz}, \, 1\text{H} \)), 6.62 (s, 2\text{H}), 5.71 (s, 1\text{H}), \, 3.93 (s, 3\text{H}). \) HRMS (ESI) calculated for \( \text{C}_{21}\text{H}_{19}\text{N}_{2}\text{O}_{3} \): calculated 347.1396; found 347.1384.

\[ \text{5-(Hydroxy(phenyl)methyl)-3-methoxy-2-}(1\text{-methyl}-1\text{H}-\text{benzo[d]imidazol-2-yl})\text{phenol (41a):} \]

The synthesis was carried out as described for \( \text{28a} \) using (3,5-dimethoxy-4-(1-methyl-1\text{H}-benzo[d]imidazol-2-yl)phenyl)(phenyl)methanol (15 mg, 0.040 mmol) and BBr\(_3\) (7.57 \( \mu \text{L}, \, 0.080 \) mmol) to give \( \text{41a} \) (8 mg, 0.022 mmol, 55\% yield) as a white solid. \(^1\text{H} \text{NMR} \) (500 MHz, CDCl\(_3\) + CD\(_3\)OD) \( \delta: \) 7.80-7.74 (m, 2\text{H}), 7.67-7.66 (m, 2\text{H}), 7.43 (d, \( J = 7.0 \, \text{Hz}, \, 2\text{H} \)), 7.38 (t, \( J = 7.5 \, \text{Hz}, \, 2\text{H} \)), 7.33-7.28 (m, 1\text{H}), 6.77 (s, 1\text{H}), 6.71 (s, 1\text{H}), 5.78 (s, 1\text{H}), \, 3.89 (s, 3\text{H}), \, 3.83 (s, 3\text{H}).

\[ \text{2-(2,6-Dimethoxy-4-(2-methyloctan-2-yl)phenyl)-1H-benzo[d]imidazole (27f):} \]

A mixture of 2,6-dimethoxy-4-(2-methyloctan-2-yl)benzaldehyde (50 mg, 0.171 mmol), benzene-1,2-diamine (20.34 mg, 0.188 mmol), \( \text{H}_2\text{O}_2 \) (0.070 mL, 0.684 mmol) and ceric ammonium nitrate (9.37 mg, 0.017 mmol) in acetonitrile (35 mL) was stirred for 6 hrs at room temperature. The reaction mixture was diluted with 100 mL of ethyl acetate, 100 mL water and 20 mL of 10\% \( \text{Na}_2\text{SO}_3 \). The organic layer was separated, washed with brine, dried over \( \text{Na}_2\text{SO}_4 \) and concentrated under reduced pressure. Purification by flash column chromatography (10-30\% ethyl acetate : hexanes)
gave desired product 27f (58.6 mg, 0.154 mmol, 90% yield) as a white solid. $^1$H NMR (500 MHz, CDCl$_3$) $\delta$: 7.55 (dd, $J = 6.0$ Hz, $J = 3.5$ Hz, 2H), 7.52 (dd, $J = 6.0$ Hz, $J = 3.5$ Hz, 2H), 6.73 (s, 2H), 3.79 (s, 6H), 1.72-1.66 (m, 2H), 1.36 (s, 6H), 1.32-1.20 (m, 6H), 1.18-1.08 (m, 2H), 0.88 (t, $J = 7.0$ Hz, 3H).

![Chemical Structure](image)

2-(2,6-Dimethoxy-4-(2-methylcyclooctan-2-yl)phenyl)-1,3-dimethyl-1H-benzo[d]imidazole-3-ium iodide (49a): To a solution of 2-(2,6-dimethoxy-4-(2-methylcyclooctan-2-yl)phenyl)-1H-benzo[d]imidazole (35 mg, 0.092 mmol) in anhydrous DMF (5 mL) was added K$_2$CO$_3$ (153 mg, 1.104 mmol) followed by iodomethane (0.058 mL, 0.920 mmol) and stirred for 16h at room temperature. The reaction solution was quenched by adding 50 mL water to obtain light yellow precipitate. The precipitate was collected by filtration and air dried to afford the desired product 49a (35 mg, 0.065 mmol, 71% yield) as light yellow solid. $^1$H NMR (500 MHz, CD$_3$OD) $\delta$: 7.98 (dd, $J = 6.5$ Hz, $J = 3.0$ Hz, 2H), 7.75 (dd, $J = 6.0$ Hz, $J = 3.0$ Hz, 2H), 6.93 (s, 2H), 3.91 (s, 6H), 3.87 (s, 6H), 1.66-1.60 (m, 2H), 1.30 (s, 6H), 1.28-1.20 (m, 6H), 1.18-1.10 (m, 2H), 0.87 (t, $J = 7.0$ Hz, 3H).
2-(2,6-Dihydroxy-4-(2-methyloctan-2-yl)phenyl)-1,3-dimethyl-1H-benzo[d]imidazol-3-ium bromide (50b): The synthesis was carried out as described for 28a using 2-(2,6-dimethoxy-4-(2-methyloctan-2-yl)phenyl)-1,3-dimethyl-1H-benzo[d]imidazol-3-ium iodide (300 mg, 0.559 mmol) and BBr₃ (0.062 mL, 0.659 mmol) to afford 50b (240 mg, 0.472 mmol, 84% yield) as a white solid. ¹H NMR (500 MHz, CD₃OD) δ: 7.96 (dd, J = 6.5 Hz, J = 3.0 Hz, 2H), 7.73 (dd, J = 6.5 Hz, J = 3.0 Hz, 2H), 6.63 (s, 2H), 3.92 (s, 6H), 1.68-1.61 (m, 2H), 1.31 (s, 6H), 1.29-1.21 (m, 6H), 1.19-1.11 (m, 2H), 0.86 (t, J = 7.0 Hz, 3H).

2-(2,6-Dihydroxy-4-(2-methyloctan-2-yl)phenyl)-1,3-dimethyl-1H-benzo[d]imidazol-3-ium chloride (50a): Compound 50b was purified by prep-HPLC eluting with acetonitrile : water (10:90) and 0.1% TFA as a modifier. 1 ml of 1N aqueous HCl was added to fractions containing desired compound and was concentrated under reduced pressure to afford desired product as chloride salt.
2-(2,6-Dihydroxy-4-(2-methyloctan-2-yl)phenyl)-1,3-dimethyl-1H-benzo[d]imidazol-3-ium bromide (50c): A solution of 2-(2,6-dimethoxy-4-(2-methyloctan-2-yl)phenyl)-1,3-dimethyl-1H-benzo[d]imidazol-3-ium iodide (300 mg, 0.559 mmol) and HI (0.421 mL, 5.59 mmol) was heated to 60 °C for overnight. Reaction was concentrated under reduced pressure to afford crude desired product. Crude product was purified by silica gel chromatography eluting with methanol : DCM (2-20%) to afford 2-(2,6-dihydroxy-4-(2-methyloctan-2-yl)phenyl)-1,3-dimethyl-1H-benzo[d]imidazol-3-ium iodide (185 mg, 0.363 mmol, 65% yield) as a white solid.

2-(2,6-Dimethoxy-4-(2-methyloctan-2-yl)phenyl)-4-methyl-1H-benzo[d]imidazole (27h): The synthesis was carried out as described for 27a using 2,6-dimethoxy-4-(2-methyloctan-2-yl)benzaldehyde (50 mg, 0.171 mmol), 3-methylbenzene-1,2-diamine (22.98 mg, 0.188 mmol), H₂O₂ (30% concentration) (0.070 mL, 0.684 mmol) and ceric ammonium nitrate (9.37 mg, 0.017 mmol) to give the desired product 27h (55 mg, 0.139 mmol, 82% yield) as a white solid. ¹H NMR (500 MHz, CDCl₃) δ: 7.69 (d, J = 8.0 Hz, 1H), 7.28 (t, J = 7.0 Hz, 1H), 7.15 (d, J = 7.0 Hz, 1H), 6.73 (s, 2H), 4.13 (s, 6H), 2.60 (s, 3H), 1.69-1.61 (m, 2H), 1.35 (s, 6H), 1.31-1.17 (m,
6H), 1.14-1.04 (m, 2H), 0.85 (t, J = 7.0 Hz, 3H). HRMS (ESI) calculated for C_{25}H_{35}N_{2}O_{2}: calculated 395.2699; found 395.2689.

---

2-(2,6-Dimethoxy-4-(2-methyloctan-2-yl)phenyl)-1,3,4-trimethyl-1H-benzo[d]imidazol-3-ium iodide (49c): The synthesis was carried out as described for 49a using 2-(2,6-dimethoxy-4-(2-methyloctan-2-yl)phenyl)-4-methyl-1H-benzo[d]imidazole (40 mg, 0.101 mmol) and K_{2}CO_{3} (28.0 mg, 0.203 mmol) followed by iodomethane (0.038 mL, 0.608 mmol) to afford the desired product 49c (42 mg, 0.076 mmol, 75% yield) as light yellow solid. ^{1}H NMR (500 MHz, CDCl_{3}) \( \delta \): 7.76 (d, J = 8.0 Hz, 1H), 7.58 (t, J = 7.0 Hz, 1H), 7.47 (d, J = 8.0 Hz, 1H), 6.93 (s, 2H), 4.04 (s, 3H), 3.90 (s, 6H), 3.81 (s, 3H), 2.91 (s, 3H), 1.80-1.72 (m, 2H), 1.42 (s, 6H), 1.36-1.24 (m, 6H), 1.22-1.14 (m, 2H), 0.88 (t, J = 7.0 Hz, 3H). HRMS (ESI) calculated for C_{27}H_{39}N_{2}O_{2}: calculated 423.3012; found 423.3008.

---

2-(2,6-Dihydroxy-4-(2-methyloctan-2-yl)phenyl)-1,3,4-trimethyl-1H-benzo[d]imidazol-3-ium bromide (50e): The synthesis was carried out as described for 50b using 2-(2,6-dimethoxy-4-(2-methyloctan-2-yl)phenyl)-1,3,4-trimethyl-1H-benzo[d]imidazol-3-ium iodide (25 mg, 0.045 mmol) in DCM (1 mL, 0.036 mmol) and BB_{3} (0.062 mL, 0.659 mmol) to afford the desired
product 50e (16 mg, 0.031 mmol, 67% yield) as a light brown oil. \(^1\)H NMR (500 MHz, CD\(_3\)OD) \(\delta\): 7.75 (d, \(J = 3.0\) Hz, 1H), 7.56 (t, \(J = 8.0\) Hz, 1H), 7.46 (d, \(J = 8.0\) Hz, 1H), 6.63 (s, 2H), 4.11 (s, 3H), 3.87 (s, 3H), 2.91 (s, 3H), 1.68-1.61 (m, 2H), 1.32 (s, 6H), 1.26 (s, 6H), 1.20-1.10 (m, 2H), 0.88 (t, \(J = 7.0\) Hz, 3H). HRMS (ESI) calculated for C\(_{25}\)H\(_{35}\)N\(_2\)O\(_2\): calculated 395.2699; found 395.2711.

\[\text{2-(2,6-Dimethoxy-4-(2-methyloctan-2-yl)phenyl)-4-fluoro-1H-benzo[d]imidazole (27i):}\]
The synthesis was carried out as described for 27a using 2,6-dimethoxy-4-(2-methyloctan-2-yl)benzaldehyde (50 mg, 0.171 mmol), 3-fluorobenzene-1,2-diamine (23.72 mg, 0.188 mmol), H\(_2\)O\(_2\) (30% concentration) (0.070 mL, 0.684 mmol) and ceric ammonium nitrate (9.37 mg, 0.017 mmol) to give the desired product 27i (54 mg, 0.138 mmol, 81% yield) as a white solid. \(^1\)H NMR (500 MHz, CDCl\(_3\)) \(\delta\): 7.59 (d, \(J = 8.5\) Hz, 1H), 7.05-6.98 (m, 1H), 6.81 (t, \(J = 8.0\) Hz, 1H), 6.65 (s, 2H), 4.10 (s, 6H), 1.70-1.62 (m, 2H), 1.36 (s, 6H), 1.32-1.18 (m, 6H), 1.16-1.07 (m, 2H), 0.88 (t, \(J = 7.5\) Hz, 3H). HRMS (ESI) calculated for C\(_{24}\)H\(_{32}\)FN\(_2\)O\(_2\): calculated 399.2448; found 399.2443.
2-(2,6-Dimethoxy-4-(2-methyloctan-2-yl)phenyl)-4-fluoro-1,3-dimethyl-1H-benzo[d]imidazol-3-ium iodide (49d): The synthesis was carried out as described for 49a using 2-(2,6-dimethoxy-4-(2-methyloctan-2-yl)phenyl)-4-fluoro-1H-benzo[d]imidazole (36 mg, 0.090 mmol) in anhydrous DMF (5 mL) was added K$_2$CO$_3$ (24.97 mg, 0.181 mmol) followed by iodomethane (0.034 mL, 0.542 mmol) to afford desired product 49d (40 mg, 0.072 mmol, 80% yield) as light yellow solid. $^1$H NMR (500 MHz, CD$_3$OD) $\delta$: 7.79 (d, $J = 8.0$ Hz, 1H), 7.76-7.67 (m, 1H), 7.51 (dd, $J = 12.0$ Hz, $J = 8.0$ Hz, 1H), 6.93 (s, 2H), 3.91 (s, 6H), 3.86 (s, 3H), 1.82-1.72 (m, 2H), 1.42 (s, 6H), 1.36-1.24 (m, 6H), 1.22-1.12 (m, 2H), 0.88 (t, $J = 7.0$ Hz, 3H). HRMS (ESI) calculated for C$_{26}$H$_{36}$FN$_2$O$_2$: calculated 427.2761; found 427.2757.

2-(2,6-Dihydroxy-4-(2-methyloctan-2-yl)phenyl)-4-fluoro-1,3-dimethyl-1H-benzo[d]imidazol-3-ium bromide (50f): The synthesis was carried out as described for 50b using 2-(2,6-dimethoxy-4-(2-methyloctan-2-yl)phenyl)-4-fluoro-1,3-dimethyl-1H-benzo[d]imidazol-3-ium iodide (20 mg, 0.036 mmol) and BBr$_3$ (0.062 mL, 0.659 mmol) to
afford the desired product 50f (12 mg, 0.023 mmol, 63% yield) as a light brown oil. \( ^1 \text{H NMR} \) (500 MHz, CD\(_2\)OD) \( \delta \): 7.80-7.74 (m, 1H), 7.72-7.62 (m, 1H), 7.52-7.42 (m, 1H), 6.64 (s, 2H), 4.06 (s, 3H), 3.93 (s, 3H), 1.68-1.58 (m, 2H), 1.32 (s, 6H), 1.28-1.21 (m, 6H), 1.20-1.08 (m, 2H), 0.88 (t, \( J = 6.5 \) Hz, 3H). HRMS (ESI) calculated for C\(_{24}\)H\(_{32}\)FN\(_2\)O\(_2\): calculated 399.2448; found 399.2439.

4-Chloro-2-(2,6-dimethoxy-4-(2-methyloctan-2-yl)phenyl)-1H-benzo[d]imidazole (27j): The synthesis was carried out as described for 27a using 2,6-dimethoxy-4-(2-methyloctan-2-yl)benzaldehyde (50 mg, 0.171 mmol), 3-chlorobenzene-1,2-diamine (26.8 mg, 0.188 mmol), H\(_2\)O\(_2\) (30% concentration) (0.070 mL, 0.684 mmol) and ceric ammonium nitrate (9.37 mg, 0.017 mmol) to give the desired product 27j (61 mg, 0.147 mmol, 86% yield) as a white solid. \( ^1 \text{H NMR} \) (500 MHz, CDCl\(_3\)) \( \delta \): 7.95 (d, \( J = 8.0 \) Hz, 1H), 7.49-7.41 (m, 2H), 6.77 (s, 2H), 4.18 (s, 6H), 1.70-1.62 (m, 2H), 1.36 (s, 6H), 1.29-1.17 (m, 6H), 1.12-1.04 (m, 2H), 0.85 (t, \( J = 7.0 \) Hz, 3H). HRMS (ESI) calculated for C\(_{24}\)H\(_{32}\)ClN\(_2\)O\(_2\): calculated 415.2152; found 415.2159.
4-Chloro-2-(2,6-dimethoxy-4-(2-methyloctan-2-yl)phenyl)-1,3-dimethyl-1H-benzo[d]imidazol-3-ium iodide (49e): The synthesis was carried out as described for 49a using 4-chloro-2-(2,6-dimethoxy-4-(2-methyloctan-2-yl)phenyl)-1H-benzo[d]imidazole (40 mg, 0.096 mmol) and K2CO3 (26.6 mg, 0.193 mmol) followed by iodomethane (0.036 mL, 0.578 mmol) to give 49e (41 mg, 0.072 mmol, 74% yield) as light yellow solid. 1H NMR (500 MHz, CD3OD) δ: 7.94 (d, J = 8.5 Hz, 1H), 7.75 (d, J = 8.5 Hz, 1H), 7.68 (t, J = 8.5 Hz, 1H), 6.93 (s, 2H), 4.11 (s, 3H), 3.91 (s, 6H), 3.86 (s, 3H), 1.80-1.73 (m, 2H), 1.42 (s, 6H), 1.36-1.25 (m, 6H), 1.23-1.13 (m, 2H), 0.88 (t, J = 7.0 Hz, 3H). HRMS (ESI) calculated for C26H36ClN2O2: calculated 443.2465; found 443.2461.

4-Chloro-2-(2,6-dihydroxy-4-(2-methyloctan-2-yl)phenyl)-1,3-dimethyl-1H-benzo[d]imidazol-3-ium bromide (50g): The synthesis was carried out as described for 50b using 4-chloro-2-(2,6-dimethoxy-4-(2-methyloctan-2-yl)phenyl)-1,3-dimethyl-1H-benzo[d]imidazole-3-ium iodide (25 mg, 0.044 mmol) and BBr3 (0.062 mL, 0.659 mmol) to afford the desired product 50g (18 mg, 0.033 mmol, 76% yield) as a light brown oil. 1H NMR (500 MHz, CD3OD) δ: 7.93 (d, J = 8.0 Hz, 1H), 7.73 (d, J = 8.0 Hz, 1H), 7.66 (t, J = 8.0 Hz,
1H), 6.64 (s, 2H), 4.18 (s, 3H), 3.92 (s, 3H), 1.68-1.60 (m, 2H), 1.32 (s, 6H), 1.26 (s, 6H), 1.20-1.09 (m, 2H), 0.88 (t, $J = 7.0$ Hz, 3H). HRMS (ESI) calculated for $C_{24}H_{32}ClN_2O_2$: calculated 415.2152; found 415.2143.

![Chemical Structure](image)

**Methyl 2-(2,6-dimethoxy-4-(2-methyloctan-2-yl)phenyl)-1H-benzo[d]imidazole-4-carboxylate (27g):** The synthesis was carried out as described for 27a using 2,6-dimethoxy-4-(2-methyloctan-2-yl)benzaldehyde (50 mg, 0.171 mmol), methyl 2,3-diaminobenzoate (31.3 mg, 0.188 mmol), $H_2O_2$ (30% concentration) (0.070 mL, 0.684 mmol) and ceric ammonium nitrate (9.37 mg, 0.017 mmol) to give product 27g (60 mg, 0.137 mmol, 80% yield) as a white solid. $^1H$ NMR (500 MHz, CDCl$_3$) $\delta$: 8.26 (d, $J = 8.5$ Hz, 1H), 8.09 (d, $J = 8.0$ Hz, 1H), 7.58 (t, $J = 7.5$ Hz, 1H), 6.79 (s, 2H), 4.21 (s, 6H), 4.08 (s, 3H), 1.69-1.62 (m, 2H), 1.36 (s, 6H), 1.29-1.18 (m, 6H), 1.11-1.05 (m, 2H), 0.85 (t, $J = 7.0$ Hz, 3H). HRMS (ESI) calculated for $C_{26}H_{35}N_2O_4$: calculated 439.2597; found 439.2603.
2-(2,6-Dimethoxy-4-(2-methyloctan-2-yl)phenyl)-4-(methoxycarbonyl)-1,3-dimethyl-1H-benzo[d]imidazol-3-ium iodide (49b): The synthesis was carried out as described for 49a using 2-(2,6-dimethoxy-4-(2-methyloctan-2-yl)phenyl)-1H-benzo[d]imidazole-4-carboxylate (35 mg, 0.080 mmol) and K$_2$CO$_3$ (22.06 mg, 0.160 mmol) followed by iodomethane (0.030 mL, 0.479 mmol) to afford the desired product 49b (30 mg, 0.050 mmol, 63% yield) as light yellow solid.

$^1$H NMR (500 MHz, CD$_3$OD) $\delta$: 8.22 (d, $J = 8.0$ Hz, 1H), 8.17 (d, $J = 8.0$ Hz, 1H), 7.81 (t, $J = 8.0$ Hz, 1H), 6.94 (s, 2H), 4.04 (s, 3H), 3.94 (s, 3H), 3.91 (s, 6H), 3.90 (s, 3H), 1.82-1.72 (m, 2H), 1.42 (s, 6H), 1.36-1.24 (m, 6H), 1.22-1.14 (m, 2H), 0.88 (t, $J = 7.0$ Hz, 3H). HRMS (ESI) calculated for C$_{29}$H$_{39}$N$_2$O$_4$: calculated 467.2910; found 467.2919.

4-Carboxy-2-(2,6-dihydroxy-4-(2-methyloctan-2-yl)phenyl)-1,3-dimethyl-1H-benzo[d]imidazol-3-ium (50d): The synthesis was carried out as described for 50b using 2-(2,6-dimethoxy-4-(2-methyloctan-2-yl)phenyl)-4-(methoxycarbonyl)-1,3-dimethyl-1H-
benzo[d]imidazol-3-ium iodide (20 mg, 0.034 mmol) and BBr₃ (0.062 mL, 0.659 mmol) to give 50d (5 mg, 9.05 µmol, 27 % yield) as a light brown oil. HRMS (ESI) calculated for C₂⁵H₃₃N₂O₄: calculated 425.2440; found 425.2445.

5-Chloro-2-(2,6-dimethoxy-4-(2-methyloctan-2-yl)phenyl)-1H-benzo[d]imidazole (27l): The synthesis was carried out as described for 27a using 2,6-dimethoxy-4-(2-methyloctan-2-yl)benzaldehyde (50 mg, 0.171 mmol), 4-chlorobenzene-1,2-diamine (26.8 mg, 0.188 mmol), H₂O₂ (30% concentration) (0.070 mL, 0.684 mmol) and ceric ammonium nitrate (9.37 mg, 0.017 mmol) to give 27l (40 mg, 0.096 mmol, 56% yield) as a white solid. ¹H NMR (500 MHz, CDCl₃) δ: 7.67 (d, J = 1.5 Hz, 1H), 7.45 (d, J = 8.5 Hz, 1H), 6.65 (s, 2H), 6.57 (dd, J = 9.0, J = 2.0 Hz, 1H), 4.13 (s, 6H), 1.72-1.64 (m, 2H), 1.39 (s, 6H), 1.32-1.22 (m, 6H), 1.20-1.12 (m, 2H), 0.86 (t, J = 7.5 Hz, 3H). HRMS (ESI) calculated for C₂₄H₃₂ClN₂O₂: calculated 415.2152; found 415.2150.

5-Chloro-2-(2,6-dimethoxy-4-(2-methyloctan-2-yl)phenyl)-1,3-dimethyl-1H-benzo[d]imidazol-3-ium iodide (49g): The synthesis was carried out as described for 49a using
5-chloro-2-(2,6-dimethoxy-4-(2-methyloctan-2-yl)phenyl)-1H-benzo[d]imidazole (20 mg, 0.048 mmol) and K$_2$CO$_3$ (13.32 mg, 0.096 mmol) followed by iodomethane (0.018 mL, 0.289 mmol) to afford desired product 49g (15 mg, 0.026 mmol, 54% yield) as light yellow solid. $^1$H NMR (500 MHz, CD$_3$OD) $\delta$: 8.13 (d, $J = 1.5$ Hz, 1H), 7.97 (d, $J = 8.5$ Hz, 1H), 7.75 (d, $J = 8.5$ Hz, $J = 1.5$ Hz, 1H), 6.93 (s, 2H), 3.91 (s, 3H), 3.86 (s, 3H), 3.84 (s, 3H), 1.80-1.73 (m, 2H), 1.42 (s, 6H), 1.28 (d, $J = 3.0$ Hz, 6H), 1.24-1.14 (m, 2H), 0.88 (t, $J = 7.0$ Hz, 3H). HRMS (ESI) calculated for C$_{26}$H$_{36}$ClN$_2$O$_2$: calculated 443.2465; found 443.2466.

![Chemical structure](attachment:image.png)

5-Chloro-2-(2,6-dihydroxy-4-(2-methyloctan-2-yl)phenyl)-1,3-dimethyl-1H-benzo[d]imidazol-3-ium bromide (50i): The synthesis was carried out as described for 50b using 5-chloro-2-(2,6-dimethoxy-4-(2-methyloctan-2-yl)phenyl)-1,3-dimethyl-1H-benzo[d]imidazol-3-ium iodide (20 mg, 0.035 mmol) and BBr$_3$ (0.062 mL, 0.659 mmol) to afford the desired product 50i (14 mg, 0.026 mmol, 73% yield) as a light brown oil. $^1$H NMR (500 MHz, CD$_3$OD) $\delta$: 8.11 (d, $J = 2.0$ Hz, 1H), 7.96 (d, $J = 9.0$ Hz, 1H), 7.73 (dd, $J = 9.0$ Hz, $J = 2.0$ Hz, 1H), 6.64 (s, 2H), 3.93 (s, 3H), 3.91 (s, 3H), 1.68-1.60 (m, 2H), 1.31 (s, 6H), 1.26 (s, 6H), 1.20-1.10 (m, 2H), 0.88 (t, $J = 7.0$ Hz, 3H). HRMS (ESI) calculated for C$_{24}$H$_{32}$ClN$_2$O$_2$: calculated 415.2152; found 415.2144.
2-(2,6-Dimethoxy-4-(2-methyloctan-2-yl)phenyl)-5-(trifluoromethyl)-1H-benzo[d]imidazole (27k): The synthesis was carried out as described for 27a using 2,6-dimethoxy-4-(2-methyloctan-2-yl)benzaldehyde (50 mg, 0.171 mmol), 4-(trifluoromethyl)benzene-1,2-diamine (33.1 mg, 0.188 mmol), H$_2$O$_2$ (30% concentration) (0.070 mL, 0.684 mmol) and ceric ammonium nitrate (9.37 mg, 0.017 mmol) to give the desired product 27k (45 mg, 0.100 mmol, 59% yield) as a white solid. $^1$H NMR (500 MHz, CDCl$_3$) $\delta$: 7.97 (s, 1H), 7.71 (d, $J = 8.0$ Hz, 1H), 7.09 (d, $J = 8.0$ Hz, 1H), 6.63 (s, 2H), 4.10 (s, 6H), 1.66-1.58 (m, 2H), 1.35 (s, 6H), 1.30-1.18 (m, 6H), 1.14-1.06 (m, 2H), 0.86 (t, $J = 7.0$ Hz, 3H). HRMS (ESI) calculated for C$_{25}$H$_{32}$F$_3$N$_2$O$_2$: calculated 449.2416; found 449.2414.

2-(2,6-Dimethoxy-4-(2-methyloctan-2-yl)phenyl)-1,3-dimethyl-5-(trifluoromethyl)-1H-benzo[d]imidazol-3-ium iodide (49f): The synthesis was carried out as described for 49a using 2-(2,6-dimethoxy-4-(2-methyloctan-2-yl)phenyl)-5-(trifluoromethyl)-1H-benzo[d]imidazole (25
mg, 0.056 mmol) and K₂CO₃ (15.41 mg, 0.111 mmol) followed by iodomethane (0.021 mL, 0.334 mmol) to afford desired product 49f (18 mg, 0.030 mmol, 53% yield) as light yellow solid.

¹H NMR (500 MHz, CD₃OD) δ: 8.46 (s, 1H), 8.19 (d, J = 8.5 Hz, 1H), 8.04 (d, J = 9.0 Hz, 1H), 6.94 (s, 2H), 3.94 (s, 3H), 3.92 (s, 3H), 3.91 (s, 6H), 1.82-1.73 (m, 2H), 1.42 (s, 6H), 1.28 (d, J = 3.5 Hz, 6H), 1.24-1.14 (m, 2H), 0.88 (t, J = 6.5 Hz, 3H). HRMS (ESI) calculated for C₂₇H₃₆F₃N₂O₂: calculated 477.2729; found 477.2723.

2-(2,6-Dihydroxy-4-(2-methyloctan-2-yl)phenyl)-1,3-dimethyl-5-(trifluoromethyl)-1H-benzo[d] imidazol-3-ium bromide (50h): The synthesis was carried out as described for 50b using 2-(2,6-dimethoxy-4-(2-methyloctan-2-yl)phenyl)-1,3-dimethyl-5-(trifluoromethyl)-1H-benzo[d]imidazol-3-ium iodide (20 mg, 0.033 mmol) and BBr₃ (0.062 mL, 0.659 mmol) to give 50h (16 mg, 0.028 mmol, 84% yield) as a light brown oil. ¹H NMR (500 MHz, CD₃OD) δ: 8.44 (s, 1H), 8.17 (d, J = 8.5 Hz, 1H), 8.02 (dd, J = 8.5 Hz, J = 1.5 Hz, 1H), 6.65 (s, 2H), 4.01 (s, 3H), 3.99 (s, 3H), 1.68-1.62 (m, 2H), 1.32 (s, 6H), 1.30-1.22 (m, 6H), 1.20-1.10 (m, 2H), 0.88 (t, J = 7.0 Hz, 3H). HRMS (ESI) calculated for C₂₅H₃₂F₃N₂O₂: calculated 449.2416; found 449.2415.
2-(2,6-Dimethoxy-4-(2-methyloctan-2-yl)phenyl)-1,3-dimethyl-1H-imidazol-3-ium iodide (49h): The synthesis was carried out as described for 49a using 2-(2,6-dimethoxy-4-(2-methyloctan-2-yl)phenyl)-1H-imidazole (30 mg, 0.091 mmol) and K₂CO₃ (151 mg, 1.089 mmol) followed by iodomethane (0.057 mL, 0.908 mmol) to afford desired product 49h (35 mg, 0.072 mmol, 79% yield) as light yellow solid. (Note: product has little impurity which was difficult to separate. Crude product was carried to next step without further purification.) HRMS (ESI) calculated for C₂₂H₃₅N₂O₂: calculated 359.2699; found 359.2690.

2-(2,6-Dihydroxy-4-(2-methyloctan-2-yl)phenyl)-1,3-dimethyl-1H-imidazol-3-ium bromide (50j): The synthesis was carried out as described for 50b using 2-(2,6-dimethoxy-4-(2-methyloctan-2-yl)phenyl)-1,3-dimethyl-1H-imidazol-3-ium iodide (20 mg, 0.041 mmol) and BBr₃ (0.062 mL, 0.659 mmol) to give 50j (12 mg, 0.026 mmol, 64% yield) as a white solid. ¹H NMR (500 MHz, CD₃OD) δ: 7.66 (s, 2H), 6.56 (s, 2H), 3.71 (s, 6H), 1.64-1.58 (m, 2H), 1.28 (s, 6H), 1.26-1.21 (m, 6H), 1.17-1.08 (m, 2H), 0.87 (t, J = 7.0 Hz, 3H). HRMS (ESI) calculated for C₂₀H₃₁N₂O₂: calculated 331.2386; found 331.2388.
2,6-Dihydroxy-4-(2-methyloctan-2-yl)benzaldehyde (52): To a solution of 5-(2-methyloctan-2-yl)benzene-1,3-diol (5 g, 21.16 mmol) in anhydrous DMF (20 mL) at 0 °C under argon atmosphere was added slowly POCl₃ (2.119 mL, 22.73 mmol). The reaction mixture was stirred for 16 hrs while letting it warm to room temperature. Reaction mixture was poured onto ice-water and pH was neutralized by adding 10% NaOH. The reaction mixture was stirred for 10 min and extracted with ethyl acetate (3x100 mL). Combined organic extract was washed with brine, dried over MgSO₄ and concentrated under reduced pressure. The crude mixture was purified by flash column chromatography (10-30% ethyl acetate : hexanes) to afford product 52 (3.41 g, 12.87 mmol, 62% yield) as yellow oil. HRMS (ESI) calculated for C₁₆H₂₅O₃: calculated 265.1804; found 265.1814.

![Chemical Structure](image)

2-(1H-Benz[d]imidazol-2-yl)-5-(2-methyloctan-2-yl)benzene-1,3-diol (28a): A mixture of 52 (2 g, 7.57 mmol), benzene-1,2-diamine (0.900 g, 8.32 mmol) and sodium metabisulfite (1.582 g, 8.32 mmol) in DMA (40 ml) was stirred at 60°C for 16 hrs. Reaction was diluted with 100 mL of ethyl acetate, 100 mL water. Organic layer was separated, washed with brine, dried over Na₂SO₄ and concentrated under reduced pressure. Purification by flash column chromatography (10-70% ethyl acetate : hexanes) gave desired product 28a (1.893 g, 5.37 mmol, 71 % yield) as a white
solid. $^1$H NMR (500 MHz, CDCl$_3$ + 2 drops CD$_3$OD) $\delta$: 7.62 (dd, $J = 6.0$ Hz, $J = 3.0$ Hz, 2H),
7.31 (dd, $J = 6.0$ Hz, $J = 3.0$ Hz, 2H), 6.74 (s, 1H), 6.33 (s, 1H), 1.54-1.49 (m, 2H), 1.21 (s, 6H),
1.20-1.11 (m, 6H), 1.04-0.96 (m, 2H), 0.77 (t, $J = 7.0$ Hz, 3H). HRMS (ESI) calculated for
C$_{22}$H$_{29}$N$_2$O$_2$: calculated 353.2229; found 353.2233.

4-((1-Adamantyl)-2,6-dimethoxybenzaldehyde (54): To a solution of (1-(3,5-
dimethoxyphenyl)adamantane (500 mg, 1.836 mmol) in THF (18mL) was added $n$-butyllithium
(1.101 mL, 2.75 mmol) at -78 °C and stirred for 2h. To the reaction mixture paraformaldehyde
(110 mg, 3.67 mmol) was added and stirred for 5 hrs at room temperature. The reaction mixture
was diluted with 100 mL DCM and 100 mL water and 10 mL saturated NH$_4$Cl solution. The
organic layer was separated, washed (2x50 mL water), dried (MgSO$_4$) and concentrated under
reduced pressure. Crude product was purified by silica gel chromatography eluting with 10-50%
ethyl acetate : hexanes to afford desired product 54 (420 mg, 1.398 mmol, 76% yield) as a
colorless oil. HRMS (ESI) calculated for C$_{19}$H$_{25}$O$_3$: calculated 301.1804; found 301.1799. $^1$H
NMR (500 MHz, CDCl$_3$) $\delta$: 10.45 (s, 1H), 6.56 (s, 2H), 3.91 (s, 6H), 2.12 (br s, 3H), 1.91 (d, $J =$
2.0 Hz, 6H), 1.81 (d, $J = 12.5$ Hz, 3H), 1.76 (d, $J = 12.5$ Hz, 3H).
2-(4-((1-Adamantyl)-2,6-dimethoxyphenyl)-1H-benzo[d]imidazole (55): To a solution of 4-((1-adamantan-1-yl)-2,6-dimethoxybenzaldehyde (100 mg, 0.333 mmol) and benzene-1,2-diamine (39.6 mg, 0.366 mmol) in acetonitrile (4 mL) was added CAN (219 mg, 0.399 mmol) followed by H₂O₂ (0.102 mL, 0.999 mmol) and stirred at room temp for overnight. The reaction mixture was diluted with 20 mL DCM and 20 mL water. The organic layer was separated, washed (2x20 mL water), dried (MgSO₄) and concentrated under reduced pressure. The crude product was purified by silica gel chromatography eluting with 10-50% ethyl acetate : hexanes to afford the desired product 55 (95 mg, 0.245 mmol, 73% yield) as a brown solid. ¹H NMR (500 MHz, CD₃OD) δ: 8.0 (dd, J = 6.5 Hz, J = 3.0 Hz, 2H), 7.45 (dd, J = 6.0 Hz, J = 3.0 Hz, 2H), 6.80 (s, 2H), 4.21 (s, 6H), 2.18 (br s, 3H), 1.96 (d, J = 3.0 Hz, 6H), 1.86 (d, J = 12.0 Hz, 3H), 1.80 (d, J = 12.0 Hz, 3H). HRMS (ESI) calculated for C₂₅H₂₉N₂O₂: calculated 389.2229; found 389.2241.

2-(4-((1-Adamantyl)-2,6-dimethoxyphenyl)-1,3-dimethyl-1H-benzo[d]imidazol-3-ium iodide (56): The synthesis was carried out as described for 49a using 2-(4-((1-Aadamantyl)-2,6-
dimethoxyphenyl)-1H-benzo[d]imidazole (50 mg, 0.129 mmol) and K$_2$CO$_3$ (21.34 mg, 0.154 mmol) followed by iodomethane (0.040 mL, 0.643 mmol) to afford the desired product 56 (55 mg, 0.101 mmol, 78% yield) as a brown solid. $^1$H NMR (500 MHz, CD$_3$OD) $\delta$: 7.98 (dd, $J = 6.0$ Hz, $J = 3.5$ Hz, 2H), 7.75 (dd, $J = 6.5$ Hz, $J = 3.5$ Hz, 2H), 6.96 (s, 2H), 3.91 (s, 6H), 3.86 (s, 6H), 2.17 (br s, 3H), 2.07 (br s, 6H), 1.93-1.85 (m, 6H).

2-(4-((1-Aadamantyl)-2,6-dihydroxyphenyl)-1,3-dimethyl-1H-benzo[d]imidazol-3-ium iodide (57): A solution of 2-(4-((1-adamantyl-1-yl)-2,6-dimethoxyphenyl)-1,3-dimethyl-1H-benzo[d]imidazol-3-ium iodide (25 mg, 0.046 mmol) in hydrogen iodide (1 mL, 0.046 mmol) was heated to 60 °C for overnight. The reaction mixture was concentrated under reduced pressure and was purified by silica gel chromatography eluting with 0-20% methanol : DCM to afford desired product 57 (15 mg, 0.029 mmol, 63% yield) as a light brown oil. $^1$H NMR (500 MHz, CD$_3$OD) $\delta$: 7.96 (dd, $J = 6.5$ Hz, $J = 3.5$ Hz, 2H), 7.73 (dd, $J = 6.5$ Hz, $J = 3.5$ Hz, 2H), 6.65 (s, 2H), 3.95 (s, 6H), 2.12 (br s, 3H), 1.96 (d, $J = 2.5$ Hz, 6H), 1.87 (d, $J = 12.5$ Hz, 3H), 1.82 (d, $J = 12.5$ Hz, 3H). HRMS (ESI) calculated for C$_{25}$H$_{29}$IN$_2$O$_2$: calculated 389.2229; found 389.2237.
Chapter 3

Allosteric Cannabinoid Modulators
3.1 Allosteric Modulators

Although, approximately 30% of Food and Drug Administration (FDA) approved small molecules are thought to target GPCRs, useful or selective ligands do not exist to modulate GPCRs. Synthetic ligands are known for only a fraction of the known GPCRs. Many attempts to find highly selective GPCRs ligand failed with high rate which could be useful as drug molecules. Many issues contribute to the challenges of discovering novel selective agonists or antagonists that bind at the orthosteric site of the receptor. Orthosteric sites for specific endogenous ligands across GPCR subtypes are often conserved, making it difficult to achieve high selectivity within subfamily of GPCRs. An alternative approach is the development of allosteric modulators of specific subtypes of GPCRs, this approach has been highly successful in case of the ligand-gated ion channels. These ligands bind to a site (allostERIC site) which is distinct from orthosteric site and potentiate or inhibit activation of receptor by its natural ligands. Three types of allosteric modulators are known; Positive Allosteric Modulators (PAMs) which increase the response of receptor, Negative Allosteric Modulators (NAMs) which decrease the response of receptor and Silent Allosteric Ligands (SAMs) which bind to the allosteric site but do not alter receptor response.

Recently remarkable progress has been made in the discovery, optimization and clinical development of allosteric modulators for multiple GPCR subtypes. There are PAMs, NAMs and SAMs for each of three major GPCR subfamilies. Such allosteric ligands offer a novel mode of action compared to orthosteric ligands. Allosteric modulators are providing major advances in developing drugs and have potential utility for the treatment of multiple human disorders, including psychiatric and neurological disorders, and several potential GPCR drug targets which were intractable using traditional orthosteric ligand approaches.
Allosteric modulators bind to GPCRs at the site distinct to the orthosteric sites leading to the change in receptor conformation. As a result, interactive properties of GPCRs with respect to orthosteric ligand(s) and cellular host environment can be modified in either a positive or negative direction. Allosteric modulators can exhibit the following pharmacological properties:

- **affinity modulation** – the resulting conformation can alter either association or dissociation rate of an orthosteric ligand;
- **efficacy modulation** – the allosteric effect can modify intracellular response, thus leading to change in signaling capacity of the orthosteric ligand;
- **agonism/inverse agonism** – the allosteric modulator can perturb receptor signaling in either a positive or negative direction, irrespective of presence of orthosteric modulator. There are number of examples of allosteric modulators that exhibit one or more of these pharmacological properties.

In 2005, the novel allosteric modulators of CB₁ receptor Org27569, Org29647 and Org27759 (Figure 3.1) were reported by Organon. These compounds displayed several common allosteric modulator characteristics. Interestingly, they behaved as allosteric enhancers of agonist binding affinity, but allosteric inhibitors of its signaling efficacy. Recently, a similar pharmacological profile for PSNCBAM-1, a structurally novel CB₁ allosteric modulator discovered through HTS, has been reported by Prosidion Limited (currently OSI Pharmaceuticals Inc). RTI-371 an inhibitor of the dopamine transporter (DAT) was shown to also behave as a PAM of the hCB₁ receptor.
Figure 3.1: Allosteric modulators of CB₁ receptor.

Recently rimonabant (Acomplia), a CB₁ inverse agonist, approved and marketed for the treatment of obesity, was withdrawn from European market. Rimonabant was responsible for CNS mediated side effects such as nausea and psychiatric disorders. A safer alternative could come from developing novel allosteric modulators that give the desired pharmacological response by inducing receptor conformational change.

Comparing Org27569 and PSNCBAM-1, the latter is the only in vivo studied CB1 NAM that possesses hypophagic activity in rats. PSNCBAM-1 showed decrease in food intake and body weight when tested in an acute rat feeding model. In CB1 receptor yeast reporter assay, agonist effect of CP55,940, WIN55212-2, anandamide (AEA) and 2-arachidonoyl was antagonized by PSNCBAM-1. Allosteric mechanism was shown for PSNCBAM-1 activity by Schild analysis. Constitutional activity of CB1 receptor was unchanged in the presence of
PSNCBAM-1. However, the *in vivo* potency was moderate and further lead optimization efforts were required.

Figure 3.2: Proposed SAR plan for PSNCBAM-1 scaffold.

To understand structural requirements for CB1 NAM activity within the PSNCBAM-1 template, we decided to first optimize chlorophenyl ring A (site I). Having optimized site I, we plan to identify suitable bioisosteres for the urea group (site II) followed by optimization of the phenyl ring B and pyridine ring C (site III). We would further probe the significance of N in the pyridine ring C as well as explore different water solubility enhancing functional groups in place of pyrrolidine ring D (Figure 3.2).

3.2 Chemistry

2,6-Dibromopyridine was treated with neat pyrrolidine (excess) to give pyrrolidine substituted bromopyridine 3 in good yield (89%). Suzuki coupling of 3 with m-nitrophenylboronic acid 4 under reflux in the presence of catalytic Pd(PPh$_3$)$_4$ gave intermediate 5 also in good yield (89%). Interestingly, potential chelation of palladium by 2-(pyrrolidin-1-yl)pyridine did not affect the coupling reaction. The nitro group was reduced to amine...
(compound 6) by catalytic hydrogenation as reported in the literature. The p-nitrophenylcarbamate of 6 was highly reactive, unstable and led to the formation of undesired symmetrical urea in significant quantities. To reduce or avoid formation of this byproduct which interfered with the purification of desired urea analogs, we decided to make a less reactive and more stable carbamate by simply changing nitrophenyl to phenyl. Desired phenylcarbamate 8 was obtained in good yield (93%) by treating amine 6 with phenylchloroformate at 0°C in the presence of triethylamine. Carbamate 8 underwent reaction with various amines 9 to give desired ureas 10 in generally good yield (29-78%).
Scheme 3.1: Site I Modification of PSNCBAM-1

![Chemical diagram with reaction steps and structures](image)

**Reagents and Conditions:** (a) neat, RT, overnight, 89%; (b) Pd(PPh₃)₄, NaHCO₃, DME, reflux, overnight, 89%; (c) H₂, Pd/C, cat. acetic acid, Ethanol, THF, RT, overnight, 16 h, 96%; (d) Et₃N, DCM, 0 °C, 3 h, 63%; (e) DIEA, DMF, 50 °C, 6 h, 29-78%.
Scheme 3.2: Site I Modification: Synthesis of Urea and Carbamate from Isocyanate Route

Reagents and Conditions: (a) Triphosgene, Et$_3$N, toluene, 70 °C, 3 h; (b) Et$_3$N, DCM, 0 °C, 3 h, 21-46%.

It was observed that electron deficient or sterically hindered anilines reacted poorly or not at all with phenyl carbamate 8 to produce desired urea. To overcome this challenge, isocyanate 11 was synthesized in good yield by treating amine 6 with triphosgene in the presence of triethylamine.\textsuperscript{102} Aniline (12a) or phenols (12b and 12c) reacted with isocyanate 11 to give desired urea 13a or carbamates (13b and 13c) respectively in good yield.

It was planned to make ortho substituted carboxylic acid derivatives of A phenyl ring. Weakly nucleophilic aniline 14 did not react with carbamate 8 or isocyanate 11. This synthetic hurdle was overcome by treating isocyanate 16 formed from aniline 14, with amine intermediate 6 to give desired urea 15 in good yield (76%). Carboxylic acid analog 16 was obtained by hydrolysis of methyl ester 15. LAH reduction of methyl ester 15 gave the corresponding benzyl alcohol analog 17 in a moderate yield.
Scheme 3.3: Attempted Synthesis of 15 from aniline 14

First attempt:

\[
\text{14} + \text{8} \rightarrow \text{15}
\]

Second attempt:

\[
\text{14} + \text{11} \rightarrow \text{15}
\]

Reagents and Conditions: (a) DIEA, DMF, 50 °C, 6 h, no rxn; (b) Et$_3$N, DCM, 0 °C, 3 h, no rxn.

Scheme 3.4: Successful synthesis of ureas from isocyanate 16

\[
\text{16} + \text{6} \rightarrow \text{15}
\]

Reagents and Conditions: (a) Et$_3$N, DCM, 0 °C, 3 h, 76%; (b) LiOH, THF, MeOH overnight, 68%; (c) LAH, THF, 0 °C, 5 h, 40%.

It was decided to make long alkyl chain alcohol group attached to the chlorophenyl ring A at ortho position to the urea group. Initial attempts to make iodosubstituted urea 21 by reacting iodoaniline 18 with carbamate 8 failed to give desired product, presumably due to steric
hindrance of the bulky iodo group (Scheme 3.5). Alternatively, the isocyanate derived from aniline 20, synthesized as previously described, gave upon treatment with amine 6 the desired ureas 21. Sonogashira coupling\textsuperscript{103,104} with alkyne containing THP protected alcohol group 22 on aryl iodides 21 gave compounds 23 in good yield. Alkynes 22 were reduced under catalytic hydrogenation condition to yield saturated alkyl chain analogs 25. THP was removed to give primary alcohol compounds 26 and 24.

**Scheme 3.5:** Synthesis Challenge to Make Urea from Sterically Hindered Anilines

\[
\begin{array}{c}
\text{Cl} & \text{I} & \text{NH}_2 \\
\text{18} & + & \text{O} \text{N} \\
\text{8} & \xrightarrow{a} & \text{Cl} & \text{I} & \text{O} & \text{N} \\
\text{19}
\end{array}
\]

**Reagents and Conditions:** (a) DIEA, DMF, 70 °C, 6 h, no rxn.
**Scheme 3.6: Modified Route to Prepare Alkyne Substituted Aryl Urea Analogs**

Reagents and Conditions: (a) Triphosgene, Et$_3$N, toluene, 70 °C, 3 h; (b) Et$_3$N, DCM, 0 °C, 3 h, 54-66%; (c) Pd(PPh$_3$)$_2$Cl$_2$, Cu(I)Cl, 90 °C, 2 h, 67-76%; (d) PTSA, methanol, room temp, 5 h, 52-76%; (e) Pd/C, H$_2$, methanol, room temp, overnight, 23-84%.

We decided to probe the role of urea group w.r.t. its receptor binding interactions in the context of H-bonding. Reductive amination on 6 with paraformaldehyde under basic conditions produced N-methylated intermediate 27 which was treated with 4-chlorophenyl isocyanate to give desired N-methylated urea compound 29. The reaction was much cleaner compared to the
earlier approaches, especially w.r.t. the formation of symmetrical urea byproduct, therefore this approach was extended to the scale-up of lead compound PSNCBAM-1 (Scheme 3.7).

**Scheme 3.7: N-Methylation of urea analogs and scale-up of PSNCBAM-1 10a**

![Scheme 3.7 diagram]

**Reagents and Conditions:** (a) paraformaldehyde, NaOCH₃, reflux, 3 h, 0 °C, NaBH₄, reflux, 1 h, 83% (b) Et₃N, DCM, 0 °C, 3 h, 72-85%.

N,N-dimethylation of urea was conducted directly on 10a by treating it first with NaH, followed by addition of iodomethane to give desired bis-methyl substituted urea 30 (Scheme 3.8). ¹⁰⁶
Scheme 3.8: Synthesis of $N,N$-Dimethylurea analog 30

Reagents and Conditions: (a) CH$_3$I, NaH, DMF, 0 °C, 3 h, 78%.

The reverse carbamate 33 was synthesized by first performing Suzuki coupling between commercially available boronic acid 31 and pyridyl bromide 3 to give intermediate 32 in good yield. Intermediate 32 was then treated with $p$-chlorophenyl isocyanate in the presence of triethylamine to give desired carbamate 33 in good yield (61%).

Scheme 3.9: Synthesis of Carbamate Analog 33

Reagents and Conditions: (a) Pd(PPh$_3$)$_4$, NaHCO$_3$, DME, reflux, overnight, 79%; (b) Et$_3$N, DCM, 0 °C, 3 h, 61%.

We tried to make thiourea 34 from final urea compound itself using Lawesson’s reagent$^{107}$. Unfortunately upon treatment under standard conditions, no product formation was observed. Thiourea analog 34 was synthesized in good yield (75%) when the amine was treated with phenyl isothiocyanate 35.
Scheme 3.10: Synthesis of Thiourea Analog 34

Reagents and Conditions: (a) Lawesson’s reagent, toluene, reflux, overnight, no rxn, (b) Et₃N, DCM, 40 °C, 3 h, 56%.

Cyanoguanidine analog 37 was synthesized by first treating diphenyl cyanocarbonimidate 36 with 4-chloroaniline to give chlorophenyl cyanocarbaminoimidate 36a in good yield, as reported by the Buschauer group.¹⁰⁸ We found poor reactivity of cyanocarbaminoimidate when treated with aniline 6 under thermal as well as microwave conditions in acetonitrile. However, changing solvent to dimethylformamide under microwave conditions, gave the desired product cyanoguanidine 37 in good yield (52%).
Scheme 3.11: Synthesis of Cyanoguanidine Challenges and Solution

Reagents and Conditions: (a) Acetonitrile, 50 °C, overnight, 74%; (b) Acetonitrile, reflux, overnight, no rxn; (c) Acetonitrile, 100 °C, MW, 30 min, no rxn; (d) DMF, MW, 150 °C, 1 h, 52%.

4-Chloroaniline was treated with diethoxycyclobutenedione 38 at room temperature in ethanol to give mono substituted ethoxycyclobutenedione aniline 39. It was found that the displacement of second ethoxy group with aniline was difficult. Reactions using conventional and microwave heating under different solvents gave no observable conversion of intermediate 39 to desired product 40. We decided to activate ethoxycyclobutenedione 39 with a Lewis acid such as trimethylaluminum. Thus, 39 was treated with amine 6 in the presence of trimethylaluminum at room temperature to give desired disubstituted anilincerobutenedione 40 in moderate yield (47%).
Scheme 3.12: Synthesis of Cyclobutenedione Analog 40

Reagents and Conditions: (a) ethanol, RT, 48 h, 90%; (b) ethanol, RT, overnight, no rxn; (c) ethanol, reflux, overnight, no rxn; (d) ethanol, MW, 100 °C, 30 min, no rxn; (e) DMF, MW, 150 °C, 30 min, no rxn; (f) DMSO, MW, 150 °C, 30 min, no rxn; (g) AlMe₃, DCM, 0 °C, 1 h, 47%.

Synthesis of oxadiazoles 44 and 47 as urea bioisosteres was planned. Thus, Suzuki coupling of boronic acid 41 with 3 in the presence of catalytic Pd(PPh₃)₄ gave the coupled biphenylmethyl carboxylic ester 42 in a good yield. The methyl ester was converted into a benzoyl hydrazide 43, as described by Dolman group. Treatment with isothiocyanate 35 gave the desired oxadiazole 44 in good yield (Scheme 3.13). The isomeric oxadiazole 47 was synthesized by first converting amine 6 into isothiocyanate 45 using thiophosgene, followed by cyclization with 4-chlorophenylhydrazide 46 (Scheme 3.14).
Scheme 3.13: Bioisosteres of Urea Group: Synthesis of Oxadiazole 44

Reagents and Conditions: (a) Pd(PPh₃)₄, NaHCO₃, DME, reflux, overnight, 68%. (b) hydrazine, ethanol, reflux, overnight; (c) THF, reflux, overnight, 38%.

Scheme 3.14: Bioisosteres of Urea group: Synthesis of Oxadiazole 47

Reagents and Conditions: (a) thiophosgene, DCM, RT; (b) THF, reflux, overnight, 30%.

We have synthesized PSNCBAM-1 by two different routes. Both routes, however, yielded variable quantities of the undesired symmetric urea, which posed problems during the purification, especially when done on a large scale. We designed an alternate route to PSNCBAM-1 by utilizing a C-N coupling reaction that could possibly be extended to other analogs. Aniline 48 was treated with 4-chlorophenyl isocyanate 28 to give desired urea containing boronic acid 49. Suzuki coupling was performed on boronic acid with bromopyridine...
3 in the presence of catalytic Pd(PPh₃)₄ to give desired final coupled urea product 10a in 62% yield (Scheme 3.15).

**Scheme 3.15: Alternate Preparation of PSNCBAM-1 10a**

\[
\begin{align*}
\text{Cl} & \quad \text{H₂N} \quad \text{B(OH)₂} \\
\text{N} & \quad \text{Cl} \\
28 & \quad \text{48} & \quad \text{Cl} \\
\text{a} & \quad \text{49} & \quad \text{3} & \quad \text{b} \\
\text{O} & \quad \text{N} & \quad \text{Cl} \\
\text{NH} & \quad \text{Cl} \\
10a & \quad \text{49} \\
\end{align*}
\]

**Reagents and Conditions:** (a) DMF, RT, 3 h, 53%; (b) Pd(PPh₃)₄, NaHCO₃, DME, reflux, overnight, 62%.

Phenyl ring B substituted analogs were prepared as shown in Scheme 3.16. Suzuki coupling between 4-nitrophenylboronic acid 50 and 3 gave nitroaryl intermediate 51. Subsequent catalytic hydrogenation followed by treatment with 4-chlorophenyl isocyanate 28 gave the desired urea 53. Urea regioisomer 57 was synthesized by following the same steps mentioned above using corresponding starting material as shown in Scheme 3.17.
Scheme 3.16: Synthesis of Analog 53

Reagents and Conditions: (a) Pd(PPh₃)₄, NaHCO₃, DME, reflux, overnight, 81%. (b) H₂, Pd/C, cat. acetic acid, ethanol, THF, RT, overnight, 84%; (c) Et₃N, DCM, 0 °C, 3 h, 34%.

Scheme 3.17: Synthesis of Analog 57

Reagents and Conditions: (a) Pd(PPh₃)₄, NaHCO₃, DME, reflux, overnight, 78%; (b) H₂, Pd/C, cat. acetic acid, ethanol, THF, RT, overnight, 90%; (c) Et₃N, DCM, 0 °C, 3 h, 54%.

The synthesis of analog 60 was attempted as shown in Scheme 3.18. Interestingly, 4-bromo-2-chloropyridine 58 showed poor reactivity when treated with pyrrolidine 2 in the presence of K₂CO₃ at 85 °C under thermal as well as microwave conditions. A neat reaction between 4-bromo-2-chloropyridine and pyrrolidine at room temperature was tried but gave no conversion.
Increasing temperature to reflux did not improve conversion. Buchwald coupling (C-N bond formation) with pyrrolidine under standard conditions failed to give desired product. We tried copper (I) iodide catalyzed C-N coupling conditions for this reaction under thermal as well as microwave conditions. Unfortunately both reactions failed to give desired product (Scheme 3.18). Finally, we decided to replace chloro by fluoro 61 and were delighted to see clean conversion of the starting material to desired product 59 in the presence of K₂CO₃ (Scheme 3.19). Suzuki coupling with previously synthesized boronic acid 49 gave desired urea 60 in good yield (74%).

**Scheme 3.18: Synthesis Challenge for Pyridine Ring Substitution**

![Reaction Scheme](image)

**Reagents and Conditions:** (a) neat, RT, overnight, no rxn; (b) reflux, overnight, no rxn (c) K₂CO₃, DMF, 100 °C, 7 hs, no rxn; (d) K₂CO₃, DMF, 100 °C, MW, 30 min, no rxn; (e) Cul, L-proline, 100 °C, overnight, no rxn; (f) Cul, L-proline, 100 °C, MW, 30 min , no rxn; (g) Pd₂(dba)₃, BINAP, t-BuONa, toluene, 80 °C, no rxn.
Scheme 3.19: Synthesis of Ring D Variation Analog 60

Reagents and Conditions: (a) K$_2$CO$_3$, DMF, 100 °C, 16 h, 59%. (b) Pd(PPh$_3$)$_4$, NaHCO$_3$, DME, reflux, overnight, 53%.

Compound 64 was synthesized as shown in Scheme 3.20. Treatment of 5-bromo-2-chloropyridine 62 with pyrrolidine in the presence of K$_2$CO$_3$ gave desired substituted pyrrolidine bromopyridine 63 (81%) which was coupled with boronic acid 49 under Suzuki conditions to give 64 in 62% yield.

Scheme 3.20: Synthesis of Analog 64

Reagents and Conditions: (a) K$_2$CO$_3$, DMF, 100 °C, 7 h, 64%; (b) Pd(PPh$_3$)$_4$, NaHCO$_3$, DME, reflux, overnight, 62%.
Synthesis of non-pyridyl compound 67 was attempted as shown in Scheme 3.21. 1,3-Dibromobenzene 65 when treated with pyrrolidine 2 under thermal condition did not yield 66. When we changed the starting material to 1-bromo-3-fluorobenzene 68, the reaction proceeded smoothly under thermal condition in the presence of K$_2$CO$_3$ to give 66 (Scheme 3.22). Subsequent coupling with previously synthesized boronic acid 49 gave final desired non-pyridyl derivative 67 in moderate yield (54%).

**Scheme 3.21: Attempted Synthesis of Non-Pyridyl Analog 67**

Reagents and Conditions: (a) K$_2$CO$_3$, DMF, 100 °C, 16 h, no rxn.
**Scheme 3.22:** Successful synthesis of non-pyridyl analog 67

![Scheme 3.22](image)

**Reagents and Conditions:** (a) K$_2$CO$_3$, DMF, 100 °C, 7 h, 70%; (b) Pd(PPh$_3$)$_4$, NaHCO$_3$, DME, reflux, overnight, 53%.

Synthesis of analogs 70 was carried out as shown in Schemes 3.23 and 3.24. Suzuki coupling was carried on boronic acid 49 and excess of dibromopyridine to give desired coupled bromopyridine product 68 in good yield (72%). Subsequent C-N Buchwald coupling under standard condition did not provide any desired product (Scheme 3.23). However, Copper (I) chloride catalyzed C-N bond formation in the presence of L-proline provided desired the morpholine and piperazine substituted products 70 in a good (32% and 39%) yields (Scheme 3.24).
Scheme 3.23: Synthesis Challenge for Modification of Aliphatic Group

Reagents and Conditions: (a) Pd(PPh₃)₄, NaHCO₃, DME, reflux, overnight, 72%; (b) Pd₂dba₃, BINAP, t-BuONa, toluene, 80°C, no rxn.

Scheme 3.24: Synthesis of Modification of Aliphatic Group

Reagents and Conditions: (a) CuI, L-proline, t-BuONa, DMF, 100 °C, overnight, 32-39%.
3.3 X-Ray Structures of Urea Analogs

We have developed crystals of two allosteric analogs 24a (solvent system: methanol:DCM), in which the alkyl chain in phenyl ring is ortho to the urea function and 24b where the alkyl chain is meta to the urea function. It was observed that orientation of hydroxy alkyne chain is important for activity at the both receptors. When alkyne chain points in the direction of oxygen of urea, it shows moderate activity and when alkyne chain points in the same side of nitrogen of urea it diminishes activity at both the receptors. These important conformational effects on activity will aid the design of future NAM allosteric probes.

Figure 3.3: Crystal Structures of 24a and 24b.
3.4 Results and Discussion

Table 3.1: EC<sub>50</sub> values of novel CB<sub>1</sub> negative allosteric modulators in cAMP Hunter assay and CNR1 PathHunter assay (β-arrestin).

<table>
<thead>
<tr>
<th>Compound No.</th>
<th>Structure</th>
<th>cAMP</th>
<th>β-arrestin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>EC&lt;sub&gt;50&lt;/sub&gt; nM</td>
<td>% inhibition</td>
</tr>
<tr>
<td>10a&lt;sup&gt;*&lt;/sup&gt;</td>
<td><img src="image" alt="Structural formula" /></td>
<td>110±11</td>
<td>136%</td>
</tr>
<tr>
<td>10a&lt;sup&gt;**&lt;/sup&gt;</td>
<td><img src="image" alt="Structural formula" /></td>
<td>231±23</td>
<td>108%</td>
</tr>
<tr>
<td>10q&lt;sup&gt;*&lt;/sup&gt; (GAT329)</td>
<td><img src="image" alt="Structural formula" /></td>
<td>501±50</td>
<td>135%</td>
</tr>
<tr>
<td>10c&lt;sup&gt;*&lt;/sup&gt; (GAT301)</td>
<td><img src="image" alt="Structural formula" /></td>
<td>782±80</td>
<td>111%</td>
</tr>
<tr>
<td>10d&lt;sup&gt;*&lt;/sup&gt; (GAT302)</td>
<td><img src="image" alt="Structural formula" /></td>
<td>&gt;10,000</td>
<td>39%</td>
</tr>
<tr>
<td>10e&lt;sup&gt;*&lt;/sup&gt; (GAT303)</td>
<td><img src="image" alt="Structural formula" /></td>
<td>218±22</td>
<td>161%</td>
</tr>
<tr>
<td>10f&lt;sup&gt;*&lt;/sup&gt; (GAT304)</td>
<td><img src="image" alt="Structural formula" /></td>
<td>242±24</td>
<td>116%</td>
</tr>
<tr>
<td>10g&lt;sup&gt;*&lt;/sup&gt; (GAT305)</td>
<td><img src="image" alt="Structural formula" /></td>
<td>1156±110</td>
<td>119%</td>
</tr>
<tr>
<td>10b&lt;sup&gt;*&lt;/sup&gt; (GAT337)</td>
<td><img src="image" alt="Structural formula" /></td>
<td>223±22</td>
<td>126%</td>
</tr>
<tr>
<td></td>
<td>Chemical Structure</td>
<td>Activity</td>
<td>Percentage</td>
</tr>
<tr>
<td>---</td>
<td>--------------------</td>
<td>----------</td>
<td>------------</td>
</tr>
<tr>
<td>10t**</td>
<td><img src="image" alt="10t** structure" /></td>
<td>148±15</td>
<td>119%</td>
</tr>
<tr>
<td>(GAT347)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10u**</td>
<td><img src="image" alt="10u** structure" /></td>
<td>330±33</td>
<td>79%</td>
</tr>
<tr>
<td>(GAT341)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10n*</td>
<td><img src="image" alt="10n* structure" /></td>
<td>764±76</td>
<td>122%</td>
</tr>
<tr>
<td>(GAT315)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10o*</td>
<td><img src="image" alt="10o* structure" /></td>
<td>483±50</td>
<td>65%</td>
</tr>
<tr>
<td>(GAT316)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10p**</td>
<td><img src="image" alt="10p** structure" /></td>
<td>1700±170</td>
<td>97%</td>
</tr>
<tr>
<td>(GAT317)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10h**</td>
<td><img src="image" alt="10h** structure" /></td>
<td>1233±120</td>
<td>97%</td>
</tr>
<tr>
<td>(GAT306)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10i*</td>
<td><img src="image" alt="10i* structure" /></td>
<td>1792±180</td>
<td>92%</td>
</tr>
<tr>
<td>(GAT307)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10j*</td>
<td><img src="image" alt="10j* structure" /></td>
<td>&gt;10,000</td>
<td>0</td>
</tr>
<tr>
<td>(GAT308)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10k*</td>
<td><img src="image" alt="10k* structure" /></td>
<td>750±75</td>
<td>91%</td>
</tr>
<tr>
<td>(GAT309)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10l*</td>
<td><img src="image" alt="10l* structure" /></td>
<td>2241±225</td>
<td>57%</td>
</tr>
<tr>
<td>(GAT310)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10m*</td>
<td><img src="image" alt="10m* structure" /></td>
<td>&gt;10,000</td>
<td>18%</td>
</tr>
<tr>
<td>(GAT311)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>10z</strong>  (GAT333)</td>
<td><img src="image1" alt="Structure" /></td>
<td>659±66</td>
<td>78%</td>
</tr>
<tr>
<td><strong>10w</strong>  (GAT312)</td>
<td><img src="image2" alt="Structure" /></td>
<td>1256±125</td>
<td>69%</td>
</tr>
<tr>
<td><strong>10x</strong>  (GAT313)</td>
<td><img src="image3" alt="Structure" /></td>
<td>1830±180</td>
<td>68%</td>
</tr>
<tr>
<td><strong>10y</strong>  (GAT314)</td>
<td><img src="image4" alt="Structure" /></td>
<td>&gt;10,000</td>
<td>2.55%</td>
</tr>
<tr>
<td><strong>10r</strong>  (GAT339)</td>
<td><img src="image5" alt="Structure" /></td>
<td>1190±120</td>
<td>42%</td>
</tr>
<tr>
<td><strong>10v</strong>  (GAT356)</td>
<td><img src="image6" alt="Structure" /></td>
<td>4808±480</td>
<td>70%</td>
</tr>
<tr>
<td><strong>17</strong>  (GAT335)</td>
<td><img src="image7" alt="Structure" /></td>
<td>1145±115</td>
<td>33%</td>
</tr>
<tr>
<td><strong>15</strong>  (GAT342)</td>
<td><img src="image8" alt="Structure" /></td>
<td>&gt;10,000</td>
<td>0.6%</td>
</tr>
<tr>
<td><strong>16</strong>  (GAT354)</td>
<td><img src="image9" alt="Structure" /></td>
<td>&gt;10,000</td>
<td>35%</td>
</tr>
<tr>
<td><strong>21a</strong>  (GAT319)</td>
<td><img src="image10" alt="Structure" /></td>
<td>1268±126</td>
<td>53%</td>
</tr>
<tr>
<td><strong>10s</strong>  (GAT340)</td>
<td><img src="image11" alt="Structure" /></td>
<td>&gt;10,000</td>
<td>18%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>21b* (GAT324)</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>&gt;10,000</td>
<td>30%</td>
</tr>
<tr>
<td>23a* (GAT320)</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>&gt;10,000</td>
<td>9%</td>
</tr>
<tr>
<td>24a* (GAT321)</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>6491±650</td>
<td>49%</td>
</tr>
<tr>
<td>25a* (GAT322)</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>820±80</td>
<td>41%</td>
</tr>
<tr>
<td>26a* (GAT323)</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>1230±120</td>
<td>82%</td>
</tr>
<tr>
<td>25c** (GAT324)</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>&gt;10,000</td>
<td>20%</td>
</tr>
<tr>
<td>23b* (GAT325)</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>&gt;10,000</td>
<td>22%</td>
</tr>
<tr>
<td>Compound</td>
<td>Structure</td>
<td>Activity</td>
<td>Metabolite</td>
</tr>
<tr>
<td>----------</td>
<td>----------</td>
<td>----------</td>
<td>------------</td>
</tr>
<tr>
<td>24b* (GAT326)</td>
<td><img src="#" alt="Structure" /></td>
<td>551±55</td>
<td>152%</td>
</tr>
<tr>
<td>25b* (GAT327)</td>
<td><img src="#" alt="Structure" /></td>
<td>354±35</td>
<td>55%</td>
</tr>
<tr>
<td>26b* (GAT328)</td>
<td><img src="#" alt="Structure" /></td>
<td>1420±140</td>
<td>111%</td>
</tr>
<tr>
<td>13a** (GAT344)</td>
<td><img src="#" alt="Structure" /></td>
<td>1643±165</td>
<td>17%</td>
</tr>
<tr>
<td>29** (GAT349)</td>
<td><img src="#" alt="Structure" /></td>
<td>527±53</td>
<td>31%</td>
</tr>
<tr>
<td>30** (GAT350)</td>
<td><img src="#" alt="Structure" /></td>
<td>1666±160</td>
<td>39%</td>
</tr>
<tr>
<td>13b** (GAT343)</td>
<td><img src="#" alt="Structure" /></td>
<td>&gt;10,000</td>
<td>3.5%</td>
</tr>
<tr>
<td>33** (GAT351)</td>
<td><img src="#" alt="Structure" /></td>
<td>&gt;10,000</td>
<td>8.8%</td>
</tr>
<tr>
<td>13c** (GAT346)</td>
<td><img src="#" alt="Structure" /></td>
<td>3046±300</td>
<td>24%</td>
</tr>
<tr>
<td>#</td>
<td>Compound</td>
<td>Structure</td>
<td>IC50 (μM)</td>
</tr>
<tr>
<td>----</td>
<td>----------</td>
<td>-----------</td>
<td>-----------</td>
</tr>
<tr>
<td>34**</td>
<td>(GAT359)</td>
<td><img src="image" alt="Structure" /></td>
<td>830±80</td>
</tr>
<tr>
<td>37**</td>
<td>(GAT357)</td>
<td><img src="image" alt="Structure" /></td>
<td>3343±335</td>
</tr>
<tr>
<td>40**</td>
<td>(GAT358)</td>
<td><img src="image" alt="Structure" /></td>
<td>&gt;10,000</td>
</tr>
<tr>
<td>47**</td>
<td>(GAT367)</td>
<td><img src="image" alt="Structure" /></td>
<td>4053±400</td>
</tr>
<tr>
<td>44**</td>
<td>(GAT366)</td>
<td><img src="image" alt="Structure" /></td>
<td>&gt;10,000</td>
</tr>
<tr>
<td>57**</td>
<td>(GAT364)</td>
<td><img src="image" alt="Structure" /></td>
<td>3400±340</td>
</tr>
<tr>
<td>53**</td>
<td>(GAT362)</td>
<td><img src="image" alt="Structure" /></td>
<td>&gt;10,000</td>
</tr>
<tr>
<td>60**</td>
<td>(GAT369)</td>
<td><img src="image" alt="Structure" /></td>
<td>9789±950</td>
</tr>
<tr>
<td>67**</td>
<td>(GAT370)</td>
<td><img src="image" alt="Structure" /></td>
<td>679±650</td>
</tr>
<tr>
<td>64**</td>
<td>(GAT368)</td>
<td><img src="image" alt="Structure" /></td>
<td>&gt;10,000</td>
</tr>
</tbody>
</table>
Earlier work from Prosidion Inc. has shown that PSNCBAM-1 is an optimized prototypical CB₁ negative allosteric modulator that increases the binding of the orthosteric agonist CP55,940 but decreases its functional activity in [³⁵S]GTPγS and cAMP. It exhibited an EC₅₀ of 74.3nM in GTPγS assay. To probe the steroelectronic requirements and also explore potential opportunities for improving the polar properties of this molecule, we synthesized three groups of CB₁ allosteric modulators. The first (Site I) includes analogs in which we varied ring A or substitutions on ring A and their relative positions. In the second group, we incorporated various bioisoteric groups of urea as well as substituted ureas. In the third group, we changed the relative positions of ring B, C and D. Here we also prepared analogs in which the nitrogen is eliminated from ring C or its relative position is changed. This group also includes analogs in
which the pyrrolidine ring was changed with various other water solubilizing rings such as piperazine and morpholine.

The SAR of novel CB₁ allosteric modulators was examined by measuring their abilities to modify orthosteric agonist CP55,940’s cAMP response. In this assay we used EC₈₀ concentration of CP55,940, developed dose response curve, and measured EC₅₀ of newly developed analogs. In addition to the cAMP assay, we also examined all analogs for their ability to modify CP-55,940’s β-arrestin recruitment using the pathHunter assay. For most analogs in the Site I variations, we used the EC₂₀ of CP55,940, whereas for remaining analogs of Site I and all analogs of Site II and Site III variations, we used EC₈₀ of CP55,940. In GPCR allostery literature, we find use of EC₂₀, EC₅₀ or EC₈₀ of orthosteric agonist to examine modulatory effects of allosteric compounds. Both these assays were run at DiscoverX and are their proprietary assays.

All novel allosteric modulators exhibited reduced potencies for the CB₁ receptor in both cAMP and β-arrestin assays.

3.4.1.1 Site I variations:

Significance of 4-chloro group: To find the significance of 4-chloro group in the lead molecule PSNCBAM-1, we evaluated analog 10q which lacked 4-chloro substituent. This compound exhibited about 4-fold reduction in both cAMP (EC₅₀ = 501nM; Eₘₐₓ = 135%) and β-arrestin potencies. Under this conditions the PSNCBAM-1, 10a, had an EC₅₀ of 110nM (Eₘₐₓ = 136%) in cAMP assay and an EC₅₀ of 27nM (Eₘₐₓ = 120%).
Relative position of chloro group: To find optimum position of chloro group in ring A, we prepared corresponding meta- and ortho- analogs. The meta-chloro analog 10c exhibited reduced potency in cAMP (EC$_{50}$ = 782nM; E$_{\text{max}}$ = 111%) and β-arrestin (EC$_{50}$ = 260nM; E$_{\text{max}}$ = 128%). The potency of the molecule (10d) was further reduced when the chloro group was present at ortho position. In this case the cAMP potency was completely lost (EC$_{50}$ = >10,000nM) with retention of some β -arrestin activity.

Other electronegative/bioisosteric groups on ring A: We evaluated analogs in which other halogens (F, I) and other strong electron withdrawing and bioisosteric –CF$_3$ group is incorporated in ring A at 4-position and also related meta- and ortho-analogs. Analog 10e bearing 4-CF$_3$ group exhibited 2-fold decrease in both cAMP (EC$_{50}$ = 218nM; E$_{\text{max}}$ = 161%) and β-arrestin (EC$_{50}$ = 51nM; E$_{\text{max}}$ =128%) potencies. Moving CF$_3$ group from para- to meta- (compound 10f) to ortho- (compound 10g) position progressively decreased both cAMP and β-arrestin activities (see Table 3.1). Replacement of 4-chloro group with 4-iodo group led to compound 10b which exhibited 2-fold decrease in cAMP activity (EC$_{50}$ = 223nM; E$_{\text{max}}$ =126%). Interestingly, there was no change in the β-arrestin potency (EC$_{50}$ = 27; E$_{\text{max}}$ = 133%). This compound thus exhibited better (8-fold) functional selectivity for β -arrestin recruitment over cAMP compared to 4-fold selectivity of PSNCBAM-1. Replacement of 4-chloro group with 4-fluoro group (compound 10t) led to improved potency in cAMP assay (EC$_{50}$ = 148nM; E$_{\text{max}}$ = 119%) and retained potency in β-arrestin modulation (EC$_{50}$ = 7.4nM; E$_{\text{max}}$ =108%). This smaller fluoro group when present at ortho-position of ring A (compound 10u), however, showed decreased potency at both cAMP and β-arrestin activity, similar to a trend observed with other substituents. Note that compounds 10t and 10u were run under conditions in which EC$_{80}$ of orthosteric agonist CP55,940 was used for both cAMP and β -arrestin assays. In this assay, the reference
compound PSNCBAM-1 exhibited an EC$_{50}$ of 231nM ($E_{\text{max}}$ =108%) for cAMP activity and an EC$_{50}$ of 5nM ($E_{\text{max}}$ =109%) for $\beta$-arrestin assay.

**Electron-donating groups in ring A:** To understand the effect of electron-donating vs. electron-withdrawing groups, we evaluated analogs in which ring A contained methoxy or methyl groups instead of electron-withdrawing chloro group. Compound 10n, containing 4-CH$_3$ group exhibited decreased activity in both cAMP and $\beta$-arrestin assays compared to the parent 4-Cl compound. The decrease in potency was more as this group was moved from *para-* to *meta-* (compound 10o) to *ortho* (compound 10p) position (see Table 3.1). Compound 10p was tested under condition b, in which the dose response curve was obtained with an EC$_{80}$ of the orthosteric agonist, CP55,940. A methoxy group at 4-position (compound 10h) also showed reduced potency in both assays (for cAMP, EC$_{50}$ = 1233nM; $E_{\text{max}}$ = 97% and for $\beta$-arrestin, EC$_{50}$ = 99nM; $E_{\text{max}}$ =99%) and a progressive decrease in activity as the group was moved from *para-* to *meta-* (compound 10i) to *ortho-* (compound 10j) position.

**Effect of Sterics in Ring A:** We further evaluated analogs in which a phenoxy group replaced the chloro substituent on ring A. Compound 10k containing 4-phenoxy group exhibited decrease in potency in both cAMP (EC$_{50}$ = 750nM; $E_{\text{max}}$ =91%) and $\beta$-arrestin assays (EC$_{50}$ = 115nM; $E_{\text{max}}$=123%) compared to PSNCBAM-1. A further decrease in functional potencies was found for compounds 10l (*meta*-phenoxy) and 10m (*ortho*-phenoxy) as shown in Table 3.1.

**Significance of Phenyl Ring A:** To find effects of replacing phenyl ring with other alicyclic or aromatic rings on molecule’s ability to modulate CB$_1$ orthosteric agonist’s response, we evaluated analogs 10z-10y. Phenyl ring was found to be essential w.r.t. activity at CB$_1$ in both assays. Replacing phenyl ring with a cyclohexyl ring (compound 10z) led to decrease in both
cAMP (EC$_{50}$ = 659nM; E$_{max}$ =78%) and β-arrestin (EC$_{50}$ = 333nM; E$_{max}$ =136%) assay. When ring A was 1-naphthyl (analog 10w) the EC$_{50}$ was 1256nM, E$_{max}$ =69% for cAMP and for β-arrestin EC$_{50}$ was 1269nM, E$_{max}$ = 109%. For 2-naphthyl ring analog 10x, there was further decrease in both cAMP and β-arrestin activity. However, the activity was completely abolished for 10y, in which ring A was tetrahydro-isoquinoline (see Table 3.1).

**Effect of different ortho-substituents on functional potency and selectivity (biased-signaling):** It was observed that several analogs bearing ortho-substitutents exhibited some functional selectivity in β-arrestin assays (e.g. 10d, 10u, 10p and 10m). To investigate role of ortho-substituents on functional potency and selectivity, we designed and evaluated analogs 10r, 10v and 15-17 bearing different substituents at the ortho- position of ring A. Analogs containing ethyl- (10r) or isopropyl-(10v) group showed significantly decreased activity compared to PSNCBAM-1. Similar trend was observed with compound 15 (methyl ester) and compound 16 (carboxylic acid). The activity was completely abolished for these two analogs. Interestingly, the decrease in the activity was least for compound 17 containing a hydroxymethyl group at the ortho- position of ring A. It exhibited an EC$_{50}$ of 1145nM (E$_{max}$ = 33%) in cAMP assay and an EC$_{50}$ of 672nM (E$_{max}$ = 106%) in β-arrestin assay.

**Effect of di-substitution on A ring:** From the biochemical evaluations of the compounds, it was clear that the 4-chloro group was essential for both cAMP and β-arrestin activity modulation. To examine effects of ortho- or meta-substituents in addition to 4-chloro group, we evaluated disubstituted analogs 21a, 10s and 21b. Compound 21a, which contains 4-chlor-2-iodo group in ring A, exhibited reduced potency in both assays with no functional selectivity. It showed an EC$_{50}$ of 1268nM (E$_{max}$ = 53%) in cAMP assay and an EC$_{50}$ of 1201 (E$_{max}$ = 118%). Compound
10s, containing 4-chloro-2-methylphenyl ring, showed no activity in the cAMP assay (EC$_{50} >$ 10,000nM) and had reduced activity in β-arrestin assay (EC$_{50} = 209$nM; E$_{\text{max}} = 109\%$). It is noteworthy that this compound had over 50-fold selectivity for β-arrestin activity over cAMP activity. Another compound in this series, containing the 4-chloro-3-iodophenyl (compound 21b) ring, also exhibited good activity at β-arrestin assay (EC$_{50} = 126$nM; E$_{\text{max}} = 120\%$) and had no activity in the cAMP assay (EC$_{50} >$ 10,000nM). It exhibited over 78-fold functional bias for β-arrestin recruitment modulation over cAMP modulation.

We further explored the SAR by evaluating di-substituted analogs containing 4-chloro group with an alkyl or alkynyl chain at 2- or 3-position. Compounds 23a and 24a containing conformationally restricted alkyne chains at 2-position had significantly diminished activities in both cAMP and β-arrestin assays. Analogs 25a and 26a containing corresponding saturated side chains exhibited better tolerance than constrained side chains (see Table 3.1), compound 26a, containing a terminal hydroxyl group, had better activity in β-arrestin assays than the THP protected compound 25a. Compound 26c, which is similar to 25a but lack critical 4-chloro functionality, exhibited completely diminished activities in both assays. When the constrained side chain was present at 3-position as in 23b and 24b, compounds showed better tolerance in β-arrestin assays compared to 2-position analogs. Compound 23b had an EC$_{50}$ of >10,000nM in cAMP assay and an EC$_{50}$ of 208nM (E$_{\text{max}} = 111\%$) in β-arrestin assays. It exhibited over 50-fold functional selectivity in favor of β-arrestin recruitment modulation. Between 23b and 24b (compound containing terminal hydroxyl group), compound 24b had better potency in both assays (EC$_{50} = 551$nM, E$_{\text{max}} = 152\%$ for cAMP assay; EC$_{50} = 149$nM, E$_{\text{max}} = 123\%$ for β-arrestin
Compounds 25b and 26b exhibited moderate potency in both assays and without any functional selectivity (Table 3.1).

Our SAR studies on Site I concluded that the 4-Chloro functionality (compound 10a) in ring A is very important but can be replaced with fluoro- (compound 10t) or iodo- (compound 10b) functionalities with minimal loss in activities. Electron-donating groups (-CH₃, -OCH₃) and bulkier groups (-OPh, 1- and 2-naphthyl) significantly decreases both activities. Di-substitution in ring A, either 3,4- or 2,4- where at 4-position a chloro group is present, impart 48- to 78- fold functional selectivity for β-arrestin activation.

3.4.1.2 Site II variations:

Significance of urea group: To determine the significance of urea group present in the lead molecule, we evaluated analogs 13a, 29, 30, 13b, 33 and 13c, in which either one or both urea nitrogen atoms are methylated or replaced by oxygen atom. Mono-methylated derivatives 13a-29, di-methylated derivative 30 as well as carbamate derivatives 13b, 33 and 13c exhibited significant decreased activity in both cAMP and β-arrestin assays. Analog 34 containing thiourea group instead of urea, exhibited reduced activity at CB₁ receptor in both assays compared to PSNCBAM-1. The reduction was much more significance for β-arrestin signaling (54-fold) compared to cAMP signaling (about 4-fold; see Table 3.1). Cyanoguanidine, a polar bioisosteric group of urea, when incorporated in the molecule (compound 37) generated significantly diminished activities in both assays. However, when cyclobutenedione group was used as urea bioisostere, the compound (40) exhibited about 90-fold functional selectivity for β-arrestin
signaling. Compound 49 had an EC\textsubscript{50} value of >10,000nM for cAMP and an EC\textsubscript{50} value of 111nM (E\textsubscript{max} = 107\%) for β-arrestin assay.

We further examined analogs in which urea group was replaced by the bioisosteric oxadiazole ring. This ring was incorporated in two different ways in the molecule, one variation involves oxadiazole ring directly appended to ring A (compound 47) and other variation contained the oxadiazole ring directly attached to ring B (compound 44). Both these analogs did not show good tolerance and exhibited reduced potencies at CB1 receptor in both assays (see Table 3.1).

To conclude SAR of Site II variations, we found the urea group is very important for activity at CB\textsubscript{1} receptor in both assays and any substitution on the urea group or its replacement by other bioisosteric groups decreased activity. One interesting finding involved, the cyclobutenedione analog 40 showed reduced activity in both assays, it exhibited remarkable selectivity (90-fold) for β-arrestin.

As site I and site II variations identified 4-chloro and urea group as very essential for activity at CB\textsubscript{1} in both assays, we retained these two groups for site III variations.

3.4.1.3 Site III Variations: In the lead molecule PSNCBAM-1 the orientation of ring B and ring D is meta to each other. This gives the tricyclic scaffold a unique V-shape. To find optimal orientation of rings C and D w.r.t. ring B, we evaluated analogs 57, 53, 60, and 67. Analog 57 contained ring C connected to ring B at the ortho- position w.r.t. the urea group. The pyrrolidine ring D was kept at same position w.r.t to ring C, so that the tricyclic scaffold (B-C-D) retained its original V-shape. It exhibited decreased activities in both assays (see Table 3.1). In another variation, we changed the connectivity of ring C on ring B to para-position w.r.t. the urea group.
The resulting compound 53 exhibited over 48-fold selectivity for β-arrestin assay. Although its activity in the cAMP assay was completely lost (EC\textsubscript{50} = >10,000nM), it retained significant activity in β-arrestin recruitment modulation (EC\textsubscript{50} = 206nM; E\textsubscript{max} = 110%). We further tested novel analogs to find the significance of nitrogen on ring C. Analog 60 in which the C ring nitrogen is moved from 2’ to 4’-position, exhibited decreased activity compared to PSNCBAM-1 (I\textsuperscript{b}). Interestingly, it exhibited over 125-fold functional selectivity for β-arrestin recruitment. Compound 67 in which the nitrogen on ring C is missing, exhibited slightly decreased activity in cAMP (5-fold decrease, EC\textsubscript{50} = 27nM, E\textsubscript{max} =110%) and β-arrestin assay (about 3-fold decrease, EC\textsubscript{50} = 679nM, E\textsubscript{max} = 84%).

Compound 64, in which the connectivity of rings C and D to B is linear, exhibited essentially no cAMP activity and moderate to weak activity in β-arrestin assay (see Table 3.1). To find better water-solubilizing rings instead of pyrrolidine D ring, we evaluated analogs 70a and 70b containing morpholino and piperazine rings respectively. Although both analogs exhibited reduced activity at CB\textsubscript{1} in both assays compared to lead molecule PSNCBAM-1, piperazine ring analogs was better tolerated than morpholino ring analog.

3.5 Conclusions

In summary, a series of Site I, II and III of PSNCBAM-1 analogs were evaluated for negative allosteric modulation of cannabinoid receptor1. Site I SAR has shown that both steric bulk around aryl ring and electronic density of aromatic ring are important for activity. Position of the chloro group was found critical at para position on the ring A. Replacement of chloro with the fluoro group provided high potency ligand in β-arresting assay while maintaining cAMP activity thus slightly improving functional selectivity. Electron donating groups and sterically
bulky substitutions on the ring A were not tolerated. Ortho or meta- substituted analogs in addition to 4-chloro group were also not tolerated. Long alkyl chain substitution in addition to 4-chloro group gave minimal loss in activity at 3 position compared to at 2 position present on the ring A. Probing urea group in site II concludes the involvent of hydrogen bonding with the receptor. Analogs lacking the ability to make hydrogen bonding with receptor as a hydrogen bond donor such as carbamate and N-methyled urea did not show any activity. Cyclobutenedione, one of the probed bioisosteric replacements of urea, showed high functional selectivity in favor of β-arresting activity. Finally, the importance of pyridine nitrogen and molecular shape required for activity are studied in site III variation analogs. Unique V-shape of PSNCBAM-1 is important for activity at both cAMP and β-arrestin. Analogs lacking V-shape that is present in the lead molecule did not show significant activity. Significant functional selectivity (125-fold) was observed when nitrogen moved from ortho to para w.r.t. phenyl in the ring C modification.

These results showed that relatively flat SAR was observed for all sites variation analogs however, functional selectivity for β-arrestin over cAMP was observed for number of analogs. Further in vitro and in vivo studies are required to elucidate the full potential of newly discovered functionally selective negative allosteric modulators of CB₁ receptor.
3.6 Experimental

2-Bromo-6-(pyrrolidin-1-yl)pyridine (3): A mixture of 2,6-dibromopyridine (10 g, 42.2 mmol) and pyrrolidine (13.87 mL, 169 mmol) was stirred overnight at room temperature. The reaction mixture was quenched with 100 mL saturated NaHCO₃ solution and diluted with 100 mL dichloromethane. The organic layer was separated, washed with water (2x50 mL), brine and dried (MgSO₄) and concentrated under reduced pressure. The resulting crude was crystallized from Methanol (50 mL) to afford the desired product 3 (8.5 g, 37.4 mmol, 89% yield) as a white solid. ¹H NMR (500 MHz, CD₃OD) δ: 7.22 (t, J = 8.0 Hz, 1H), 6.64 (d, J = 7.5 Hz, 1H), 6.23 (d, J = 8.0 Hz, 1H), 3.43 (t, J = 6.5 Hz, 4H), 1.99 (quintet, J = 3.5 Hz, 4H).

2-(3-Nitrophenyl)-6-(pyrrolidin-1-yl)pyridine (5): To a solution of 2-bromo-6-(pyrrolidin-1-yl)pyridine (5 g, 22.02 mmol) and 3-nitrophenylboronic acid (3.68 g, 22.02 mmol) in 1,2-Dimethoxyethane (22 mL) was added NaHCO₃ (5.55 g, 66.0 mmol) and water (22 mL). Argon was bubbled through the reaction solution for 5 min. To the degassed reaction solution was added Pd(Ph₃P)₄ (0.254 g, 0.22 mmol) and the resulting mixture was heated to reflux for 4 h. The reaction mixture was diluted with 100 mL ethyl acetate and 100 mL water. The organic layer
was separated, washed with water (2x50 mL), brine and dried over MgSO$_4$ and concentrated under reduced pressure. Crude product was purified by silica gel chromatography by eluting with 2-70% ethyl acetate : hexanes to give 5 (5.25 g, 19.50 mmol, 89 % yield) as a yellow solid. $^1$H NMR (500 MHz, DMSO) δ: 8.57 (t, $J = 1.5$ Hz, 1H), 8.50 (td, $J = 8.0$ Hz, $J = 1.5$ Hz, 1H), 8.23 (ddd, $J = 8.0$ Hz, $J = 2.5$ Hz, $J = 1.0$ Hz, 1H), 7.74 (t, $J = 8.0$ Hz, 1H), 7.63 (dd, $J = 8.0$ Hz, $J = 7.0$ Hz, 1H), 7.28 (d, $J = 7.0$ Hz, 1H), 6.51 (d, $J = 8.0$ Hz, 1H), 3.49 (t, $J = 6.5$ Hz, 4H), 1.98 (quintet, $J = 3.0$ Hz, 4H). MS m/z (M$^+$+1): 270.1.

3-(6-(Pyrrolidin-1-yl)pyridin-2-yl)aniline (6): To a solution of 5 (5 g, 18.57 mmol) in ethanol (30 mL) and THF (15 mL) was added Pd/C (1.976 g, 0.928 mmol) under argon atmosphere. To this stirred reaction mixture was added catalytic acetic acid and the resultant solution was stirred for overnight under hydrogen atmosphere through balloon pressure. Pd/C was filtered off and filtrate was concentrated and purified by silica gel chromatography by eluting with 2-70% ethyl acetate: hexanes to give 6 (4.25 g, 17.76 mmol, 96% yield) as a light yellow oil. $^1$H NMR (500 MHz, CDCl$_3$) δ: 7.52-7.48 (m, 1H), 7.46 (t, $J = 2.0$ Hz, 1H), 7.45-7.41 (m, 1H), 7.29 (s, 1H), 7.23 (t, $J = 8.0$ Hz, 1H), 6.99 (d, $J = 7.0$ Hz, 1H), 6.72 (ddd, $J = 5.5$ Hz, $J = 2.5$ Hz, $J = 1.0$ Hz, 1H), 3.75 (br s, 2H), 3.57 (t, $J = 6.5$ Hz, 4H), 2.08-1.80 (m, 4H). MS m/z (M$^+$+1): 240.2.
Phenyl (3-(6-(pyrrolidin-1-yl)pyridin-2-yl)phenyl)carbamate (8): To a solution of 6 (300 mg, 1.254 mmol) in DCM (8 mL) was added phenyl carbonochloridate (216 mg, 1.379 mmol) followed by Et$_3$N (0.349 mL, 2.507 mmol) at 0 °C and stirred for 3 h. Complete conversion of Starting material was observed by TLC (ethyl acetate/hexanes = 30/70). The reaction mixture was diluted with 50 mL DCM and 50 mL water. The organic layer was separated, washed with water (2x50 mL), dried (MgSO$_4$) and concentrated under reduced pressure. Crude product was purified by silica gel chromatography with 10-50% ethyl acetate : hexanes to afford the desired product 8 (284 mg, 0.79 mmol, 63% yield) as a white solid. $^1$H NMR (500 MHz, CDCl$_3$) δ: 8.06 (t, $J = 2.0$ Hz, 1H), 7.78 (td, $J = 8.0$ Hz, $J = 1.0$ Hz, 1H), 7.62-7.52 (m, 1H), 7.48 (dd as t, $J = 8.0$ Hz, 1H), 7.43-7.36 (m, 3H), 7.26-7.18 (m, 3H), 7.05 (br s, 1H), 7.01 (d, $J = 7.5$ Hz, 1H), 6.32 (d, $J = 8.5$ Hz, 1H), 3.54 (t, $J = 7.0$ Hz, 4H), 2.01 (quintet, $J = 3.0$ Hz, 4H). MS m/z (M$^+$+1): 360.5

1-(4-Chlorophenyl)-3-(3-(6-(pyrrolidin-1-yl)pyridin-2-yl)phenyl)urea (10a): To a solution of 8 (200 mg, 0.556 mmol) in DMF (4 mL) was added 4-chloroaniline (78 mg, 0.612 mmol) followed by DIEA (0.292 mL, 1.669 mmol) and heated to 50 °C for overnight. Complete conversion of starting material was observed by TLC. The reaction mixture was diluted in 100 mL DCM and 100 mL water. Organic layer was separated, washed with water (2x50 mL), dried
(MgSO₄) and concentrated under reduced pressure. The crude product was purified by silica gel chromatography eluting with 10-50% ethyl acetate: hexanes to afford the desired product 10a (118 mg, 0.300 mmol, 54 % yield) as white solid. ¹H NMR (500 MHz, DMSO) δ: 8.83 (d, J = 20 Hz, 1H), 8.07 (s, 1H), 7.63 (d, J = 7.5 Hz, 1H), 7.56 (dd, J = 8.0 Hz, J = 1.0 Hz, 2H), 7.50 (dd, J = 7.0 Hz, J = 2.0 Hz, 2H), 7.38-7.31 (m, 3H), 7.05 (d, J = 7.0 Hz, 1H), 6.42 (d, J = 8.0 Hz, 1H), 3.48 (br s, 4H), 1.98 (quintet, J = 3.0 Hz, 4H). MS m/z (M+1): 393.2.

1-(4-Chlorophenyl)-3-(3-(6-(pyrrolidin-1-yl)pyridin-2-yl)phenyl)urea (10a) as per scheme 3.7: To the solution of 6 in DCM (10 mL) was added 1-chloro-4-isocyanatobenzene (321 mg, 2.089 mmol) followed by Et₃N (0.582 mL, 4.18 mmol) and stirred at room temperature for 16 hrs. Reaction was concentrated under reduced pressure and crude was dissolved in methanol. DCM was added and stirred for 30 min to obtained precipitate. Solid was collected by filtration to give desired product 10a (700 mg, 1.77 mmol, 85 % yield) as a white solid.

1-(4-Chlorophenyl)-3-(3-(6-(pyrrolidin-1-yl)pyridin-2-yl)phenyl)urea (10a) as per scheme 3.15: To a solution of 49 (500 mg, 1.721 mmol) and 3 (391 mg, 1.721 mmol) in 1,2-Dimethoxyethane (10 mL) was added sodium bicarbonate (289 mg, 3.44 mmol) and water (10.00 mL). Argon was bubbled through reaction solution for 5 min. Pd(Ph₃P)₄ (9.94 mg, 8.61 µmol) was added to the reaction mixture and heated to refluxed for 4 hrs. Reaction was diluted with 50 ml ethyl acetate and 50 ml water. Organic layer was separated, washed with 2x50ml water, dried over MgSO₄ and concentrated under reduced pressure. Crude product was purified by silica gel chromatography eluting with 10-90% ethyl acetate: hexanes to give 10a (420 mg, 1.069 mmol, 62 % yield) as a white solid.
1-(4-Iodophenyl)-3-(3-(6-(pyrrolidin-1-yl)pyridin-2-yl)phenyl)urea (10b): The synthesis was carried out same as 10a using 8 (50 mg, 0.139 mmol) and 4-iodoaniline (33.5 mg, 0.153 mmol) in DMF (1.5 mL) followed by DIEA (0.049 mL, 0.278 mmol) to afford the desired product 10b (32 mg, 0.072 mmol, 52% yield) as light brown solid. $^1$H NMR (500 MHz, CD$_3$OD) δ: 8.06 (t, $J = 2.0$ Hz, 1H), 7.66 (d, $J = 8.0$ Hz, 1H), 7.60 (d, $J = 9.0$ Hz, 2H), 7.54 (dd, $J = 8.5$ Hz, $J = 7.0$ Hz, 1H), 7.49 (ddd, $J = 8.0$ Hz, $J = 2.0$ Hz, $J = 1.0$ Hz, 1H), 7.35 (t, $J = 8.0$ Hz, 1H), 7.28 (d, $J = 8.5$ Hz, 2H), 7.04 (d, $J = 7.0$ Hz, 1H), 6.41 (d, $J = 8.5$ Hz, 1H), 3.54 (t, $J = 7.0$ Hz, 4H), 2.04 (quintet, $J = 3.5$ Hz, 4H). MS m/z (M$^+$+1): 485.0.

1-(3-Chlorophenyl)-3-(3-(6-(pyrrolidin-1-yl)pyridin-2-yl)phenyl)urea (10c): The synthesis was carried out as described for 10a using 8 (25 mg, 0.070 mmol) and 3-chloroaniline (9.76 mg, 0.077 mmol) in DMF (1 mL) followed by DIEA (0.036 mL, 0.209 mmol) to afford the desired product 10c (15 mg, 0.038 mmol, 55% yield) as white solid. $^1$H NMR (500 MHz, CD$_3$OD) δ: 8.03-7.99 (m, 1H), 7.95 (dd, $J = 9.5$ Hz, $J = 7.5$ Hz, 1H), 7.67 (t, $J = 2.0$ Hz, 1H), 7.52-7.48 (m, 2H), 7.44-7.39 (m, 1H), 7.30-7.26 (m, 2H), 7.07-6.99 (m, 3H), 3.69 (t, $J = 6.5$ Hz, 4H), 2.01 (quintet, $J = 3.0$ Hz, 4H). MS m/z (M$^+$+1): 393.3.
1-(2-Chlorophenyl)-3-(3-(6-(pyrrolidin-1-yl)pyridin-2-yl)phenyl)urea: (10d): The synthesis was carried out as described for 10a using 8 (25 mg, 0.070 mmol) and chloroaniline (9.76 mg, 0.077 mmol) in DMF (1 mL) followed by DIEA (0.036 mL, 0.209 mmol) to afford the desired product 10d (18 mg, 0.046 mmol, 66% yield) as white solid. $^1$H NMR (500 MHz, CD$_3$OD) $\delta$: 8.11 (dd, $J = 8.5$ Hz, $J = 2.0$ Hz, 1H), 8.08 (t, $J = 2.0$ Hz, 1H), 7.98 (dd, $J = 9.5$ Hz, $J = 7.5$ Hz, 1H), 7.52 (t, $J = 8.0$ Hz, 1H), 7.46 (td, $J = 8.0$ Hz, $J = 1.0$ Hz, 1H), 7.43 (dd, $J = 8.5$ Hz, $J = 1.5$ Hz, 2H), 7.29 (dt, $J = 8.0$ Hz, $J = 1.5$ Hz, 1H), 7.09-7.03 (m, 3H), 3.70 (t, $J = 6.5$ Hz, 4H), 2.16 (quintet, $J = 3.0$ Hz, 4H). MS m/z (M$^+$+1): 393.4.

1-(3-(6-(Pyrrolidin-1-yl)pyridin-2-yl)phenyl)-3-(4-(trifluoromethyl)phenyl)urea (10e): The synthesis was carried out as described for 10a using 8 (25 mg, 0.070 mmol) and 4-(trifluoromethyl)aniline (12.33 mg, 0.077 mmol) in DMF (1 mL) followed by DIEA (0.036 mL, 0.209 mmol) to afford the desired product 10e (17 mg, 0.042 mmol, 60% yield) as white solid. $^1$H NMR (500 MHz, CD$_3$OD) $\delta$: 8.05 (t, $J = 1.5$ Hz, 1H), 8.0 (dd, $J = 9.0$ Hz, $J = 7.0$ Hz, 1H), 7.66 (d, $J = 9.0$ Hz, 2H), 7.53 (t, $J = 8.5$ Hz, 1H), 7.47 (ddd, $J = 8.5$ Hz, $J = 2.0$ Hz, $J = 1.0$ Hz, 1H), 7.41 (ddd, $J = 8.5$ Hz, $J = 2.0$ Hz, $J = 1.0$ Hz, 1H), 7.07 (dt, $J = 9.0$ Hz, $J = 1.0$ Hz, 2H), 3.72 (t, $J = 7.0$ Hz, 4H), 2.17 (quintet, $J = 3.0$ Hz, 4H). MS m/z (M$^+$+1): 427.1.
1-(3-(6-(Pyrrolidin-1-yl)pyridin-2-yl)phenyl)-3-(3-(trifluoromethyl)phenyl)urea (10f): The synthesis was carried out as described for 10a using 8 (25 mg, 0.070 mmol) and 3-(trifluoromethyl)aniline (12.33 mg, 0.077 mmol) in DMF (1 mL) followed by DIEA (0.036 mL, 0.209 mmol) to afford the desired product 10f (20 mg, 0.047 mmol, 67% yield) as white solid. 

$^1$H NMR (500 MHz, CD$_3$OD) $\delta$: 8.04 (t, $J = 2.0$ Hz, 1H), 7.99 (dd, $J = 8.5$ Hz, $J = 2.0$ Hz, 1H), 7.95 (br s, 1H), 7.62 (dd, $J = 8.0$ Hz, $J = 2.0$ Hz, 1H), 7.54-7.45 (m, 3H), 7.42 (td, $J = 6.5$ Hz, $J = 2.0$ Hz, 1H), 7.31 (dd, $J = 2.0$ Hz, $J = 1.0$ Hz, 1H), 7.07 (d, $J = 1.5$ Hz, 1H), 7.06 (s, 1H), 3.71 (t, $J = 7.0$ Hz, 4H), 2.17 (quintet, $J = 3.5$ Hz, 4H). MS m/z (M$^+$+1): 427.1.

1-(3-(6-(Pyrrolidin-1-yl)pyridin-2-yl)phenyl)-3-(2-(trifluoromethyl)phenyl)urea (10g): The synthesis was carried out as described for 10a using 8 (25 mg, 0.070 mmol) and 3-(trifluoromethyl)aniline (12.33 mg, 0.077 mmol) in DMF (1 mL) followed by DIEA (0.036 mL, 0.209 mmol) to afford the desired product 10g (15 mg, 0.035 mmol, 50% yield) as white solid. 

$^1$H NMR (500 MHz, CD$_3$OD) $\delta$: 8.08 (t, $J = 2.0$ Hz, 1H), 7.98 (dd, $J = 9.0$ Hz, $J = 7.0$ Hz, 1H), 7.92 (d, $J = 8.5$ Hz, 1H), 7.69 (d, $J = 8.0$ Hz, 1H), 7.63 (dt, $J = 8.0$ Hz, $J = 1.0$ Hz, 1H), 7.53 (t, $J = 8.0$ Hz, 1H), 7.47-7.40 (m, 2H), 7.33 (t, $J = 8.0$ Hz, 1H), 7.07 (d, $J = 2.0$ Hz, 1H), 7.05 (s, 1H), 3.70 (t, $J = 6.5$ Hz, 4H), 2.17 (quintet, $J = 3.5$ Hz, 4H). MS m/z (M$^+$+1): 427.3.
1-(4-Methoxyphenyl)-3-(3-(6-(pyrrolidin-1-yl)pyridin-2-yl)phenyl)urea (10h): The synthesis was carried out as described for 10a using 8 (50 mg, 0.139 mmol) and 4-methoxylaniline (18.85 mg, 0.153 mmol) in DMF (2 mL) followed by DIEA (53.5 µl, 0.306 mmol) to afford the desired product 10h (30 mg, 0.077 mmol, 55% yield) as white solid. $^1$H NMR (500 MHz, CD$_3$OD) $\delta$: 8.03-7.95 (m, 2H), 7.50 (dd as t, $J = 8.0$ Hz, 1H), 7.42 (ddd, $J = 8.0$ Hz, $J = 2.0$ Hz, $J = 1.0$ Hz, 1H), 7.39 (ddd, $J = 8.0$ Hz, $J = 2.0$ Hz, $J = 1.0$ Hz, 1H), 7.35-7.28 (m, 2H), 7.09-6.99 (m, 2H), 6.91-6.83 (m, 2H), 3.77 (s, 3H), 3.71 (t, $J = 6.0$ Hz, 4H), 2.17 (quintet, $J = 3.0$ Hz, 4H). MS m/z (M$^+$+1): 389.2.

1-(3-Methoxyphenyl)-3-(3-(6-(pyrrolidin-1-yl)pyridin-2-yl)phenyl)urea (10i): The synthesis was carried out as described for 10a using 8 (50 mg, 0.139 mmol) and 3-methoxylaniline (18.85 mg, 0.153 mmol) in DMF (2 mL) followed by DIEA (53.5 µl, 0.306 mmol) to afford the desired product 10i (36 mg, 0.093 mmol, 67% yield) as white solid. $^1$H NMR (500 MHz, CD$_3$OD) $\delta$: 8.01 (t, $J = 2.0$ Hz, 1H), 7.96 (dd, $J = 9.0$ Hz, $J = 7.5$ Hz, 1H), 7.50 (dd as t, $J = 7.5$ Hz, 1H), 7.47 (td, $J = 8.0$ Hz, $J = 2.0$ Hz, 1H), 7.40 (td, $J = 7.5$ Hz, $J = 2.0$ Hz, 1H), 7.19-7.15 (m, 2H), 7.03 (t, $J = 7.5$ Hz, 2H), 6.96-6.91 (m, 1H), 6.61 (ddd, $J = 8.5$ Hz, $J = 2.0$ Hz, $J = 1.0$ Hz, 1H), 3.78 (s, 3H), 3.71 (t, $J = 6.0$ Hz, 4H), 2.17 (quintet, $J = 3.0$ Hz, 4H). MS m/z (M$^+$+1): 389.2.
1-(2-Methoxyphenyl)-3-(3-(6-(pyrrolidin-1-yl)pyridin-2-yl)phenyl)urea (10j): The synthesis was carried out as described for 10a using 8 (50 mg, 0.139 mmol) and 2-methoxyaniline (18.85 mg, 0.153 mmol) in DMF (2 mL) followed by DIEA (53.5 µl, 0.306 mmol) to afford the desired product 10j (37 mg, 0.095 mmol, 68% yield) as white solid. $^1$H NMR (500 MHz, CD$_3$OD) δ: 8.09 (m, 2H), 7.99 (dd, $J = 9.0$ Hz, $J = 7.5$ Hz, 1H), 7.51 (t, $J = 7.5$ Hz, 1H), 7.40 (dt, $J = 7.5$ Hz, $J = 1.0$ Hz, 2H), 7.07 (d, $J = 2.5$ Hz, 1H), 7.06 (d, $J = 1.0$ Hz, 1H), 7.02-6.98 (m, 2H), 6.94-6.89 (m, 1H), 3.92 (s, 3H), 3.71 (t, $J = 7.0$ Hz, 4H), 2.17 (quintet, $J = 3.5$ Hz, 4H). MS m/z (M$^+$+1): 389.2.

1-(4-Phenoxyphenyl)-3-(3-(6-(pyrrolidin-1-yl)pyridin-2-yl)phenyl)urea (10k): The synthesis was carried out as described for 10a using 8 (30 mg, 0.083 mmol) and 4-phenoxyaniline (17.01 mg, 0.092 mmol) in DMF (2 mL) followed by DIEA (0.032 mL, 0.184 mmol) to afford the desired product 10k (24 mg, 0.053 mmol, 64% yield) as white solid. $^1$H NMR (500 MHz, CD$_3$OD) δ: 8.04 (t, $J = 9.0$ Hz, 1H), 7.99 (dd, $J = 9.0$ Hz, $J = 7.5$ Hz, 1H), 7.51 (t, $J = 7.5$ Hz, 1H), 7.46-7.42 (m, 3H), 7.40 (ddd, $J = 8.0$ Hz, $J = 2.0$ Hz, $J = 1.0$ Hz, 1H), 7.35-7.30 (m, 2H), 7.08-7.03 (m, 3H), 6.98-6.93 (m, 4H), 3.71 (t, $J = 7.0$ Hz, 4H), 2.17 (quintet, $J = 3.5$ Hz, 4H). MS m/z (M$^+$+1): 451.5.
1-(3-Phenoxyphenyl)-3-(3-(6-(pyrrolidin-1-yl)pyridin-2-yl)phenyl)urea (10l): The synthesis was carried out as described for 10a using 8 (30 mg, 0.083 mmol) and 3-phenoxyaniline (17.01 mg, 0.092 mmol) in DMF (2 mL) followed by DIEA (0.032 mL, 0.184 mmol) to afford the desired product 10l (22 mg, 0.049 mmol, 59% yield) as white solid. $^1$H NMR (500 MHz, CD$_3$OD) $\delta$: 8.02 (t, $J = 9.0$ Hz, 1H), 7.98 (dd, $J = 9.0$ Hz, $J = 7.0$ Hz, 1H), 7.50 (t, $J = 8.0$ Hz, 1H), 7.43-7.38 (m, 2H), 7.37-7.33 (m, 2H), 7.29-7.26 (m, 2H), 7.12 (dd, $J = 2.0$ Hz, $J = 1.0$ Hz, 1H), 7.11-7.09 (m, 1H), 7.06 (d, $J = 3.5$ Hz, 1H), 7.04 (d, $J = 2.0$ Hz, 1H), 7.00 (dd, $J = 9.0$ Hz, $J = 1.0$ Hz, 2H), 6.65 (ddd, $J = 7.5$ Hz, $J = 2.5$ Hz, $J = 1.0$ Hz, 1H), 3.71 (t, $J = 7.0$ Hz, 4H), 2.17 (quintet, $J = 3.5$ Hz, 4H). MS m/z (M$^+$+1): 451.1.

1-(2-Phenoxyphenyl)-3-(3-(6-(pyrrolidin-1-yl)pyridin-2-yl)phenyl)urea (10m): The synthesis was carried out as described for 10a using 8 (30 mg, 0.083 mmol) and 2-phenoxyaniline (17.01 mg, 0.092 mmol) in DMF (2 mL) followed by DIEA (0.032 mL, 0.184 mmol) to afford the desired product 10m (25 mg, 0.055 mmol, 66% yield) as white solid. $^1$H NMR (500 MHz, CD$_3$OD) $\delta$: 8.20 (dd, $J = 8.5$ Hz, $J = 1.5$ Hz, 1H), 8.06 (t, $J = 3.0$ Hz, 1H), 7.99 (dd, $J = 9.0$ Hz, $J = 7.0$ Hz, 1H), 7.49 (t, $J = 8.5$ Hz, 1H), 7.41-7.34 (m, 4H), 7.13 (ddd, $J = 6.0$ Hz, $J = 3.0$ Hz, 1H),
= 1.0 Hz, 2H), 7.09-6.98 (m, 5H), 6.87 (dd, J = 8.5 Hz, J = 1.5 Hz, 1H), 3.71 (t, J = 7.0 Hz, 4H), 2.17 (quintet, J = 3.5 Hz, 4H). MS m/z (M+1): 451.2.

1-(3-(6-(Pyrrolidin-1-yl)pyridin-2-yl)phenyl)-3-(p-tolyl)urea (10n): The synthesis was carried out as described for 10a using 8 (50 mg, 0.139 mmol) and p-toluidine (16.40 mg, 0.153 mmol) in DMF (2 mL) followed by DIEA (0.053 mL, 0.306 mmol) to afford the desired product 10n (33 mg, 0.089 mmol, 64% yield) as white solid. 1H NMR (500 MHz, CD3OD) δ: 8.03 (d, J = 2.0 Hz, 1H), 7.98 (dd, J = 9.5 Hz, J = 7.5 Hz, 1H), 7.50 (t, J = 8.0 Hz, 1H), 7.42 (ddd, J = 8.0 Hz, J = 2.0 Hz, J = 1.0 Hz, 1H), 7.39 (ddd, J = 8.0 Hz, J = 2.0 Hz, J = 1.0 Hz, 1H), 7.31 (d, J = 9.0 Hz, 2H), 7.12 (d, J = 7.5 Hz, 2H), 7.07 (d, J = 3.0 Hz, 1H), 7.05 (d, J = 1.5 Hz, 1H), 3.71 (t, J = 6.5 Hz, 4H), 2.30 (s, 3H), 2.17 (quintet, J = 3.5 Hz, 4H). MS m/z (M+1): 373.2.

1-(3-(6-(Pyrrolidin-1-yl)pyridin-2-yl)phenyl)-3-(m-tolyl)urea (10o): The synthesis was carried out as described for 10a using 8 (50 mg, 0.139 mmol) and m-toluidine (16.40 mg, 0.153 mmol) in DMF (2 mL) followed by DIEA (0.053 mL, 0.306 mmol) to afford the desired product 10o (36 mg, 0.097 mmol, 69% yield) as white solid. 1H NMR (500 MHz, CD3OD) δ: 8.04 (t, J = 2.0 Hz, 1H), 7.98 (dd, J = 11.0 Hz, J = 9.5 Hz, 1H), 7.49 (d, J = 9.5 Hz, 1H), 7.46-7.38 (m, 2H), 7.29-7.22 (m, 2H), 7.17 (t, J = 9.5 Hz, 1H), 7.07 (s, 1H), 7.04 (d, J = 2.5 Hz, 1H), 6.91-6.85 (m, 1H), 3.71 (t, J = 6.5 Hz, 4H), 2.32 (s, 3H), 2.16 (quintet, J = 3.0 Hz, 4H). MS m/z (M+1): 373.1.
1-(3-(6-(Pyrrolidin-1-yl)pyridin-2-yl)phenyl)-3-(o-tolyl)urea (10p): The synthesis was carried out as described for 10a using 8 (50 mg, 0.139 mmol) and o-toluidine (16.40 mg, 0.153 mmol) in DMF (2 mL) followed by DIEA (0.053 mL, 0.306 mmol) to afford the desired product 10p (15 mg, 0.040 mmol, 29% yield) as white solid. $^1$H NMR (500 MHz, CD$_3$OD) $\delta$: 8.07 (t, $J = 2.5$ Hz, 1H), 7.98 (dd, $J = 11.0$ Hz, $J = 9.5$ Hz, 1H), 7.63 (dd, $J = 9.5$ Hz, $J = 1.0$ Hz, 1H), 7.50 (d, $J = 10.5$ Hz, 1H), 7.46-7.38 (m, 2H), 7.25-7.15 (m, 2H), 7.10-7.03 (m, 3H), 3.67 (t, $J = 7.0$ Hz, 4H), 2.31 (s, 3H), 2.16 (quintet, $J = 3.5$ Hz, 4H). MS $m/z$ (M$^+$$+1$): 373.3.

1-Phenyl-3-(3-(6-(pyrrolidin-1-yl)pyridin-2-yl)phenyl)urea (10q): The synthesis was carried out as described for 10a using 8 (50 mg, 0.139 mmol) and aniline (14.25 mg, 0.153 mmol) in DMF (1 mL), followed by DIEA (0.053 mL, 0.306 mmol) to afford the desired product 10q (15 mg, 0.042 mmol, 30% yield) as white solid. $^1$H NMR MS $m/z$ (M$^+$$+1$): 359.2.
1-(2-Ethylphenyl)-3-(3-(6-(pyrrolidin-1-yl)pyridin-2-yl)phenyl)urea (10r): The synthesis was carried out as described for 10a using 8 (50 mg, 0.139 mmol) and 2-ethylaniline (16.86 mg, 0.139 mmol) followed by DIEA (0.049 mL, 0.278 mmol) to afford the desired product 10r (42 mg, 0.109 mmol, 78% yield) as white solid. \(^1\)H NMR (500 MHz, CD\(_3\)OD) \(\delta\): 8.04 (t, \(J = 2.0 \) Hz, 1H), 7.95 (dd, \(J = 9.5 \) Hz, \(J = 2.5 \) Hz, 1H), 7.58 (dd, \(J = 8.0 \) Hz, \(J = 1.0 \) Hz, 1H), 7.49 (t, \(J = 8.0 \) Hz, 1H), 7.45 (td, \(J = 8.0 \) Hz, \(J = 1.5 \) Hz, 1H), 7.39 (td, \(J = 8.0 \) Hz, \(J = 1.0 \) Hz, 1H), 7.24 (dd, \(J = 7.0 \) Hz, \(J = 1.5 \) Hz, 1H), 7.18 (dd, \(J = 8.0 \) Hz, \(J = 1.5 \) Hz, 1H), 7.11 (dd, \(J = 8.0 \) Hz, \(J = 1.5 \) Hz, 1H), 7.03 (dd as t, \(J = 8.0 \) Hz, 2H), 3.68 (t, \(J = 7.0 \) Hz, 4H), 2.68 (q, \(J = 8.0 \) Hz, 2H), 2.14 (quintet, \(J = 3.0 \) Hz, 4H), 1.23 (t, \(J = 8.0 \) Hz, 3H).

1-(4-Chloro-2-methylphenyl)-3-(3-(6-(pyrrolidin-1-yl)pyridin-2-yl)phenyl)urea (10s): The synthesis was carried out as described for 10a using 8 (50 mg, 0.139 mmol) and 4-chloro-2-methylaniline (19.70 mg, 0.139 mmol) in DMF (1 mL) followed by DIEA (0.049 mL, 0.278 mmol) to afford the desired product 10s (32 mg, 0.079 mmol, 56% yield) as white solid. \(^1\)H NMR (500 MHz, CD\(_3\)OD) \(\delta\): 8.05 (t, \(J = 2.0 \) Hz, 1H), 7.97 (dd, \(J = 9.0 \) Hz, \(J = 2.0 \) Hz, 1H), 7.65 (d, \(J = 8.5 \) Hz, 1H), 7.50 (t, \(J = 8.0 \) Hz, 1H), 7.44 (ddd, \(J = 8.0 \) Hz, \(J = 2.0 \) Hz, \(J = 1.0 \) Hz, 1H), 7.40 (td, \(J = 8.0 \) Hz, \(J = 1.5 \) Hz, 1H), 7.24 (d, \(J = 2.0 \) Hz, 1H), 7.17 (dd, \(J = 8.5 \) Hz, \(J = 2.5 \) Hz,
1H), 7.05 (d, J = 1.0 Hz, 1H), 7.03 (d, J = 3.0 Hz, 1H), 3.69 (t, J = 6.5 Hz, 4H), 2.29 (s, 3H), 2.16 (quintet, J = 3.0 Hz, 4H).

1-(4-Fluorophenyl)-3-(3-(6-(pyrrolidin-1-yl)pyridin-2-yl)phenyl)urea (10t): The synthesis was carried out as described for 10a using 8 (25 mg, 0.070 mmol) in DMF (1 mL) and 4-fluoroaniline (8.5 mg, 0.077 mmol) followed by DIEA (0.036 mL, 0.209 mmol) to afford the desired product 10t (11 mg, 0.03 mmol, 43% yield) as white solid. $^1$H NMR (500 MHz, CD$_3$OD) $\delta$: 8.10-7.96 (m, 2H), 7.60-7.38 (m, 5H), 7.16-7.02 (m, 4H), 3.70 (t, J = 7.0 Hz, 4H), 2.17 (quintet, J = 3.0 Hz, 4H).

1-(2-Fluorophenyl)-3-(3-(6-(pyrrolidin-1-yl)pyridin-2-yl)phenyl)urea (10u): The synthesis was carried out as described for 10a using 8 (50 mg, 0.139 mmol) and 2-fluoroaniline (15.46 mg, 0.139 mmol) in DMF (1 mL) followed by DIEA (0.049 mL, 0.278 mmol) to afford the desired product 10u (22 mg, 0.058 mmol, 42% yield) as white solid. $^1$H NMR (500 MHz, CD$_3$OD) $\delta$: 8.06 (td, J = 8.0 Hz, J = 1.5 Hz, 2H), 7.95 (dd, J = 9.0 Hz, J = 7.5 Hz, 1H), 7.51 (t, J = 7.5 Hz, 1H), 7.45 (ddd, J = 8.0 Hz, J = 2.0 Hz, J = 1.0 Hz, 1H), 7.42 (td, J = 7.5 Hz, J = 1.5 Hz, 1H), 7.18-7.11 (m, 2H), 7.08-6.99 (m, 3H), 3.70 (t, J = 6.5 Hz, 4H), 2.16 (quintet, J = 3.5 Hz, 4H).
1-(2-Isopropylphenyl)-3-(3-(6-(pyrrolidin-1-yl)pyridin-2-yl)phenyl)urea (10v): The synthesis was carried out as described for 10a using a solution of 8 (50 mg, 0.139 mmol) in DMF (3 mL), 2-isopropylaniline (18.81 mg, 0.139 mmol) followed by Et$_3$N (0.039 mL, 0.278 mmol) to afford the desired product 10v (33 mg, 0.082 mmol, 59% yield) as white solid. $^1$H NMR (500 MHz, CD$_3$OD) $\delta$: 8.07 (br s, 1H), 7.93-7.86 (m, 1H), 7.48 (d, $J = 6.0$ Hz, 1H), 7.47 (d, $J = 4.0$ Hz, 1H), 7.45-7.41 (m, 2H), 7.36-7.31 (m, 1H), 7.19 (dd, $J = 6.5$ Hz, $J = 4.0$ Hz, 2H), 7.05 (d, $J = 7.5$ Hz, 1H), 6.95 (br s, 1H), 3.67 (t, $J = 6.5$ Hz, 4H), 3.23 (septet, $J = 2.0$ Hz, 1H), 2.13 (quintet, $J = 3.0$ Hz, 4H), 1.27 (s, 3H), 1.26 (s, 3H).

1-(Naphthalen-1-yl)-3-(3-(6-(pyrrolidin-1-yl)pyridin-2-yl)phenyl)urea (10w): The synthesis was carried out as described for 10a using 8 (50 mg, 0.139 mmol) and naphthalen-1-amine (21.91 mg, 0.153 mmol) in DMF (2 mL) followed by DIEA (53.5 µl, 0.306 mmol) to afford the desired product 10w (31 mg, 0.076 mmol, 54% yield) as white solid. $^1$H NMR (500 MHz, CD$_3$OD) $\delta$: 8.1 (t, $J = 1.5$ Hz, 1H), 7.96 (dd, $J = 9.5$ Hz, $J = 7.5$ Hz, 1H), 7.90 (dd, $J = 8.0$ Hz, $J = 1.5$ Hz, 1H), 7.79 (dd, $J = 8.0$ Hz, $J = 1.5$ Hz, 1H), 7.72 (d, $J = 8.0$ Hz, 1H), 7.58-7.46 (m, 5H), 7.41 (td, $J = 7.0$ Hz, $J = 1.5$ Hz, 1H), 7.04 (dd, $J = 5.5$ Hz, $J = 1.0$ Hz, 1H), 7.02 (dd, $J = 7.0$ Hz, $J = 1.0$ Hz, 1H), 3.71 (t, $J = 7.0$ Hz, 4H), 2.17 (quintet, $J = 3.5$ Hz, 4H). MS $m/\ell$ (M$^+$$+$$1$): 409.
1-(Naphthalen-2-yl)-3-(3-(6-(pyrrolidin-1-yl)pyridin-2-yl)phenyl)urea (10x): The synthesis was carried out as described for 10a using 8 (50 mg, 0.139 mmol) and naphthalen-2-amine (21.91 mg, 0.153 mmol) in DMF (2 mL) followed by DIEA (53.5 µl, 0.306 mmol) to afford the desired product 10x (35 mg, 0.086 mmol, 61% yield) as white solid. $^1$H NMR (500 MHz, CD$_3$OD) δ: 8.09 (t, $J = 1.5$ Hz, 1H), 8.04 (d, $J = 2.5$ Hz, 1H), 7.99 (dd, $J = 9.0$ Hz, $J = 7.5$ Hz, 1H), 7.83-7.73 (m, 3H), 7.55-7.47 (m, 3H), 7.46-7.40 (m, 2H), 7.37 (dt, $J = 7.5$ Hz, $J = 1.5$ Hz, 1H), 7.07 (dd, $J = 2.5$ Hz, $J = 1.0$ Hz, 1H), 7.06 (dd, $J = 5.0$ Hz, $J = 1.0$ Hz, 1H), 3.71 (t, $J = 7.0$ Hz, 4H), 2.17 (quintet, $J = 3.5$ Hz, 4H). MS m/z (M$^+$+1): 409.1.

$N$-(3-(6-(Pyrrolidin-1-yl)pyridin-2-yl)phenyl)-3,4-dihydroisoquinoline-2(1H)-carboxamide (10y): The synthesis was carried out as described for 10a using 8 (50 mg, 0.139 mmol) and 1,2,3,4-tetrahydroisoquinoline (18.53 mg, 0.139 mmol) in DMF (2 mL) followed by DIEA (53.5 µl, 0.306 mmol) to afford the desired product 10y (35 mg, 0.088 mmol, 63% yield) as white solid. $^1$H NMR (500 MHz, CD$_3$OD) δ: 7.97 (dd, $J = 9.0$ Hz, $J = 7.0$ Hz, 1H), 7.87 (t, $J = 2.0$ Hz, 1H), 7.52 (td, $J = 8.5$ Hz, $J = 1.5$ Hz, 1H), 7.49 (t, $J = 7.0$ Hz, 1H), 7.40 (td, $J = 8.0$ Hz, $J = 1.5$ Hz, 1H), 7.21-7.16 (m, 4H), 7.04 (dt, $J = 9.5$ Hz, $J = 1.0$ Hz, 2H), 4.72 (s, 2H), 3.79 (t, $J = 6.0$ Hz, 2H), 3.70 (t, $J = 6.5$ Hz, 4H), 2.94 (t, $J = 6.0$ Hz, 2H), 2.16 (quintet, $J = 3.0$ Hz, 4H). MS m/z (M$^+$+1): 399.4.
1-Cyclohexyl-3-(3-(6-(pyrrolidin-1-yl)pyridin-2-yl)phenyl)urea (10z): The synthesis was carried out as described for 10a using 8 (50 mg, 0.139 mmol) and cyclohexanamine (15.18 mg, 0.153 mmol) in DMF (2 mL) followed by DIEA (0.053 mL, 0.306 mmol) to afford the desired product 10z (27 mg, 0.074 mmol, 53% yield) as white solid. $^1$H NMR (500 MHz, CD$_3$OD) $\delta$: 7.97 (t, $J$ = 2.0 Hz, 1H), 7.59 (td, $J$ = 7.5 Hz, $J$ = 1.5 Hz, 1H), 7.52 (dd, $J$ = 8.5 Hz, $J$ = 7.5 Hz, 1H), 7.40 (ddd, $J$ = 9.0 Hz, $J$ = 2.0 Hz, $J$ = 1.0 Hz, 1H), 7.30 (t, $J$ = 8.0 Hz, 1H), 7.0 (d, $J$ = 7.5 Hz, 1H), 6.39 (d, $J$ = 7.0 Hz, 1H), 3.62-3.56 (m, 1H), 3.53 (t, $J$ = 6.5 Hz, 4H), 2.04 (quintet, $J$ = 3.5 Hz, 4H), 1.94 (dd, $J$ = 12.0 Hz, $J$ = 3.5 Hz, 2H), 1.75 (dt, $J$ = 13.5 Hz, $J$ = 3.5 Hz, 2H), 1.66-1.59 (m, 1H), 1.46-1.35 (m, 2H), 1.30-1.18 (m, 3H). MS m/z (M$^+$+1): 365.3.

2-(3-Isocyanatophenyl)-6-(pyrrolidin-1-yl)pyridine (11): To a solution of triphosgene (0.868 g, 2.93 mmol) in toluene (15 mL) was added 6 (2 g, 8.36 mmol) followed by Et$_3$N (13.98 mL, 100 mmol) under inert atmosphere and heated to 70 °C for 3 h under argon atmosphere. The reaction was concentrated under reduced pressure and carried forward to the next step without any purification. $^1$H NMR (500 MHz, CD$_3$OD) $\delta$: 8.02 (dd, $J$ = 9.0 Hz, $J$ = 2.5 Hz, 1H), 7.97 (br s, 1H), 7.61-7.56 (m, 1H), 7.52 (t, $J$ = 8.0 Hz, 1H), 7.45-7.40 (m, 1H), 7.10 (d, $J$ = 9.0 Hz, 1H), 7.06 (d, $J$ = 7.5 Hz, 1H), 3.74 (t, $J$ = 6.5 Hz, 4H), 2.20 (quintet, $J$ = 3.0 Hz, 4H).
1-(4-Chlorophenyl)-1-methyl-3-(3-(6-(pyrrolidin-1-yl)pyridin-2-yl)phenyl)urea (13a): To a solution of 11 (50 mg, 0.188 mmol) in DCM (2 mL) was added 4-chloro-N-methylaniline (26.7 mg, 0.188 mmol) followed by Et₃N (0.079 mL, 0.565 mmol) and stirred at 0 °C for 6 h. The reaction mixture was diluted in 20 mL DCM and 20 mL water. The organic layer was separated, washed with water (2x20 mL), dried (MgSO₄) and concentrated under reduced pressure. The crude product was purified by silica gel chromatography by eluting with 10-50% ethyl acetate : hexanes to afford the desired product 13a (35 mg, 0.086 mmol, 46% yield) as white solid. ¹H NMR (500 MHz, CD₃OD) δ: 7.98 (td, J = 8.0 Hz, J = 1.5 Hz, 1H), 7.86-7.84 (m, 1H), 7.48 (d, J = 1.5 Hz, 1H), 7.46 (td, J = 4.0 Hz, J = 1.5 Hz, 3H), 7.43-7.39 (m, 1H), 7.38 (d, J = 1.5 Hz, 1H), 7.36 (t, J = 1.5 Hz, 1H), 7.06 (dd, J = 9.0 Hz, J = 1.0 Hz, 1H), 7.02 (dd, J = 7.0 Hz, J = 1.0 Hz, 1H), 3.70 (t, J = 7.0 Hz, 4H), 3.35 (s, 3H), 2.16 (quintet, J = 3.0 Hz, 4H).

4-Chlorophenyl (3-(6-(pyrrolidin-1-yl)pyridin-2-yl)phenyl)carbamate (13b): To a solution of 11 (25 mg, 0.094 mmol) in DCM (2 mL) was added 4-chlorophenol (12.11 mg, 0.094 mmol) followed by Et₃N (0.026 mL, 0.188 mmol) and stirred at 0 °C for 16 h. The organic layer was separated, washed with 2x20 mL water and brine, dried (MgSO₄) and concentrated under reduced pressure. The crude product was purified by silica gel chromatography with 10-50%
ethyl acetate : hexanes to afford the desired product **13a** (15 mg, 0.038 mmol, 40% yield) as white solid. $^1$H NMR (500 MHz, CD$_3$OD) δ: 8.01 (br s, 1H), 7.97 (dd, $J = 9.0$ Hz, $J = 7.5$ Hz, 1H), 7.61 (ddd, $J = 8.5$ Hz, $J = 2.5$ Hz, $J = 1.0$ Hz, 1H), 7.54 (t, $J = 8.5$ Hz, 1H), 7.46 (td, $J = 8.5$ Hz, $J = 1.0$ Hz, 1H), 7.41 (d, $J = 8.5$ Hz, 2H), 7.21 (d, $J = 9.0$ Hz, 2H), 7.12 (d, $J = 8.5$ Hz, 1H), 7.04 (t, $J = 7.0$ Hz, 2H), 6.30 (d, $J = 8.5$ Hz, 1H), 3.69 (t, $J = 6.5$ Hz, 4H), 2.15 (quintet, $J = 3.0$ Hz, 4H).

![Chemical Structure](image)

**4-Chloro-2-methylphenyl (3-(6-(pyrrolidin-1-yl)pyridin-2-yl)phenyl)carbamate (13c):** The synthesis was carried out as described for **13a** using a solution of **11** (25 mg, 0.094 mmol) in DCM (3 mL) and 4-chloro-2-methylphenol (13.44 mg, 0.094 mmol) to afford the desired product **13c** (8 mg, 0.02 mmol, 21% yield) as white solid. $^1$H NMR (500 MHz, CD$_3$OD) δ: 8.03-7.97 (m, 2H), 7.65 (ddd, $J = 8.5$ Hz, $J = 2.5$ Hz, $J = 1.5$ Hz, 1H), 7.55 (t, $J = 8.0$ Hz, 1H), 7.47 (dd, $J = 8.0$ Hz, $J = 1.0$ Hz, 1H), 7.31 (d, $J = 2.5$ Hz, 1H), 7.23 (dd, $J = 8.5$ Hz, $J = 2.5$ Hz, 1H), 7.12 (d, $J = 8.5$ Hz, 1H), 7.08 (dd, $J = 9.0$ Hz, $J = 1.0$ Hz, 1H), 7.04 (d, $J = 7.0$ Hz, 1H), 3.70 (t, $J = 7.0$ Hz, 4H), 2.25 (s, 3H), 2.16 (quintet, $J = 3.0$ Hz, 4H).
Methyl 2-(3-(3-(6-(pyrrolidin-1-yl)pyridin-2-yl)phenyl)ureido)benzoate (15): The synthesis was carried out as described for 10a using 8 (50 mg, 0.139 mmol) and methyl 2-aminobenzoate (21.03 mg, 0.139 mmol) in DMF (1 mL) followed by DIEA (0.049 mL, 0.278 mmol). No desired product 15 was observed and the reaction was discarded (symmetric urea was formed).

Methyl 2-(3-(3-(6-(pyrrolidin-1-yl)pyridin-2-yl)phenyl)ureido)benzoate (15): To a solution of 11 (50 mg, 0.188 mmol) in DCM (3 mL) was added ethyl 2-aminobenzoate (31.1 mg, 0.188 mmol) followed by Et₃N (0.026 mL, 0.188 mmol) and stirred at 0 °C for 16 h. No desired product 15 was observed and the reaction was discarded (symmetric urea was formed).

Methyl 2-(3-(3-(6-(pyrrolidin-1-yl)pyridin-2-yl)phenyl)ureido)benzoate (15): To a solution of 6 (50 mg, 0.209 mmol) in DCM (3 mL) was added 16 (37 mg, 0.209 mmol) followed by Et₃N (0.058 mL, 0.418 mmol) and stirred at room temperature for 16 h. Reaction was diluted in 20 mL DCM and 20 mL water. The organic layer was separated, washed with 2x20 mL water and brine, dried (MgSO₄) and concentrated under reduced pressure. The crude product was purified by silica gel chromatography with 10-50% ethyl acetate : hexanes to afford the desired product 15 (66 mg, 0.159 mmol, 76% yield) as white solid. ¹H NMR (500 MHz, CD₃OD) δ: 8.37 (d, J = 8.5 Hz, 1H), 8.06 (d, J = 1.0 Hz, 1H), 8.04 (d, J = 1.5 Hz, 1H), 7.99 (dd, J = 9.0 Hz, J = 7.5 Hz, 1H), 7.58-7.51(m, 3H), 7.42 (td, J = 6.5 Hz, J = 2.0 Hz, 1H), 7.11-7.0 (m, 3H), 4.42 (q, J = 7.0 Hz, 2H), 3.71 (t, J = 6.5 Hz, 4H), 2.17 (quintet, J = 3.5 Hz, 4H), 1.42 (t, J = 7.0 Hz, 3H).
2-(3-(3-(6-(Pyrrolidin-1-yl)pyridin-2-yl)phenyl)ureido)benzoic acid (16): To a solution of 15 (25 mg, 0.058 mmol) in THF (3 mL) and MeOH (3 mL) was added 1M LiOH (0.290 mL, 0.290 mmol) and stirred for 16 h at room temperature. The reaction mixture was concentrated and the crude product was purified by silica gel chromatography using 10-50% ethyl acetate : hexanes to afford the desired product 16 (16 mg, 0.040 mmol, 68% yield) as a white solid. $^1$H NMR (500 MHz, CD$_3$OD) δ: 8.05 (dd, $J = 8.0$ Hz, $J = 1.5$ Hz, 1H), 7.98 (dd, $J = 9.0$ Hz, $J = 7.5$ Hz, 1H), 7.83 (ddd as td, $J = 8.0$ Hz, $J = 1.0$ Hz, 1H), 7.75 (t, $J = 2.0$ Hz, 1H), 7.71 (tt, $J = 7.5$ Hz, $J = 1.5$ Hz, 2H), 7.57 (ddd, $J = 8.5$ Hz, $J = 2.0$ Hz, $J = 1.0$ Hz, 1H), 7.27 (dt, $J = 7.5$ Hz, $J = 1.0$ Hz, 1H), 7.24 (d, $J = 7.5$ Hz, 1H), 7.08 (dd, $J = 9.0$ Hz, $J = 1.0$ Hz, 1H), 7.05 (d, $J = 7.5$ Hz, 1H), 3.69 (t, $J = 6.5$ Hz, 4H), 2.15 (quintet, $J = 3.0$ Hz, 4H).

1-(2-(Hydroxymethyl)phenyl)-3-(3-(6-(pyrrolidin-1-yl)pyridin-2-yl)phenyl)urea (17): To a solution of 15 (50 mg, 0.116 mmol) in THF (5 mL) at 0 °C was added LAH (0.116 mL, 0.116 mmol) and stirred for 5 h. The reaction mixture was diluted with 50 mL DCM and 50 mL 10% Rochelle salt solution. The organic layer was separated, washed with water (2x50 mL), dried (MgSO$_4$) and concentrated under reduced pressure. The crude product was purified using silica gel chromatography eluting with 10-50% ethyl acetate : hexanes to afford the desired product 17.
(18 mg, 0.046 mmol, 40% yield) as a white solid. \(^1\)H NMR (500 MHz, CD\(_3\)OD) \(\delta\): 8.03 (s, 1H), 7.98 (dd, \(J = 9.0\) Hz, \(J = 2.0\) Hz, 1H), 7.75 (d, \(J = 8.0\) Hz, 1H), 7.50 (d, \(J = 6.0\) Hz, 2H), 7.39 (ddd as td, \(J = 6.0\) Hz, \(J = 2.0\) Hz, 1H), 7.33 (d, \(J = 7.5\) Hz, 1H), 7.27 (dt, \(J = 7.5\) Hz, \(J = 1.5\) Hz, 1H), 7.10 (t, \(J = 7.5\) Hz, 1H), 7.06 (d, \(J = 4.5\) Hz, 1H), 7.04 (d, \(J = 3.0\) Hz, 1H), 4.67 (s, 2H), 3.70 (t, \(J = 6.5\) Hz, 4H), 2.15 (quintet, \(J = 3.0\) Hz, 4H).

1-(4-Chloro-2-iodophenyl)-3-(3-(6-(pyrrolidin-1-yl)pyridin-2-yl)phenyl)urea (21a) : The synthesis was carried out as described for 10a using 8 (355 mg, 0.986 mmol) in DMF (10 mL) and 4-chloro-2-iodoaniline (250 mg, 0.986 mmol) followed by DIEA (0.345 mL, 1.973 mmol). No desired product was observed. Complete conversion of starting material to undesired symmetric urea byproduct was observed.

1-(4-Chloro-2-iodophenyl)-3-(3-(6-(pyrrolidin-1-yl)pyridin-2-yl)phenyl)urea (21a) : To a solution of triphosgene (222 mg, 0.750 mmol) in toluene (10 mL) was added a solution of 4-chloro-2-iodoaniline (500 mg, 1.973 mmol), Et\(_3\)N (3.3 mL, 23.68 mmol) in toluene (10 mL) under argon atmosphere. The resulting solution was heated to 70 °C for 3 h. The reaction mixture was filtered through a pad of celite and eluted with Et\(_2\)O. The filtrate was concentrated under reduced pressure to give the crude product as brown solid which was used for the next step without further purification. To the solution of crude intermediate in DCM (25 mL) was added 3-(6-(pyrrolidin-1-yl)pyridin-2-yl)aniline (472 mg, 1.973 mmol) followed by Et\(_3\)N (3.3 mL, 23.68 mmol)
mmol) and stirred for 6 hours at 0 °C. The reaction mixture was diluted with 100 mL DCM and 100 mL water. The organic layer was separated, washed with 2x50 mL water and brine, dried (MgSO\textsubscript{4}) and concentrated under reduced pressure. The crude product was purified by silica gel chromatography eluting with 10-50% ethyl acetate : hexanes to afford the desired product 21a (676 mg, 1.302 mmol, 66% yield) as a white solid. \(^1\)H NMR (500 MHz, DMSO) \(\delta\): 9.57 (s, 1H), 8.09 (t, \(J = 2.0\) Hz, 1H), 7.98 (s, 1H), 7.91 (d, \(J = 2.5\) Hz, 1H), 7.88 (d, \(J = 8.5\) Hz, 1H), 7.66 (td, \(J = 8.0\) Hz, \(J = 1.5\) Hz, 1H), 7.56 (dd, \(J = 8.5\) Hz, \(J = 7.5\) Hz, 2H), 7.44 (dd, \(J = 7.0\) Hz, \(J = 2.5\) Hz, 1H), 7.36 (t, \(J = 8.0\) Hz, 1H), 7.06 (d, \(J = 7.5\) Hz, 1H), 6.42 (d, \(J = 8.5\) Hz, 1H), 3.48 (t, \(J = 6.5\) Hz, 4H), 1.97 (quintet, \(J = 3.0\) Hz, 4H). MS \(m/z\) (M\(^+\)+1): 519.0.

1-(4-Chloro-3-iodophenyl)-3-(3-(6-(pyrrolidin-1-yl)pyridin-2-yl)phenyl)urea (21b): The synthesis was carried out as described for 21a using a solution of triphosgene (267 mg, 0.900 mmol) in toluene (10 mL), 4-chloro-3-iodoaniline (600 mg, 2.367 mmol) and Et\(_3\)N (3.96 mL, 28.4 mmol) in toluene (10 mL) followed by 6 (567 mg, 2.367 mmol) and Et\(_3\)N (3.96 mL, 28.4 mmol) to afford the desired product 21b (660 mg, 1.272 mmol, 54% yield) as a white solid. \(^1\)H NMR (500 MHz, CD\(_3\)OD + CDCl\(_3\)) \(\delta\): 8.12 (d, \(J = 2.5\) Hz, 1H), 8.05 (t, \(J = 1.5\) Hz, 1H), 7.67 (td, \(J = 8.5\) Hz, \(J = 4.0\) Hz, 1H), 7.54 (dd, \(J = 8.5\) Hz, \(J = 7.0\) Hz, 1H), 7.52-7.48 (m, 1H), 7.43 (dd, \(J = 9.0\) Hz, \(J = 2.5\) Hz, 1H), 7.40 (s, 1H), 7.38-7.35 (m, 1H), 7.03 (d, \(J = 7.0\) Hz, 1H), 6.41 (d, \(J = 8.5\) Hz, 1H), 3.54 (t, \(J = 7.0\)Hz, 4H), 2.04 (quintet, \(J = 3.0\) Hz, 4H).
1-(4-Chloro-2-(4-((tetrahydro-2H-pyran-2-yl)oxy)but-1-yn-1-yl)phenyl)-3-(3-(6-(pyrrolidin-1-yl)pyridin-2-yl)phenyl)urea (23a): A solution of 21a (500 mg, 0.964 mmol) and 2-(but-3-ynyloxy)tetrahydro-2H-pyran (163 mg, 1.060 mmol) in DMF (10 mL) was treated with Pd(PPh₃)₂Cl₂ (33.8 mg, 0.048 mmol) and copper(I) iodide (12.84 mg, 0.067 mmol), and the reaction was heated to 90 °C for 2 h. After cooling to the room temperature, the reaction mixture was diluted with 100 mL DCM and 100 mL water. The organic layer was separated, washed with water (2x50 mL), dried (MgSO₄) and concentrated under reduced pressure. The crude product was purified by silica gel chromatography using 10-50% ethyl acetate : hexanes to afford the desired product 23a (350 mg, 0.642 mmol, 67% yield) as a white solid. ¹H NMR (500 MHz, CD₃OD) δ: 8.11 (d, J = 9.0 Hz, 1H), 8.09 (t, J = 2.0 Hz, 1H), 7.68 (d, J = 8.0 Hz, 1H), 7.55 (dd, J = 7.5 Hz, J = 2.5 Hz, 2H), 7.38 (t, J = 8.0 Hz, 1H), 7.33 (d, J = 2.5 Hz, 1H), 7.27 (dd, J = 9.5 Hz, J = 2.5 Hz, 1H), 7.04 (d, J = 7.0 Hz, 1H), 6.43 (d, J = 8.5 Hz, 1H), 4.69 (dd, J = 5.0 Hz, J = 2.5 Hz, 1H), 3.96-3.87 (m, 2H), 3.71-3.64 (m, 1H), 3.54 (t, J = 7.0 Hz, 4H), 3.52-3.47 (m, 1H), 2.81 (t, J = 7.0 Hz, 2H), 2.04 (quintet, J = 3.5 Hz, 4H), 1.85-1.78 (m, 1H), 1.75-1.67 (m, 1H), 1.62-1.42 (m, 4H). MS m/z (M⁺+1): 545.2.
1-(4-Chloro-3-(4-((tetrahydro-2H-pyran-2-yl)oxy)but-1-yn-1-yl)phenyl)-3-(3-(6-(pyrrolidin-1-yl)pyridin-2-yl)phenyl)urea (23b): The synthesis was carried out as described for 23a using 21b (500 mg, 0.964 mmol) and 2-(but-3-ynyloxy)tetrahydro-2H-pyran (163 mg, 1.060 mmol) in DMF (10 mL), Pd(PPh₃)₂Cl₂ (33.8 mg, 0.048 mmol) and copper(I)iodide (12.84 mg, 0.067 mmol) to afford the desired product 23b (400 mg, 0.734 mmol, 76% yield) as a white solid. ¹H NMR (500 MHz, CD₃OD) δ: 8.06 (t, J = 2.0 Hz, 1H), 7.66 (td, J = 8.0 Hz, J = 2.0 Hz, 1H), 7.63 (d, J = 2.5 Hz, 1H), 7.54 (dd as t, J = 8.0 Hz, 1H), 7.51-7.48 (m, 1H), 7.37-7.36 (m, 1H), 7.35 (d, J = 2.5 Hz, 1H), 7.32 (t, J = 8.0 Hz, 1H), 7.03 (d, J = 7.0 Hz, 1H), 6.41 (d, J = 8.5 Hz, 1H), 4.74 (t, J = 3.5 Hz, 1H), 4.0-3.86 (m, 2H), 3.69-3.62 (m, 1H), 3.54 (t, J = 6.5 Hz, 4H), 3.16 (quintet, J = 1.5 Hz, 1H), 2.76 (t, J = 6.5 Hz, 2H), 2.04 (quintet, J = 3.0 Hz, 4H), 1.92-1.82 (m, 1H), 1.78-1.68 (m, 1H), 1.65-1.48 (m, 4H). MS m/z (M⁺+1): 545.3.
1-(4-Chloro-2-(4-hydroxybut-1-yn-1-yl)phenyl)-3-(3-(6-(pyrrolidin-1-yl)pyridin-2-yl)phenyl)urea (24a): To a solution of 23a (50 mg, 0.092 mmol) in methanol (5 mL) was added p-toluenesulfonic acid monohydrate (87 mg, 0.459 mmol) and stirred for 5 h at room temperature. The reaction solution was diluted with 100 mL DCM and 100 mL 10% NaHCO₃. The organic layer was separated, washed with 2x50 mL water and brine, dried (MgSO₄) and concentrated under reduced pressure. The crude product was purified by silica gel chromatography by eluting with 10-50% ethyl acetate : hexanes to afford the desired product 23a (32 mg, 0.069 mmol, 76% yield) as a white solid. ¹H NMR (500 MHz, CD₃OD) δ: 8.15 (d, J = 9.0 Hz, 1H), 8.09 (t, J = 2.0 Hz, 1H), 7.75 (dt, J = 7.5 Hz, J = 2.0 Hz, 1H), 7.56-7.52 (m, 2H), 7.39-7.34 (m, 2H), 7.27 (dd, J = 9.0 Hz, J = 2.5 Hz, 1H), 7.03 (d, J = 7.0 Hz, 1H), 6.42 (d, J = 8.5 Hz, 1H), 3.81 (t, J = 6.0 Hz, 2H), 3.54 (t, J = 7.0 Hz, 4H), 2.75 (t, J = 6.0 Hz, 2H), 2.04 (quintet, J = 3.5 Hz, 4H). MS m/z (M⁺+1): 461.2.
1-(4-Chloro-3-(4-hydroxybut-1-yn-1-yl)phenyl)-3-(3-(6-(pyrrolidin-1-yl)pyridin-2-yl)phenyl)urea (24b): The synthesis was carried out as described for 24a by adding p-toluenesulfonic acid monohydrate (87 mg, 0.459 mmol), 23b (50 mg, 0.092 mmol) in methanol (5 mL) to afford the desired product 24b (30 mg, 0.065 mmol, 71% yield) as a white solid. $^1$H NMR (500 MHz, CD$_3$OD) $\delta$: 8.06 (t, $J = 2.0$ Hz, 1H), 7.67 (td, $J = 7.5$ Hz, $J = 1.50$ Hz, 1H), 7.62 (d, $J = 2.5$ Hz, 1H), 7.54 (t, $J = 8.0$ Hz, 1H), 7.50 (ddd, $J = 5.5$ Hz, $J = 2.5$ Hz, $J = 1.0$ Hz, 1H), 7.37 (dd, $J = 5.0$ Hz, $J = 2.0$ Hz, 1H), 7.35 (d, $J = 2.0$ Hz, 1H), 7.32 (t, $J = 8.0$ Hz, 1H), 7.03 (d, $J = 7.0$ Hz, 1H), 6.41 (d $J = 8.5$ Hz, 1H), 3.76 (t, $J = 7.0$ Hz, 2H), 3.54 (t, $J = 7.0$ Hz, 4H), 2.68 (t, $J = 7.0$ Hz, 2H), 2.04 (quintet, $J = 3.5$ Hz, 4H). MS $m/z$ (M$^+$+1): 461.2
1-(4-Chloro-2-((tetrahydro-2H-pyran-2-yl)oxy)butyl)phenyl)-3-(3-(6-(pyrrolidin-1-yl)pyridin-2-yl)phenyl)urea (25a): To a solution of 23a (100 mg, 0.183 mmol) in methanol (25 mL) was added Pd/C (9.76 mg, 9.17 µmol) and the reaction mixture was stirred under hydrogen atmosphere for 12 h. The desired product along with dechlorinated byproduct was observed. Pd/C was filtered off and filtrate was concentrated under reduced pressure. The crude product was purified by silica gel chromatography by eluting with 5-50% ethyl acetate : hexanes to afford the desired product 25a (70 mg, 0.127 mmol, 69% yield) as a white solid. $^1$H NMR (500 MHz, CD$_3$OD) δ: 8.08 (t, J = 2.0 Hz, 1H), 7.65 (td, J = 8.5 Hz, J = 2.0 Hz, 1H), 7.60 (dd, J = 8.0 Hz, J = 1.0 Hz, 1H), 7.55-7.51 (m, 2H), 7.34 (t, J = 8.0 Hz, 1H), 7.24-7.20 (m, 1H), 7.19 (dd, J = 8.0 Hz, J = 2.0 Hz, 1H), 7.09 (dt, J = 8.0 Hz, J = 1.5 Hz, 1H), 7.03 (d, J = 7.0 Hz, 1H), 7.40 (d, J = 8.5 Hz, 1H), 4.53 (t, J = 4.0 Hz, 1H), 3.84-3.74 (m, 3H), 3.54 (t, J = 7.0 Hz, 4H), 3.48-3.38 (m, 2H), 2.70 (t, J = 8.0 Hz, 2H), 2.04 (quintet, J = 3.0 Hz, 4H), 1.80-1.58 (m, 6H), 1.54-1.38 (m, 4H).
1-(4-Chloro-3-(4-((tetrahydro-2H-pyran-2-yl)oxy)butyl)phenyl)-3-(3-(6-(pyrrolidin-1-yl)pyridin-2-yl)phenyl)urea (25b): The synthesis was carried out as described for 25a using 23b (80 mg, 0.147 mmol) in methanol (25 mL) and Pd/C (15.62 mg, 0.015 mmol) under hydrogen atmosphere to afford the desired product 25b (68 mg, 0.124 mmol, 84% yield) as a white solid. $^1$H NMR (500 MHz, CD$_3$OD) δ: 8.08 (t, $J = 2.0$ Hz, 1H), 7.67 (d, $J = 8.0$ Hz, 1H), 7.54 (t, $J = 7.0$ Hz, 1H), 7.49 (d, $J = 7.5$ Hz, 1H), 7.4 (d, $J = 2.0$ Hz, 1H), 7.35 (t, $J = 8.0$ Hz, 1H), 7.29-7.24 (m, 2H), 7.01 (d, $J = 7.0$ Hz, 1H), 6.41 (d, $J = 8.5$ Hz, 1H), 4.59 (t, $J = 4.0$ Hz, 1H), 3.89-3.83 (m, 1H), 3.80-3.73 (m, 1H), 3.53 (t, $J = 6.5$ Hz, 4H), 3.52-3.46 (m, 1H), 3.46-3.41 (m, 1H), 2.75 (t, $J = 7.0$ Hz, 2H), 2.04 (quintet, $J = 3.0$ Hz, 4H), 1.86-1.78 (m, 1H), 1.76-1.63 (m, 5H), 1.59-1.48 (m, 5H).
1-(3-(6-(Pyrrolidin-1-yl)pyridin-2-yl)phenyl)-3-(2-(4-((tetrahydro-2H-pyran-2-yl)oxy)butyl)phenyl)urea (25c): Synthesis of 25a gave byproduct 25c (22 mg, 0.043 mmol, 23.3% yield) as a white solid. ¹H NMR (500 MHz, DMSO) δ: 9.06 (s, 1H), 8.07 (t, J = 2.0 Hz, 1H), 7.91 (s, 1H), 7.76 (d, J = 8.0 Hz, 1H), 7.62 (d, J = 8.0 Hz, 1H), 7.60-7.54 (m, 2H), 7.34 (t, J = 8.0 Hz, 1H), 7.18 (d, J = 7.5 Hz, 1H), 7.15 (d, J = 7.5 Hz, 1H), 7.05 (d, J = 7.5 Hz, 1H), 7.01 (t, J = 7.5 Hz, 1H), 6.42 (d, J = 8.5 Hz, 1H), 4.51 (t, J = 3.5 Hz, 1H), 3.70 (tt, J = 12.5 Hz, J = 3.0 Hz, 1H), 3.66-3.61 (m, 1H), 3.48 (t, J = 6.5 Hz, 4H), 3.41-3.34 (m, 2H), 2.63 (t, J = 7.0 Hz, 2H), 1.97 (quintet, J = 3.0 Hz, 4H), 1.70-1.50 (m, 6H), 1.46-1.32 (m, 4H).

1-(4-Chloro-2-(4-hydroxybutyl)phenyl)-3-(3-(6-(pyrrolidin-1-yl)pyridin-2-yl)phenyl)urea (26a): The synthesis was carried out as described for 24a using 25a (25 mg, 0.046 mmol) in methanol (5 mL) and p-toluenesulfonic acid monohydrate (43.3 mg, 0.228 mmol) to afford the desired product 26a (12 mg, 0.026 mmol, 57% yield) as a white solid. NMR
1-(4-Chloro-3-(4-hydroxybutyl)phenyl)-3-(3-(6-(pyrrolidin-1-yl)pyridin-2-yl)phenyl)urea (26b): The synthesis was carried out as described for 24a by adding p-toluenesulfonic acid monohydrate (43.3 mg, 0.228 mmol) and a solution of 25b (25 mg, 0.046 mmol) in methanol (5 mL) to afford the desired product 26b (11 mg, 0.024 mmol, 52% yield) as a white solid. $^1$H NMR (500 MHz, CD$_3$OD) δ: 8.08 (t, $J = 2.0$ Hz, 1H), 7.67 (d, $J = 8.0$ Hz, 1H), 7.64 (t, $J = 1.5$ Hz, 1H), 7.56-7.50 (m, 2H), 7.35 (t, $J = 8.0$ Hz, 1H), 7.23 (d, $J = 2.5$ Hz, 1H), 7.18 (dd, $J = 9.0$ Hz, $J = 2.5$ Hz, 1H), 7.03 (d, $J = 7.0$ Hz, 1H), 6.41 (d, $J = 8.5$ Hz, 1H), 3.60 (t, $J = 6.0$ Hz, 2H), 3.58 (t, $J = 6.0$ Hz, 4H), 2.67 (t, $J = 7.5$ Hz, 2H), 2.04 (quintet, $J = 3.5$ Hz, 4H), 1.73-1.65 (m, 2H), 1.65-1.58 (m, 2H).
**N-Methyl-3-(6-(pyrrolidin-1-yl)pyridin-2-yl)aniline (27):** To a solution of 6 (1 g, 4.18 mmol) in methanol (50 mL) was added paraformaldehyde (0.627 g, 20.89 mmol) and sodium methoxide (1.129 g, 20.89 mmol). The reaction mixture was refluxed for 3 h and then was cooled to 0 °C and NaBH₄ (0.790 g, 20.89 mmol) was added. The reaction mixture was heated to reflux for 1 h and then poured into the ice. Aqueous layer was extracted with 50 mL DCM. The organic layer was washed with water (2x50 mL), dried (MgSO₄) and concentrated under reduced pressure. The crude product was purified by silica gel chromatography eluting with 10-50% ethyl acetate : hexanes to afford the desired product 27 (0.88 g, 3.47 mmol, 83% yield) as a colorless oil. \(^1\)H NMR (500 MHz, CD₃OD) \(\delta\): 7.47 (dd, \(J = 8.5\) Hz, \(J = 7.5\) Hz, 1H), 7.31 (t, \(J = 2.0\) Hz, 1H), 7.25 (dd, \(J = 7.5\) Hz, \(J = 1.0\) Hz, 1H), 7.18 (t, \(J = 8.0\) Hz, 1H), 6.95 (d, \(J = 7.5\) Hz, 1H), 6.64 (ddd, \(J = 7.5\) Hz, \(J = 2.0\) Hz, \(J = 1.0\) Hz, 1H), 6.33 (d, \(J = 8.5\) Hz, 1H), 3.48 (t, \(J = 6.0\) Hz, 4H), 2.80 (s, 3H), 1.99 (quintet, \(J = 3.0\) Hz, 4H). MS m/z (M⁺+1): 254.1

**3-(4-Chlorophenyl)-1-methyl-1-(3-(6-(pyrrolidin-1-yl)pyridin-2-yl)phenyl)urea (29):** The synthesis was carried out as described for 21a using a solution of 27 (25 mg, 0.099 mmol) in DCM (3 mL), 1-chloro-4-isocyanatobenzene (15.15 mg, 0.099 mmol) followed by Et₃N (0.014 mL, 0.099 mmol) to afford the desired product 29 (29 mg, 0.071 mmol, 72% yield) as white solid. \(^1\)H NMR (500 MHz, CD₃OD) \(\delta\): 7.96 (dd, \(J = 8.5\) Hz, \(J = 7.0\) Hz, 1H), 7.77 (d, \(J = 2.0\) Hz,
1H), 7.68 (d, J = 8.0 Hz, 1H), 7.64 (t, J = 8.0 Hz, 1H), 7.57 (dd, J = 8.0 Hz, J = 2.0 Hz, 1H), 7.39 (d, J = 9.0 Hz, 2H), 7.25 (d, J = 8.5 Hz, 2H), 7.06 (d, J = 7.5 Hz, 1H), 7.04 (d, J = 9.0 Hz, 1H), 3.68 (t, J = 7.0 Hz, 4H), 3.44 (s, 3H), 2.15 (quintet, J = 3.0 Hz, 4H).

1-(4-Chlorophenyl)-1,3-dimethyl-3-(3-(6-(pyrrolidin-1-yl)pyridin-2-yl)phenyl)urea (30): To the solution of 10a (50 mg, 0.127 mmol) in DMF (3 mL) was added NaH (9.16 mg, 0.382 mmol) at 0 °C and stirred for 30 min. To the reaction mixture was added iodomethane (0.018 mL, 0.280 mmol) and stirred for 3 hrs. The reaction mixture was diluted in 20 mL DCM and 20 mL water. The organic layer was separated, washed with water (2x20 mL) and brine, dried (MgSO₄) and concentrated under reduced pressure. The crude product was purified by silica gel chromatography using 10-50% ethyl acetate : hexanes to afford the desired product 10a (42 mg, 0.100 mmol, 78% yield) as white solid. ¹H NMR (500 MHz, CD₃OD) δ: 7.96 (dd, J = 9.0 Hz, J = 2.0 Hz, 1H), 7.38 (t, J = 1.5 Hz, 1H), 7.37 (d, J = 1.5 Hz, 1H), 7.19 (dd, J = 5.5 Hz, J = 2.0 Hz, 1H), 7.17 (br s, 1H), 7.10 (d, J = 2.0 Hz, 1H), 7.08 (d, J = 2.0 Hz, 1H), 7.06 (s, 1H), 6.87 (dd, J = 7.0 Hz, J = 2.0 Hz, 2H), 6.82 (d, J = 7.0 Hz, 1H), 3.68 (t, J = 7.0 Hz, 4H), 3.23 (s, 3H), 3.20 (s, 3H), 2.16 (quintet, J = 3.5 Hz, 4H).
3-(6-(Pyrrolidin-1-yl)pyridin-2-yl)phenol (32): To a solution of 2-bromo-6-(pyrrolidin-1-yl)pyridine (3 g, 13.21 mmol) and 3-hydroxyphenylboronic acid (1.822 g, 13.21 mmol) in 1,2-dimethoxyethane (22 mL) was added sodium bicarbonate (3.33 g, 39.6 mmol) and water (22 mL). Argon was bubbled through the reaction solution for 2 min. Pd(Ph₃P)₄ (0.153 g, 0.132 mmol) was added to the reaction mixture and heated to reflux for 4 h. The reaction mixture was diluted with 100 mL ethyl acetate and 100 mL water. The organic layer was separated, washed with 2x50mL water and brine, dried over MgSO₄ and concentrated under reduced pressure. The crude product was purified using silica gel chromatography by eluting with 2-70% ethyl acetate : hexanes to afford 32 (2.52 g, 10.49 mmol, 79 % yield). ¹H NMR (500 MHz, CD₃OD) δ: 7.55-7.45 (m, 2H), 7.43 (d, J = 8.0 Hz, 1H), 7.22 (t, J = 8.0 Hz, 1H), 6.96 (dd, J = 7.5 Hz, J = 5.5 Hz, 1H), 6.79 (d, J = 7.5 Hz, 1H), 6.40-6.32 (m, 1H), 3.50 (br s, 4H), 2.0 (br s, 4H).

3-(6-(Pyrrolidin-1-yl)pyridin-2-yl)phenyl (4-chlorophenyl)carbamate (33): To a solution 32 (25 mg, 0.104 mmol) in DCM (3 mL) was added 1-chloro-4-isocyanatobenzene (15.98 mg, 0.104 mmol) and stirred at 0 °C for 16 h. Reaction was diluted in 20 mL DCM and 20 mL water. The organic layer was separated, washed with water (2x20 mL), dried (MgSO₄) and concentrated under reduced pressure. The crude product was purified using silica gel chromatography by eluting with 10-50% ethyl acetate : hexanes to afford the desired product 33 (25 mg, 0.063
mmol, 61% yield) as white solid. $^1$H NMR (500 MHz, CD$_3$OD) $\delta$: 7.97 (dd, $J = 9.0$ Hz, $J = 7.5$ Hz, 1H), 7.66 (d, $J = 2.0$ Hz, 1H), 7.65 (dd, $J = 3.5$ Hz, $J = 2.0$ Hz, 1H), 7.62 (d, $J = 8.5$ Hz, 1H), 7.51 (d, $J = 8.5$ Hz, 2H), 7.44 (td, $J = 7.5$ Hz, $J = 2.0$ Hz, 1H), 7.31 (d, $J = 9.5$ Hz, 2H), 7.06 (d, $J = 4.0$ Hz, 1H), 7.05 (d, $J = 2.5$ Hz, 1H), 3.69 (t, $J = 6.5$ Hz, 4H), 2.15 (quintet, $J = 3.5$ Hz, 4H).

1-(4-Chlorophenyl)-3-(3-(6-(pyrrolidin-1-yl)pyridin-2-yl)phenyl)thiourea (34): To a solution of 10a (50 mg, 0.127 mmol) in toluene (3 mL) was added Lawesson's Reagent (61.8 mg, 0.153 mmol) and refluxed for 16 hs. Only unreacted starting material was observed.

1-(4-Chlorophenyl)-3-(3-(6-(pyrrolidin-1-yl)pyridin-2-yl)phenyl)thiourea (34): To a solution of 6 (25 mg, 0.104 mmol) in DCM (5 mL) was added 1-chloro-4-isothiocyanatobenzene (17.72 mg, 0.104 mmol) followed by Et$_3$N (0.015 mL, 0.104 mmol) and stirred at room temperature for overnight. The reaction was diluted with 20 mL DCM and 20 mL water. The organic layer was separated, washed with water (2x20 mL), dried (MgSO$_4$) and concentrated under reduced pressure. The crude product was purified by silica gel chromatography eluting with 10-50% ethyl acetate : hexanes to afford the desired product 34 (24 mg, 0.059 mmol, 56% yield) as a white solid. $^1$H NMR (500 MHz, CD$_3$OD) $\delta$: 8.03 (s, 1H), 7.94 (dd, $J = 9.0$ Hz, $J = 7.0$ Hz, 1H), 7.60 (td, $J = 7.0$ Hz, $J = 2.0$ Hz, 1H), 7.58-7.51 (m, 2H), 7.48 (d, $J = 9.0$ Hz, 2H), 7.35 (d, $J = 9.0$ Hz, 2H), 7.01 (t, $J = 8.0$ Hz, 2H), 3.66 (t, $J = 6.0$ Hz, 4H), 2.15 (quintet, $J = 3.0$ Hz, 4H).
(Z)-Phenyl N-(4-chlorophenyl)-N’-cyanocarbamimidate (36a): To a solution of 4-chloroaniline (268 mg, 2.099 mmol) in acetonitrile (21 mL) was added diphenyl cyanocarbonimidate (500 mg, 2.099 mmol) and heated to 50 °C for overnight. The reaction was concentrated and crystallized from 50 mL diethyl ether to afford the desired product 36a (420 mg, 1.546 mmol, 74% yield) as a white solid. $^1$H NMR (500 MHz, DMSO) $\delta$: 10.92 (s, 1H), 7.51 (d, $J = 8.5$ Hz, 2H), 7.48 (t, $J = 2.5$ Hz, 1H), 7.46 (d, $J = 2.5$ Hz, 1H), 7.44 (d, $J = 8.5$ Hz, 2H), 7.33-7.28 (m, 3H).

(Z)-1-(4-Chlorophenyl)-2-cyano-3-(3-(6-(pyrrolidin-1-yl)pyridin-2-yl)phenyl)guanidine (37): 36a (50 mg, 0.184 mmol) and 6 (44.0 mg, 0.184 mmol) were dissolved in acetonitrile (5 mL) and refluxed for overnight. No conversion of starting material was observed and the reaction mixture was discarded.

(Z)-1-(4-Chlorophenyl)-2-cyano-3-(3-(6-(pyrrolidin-1-yl)pyridin-2-yl)phenyl)guanidine (37): 36a (50 mg, 0.184 mmol) and 6 (44.0 mg, 0.184 mmol) were dissolved in acetonitrile (3 mL) and irradiated at 140 °C for 30 min. No conversion of starting material was observed and the reaction mixture was discarded.
(Z)-1-(4-chlorophenyl)-2-cyano-3-(3-(6-(pyrrolidin-1-yl)pyridin-2-yl)phenyl)guanidine (37): 36a and 6 (44.0 mg, 0.184 mmol) were dissolved in DMF (3 mL) and irradiated at 150 °C for 30 min. No conversion of starting material was observed and reaction was discarded.

(Z)-1-(4-Chlorophenyl)-2-cyano-3-(3-(6-(pyrrolidin-1-yl)pyridin-2-yl)phenyl)guanidine (37): 36a (50 mg, 0.184 mmol) and 6 (44.0 mg, 0.184 mmol) were dissolved in DMF (3 mL) and irradiated at 170 °C for 1 h. Major desired product was observed with LC-MS. The reaction mixture was diluted with 20 mL DCM and 20 mL water. The organic layer was separated, washed with water (2x20 mL), dried (MgSO₄) and concentrated under reduced pressure. The crude product was purified by silica gel chromatography eluting with 10-50% ethyl acetate : hexanes to afford the desired product 37 (20 mg, 0.048 mmol, 52% yield) as a white solid. ¹H NMR (500 MHz, CD₃OD) δ: 8.0-7.92 (m, 1H), 7.86 (br s, 1H), 7.76 (d, J = 8.0 Hz, 1H), 7.64 (t, J = 8.0 Hz, 1H), 7.58-7.55 (m, 1H), 7.52 (t, J = 2.5 Hz, 1H), 7.51-7.49 (m, 1H), 7.43 (d, J = 9.0 Hz, 1H), 7.37 (d, J = 2.5 Hz, 1H), 7.07-7.0 (m, 2H), 3.68 (t, J = 7.0 Hz, 4H), 2.14 (quintet, J = 3.5 Hz, 4H).

3-((4-Chlorophenyl)amino)-4-ethoxycyclobut-3-ene-1,2-dione (39): To a solution of 4-chloroaniline (375 mg, 2.94 mmol) in ethanol (10 mL) was added 3,4-diethoxycyclobut-3-ene-1,2-dione (500 mg, 2.94 mmol) and stirred for 48 h at room temperature. The reaction mixture was concentrated and the product was crystallized by adding 20 mL diethyl ether to afford white solid 39 (668 mg, 2.65 mmol, 90% yield).
3-((4-Chlorophenyl)amino)-4-((3-(6-(pyrrolidin-1-yl)pyridin-2-yl)phenyl)amino)cyclobut-3ene-1,2-dione (40): A mixture of 39 (25 mg, 0.099 mmol) and 6 (23.77 mg, 0.099 mmol) were dissolved in ethanol (5 mL) and stirred at room temperature for overnight. No conversion of starting material was observed and starting material was recovered from the reaction mixture.

3-((4-Chlorophenyl)amino)-4-((3-(6-(pyrrolidin-1-yl)pyridin-2-yl)phenyl)amino)cyclobut-3ene-1,2-dione (40): A mixture of 39 (25 mg, 0.099 mmol) and 6 (23.77 mg, 0.099 mmol) were dissolved in ethanol (5 mL) and heated to 80 °C for overnight. No conversion of starting material was observed and starting material was recovered from the reaction mixture.

3-((4-Chlorophenyl)amino)-4-((3-(6-(pyrrolidin-1-yl)pyridin-2-yl)phenyl)amino)cyclobut-3ene-1,2-dione (40): A mixture of 39 (25 mg, 0.099 mmol) and 6 (23.77 mg, 0.099 mmol) were dissolved in ethanol (5 mL) and irradiated at 100 °C for 30 min. No conversion of starting material was observed and starting material was recovered from the reaction mixture.

3-((4-Chlorophenyl)amino)-4-((3-(6-(pyrrolidin-1-yl)pyridin-2-yl)phenyl)amino)cyclobut-3ene-1,2-dione (40): A mixture of 39 (25 mg, 0.099 mmol) and 6 (23.77 mg, 0.099 mmol) were dissolved in DMF (3 mL) and irradiated at 150 °C for 30 min. No conversion of starting material was observed and the starting material was recovered from the reaction mixture.

3-((4-Chlorophenyl)amino)-4-((3-(6-(pyrrolidin-1-yl)pyridin-2-yl)phenyl)amino)cyclobut-3ene-1,2-dione (40): A mixture of 39 (25 mg, 0.099 mmol) and 6 (23.77 mg, 0.099 mmol) was
dissolved in DMSO (3 mL) and irradiated at 150 °C for 30 min. No conversion of starting material was observed and the starting material was recovered from the reaction mixture.

3-((4-Chlorophenyl)amino)-4-((3-(6-(pyrrolidin-1-yl)pyridin-2-yl)phenyl)amino)cyclobut-3-ene-1,2-dione (40): To the solution of 39 (25 mg, 0.099 mmol) and 6 (23.77 mg, 0.099 mmol) in DCM (5 mL) was added trimethylaluminum (0.099 mL, 0.099 mmol) at 0 °C and stirred for 1 h. The complete conversion of starting material was observed. The reaction mixture was diluted with 20 mL DCM and 20 mL 10% Rochelle salt solution. The organic layer was separated, washed with water (2x50 mL), dried (MgSO₄) and concentrated under reduced pressure. The crude product was purified by silica gel chromatography eluting with 10-50% ethyl acetate : hexanes to afford the desired product 40 (21 mg, 0.047 mmol, 47% yield) as a white solid. ¹H NMR (500 MHz, CD₃OD) δ: 8.35 (s, 1H), 7.88 (t,  J = 6.0 Hz, 1H), 7.58 (d,  J = 8.0 Hz, 1H), 7.55-7.50 (m, 3H especially d, 7.53,  J = 2.5 Hz, 1H), 7.45 (d,  J = 6.5 Hz, 1H), 7.36 (d,  J = 9.0 Hz, 2H), 7.13 (d,  J = 7.0 Hz, 1H), 6.95-6.88 (m, 1H), 3.71 (br s, 4H), 2.15 (quintet,  J = 3.5 Hz, 4H).

Methyl 3-(6-(pyrrolidin-1-yl)pyridin-2-yl)benzoate (42): To a solution of 2-bromo-6-(pyrrolidin-1-yl)pyridine (250 mg, 1.101 mmol) and 3-(methoxycarbonyl)phenylboronic acid (198 mg, 1.101 mmol) in 1,2-Dimethoxyethane (5 mL) was added sodium bicarbonate (185 mg, 2.202 mmol) and Water (5 mL). Argon was bubbled through reaction solution for 5 min. Pd(Ph₃P)₄ (6.36 mg, 5.50 µmol) was added to the reaction mixture and heated to refluxed for 6
hrs. Reaction was diluted with 50 mL ethyl acetate and 50 mL water. The organic layer was separated, washed with 2x50 mL water, dried over MgSO₄ and concentrated under reduced pressure. Crude product was purified by silica gel chromatography eluting with 2-70% ethyl acetate : hexanes to afford the desired product 42 (210 mg, 0.744 mmol, 68% yield) as a white solid. ¹H NMR (500 MHz, CD₃OD) δ: 8.38 (t, J = 2.0 Hz, 1H), 8.23 (td, J = 7.5 Hz, J = 1.5 Hz, 1H), 8.02-7.97 (m, 2H), 7.71 (t, J = 8.0 Hz, 1H), 7.08 (t, J = 8.0 Hz, 2H), 3.96 (s, 3H), 3.71 (t, J = 7.0 Hz, 4H), 2.17 (quintet, J = 3.5 Hz, 4H).

3-(6-(Pyrrolidin-1-yl)pyridin-2-yl)benzohydrazide (43): To a solution of 42 (100 mg, 0.354 mmol) in ethanol (5 mL) was added hydrazine hydrate (0.056 mL, 1.771 mmol) and refluxed for 6 h. The reaction was concentrated under reduced pressure to afford 43 and carried to the next step without any purification. ¹H NMR (500 MHz, DMSO) δ: 9.87 (s, 1H), 8.45 (t, J = 1.5 Hz, 1H), 8.20 (td, J = 8.0 Hz, J = 1.5 Hz, 1H), 7.82 (td, J = 8.0 Hz, J = 1.5 Hz, 1H), 7.59 (t, J = 8.0 Hz, 1H), 7.52 (t, J = 8.0 Hz, 1H), 7.19 (d, J = 7.5 Hz, 1H), 6.44 (d, J = 8.0 Hz, 1H), 4.58 (s, 2H, NH₂), 3.48 (t, J = 6.0 Hz, 4H), 1.97 (quintet, J = 3.0 Hz, 4H).
**N-(4-Chlorophenyl)-5-(3-(6-(pyrrolidin-1-yl)pyridin-2-yl)phenyl)-1,3,4-oxadiazol-2-amine (44):** A mixture of 43 (25 mg, 0.089 mmol) and 1-chloro-4-isothiocyanatobenzene (15.0 mg, 0.089 mmol) in THF (2 mL) was heated at refluxed for overnight. The reaction was concentrated under reduced pressure and crude product was purified by silica gel chromatography eluting with 10-50% ethyl acetate : hexanes to afford the desired product 44 (14 mg, 0.034 mmol, 38% yield) as white solid. \(^1\)H NMR (500 MHz, CD\(_3\)OD + CDCl\(_3\)) \(\delta\): 8.62 (t, \(J = 1.0\) Hz, 1H), 8.17 (dd, \(J = 3.0\) Hz, \(J = 1.0\) Hz, 1H), 7.97 (td, \(J = 2.5\) Hz, \(J = 1.5\) Hz, 1H), 7.59 (dt, \(J = 7.5\) Hz, \(J = 1.0\) Hz, 2H), 7.55 (dd, \(J = 7.0\) Hz, \(J = 2.0\) Hz, 2H), 7.34 (dd, \(J = 6.5\) Hz, \(J = 2.5\) Hz, 2H), 7.08 (d, \(J = 7.5\) Hz, 1H), 6.43 (d, \(J = 8.0\) Hz, 1H), 3.58 (t, \(J = 6.5\) Hz, 4H), 1.70 (quintet, \(J = 3.0\) Hz, 4H).

**2-(3-Isothiocyanatophenyl)-6-(pyrrolidin-1-yl)pyridine (45):** To a solution of 6 (100 mg, 0.418 mmol) in DCM (5 mL) was added thiophosgene (0.032 mL, 0.418 mmol) at 0 °C and stirred for 3 h. The reaction mixture was concentrated under reduced pressure to afford the desired product 45 (80 mg, 0.284 mmol, 68% yield) which was carried to the next step without any purification.
5-(4-Chlorophenyl)-N-(3-(6-(pyrrolidin-1-yl)pyridin-2-yl)phenyl)-1,3,4-oxadiazol-2-amine (47): A mixture of 45 (25 mg, 0.089 mmol) and 4-chlorobenzohydrazide (15 mg, 0.089 mmol) in THF (2 mL) was heated at refluxed for overnight. The reaction was concentrated under reduced pressure and the crude product was purified by silica gel chromatography eluting with 10-50% ethyl acetate : hexanes to afford the desired product 47 (11 mg, 0.026 mmol, 30% yield) as a white solid. $^1$H NMR (500 MHz, CD3OD + CDCl3) $\delta$: 8.26 (d, $J = 1.5$ Hz, 1H), 7.97 (dd, $J = 6.5$ Hz, $J = 2.0$ Hz, 2H), 7.71 (dd, $J = 6.5$ Hz, $J = 1.0$ Hz, 1H), 7.65-7.50 (m, 4H), 7.44 (t, $J = 8.0$ Hz, 1H), 7.08 (d, $J = 8.0$ Hz, 1H), 6.44 (d, $J = 8.0$ Hz, 1H), 4.28 (t, $J = 6.5$ Hz, 3H), 3.85 (t, $J = 5.0$ Hz, 4H), 3.61 (t, $J = 5.0$ Hz, 4H).

(3-(3-(4-Chlorophenyl)ureido)phenyl)boronic acid (49): To a solution of 3-aminophenylboronic acid (250 mg, 1.826 mmol) in DCM (10 mL) was added 1-chloro-4-isocyanatobenzene (280 mg, 1.826 mmol) at room temperature. Poor solubility of starting material was observed. DMF (10 mL) was added to the reaction mixture to obtained clear solution and stirred for 3 h at room temperature. The reaction mixture was diluted with 50 mL DCM and 50 mL water. The organic layer was separated, washed with water (2x50 mL), dried (MgSO$_4$) and concentrated under reduced pressure. The crude product was purified by silica gel
chromatography eluting with 10-50% ethyl acetate : hexanes to afford the desired product 49 (280 mg, 0.964 mmol, 53% yield). $^1$H NMR (500 MHz, DMSO) $\delta$: 8.77 (s, 1H), 8.60 (s, 1H), 8.01 (s, 2H), 7.67 (s, 1H), 7.60 (dd, $J = 8.0$ Hz, $J = 1.0$ Hz, 1H), 7.49 (d, $J = 9.0$ Hz, 2H), 7.41 (d, $J = 7.0$ Hz, 1H), 7.32 (d, $J = 9.0$ Hz, 2H), 7.25 (t, $J = 7.0$ Hz, 1H).

![Chemical Structure](attachment:image.png)

**2-(4-Nitrophenyl)-6-(pyrrolidin-1-yl)pyridine (51):** To a solution of 2-bromo-6-(pyrrolidin-1-yl)pyridine (250 mg, 1.101 mmol) and 4-nitrophenylboronic acid (184 mg, 1.101 mmol) in 1,2-dimethoxyethane (5 mL) was added sodium bicarbonate (185 mg, 2.202 mmol) and water (5 mL). Argon was bubbled through the reaction solution for 5 min. Pd(Ph$_3$P)$_4$ (6.36 mg, 5.50 µmol) was added to the reaction mixture and heated to reflux for 6 h. The reaction mixture was diluted with 50 mL ethyl acetate and 50 mL water. The organic layer was separated, washed with 2x50 mL water and brine, dried over MgSO$_4$ and concentrated under reduced pressure. The crude product was purified by silica gel chromatography eluting with 2-70% ethyl acetate : hexanes to give 51 (240 mg, 0.891 mmol, 81% yield) as a yellow solid. $^1$H NMR (500 MHz, DMSO) $\delta$: 8.33-8.26 (m, 4H), 7.63 (dd, $J = 8.0$ Hz, $J = 7.0$ Hz, 1H), 7.27 (d, $J = 7.5$ Hz, 1H), 6.52 (d, $J = 8.0$ Hz, 1H), 3.47 (t, $J = 6.0$ Hz, 4H), 1.97 (quintet, $J = 3.0$ Hz, 4H).
4-(6-(Pyrrolidin-1-yl)pyridin-2-yl)aniline (52): To the solution of 2-(4-nitrophenyl)-6-(pyrrolidin-1-yl)pyridine (100 mg, 0.371 mmol) in ethanol (20 mL) and THF (10 mL) was added Pd/C (3.95 mg, 3.71 µmol) under argon atmosphere. Catalytic Acetic acid was added to the reaction mixture and stirred for overnight under hydrogen gas atmosphere under balloon pressure. Pd/C was filtered off and the filtrate was concentrated and was purified by silica gel chromatography eluting with 2-70% ethyl acetate : hexanes to give 52 (75 mg, 0.313 mmol, 84% yield) as a light yellow oil. $^1$H NMR (500 MHz, DMSO) δ: 7.75 (d, $J = 8.5$ Hz, 2H), 7.44 (t, $J = 8.0$ Hz, 1H), 6.91 (d, $J = 7.5$ Hz, 1H), 6.59 (d, $J = 8.5$ Hz, 2H), 6.23 (d, $J = 8.0$ Hz, 1H), 5.29 (s, 2H), 3.43 (t, $J = 6.5$ Hz, 4H), 1.95 (quintet, $J = 3.0$ Hz, 4H).

1-(4-Chlorophenyl)-3-(4-(6-(pyrrolidin-1-yl)pyridin-2-yl)phenyl)urea (53): A mixture of 52 (25 mg, 0.104 mmol), Et$_3$N (0.044 mL, 0.313 mmol) and 1-chloro-4-isocyanatobenzene (16.04 mg, 0.104 mmol) in THF (3 mL) was stirred at room temperature for 3 h under inert atmosphere. Reaction mixture was concentrated and purified by silica gel chromatography eluting with 10-50% ethyl acetate : hexanes to afford the desired product 53 (14 mg, 0.036 mmol, 34% yield) as a white solid. $^1$H NMR (500 MHz, CD$_3$OD) δ: 7.96 (d, $J = 9.5$ Hz, 2H), 7.53-7.48 (m, 3H), 7.44 (dd, $J = 7.0$ Hz, $J = 2.0$ Hz, 2H), 7.28 (dd, $J = 6.5$ Hz, $J = 2.0$ Hz, 2H), 7.0 (d, $J = 2.5$ Hz, 1H), 6.36 (d, $J = 8.5$ Hz, 1H), 3.53 (t, $J = 7.0$ Hz, 4H), 2.04 (quintet, $J = 3.5$ Hz, 4H).
2-(2-Nitrophenyl)-6-(pyrrolidin-1-yl)pyridine (55): To a solution of 3 (250 mg, 1.101 mmol) and 2-nitrophenylboronic acid (184 mg, 1.101 mmol) in 1,2-Dimethoxyethane (5 mL) was added sodium bicarbonate (185 mg, 2.202 mmol) and water (5 mL). Argon was bubbled through reaction solution for 5 min. Pd(Ph$_3$P)$_4$ (6.36 mg, 5.50 µmol) was added to the reaction mixture and heated to refluxed for 6 h. The reaction mixture was diluted with 50 mL ethyl acetate and 50 mL water. The organic layer was separated, washed with 2x50mL water and brine, dried over MgSO$_4$ and concentrated under reduced pressure. The crude product was purified by silica gel chromatography eluting with 2-70% ethyl acetate : hexanes to give 55 (230 mg, 0.854 mmol, 78% yield) as a yellow solid. $^1$H NMR (500 MHz, CD$_3$OD) δ: 8.29 (dd, $J$ = 8.0 Hz, $J$ = 1.0 Hz, 1H), 7.96 (dd, $J$ = 8.5 Hz, $J$ = 6.5 Hz, 1H), 7.89 (dt, $J$ = 8.0 Hz, $J$ = 1.5 Hz, 1H), 7.83 (dt, $J$ = 8.0 Hz, $J$ = 1.5 Hz, 1H), 7.70 (dd, $J$ = 8.0 Hz, $J$ = 1.5 Hz, 1H), 7.09 (d, $J$ = 9.5 Hz, 1H), 6.84 (d, $J$ = 7.5 Hz, 1H), 3.59 (t, $J$ = 7.0 Hz, 4H), 2.14 (quintet, $J$ = 3.5 Hz, 4H).

2-(6-(Pyrrolidin-1-yl)pyridin-2-yl)aniline (56): The reaction was carried out similar to 52 using a solution of 55 (100 mg, 0.371 mmol) in ethanol (20 mL) and THF (10 mL), Pd/C (3.95 mg, 3.71 µmol) and catalytic acetic acid under hydrogen gas atmosphere under balloon pressure to afford 56 (80 mg, 0.334 mmol, 90% yield) as a light yellow oil. $^1$H NMR (500 MHz, DMSO) δ: 7.92 (dd, $J$ = 8.5 Hz, $J$ = 7.0 Hz, 1H), 7.46 (dd, $J$ = 7.5 Hz, $J$ = 1.5 Hz, 1H), 7.34 (dt, $J$ = 8.5 Hz,
$J = 1.5\text{Hz, 1H}$, 7.06-6.99 (m, 3H), 6.91 (d, $J = 9.5\text{ Hz, 1H}$), 3.60 (t, $J = 6.5\text{ Hz, 4H}$), 2.14 (quintet, $J = 3.5\text{ Hz, 4H}$).

![Chemical structure]

1-(4-Chlorophenyl)-3-(2-(6-(pyrrolidin-1-yl)pyridin-2-yl)phenyl)urea (57): A mixture of 56 (25 mg, 0.104 mmol) and 1-chloro-4-isocyanatobenzene (16.04 mg, 0.104 mmol) was stirred at room temperature for 3 h under inert atmosphere. The reaction mixture was concentrated and purified by silica gel chromatography eluting with 10-50% ethyl acetate : hexanes to afford the desired product 57 (22 mg, 0.056 mmol, 54% yield) as a white solid. $^1$H NMR (500 MHz, CD$_3$OD) $\delta$: 7.95 (dd, $J = 9.5\text{ Hz, } J = 7.5\text{ Hz, 1H}$), 7.68 (dd, $J = 8.5\text{ Hz, } J = 1.5\text{ Hz, 1H}$), 7.57 (dt, $J = 8.0\text{ Hz, } J = 2.0\text{ Hz, 1H}$), 7.52 (dd, $J = 8.0\text{ Hz, } J = 1.5\text{ Hz, 2H}$), 7.36 (dd, $J = 7.5\text{ Hz, } J = 1.0\text{ Hz, 2H}$), 7.33 (dd, $J = 6.5\text{ Hz, } J = 2.0\text{ Hz, 2H}$), 7.01 (d, $J = 9.0\text{ Hz, 1H}$), 6.89 (d, $J = 7.0\text{ Hz, 1H}$), 3.59 (t, $J = 7.0\text{ Hz, 4H}$), 2.07 (quintet, $J = 3.5\text{ Hz, 4H}$).
4-Bromo-2-(pyrrolidin-1-yl)pyridine (59): To a solution of 4-bromo-2-chloropyridine (500 mg, 2.60 mmol) in DMF (5 mL) was added pyrrolidine (277 mg, 3.90 mmol) followed by K₂CO₃ (718 mg, 5.20 mmol) and heated to 100 °C for 7 h. No conversion of starting material to the desired product was observed and reaction was discarded.

4-Bromo-2-(pyrrolidin-1-yl)pyridine (59): To a solution of 4-bromo-2-chloropyridine (100 mg, 0.520 mmol) in DMF (3 mL) was added pyrrolidine (44.3 mg, 0.624 mmol) followed by K₂CO₃ (144 mg, 1.039 mmol) and irradiated at 160 °C for 3 h. No conversion of starting material to the desired product was observed and reaction was discarded.

4-Bromo-2-(pyrrolidin-1-yl)pyridine (59): To a solution of 4-bromo-2-chloropyridine (100 mg, 0.520 mmol), pyrrolidine (44.3 mg, 0.624 mmol) and sodium tert-butoxide (110 mg, 1.143 mmol) in DMF (3 mL) was added Pd₂(dba)₃ (9.52 mg, 10.39 µmol) and BINAP (12.94 mg, 0.021 mmol) under inert atmosphere and heated to 100 °C for overnight. Very low conversion of starting material was observed and reaction was discarded.

4-Bromo-2-(pyrrolidin-1-yl)pyridine (59): To a solution of 4-bromo-2-chloropyridine (100 mg, 0.520 mmol), pyrrolidine (44.3 mg, 0.624 mmol) and sodium tert-butoxide (110 mg, 1.143 mmol) in DMF (3 mL) was added Pd₂(dba)₃ (9.52 mg, 10.39 µmol) and BINAP (12.94 mg, 0.021 mmol) under inert atmosphere and irradiated at 100 °C for 1 h. Very low conversion of starting material was observed and reaction was discarded.
4-Bromo-2-(pyrrolidin-1-yl)pyridine (59): To a solution of 4-bromo-2-chloropyridine (100 mg, 0.520 mmol), pyrrolidine (44.3 mg, 0.624 mmol), L-proline (2.99 mg, 0.026 mmol) and K$_2$CO$_3$ (144 mg, 1.039 mmol) in DMF (3 mL) was added copper(I) iodide (4.95 mg, 0.026 mmol) under inert condition and heated to 100 °C for overnight. No conversion of starting material to the desired product was observed and reaction was discarded.

4-Bromo-2-(pyrrolidin-1-yl)pyridine (59): To a solution of 4-bromo-2-chloropyridine (100 mg, 0.520 mmol), pyrrolidine (44.3 mg, 0.624 mmol), L-proline (2.99 mg, 0.026 mmol) and K$_2$CO$_3$ (144 mg, 1.039 mmol) in DMF (3 mL) was added copper(I) iodide (4.95 mg, 0.026 mmol) under inert condition and irradiated at 100 °C for 1h. No conversion of starting material to the desired product was observed and reaction was discarded.

4-Bromo-2-(pyrrolidin-1-yl)pyridine (59): A mixture of 4-bromo-2-chloropyridine (100 mg, 0.520 mmol) and pyrrolidine (73.9 mg, 1.039 mmol) was stirred at room temp for overnight. No conversion of starting material to the desired product was observed and reaction was discarded.

4-Bromo-2-(pyrrolidin-1-yl)pyridine (59): A mixture of 4-bromo-2-chloropyridine (100 mg, 0.520 mmol) and pyrrolidine (73.9 mg, 1.039 mmol) was refluxed for overnight. No conversion of starting material to the desired product was observed and reaction was discarded.

4-Bromo-2-(pyrrolidin-1-yl)pyridine (59): To a mixture of 4-bromo-2-chloropyridine (100 mg, 0.520 mmol), pyrrolidine (44.3 mg, 0.624 mmol), L-proline (2.99 mg, 0.026 mmol) and K$_2$CO$_3$ (144 mg, 1.039 mmol) was added copper(I) iodide (4.95 mg, 0.026 mmol) under inert condition and heated to 100 °C for overnight. No conversion of starting material to the desired product was observed and reaction was discarded.
**4-Bromo-2-(pyrrolidin-1-yl)pyridine (59):** To a solution of 4-bromo-2-fluoropyridine (250 mg, 1.421 mmol) in DMF (Volume: 5 mL) was added pyrrolidine (101 mg, 1.421 mmol) followed by K₂CO₃ (196 mg, 1.421 mmol) and heated to 100 °C for 6 h. The reaction mixture was diluted with 50 mL DCM and 50 mL water. The organic layer was separated, washed with water (2x20 mL), dried (MgSO₄) and concentrated under reduced pressure. The crude product was purified by silica gel chromatography eluting with 10-50% ethyl acetate : hexanes to afford the desired product 59 (190 mg, 0.837 mmol, 59% yield). ¹H NMR (500 MHz, DMSO) δ: 7.93 (d, J = 5.0 Hz, 1H), 6.71 (dd, J = 5.5 Hz, J = 1.5 Hz, 1H), 6.62 (d, J = 1.5 Hz, 1H), 3.36 (t, J = 6.5 Hz, 4H), 1.92 (quintet, J = 3.0 Hz, 4H).

![Chemical Structure of 4-Bromo-2-(pyrrolidin-1-yl)pyridine](image)

**1-(4-Chlorophenyl)-3-(3-(2-(pyrrolidin-1-yl)pyridin-4-yl)phenyl)urea (60):** To a solution of 49 (25 mg, 0.086 mmol) and 59 (23.45 mg, 0.103 mmol) in 1,2-dimethoxyethane (3 mL) was added sodium bicarbonate (14.46 mg, 0.172 mmol) and water (3 mL). Argon was bubbled through the reaction solution for 5 min. Pd(Ph₃P)₄ (4.97 mg, 4.30 µmol) was added to the reaction mixture and heated to refluxed for 4 h. The reaction solution was diluted with 20 mL ethyl acetate and 20 mL water. The organic layer was separated, washed with 2x20 mL water, dried over MgSO₄ and concentrated under reduced pressure. The crude product was purified by silica gel chromatography eluting with 2-70% ethyl acetate: hexanes to give 60 (18 mg, 0.046 mmol, 53% yield) as a solid. ¹H NMR (500 MHz, CD₃OD) δ: 8.04 (d, J = 5.0 Hz, 1H), 7.85 (td, J = 2.0 Hz, 1H), 7.44 (dd, J = 7.0 Hz, J = 2.0 Hz, 1H), 7.43 (t, J = 2.0 Hz, 1H), 7.42-7.40 (m,
1H), 7.38 (d, \( J = 8.5 \) Hz, 1H), 7.35 (td, \( J = 7.0 \) Hz, \( J = 1.5 \) Hz, 1H), 7.28 (dd, \( J = 7.0 \) Hz, \( J = 2.0 \) Hz, 2H), 6.83 (dd, \( J = 5.5 \) Hz, \( J = 1.5 \) Hz, 1H), 6.68 (d, \( J = 1.0 \) Hz, 1H), 3.50 (t, \( J = 6.5 \) Hz, 4H), 2.06 (quintet, \( J = 3.5 \) Hz, 4H).

5-Bromo-2-(pyrrolidin-1-yl)pyridine (63): To a solution of 5-bromo-2-chloropyridine (500 mg, 2.60 mmol) in DMF (Volume: 3 mL) was added pyrrolidine (222 mg, 3.12 mmol) followed by \( \text{K}_2\text{CO}_3 \) (718 mg, 5.20 mmol) and heated to 100 °C for 7 h. The reaction mixture was diluted with 50 mL DCM and 50 mL water. The organic layer was separated, washed with water (2x50 mL), dried (\( \text{MgSO}_4 \)) and concentrated under reduced pressure. The crude product was purified by silica gel chromatography eluting with 10-50% ethyl acetate : hexanes to afford the desired product 63 (380 mg, 1.673 mmol, 64% yield). \(^1\)H NMR (500 MHz, DMSO) \( \delta \): 8.10 (d, \( J = 2.5 \) Hz, 1H), 7.61 (dd, \( J = 9.0 \) Hz, \( J = 2.5 \) Hz, 1H), 6.42 (d, \( J = 9.0 \) Hz, 1H), 1.93 (quintet, \( J = 3.5 \) Hz, 4H).
1-(4-Chlorophenyl)-3-(3-(6-(pyrrolidin-1-yl)pyridin-3-yl)phenyl)urea (64): To a solution of 49 (50 mg, 0.172 mmol) and 63 (46.9 mg, 0.207 mmol) in 1,2-dimethoxyethane (3 mL) was added sodium bicarbonate (28.9 mg, 0.344 mmol) and water (3 mL). Argon was bubbled through reaction solution for 5 min. Pd(Ph₃P)₄ (13.92 mg, 0.012 mmol) was added to the reaction mixture and heated to refluxed for 4 h. The reaction mixture was diluted with 20 mL ethyl acetate and 20 mL water. The organic layer was separated, washed with 2x10 mL water, dried over MgSO₄ and concentrated under reduced pressure. Crude product was purified by silica gel chromatography eluting with 2-70% ethyl acetate: hexanes to give 64 (42 mg, 0.107 mmol, 62% yield) as a white solid. ¹H NMR (500 MHz, CD₃OD) δ: 8.28 (dd, J = 9.5 Hz, J = 2.5 Hz, 1H), 8.06 (d, J = 2.0 Hz, 1H), 7.91 (t, J = 2.0 Hz, 1H), 7.45 (dd, J = 7.0 Hz, J = 2.0 Hz, 2H), 7.42 (t, J = 8.0 Hz, 1H), 7.31 (d, J = 2.0 Hz, 1H), 7.30-7.28 (m, 2H), 7.27 (d, J = 4.0 Hz, 1H), 7.20 (d, J = 9.5 Hz, 1H), 3.65 (t, J = 6.5 Hz, 4H), 2.19 (quintet, J = 3.0 Hz, 4H).

1-(3-Bromophenyl)pyrrolidine (66): To a solution of 1,3-dibromobenzene (250 mg, 1.060 mmol) in DMF (5 mL) was added pyrrolidine (113 mg, 1.590 mmol) followed by K₂CO₃ (293 mg, 2.120 mmol) and heated to 100 °C for 7 h. No conversion of starting material to the desired product was observed and the reaction mixture was discarded.
**1-(3-Bromophenyl)pyrrolidine (66):** To a solution of 1-bromo-3-fluorobenzene (250 mg, 1.429 mmol) in DMF (Volume: 5 mL) was added pyrrolidine (122 mg, 1.714 mmol) followed by K$_2$CO$_3$ (395 mg, 2.86 mmol) and heated to 100 °C for 7 h. The reaction was diluted with 50 mL DCM and 50 mL water. The organic layer was separated, washed with water (2x20 mL), dried (MgSO$_4$) and concentrated under reduced pressure. The crude product was purified by silica gel chromatography eluting with 10-50% ethyl acetate : hexanes to afford the desired product 66 (226 mg, 1.000 mmol, 70% yield). $^1$H NMR (500 MHz, CD$_3$OD) $\delta$: 7.02 (t, $J = 8.0$ Hz, 1H), 6.68 (dd, $J = 8.0$ Hz, $J = 1.0$ Hz, 1H), 6.65 (t, $J = 2.0$ Hz, 1H), 6.48 (dd, $J = 8.5$ Hz, $J = 2.5$ Hz, 1H), 3.23 (t, $J = 6.5$ Hz, 4H), 2.01 (quintet, $J = 3.5$ Hz, 4H).

![Chemical structure](image)

**1-(4-Chlorophenyl)-3-(3'-(pyrrolidin-1-yl)-[1,1'-biphenyl]-3-yl)urea (67):** To a solution of 49 (25 mg, 0.086 mmol) and 66 (19.46 mg, 0.086 mmol) in 1,2-dimethoxyethane (3 mL) was added sodium bicarbonate (14.46 mg, 0.172 mmol) and Water (3 mL). Argon was bubbled through the reaction solution for 5 min. Pd(Ph$_3$P)$_4$ (4.97 mg, 4.30 µmol) was added to the reaction mixture and heated to refluxed for 4 h. The reaction was diluted with 20 mL ethyl acetate and 20 mL water. The organic layer was separated, washed with 2x20mL water, dried over MgSO$_4$ and concentrated under reduced pressure. The crude product was purified by silica gel chromatography eluting with 2-70% ethyl acetate: hexanes to give 67 (18 mg, 0.046 mmol, 53% yield) as a solid. $^1$H NMR (500 MHz, CD$_3$OD) $\delta$: 7.70 (t, $J = 1.5$ Hz, 1H), 7.44 (dd, $J = 2.0$ Hz, 2H), 7.36 (td, $J = 8.5$ Hz, $J = 2.0$ Hz, 1H), 7.32 (d, $J = 8.5$ Hz, 1H), 7.30-7.25 (m, 3H), 7.23 (t, $J
= 8.5 Hz, 1H), 6.86 (d, J = 7.0 Hz, 1H), 6.78 (d, J = 2.0 Hz, 1H), 6.58 (dd, J = 8.5 Hz, J = 2.0 Hz, 1H), 3.50 (t, J = 7.0 Hz, 4H), 2.04 (quintet, J = 3.5 Hz, 4H).

![Chemical Structure](attachment:image.png)

1-(3-(6-Bromopyridin-2-yl)phenyl)-3-(4-chlorophenyl)urea (68): To a solution of 49 (250 mg, 0.861 mmol) and 2,6-dibromopyridine (408 mg, 1.721 mmol) in 1,2-Dimethoxyethane (10 mL) was added sodium bicarbonate (217 mg, 2.58 mmol) and water (10 mL). Argon was bubbled through the reaction solution for 5 min. Pd(Ph₃P)₄ (6.96 mg, 6.02 µmol) was added to the reaction mixture and heated to refluxed for 6 h. The reaction mixture was diluted with 100 mL ethyl acetate and 100 mL water. The organic layer was separated, washed with 2x50mL water and brine, dried over MgSO₄ and concentrated under reduced pressure. The crude product was purified by silica gel chromatography eluting with 2-70% ethyl acetate: hexanes to give 68 (250 mg, 0.621 mmol, 72.1 % yield) as a white solid. ¹H NMR (500 MHz, CD₃OD) δ: 8.10 (t, J = 2.0 Hz, 1H), 7.85 (d, J = 8.0 Hz, 1H), 7.74 (t, J = 8.0 Hz, 1H), 7.67 (td, J = 8.0 Hz, J = 2.0 Hz, 1H), 7.56 (ddd, J = 8.5 Hz, J = 2.0 Hz, J = 1.0 Hz, 1H), 7.51 (d, J = 8.0 Hz, 1H), 7.45 (d, J = 9.0 Hz, 2H), 7.43 (t, J = 3.0 Hz, 1H), 7.41 (d, J = 1.5 Hz, 1H), 7.31 -7.27 (m, 2H especially d, 7.28, J = 2.0 Hz, 1H).
1-(4-Chlorophenyl)-3-(3-(6-morpholinopyridin-2-yl)phenyl)urea (70a): To a solution of 68 (50 mg, 0.124 mmol), morpholine (16.23 mg, 0.186 mmol) and sodium tert-butoxide (26.3 mg, 0.273 mmol) in toluene (2 mL) were added Pd$_2$(dba)$_3$ (2.274 mg, 2.483 µmol) and BINAP (3.09 mg, 4.97 µmol) under inert atmosphere and heated to 100 °C for overnight. Very low conversion of starting material was observed and the reaction was discarded.

1-(4-Chlorophenyl)-3-(3-(6-morpholinopyridin-2-yl)phenyl)urea (70a): To a solution of 68 (25 mg, 0.062 mmol), K$_2$CO$_3$ (12.87 mg, 0.093 mmol), L-proline (0.5 mg, 4.35 µmol) and morpholine (8.11 mg, 0.093 mmol) in DMF (Volume: 2 mL) was added copper(I)iodide (0.82 mg, 4.32 µmol) and irradiated at 150 °C for 30 min. The reaction was diluted with 20 mL DCM and 20 mL water. The organic layer was separated, washed with water (2x50 mL), dried (MgSO$_4$) and concentrated under reduced pressure. The crude product was purified by silica gel chromatography eluting with 10-50% ethyl acetate : hexanes to afford the desired product 70a (10 mg, 0.024 mmol, 39% yield) as a white solid. $^1$H NMR (500 MHz, CD$_3$OD + CDCl$_3$) δ: 8.08 (t, $J = 2.0$ Hz, 1H), 7.70-7.60 (m, 2H), 7.55 (dt, $J = 8.0$ Hz, $J = 3.5$ Hz, 1H), 7.48 (td, $J = 8.0$ Hz, $J = 1.5$ Hz, 1H), 7.46-7.41 (m, 2H), 7.37 (t, $J = 8.0$ Hz, 1H), 7.28 (t, $J = 3.0$ Hz, 1H), 7.20 (d, $J = 7.5$ Hz, 1H), 6.73 (d, $J = 8.0$ Hz, 1H), 3.85 (t, $J = 5.0$ Hz, 4H), 3.61 (t, $J = 5.0$ Hz, 4H).
1-(4-Chlorophenyl)-3-(3-(6-(piperazin-1-yl)pyridin-2-yl)phenyl)urea (70b): To a solution of 68 (50 mg, 0.124 mmol), piperazine (16.04 mg, 0.186 mmol) and sodium tert-butoxide (26.3 mg, 0.273 mmol) in toluene (2 mL) were added Pd$_2$(dba)$_3$ (2.274 mg, 2.483 µmol) and and BINAP (3.09 mg, 4.97 µmol) under inert atmosphere and heated to 100 °C for overnight. Very low conversion of starting material was observed and the starting material was recovered from the reaction mixture.

1-(4-Chlorophenyl)-3-(3-(6-(piperazin-1-yl)pyridin-2-yl)phenyl)urea (70b): The synthesis was carried out similar to 70a using a solution of 68 (25 mg, 0.062 mmol), K$_2$CO$_3$ (12.87 mg, 0.093 mmol), L-proline (0.5 mg, 4.35 µmol), copper(I)iodide (0.82 mg, 4.32 µmol) and piperazine (8.0 mg, 0.093 mmol) in DMF (Volume: 2 mL) was added to afford the desired product 70b (8 mg, 0.020 mmol, 32% yield) as a white solid. $^1$H NMR (500 MHz, CD$_3$OD) δ: 8.04 (t, $J = 1.5$ Hz, 1H), 7.66 (td, $J = 8.0$ Hz, $J = 1.5$ Hz, 1H), 7.56 (dd, $J = 8.5$ Hz, $J = 7.5$ Hz, 1H), 7.51 (ddd, $J = 8.5$ Hz, $J = 2.5$ Hz, $J = 1.0$ Hz, 1H), 7.44 (dd, $J = 7.0$ Hz, $J = 2.0$ Hz, 2H), 7.35 (t, $J = 8.0$ Hz, 1H), 7.30 (dd, $J = 7.0$ Hz, $J = 2.0$ Hz, 2H), 7.10 (d, $J = 8.0$ Hz, 1H), 6.71 (d, $J = 9.0$ Hz, 1H), 3.64 (d, $J = 5.0$ Hz, 4H), 1.70 (br s, 4H).
Appendix

All Binding Assays:

**rCB₁, mCB₂ and hCB₂ binding assay:**

Competitive binding assays were performed using rat brain containing CB₁ (rCB₁) or HEK293 cells transfected with mouse or human CB₂ (mCB₂ or hCB₂ respectively); membrane preparation has been previously described. Compounds to be tested (10 mM in DMSO) were diluted in TME buffer (50 mM Tris-HCl, 3 mM MgCl₂, 100 mM NaCl, 0.2 mM EDTA, pH 7.4) with 0.1% BSA and transferred to 96 well plates containing [3H]-CP55940 at a final concentration of 0.76 nM. Non-specific binding was assessed in the presence of 100 nM P55940. Binding was initiated with the addition of the respective membrane suspension (25 µg membrane protein/well) followed by incubation at 30°C in a shaking water bath for 1 hour. The interaction was terminated by rapid filtration of the membrane suspension over Unifilter GF/B- 96 Well Filter Plates (Perkin Elmer Life Sciences) using a Packard Filtermate-196 Cell Harvester. Filter plates were washed five times with ice-cold wash buffer (50 mM Tris-base, 5 mM MgCl₂ with 0.5% BSA) and bound radioactivity was determined using a Packard TopCount Scintillation Counter. The results were analyzed from one triplicate experiment using nonlinear regression to determine the IC50 of the ligand (Prizm by GraphPad Software, Inc.) and the Ki values were calculated by Prism from the IC50.
GPCR Functional assay:

cAMP assay:

**Human cannabinoid CB₁ receptor – agonist effect:**

The cells are suspended in HBSS buffer (Invitrogen) complemented with 20mM HEPES (pH 7.4), then distributed in microplates at a density of 104 cells/well in the presence of either of the following: HBSS (basal control), the reference agonist at 100 nM (stimulated control) or various concentrations (EC50 determination), or the test compounds. Thereafter, the adenylyl cyclase activator NKH 477 is added at a final concentration of 3 μM. Following 10 min incubation at 37°C, the cells are lysed and the fluorescence acceptor (D2-labeled cAMP) and fluorescence donor (anti cAMP antibody labeled with europium cryptate) are added. After 60 min at room temperature, the fluorescence transfer is measured at λex = 337 nm and λem = 620 and 665 nm using a microplate reader (Rubystar, BMG). The cAMP concentration is determined by dividing the signal measured at 665 nm by that measured at 620 nm (ratio). The results are expressed as a percent of the control response to 100 nM CP55940. The standard reference agonist is CP55940, which is tested in each experiment at several concentrations to generate a concentration response curve from which its EC50 value is calculated.

**Human cannabinoid CB₁ receptor – antagonist effect :**

The cells are suspended in HBSS buffer (Invitrogen) complemented with 20mM HEPES (pH 7.4), then distributed in microplates at a density of 104 cells/well and preincubated for 5 min at room temperature in the presence of either of the following: HBSS (stimulated control), the reference antagonist AM 281 at 3 μM (basal control) or various concentrations (IC50 determination), or the test compounds. Thereafter, the reference agonist CP55940 and the adenylyl cyclase activator NKH 477 are added at respective final concentrations of 10nM and 3
μM AM 281. For basal control measurements, CP55940 is omitted from the wells containing 3194 μM AM 281. Following 10 min incubation at 37°C, the cells are lysed and the fluorescence acceptor (D2-labeled cAMP) and fluorescence donor (anti-cAMP antibody labeled with europium cryptate) are added. After 60 min at room temperature, the fluorescence transfer is measured at λex = 337 nm and λem = 620 and 665 nm using a microplate reader (Rubystar, BMG). The cAMP concentration is determined by dividing the signal measured at 665 nm by that measured at 620 nm (ratio). The results are expressed as a percent inhibition of the control response to 10 nM CP55940. The standard reference antagonist is AM 281, which is tested in each experiment at several concentrations to generate a concentration-response curve from which its IC50 value is calculated.

**Human cannabinoid CB2 receptor – agonist effect:**

The cells are suspended in HBSS buffer (Invitrogen) complemented with 20 mM HEPES (pH 7.4), then distributed in microplates at a density of 7.5x10^3 cells/well in the presence of either of the following: HBSS (basal control), the reference agonist at 100 nM (stimulated control) or various concentrations (EC50 determination), or the test compounds. Thereafter, the adenylyl cyclase activator NKH 477 is added at a final concentration of 3 μM. Following 10 min incubation at 37°C, the cells are lysed and the fluorescence acceptor (D2-labeled cAMP) and fluorescence donor (anti-cAMP antibody labeled with europium cryptate) are added. After 60 min at room temperature, the fluorescence transfer is measured at λex = 337 nm and λem = 620 and 665 nm using a microplate reader (Rubystar, BMG). The cAMP concentration is determined by dividing the signal measured at 665 nm by that measured at 620 nm (ratio). The results are expressed as a percent of the control response to 100 nM WIN 55212-2. The standard reference
195 agonist is WIN 55212-2, which is tested in each experiment at several concentrations to generate a concentration-response curve from which its EC50 value is calculated.

**Human cannabinoid CB₂ receptor – antagonist effect:**

The cells are suspended in HBSS buffer (Invitrogen) complemented with 20 mM HEPES (pH 7.4), then distributed in microplates at a density of 7.5x10³ cells/well and preincubated for 5 min at room temperature in the presence of either of the following: HBSS (basal control), the reference antagonist AM 630 at 100 μM (basal control) or various concentrations (IC50 determination), or the test compounds. Thereafter, the reference agonist WIN55212-2 and the adenylyl cyclase activator NKH 477 are added at respective final concentrations of 10 nM and 3 μM. For basal control measurements, WIN 55212-2 is omitted from the wells containing 100 μM AM 630. Following 10 min incubation at 37°C, the cells are lysed and the fluorescence acceptor (D2-labeled cAMP) and fluorescence donor (anti-cAMP antibody labeled with europium cryptate) are added. After 60 min at room temperature, the fluorescence transfer is measured at λex = 337 nm and λem = 620 and 665 nm using a microplate reader (Rubystar, BMG). The cAMP concentration is determined by dividing the signal measured at 665 nm by that measured at 620 nm (ratio). The results are expressed as a percent of the control response to 100 nM WIN 55212-2. The standard reference agonist is AM 630, which is tested in each experiment at several concentrations to generate a concentration response curve from which its IC50 value is calculated.

**Beta-arrestin assay:**

Beta-arrestin2 translocation was examined in both agonist and antagonist modes using U2OS cells permanently expression human cannabinoid1 receptor or human cannabinoid2 receptor and rat beta-arrestin2-GFP. Cells were placed in serum free medium for 3-4 hrs, and then treated
with varying concentrations of compounds over a range of 0.1 nanomolar to 100 micromolar to obtain a dose response. After 45 min of treatment at 37°C, cells were fixed with 1% PFA overnight at 4°C. Each of two independent experiments was performed in duplicate at each concentration of compound. WIN55212 was used for antagonist assays with dosage of 1 M for CB₁ and 0.1 M for CB₂, respectively.

Data Analysis: The readout was formation of or loss of (agonist/antagonist respectively) fluorescent intracellular aggregates containing cannabinoid receptor/beta-arrestin-GFP complexes. Duplicate points were collected for each concentration to enable determination of a dose response curve. Image-based wavelet analysis was performed using the Duke Batchmode computer software to assess the response at each point. Dose response data were fit by 4 nonlinear regression analysis using GraphPad Prism to determine potency and efficacy of the compounds.

In Vivo Pharmacology of AM9405: The ability of AM9405 to cross blood-brain barrier (BBB) was studied by Dr. Jodi Wood at CDD/Northeastern University. The hypothermic effects of AM9405 were evaluated by Dr. Carol Paronis at Northeastern University. The effects of novel and peripherally restricted CB₁/CB₂ agonist, AM9405 on gastrointestinal tract (GI) were carried out in Dr. Martin Storr’s lab at the University of Calgary, Canada.

Methods and Materials:

Hypothermia Studies: Female Sprague-Dawley rats (n=5-7/group), weighing between 235 and 350g (Charles River, Wilmington MA). Rats were tested repeatedly with at least seven days
Intervening between drug sessions. Outside of experimental sessions rats were group housed (2/cage) in a climate controlled vivarium with unrestricted access to food and water.

**Drugs:** All compounds were initially dissolved in a solution of 20% ethanol, 20% emulphor, and 60% saline, and were further diluted with saline. Injections were administered s.c. in a volume of 1.0 ml/kg.

**Gastrointestinal Studies:** Female C57BL/6N mice (17-25g) and male CD1 mice (25-37g) were purchased from Charles River Canada (Montreal), QC, Canada. All mice were housed in plastic cages with sawdust floors and allowed free access to tap water and standard laboratory chow, unless otherwise stated. All experimental procedures were approved by the University of Calgary Animal Care Committee and carried out in accordance with the guidelines of the Canadian Council on Animal Care.

**Drugs:** AM9405 was synthesized by Ritesh Tichkule. The CB₁ receptor antagonists/inverse agonist N-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide (AM251), and the CB₁/CB₂ receptor agonist (R)-(+)-[2,3-dihydro-5-methyl-3-(4-morpholinylmethyl)pyrrolo[1,2,3-de]-1,4-benzoxazin-6-yl]-1-naphthalenylmethanone mesylate (WIN55212-2) were purchased from Tocris Bioscience (Ellisville, MI). All drugs were dissolved in a vehicle of 2% DMSO and 1% Tween 80 in physiological saline. Injections were administered intraperitoneally at 4 ml/g body weight.
Preliminary Distribution in Brain/Plasma and Drug Oral Bioavailability:

Mice (CD-1, weighing 25-30 g) are injected intravenously or orally with 0.1-2 mg/kg of the compound mixture. Fifteen minutes post-IV injection, or 30 and 60 minutes post-oral administration, the animals are sacrificed by decapitation followed by blood collection (~500 µL) and tissue dissection; samples are flash frozen with liquid nitrogen to prevent post-mortem degradation of the compounds. Tissues (plasma or brain) are extracted following published procedures and analyzed using a Thermo-Finnigan Quantum Ultra triple quadrupole mass spectrometer in SRM mode with an Agilent 1100 HPLC front-end with internal standards used for quantitation.

Hypothermia Studies:

Temperature was recorded using a thermistor probe (Model 401, Measurement Specialties, Inc., Dayton, OH) inserted to a depth of 7cm and secured to the tail with micropore tape. Rats were minimally restrained and isolated in 38x50x10cm plastic stalls. Temperature was read to the nearest 0.01°C using a Thermometer (Model 4000A, Measurement Specialties, Inc.). Two baseline temperature measures were recorded at 15 min intervals, and drugs were injected immediately after the second baseline was recorded. After injection, temperature was recorded every 30 min for three hours and every hour thereafter for a total of six hours. The change in temperature was determined for each rat by subtracting temperature readings from the average of the two baseline measures.
In Vivo Transit Studies:

Whole Gut Transit Studies: Mice were housed in individual cages 72 hours prior to the experiment. On the day of the experiment they were acclimated to an empty cage (devoid of bedding) for 1 hour prior to drug treatment. Twenty minutes after ip administration of drugs (or vehicle) 0.2 ml of 5% evans blue suspension in 5% gum arabic was given by gastric gavage. The time to the first blue bowel movement was measured in minutes and constituted the whole gut transit time.

Colonic Propulsion: Mice were fasted overnight before the start of the experiment. These mice were administered an intra-peritoneal injection (i.p.) of vehicle, AM9405 (1.0 mg/Kg), WIN (1.0 mg/Kg). (i.p., max volume 100 µL) and 20 min later a pre-warmed (37ºC) glass bead (2 mm) was inserted 2 cm into the distal colon using a silicone pusher. After the bead insertion, mice were placed in individual cages and the time to bead expulsion was determined. Mice that did not expel the bead within 30 min were sacrificed to confirm the presence of the bead in the lumen of the intestine.

In separate experiments antagonists were injected 15 min prior to AM9405 or WIN administration and the bead expulsion was then determined.

Bowel Movement Studies:

Stress-induced Bowel Movement Studies: Mice were placed in separate gages and the new environment caused stress led increased bowel movement was measured (number of pellets per
hour). These mice were administered an intra-peritoneal injection of vehicle, AM9405 (1.0 mg/Kg), WIN (1.0 mg/Kg) (i.p., max volume 100 µL).

**Castor Oil Induced Diarrhea:** Mice were given castor oil orally. These mice were then administered an intra-peritoneal injection of AM9405 (1.0 mg/Kg), WIN (1.0 mg/Kg) (i.p., max volume 100 µL).

**In Vivo Pain Studies:**

**Mustard Oil Induced Pain Studies:** Mice were randomly assigned to experimental groups. The animals were habituated to a raised wire mesh (5 x 5 mm apertures) under a clear plastic box (20x20x15 cm) for 20 min one day before the assay and again 20 min prior to the experiment. Behavioral responses to i.c. oil of mustard (OM) were determined based on the methods described by Laird et al. For the assessment of the OM-induced pain behaviors, 50 µl of OM (0.25 and 1% v/v in 70% ethanol) or vehicle was administered i.c. (intracolnic) under isoflurane anaesthesia. Vaseline was applied to the perianal area to avoid stimulation of perianal somatic areas. After 5 min of recovery, spontaneous behaviors were counted for 20 min.
References:


5. Lindgren, J. E., Quantification of delta 1-tetrahydrocannabinol in tissues and body fluids. Archives of Toxicology 1983, 6, 74-80.


49. Zhang, Q.; Ma, P.; Iszard, M.; Cole, R. B.; Wang, W.; Wang, G., In Vitro Metabolism of R(+)-[2,3-Dihydro-5-methyl-3-[(morpholinyl)methyl]pyrrolo[1,2,3-de]1,4-benzoxazinyl]-(1-naphthalenyl) methanone mesylate, a Cannabinoid Receptor Agonist. *Drug Metabolism and Disposition* **2002**, *30* (10), 1077-1086.


63. Hurst, D. P.; Lynch, D. L.; Barnett-Norris, J.; Hyatt, S. M.; Seltzman, H. H.; Zhong, M.; Song, Z. H.; Nie, J.; Lewis, D.; Reggio, P. H., N-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole -3-carboxamide (SR141716A) interaction with LYS 3.28(192) is crucial for its inverse agonism at the cannabinoid CB1 receptor. *Molecular Pharmacology* 2002, 62 (6), 1274-87.


replacement of the pyrazole 5-aryl moiety of N-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-
dichlorophenyl)-4-methyl-1H-pyrazole -3-carboxamide (SR141716A). A novel series of
alkynylthiophenes as potent and selective cannabinoid-1 receptor antagonists. *Journal of Medicinal
Chemistry* **2008**, *51* (17), 5397-412.

S.; Hung, M. S., Biphasic suppression of appetite by cannabinoid CB1 receptor antagonists with distinct

Makriyannis, A.; Sharkey, K. A., A neutral CB1 receptor antagonist reduces weight gain in rat. *American
Journal of Physiology - Regulatory, Integrative and Comparative Physiology* **2007**, *293* (6), R2185-
R2193.

CB(1) Receptor Neutral Antagonist AM4113 Suppresses Food Intake and Food-Reinforced Behavior but
Does not Induce Signs of Nausea in Rats. *Neuropsychopharmacology : Official Publication of the
American College of Neuropsychopharmacology* **2007**.

69. Tam, J.; Vemuri, V. K.; Liu, J.; Bátkai, S.; Mukhopadhyay, B.; Godlewska, G.; Osei-Hyiaman,
D.; Ohnuna, S.; Ambudkar, S. V.; Pickel, J.; Makriyannis, A.; Kunos, G., Peripheral CB1 cannabinoid
receptor blockade improves cardiometabolic risk in mouse models of obesity. *The Journal of Clinical
Investigation* **2010**, *120* (8), 2953-2966.

70. Cluny, N. L.; Vemuri, V. K.; Chambers, A. P.; Limebeer, C. L.; Bedard, H.; Wood, J. T.; Lutz,
 cannabinoid receptor antagonist, AM6545, reduces food intake and body weight, but does not cause

71. LoVerme, J.; Duranti, A.; Tontini, A.; Spadoni, G.; Mor, M.; Rivara, S.; Stella, N.; Xu, C.;
Tarzia, G.; Piomelli, D., Synthesis and characterization of a peripherally restricted CB1 cannabinoid
antagonist, URB447, that reduces feeding and body-weight gain in mice. *Bioorganic & Medicinal


