Design and Synthesis of Novel Cannabinergic Analogs with Controlled Detoxification

Thesis Presented

by

Rishi Sharma

to

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in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Pharmaceutical Sciences with specialization in Medicinal Chemistry and Drug Development

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Abstract

Cannabis has been used medicinally and recreationally for several centuries, commonly in the form of the plant’s dried leaf/flower (“marijuana”). Δ⁹-Tetrahydrocannabinol (Δ⁹-THC) is the primary psychoactive component of cannabis and is prescribed as a pharmaceutical (Dronabinol®) to stimulate appetite in AIDS patients, and to treat nausea and vomiting for patients undergoing chemotherapy. Δ⁹-THC is also under clinical investigation as an agonist-based therapy to combat cannabis dependence and addiction. Nabilone® is a synthetic Δ⁹-THC analog sold as a prescription medication for treating emesis and as an analgesic for neuropathic pain. However, Dronabinol® and Nabilone® therapies suffer from several drawbacks, including unpredictable duration of action, poor bioavailability, and variable efficacy and detoxification, primarily due to high lipophilicity and the production of pharmacologically active metabolites after metabolic biotransformation.

In order to address the current unmet medical need for clinically viable, cannabis-based medications, novel cannabinergic analogs were designed, synthesized and pharmacologically evaluated using a ‘controlled inactivation approach’. This approach integrates ‘soft drug design’ and ‘modulation of polarity’ within the key pharmacophoric sites of a compound to facilitate enzymatic inactivation in a predictable manner after producing a desired pharmacological response. In addition, the metabolites formed after enzymatic inactivation had no (or minimal) activity at cannabinoid receptors.

An ester group hydrolyzable by ubiquitous plasma esterases was incorporated within the key pharmacophoric sites of dimethylheptyl (DMH)-Δ⁸-THC, in such a manner that the resulting
novel compound demonstrated desired pharmacological responses at cannabinoid receptors and underwent enzymatic inactivation by plasma esterases in a predictable manner. Various polar functional groups were also incorporated at strategic positions of DMH-Δ⁸-THC ester analogs to modulate the compound polarity and lipophilicity, duration of action, and CNS penetration.

In conjunction with pharmacological characterization, the synthetic endeavor resulted in two lead cannabinoid agonists [compounds 57 (AM 7418) and 54 (AM 7499)] with predictable duration of action, improved efficacy and controlled detoxification. Preliminary pharmacological evaluation (in vivo hypothermia) suggested that two cannabinoid agonists [compounds 17 (AM 7428) and 18 (AM 7488)] may be peripherally restricted, for they did not elicit hypothermia in spite of their high in vitro cannabinoid receptor affinity and functional activity at cannabinoid receptors. Additional pharmacological evaluation of these peripherally restricted agonists in select in vivo models is underway.
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Ph.D. Thesis dedicated to my wife

Shafali Khajuria
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List of Abbreviations:

THC  Tetrahydrocannabinol
Δ⁹-THC  Δ⁹-Tetrahydrocannabinol
DMH- Δ⁹-THC  Dimethylheptyl-Delta-9-tetrahydrocannabinol
CB  Cannabinoid
CBD  Cannabidiol
CBN  Cannabinol
CBG  Cannabigerol
CBC  Cannabichromene
CBL  Cannabicyclol
CBE  Cannabielsoin
CBND  Cannabinodiol
CBT  Cannabitriol
CNS  Central Nervous System
CB  Cannabinoid
GPCRs  G-protein coupled receptors
AEA  Anandamide
2-AG  2-arachidonoyl Glycerol
AC  Adenyl Cyclase
TRPV1  Vanilloid Receptor-Type 1 TRPV1
MAPK  Mitogen-Activated Protein Kinases
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<td>PLD</td>
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<td>PLC</td>
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<td>MGL</td>
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<td>THCV</td>
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<td>cyp 450</td>
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<td>LD$_{50}$</td>
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<td>NaHMDS</td>
<td>Sodium hexamethyldisilyl amide</td>
</tr>
<tr>
<td>TMSOTf</td>
<td>Trimethylsilyl trifluoromethanesulfonate</td>
</tr>
<tr>
<td>K-selectride</td>
<td>Potassium tri-sec-butylborohydride</td>
</tr>
<tr>
<td>TBDMS-Cl</td>
<td>tert-Butyldimethylchlorosilane</td>
</tr>
<tr>
<td>mCPBA</td>
<td>Meta chloroperoxybenzoic acid</td>
</tr>
<tr>
<td>DMAP</td>
<td>Dimethylaminopyridine</td>
</tr>
<tr>
<td>DME</td>
<td>Dimethoxyethane</td>
</tr>
<tr>
<td>tPSA</td>
<td>Total Polar Surface Area</td>
</tr>
<tr>
<td>cAMP</td>
<td>Cyclic Adenosine Monophosphate</td>
</tr>
<tr>
<td>cLogP</td>
<td>Calculated Logarithm of Partition Coefficient</td>
</tr>
</tbody>
</table>
Chapter 1: Introduction

Historical evidence indicative of medical and recreational use of cannabis goes beyond millennia.\textsuperscript{1} Cannabis, also known as hemp, belongs to the family ‘\textit{Cannabaceae}’, which has only one genus, ‘\textit{Cannabis}’ and only one species, ‘\textit{sativa}’:\textsuperscript{2} The dried flowers and leaves of cannabis (also known as marijuana) can be smoked, and blocks of cannabis resin (also known as hashish) can be eaten to get the desired effect. The earliest record of therapeutic use of cannabis can be traced back to China in 2737 BC, but the first documented evidence indicating its therapeutic application was found in mid 19\textsuperscript{th} century, with the pioneering work of Dr. William O’Shaughnessy. He used cannabis extracts as analgesics for rheumatoid arthritis and as an anticonvulsant while working as a surgeon with British East India Company and as Professor at University of Calcutta, India:\textsuperscript{3} This finding led researchers from Britain and North America to explore the therapeutic role of cannabis as a muscle relaxant and as an anti-spasmodic agent.\textsuperscript{3} Cannabis was used in United States for almost 150 years and was available in US Pharmacopeia until 1942, when federal legislation made cannabis illegal.

Although there is a wide array of proposed indications for cannabis, clinical evidence exists for relatively few, as shown in Table 1.1. All cannabis therapeutic applications gained pharmacological context in 1964, when the primary psychoactive constituent of cannabis, i.e., $\Delta^9$-tetrahydrocannabinol ($\Delta^9$-THC), was isolated and characterized.\textsuperscript{4} Currently cannabis is classified as a Schedule 1 controlled substance in the USA and is under strict regulation by the USA Drug Enforcement Administration (DEA). The first clinically documented evidence of cannabis as an antiemetic was provided in 1975 from a placebo controlled clinical trial.\textsuperscript{5} In this trial, THC dissolved in sesame seed oil was administered orally in capsules at a dose of 15-20
mg to 20 patients undergoing chemotherapy and resulted in statistically significant antiemetic effect of THC in 14 of the patients.5

Table 1.1: Proposed Therapeutic Indications of Marijuana

1) *Antiemetic  2) *Appetite Stimulation
2) *Antispasmodic, muscle relaxant  4) *Analgesic
5) *Bronchodilator  6) *Anticonvulsant
7) Sedative-hypnotic  8) Opiate, alcohol withdrawal
9) Anti hypertensive  10) Neuralgia, Antitussive
11) Anti neo-plastic  12) Anti pyretic, Anti inflammatory

* Evidence for clinical efficacy is available.

Clinical evidence indicating antinociceptive effects of THC were also demonstrated in 1975 by Noyes et al.6 who compared 10 mg and 20 mg doses of cannabis with 60 mg and 100 mg dose of codeine. It was seen that 20 mg dose of THC was comparable to both the doses of codeine, while the 10 mg dose of THC was better tolerated but less efficacious than both the doses of codeine. Despite its anecdotal use for other indications, there is a sparse evidence of efficacy available for therapeutic application of cannabis for the treatment of other disorders such as glaucoma, convulsions, bronchial asthma or insomnia.

1.1 Chemical Constituents of Cannabis

The key constituents of Cannabis sativa L. include cannabinoids, terpenoids and flavanoids. The contents of these constituents varies depending upon the plant genetics, time of harvest and
Table 1.2: Chemical constituents of Cannabis

[Taken from Analytica Chimica Acta 2002, 468, 245-254]

<table>
<thead>
<tr>
<th>Constituents of C. sativa L. by chemical class</th>
<th>1980</th>
<th>1995</th>
<th>2005</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cannabinoids</td>
<td>61</td>
<td>66</td>
<td>70</td>
</tr>
<tr>
<td>CBG type</td>
<td>6</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>CBC type</td>
<td>4</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>CBD type</td>
<td>7</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Δ^2-THC type</td>
<td>9</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>Δ^3-THC type</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>CBL type</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>CBE type</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>CBN type</td>
<td>6</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>CBND type</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>CBT type</td>
<td>6</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>Misc type</td>
<td>11</td>
<td>12</td>
<td>14</td>
</tr>
<tr>
<td>Nitrogenous compounds</td>
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<td>27</td>
</tr>
<tr>
<td>Amino acids</td>
<td>18</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>Proteins, enzymes and glycoproteins</td>
<td>11</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>Sugars and related compounds</td>
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<tr>
<td>Hydrocarbons</td>
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</tr>
<tr>
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<td>7</td>
</tr>
<tr>
<td>Simple aldehydes</td>
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<td>12</td>
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<tr>
<td>Simple ketones</td>
<td>13</td>
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</tr>
<tr>
<td>Simple acids</td>
<td>20</td>
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</tr>
<tr>
<td>Fatty acids</td>
<td>12</td>
<td>23</td>
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</tr>
<tr>
<td>Simple esters and lactones</td>
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<tr>
<td>Steroids</td>
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<td>11</td>
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<tr>
<td>Terpenes</td>
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<td>120</td>
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<tr>
<td>Non-cannabinoid phenols</td>
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<td>25</td>
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<tr>
<td>Flavonoids</td>
<td>19</td>
<td>21</td>
<td>23</td>
</tr>
<tr>
<td>Vitamins</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Pigments</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Elements</td>
<td>0</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>Total</td>
<td>423</td>
<td>483</td>
<td>489</td>
</tr>
</tbody>
</table>

1 The total given in the 1980 review was 421. The 2 glycoproteins of unknown structures, however, make the total 423.

2 The simple acids and fatty acids in the 1995 review are given as 21 and 22, respectively. They should, however, be 20 and 23, respectively, which leaves the total unchanged.

3 The CBC-and miscellaneous-type cannabinoids in the 1995 review are given as 5 and 11, respectively. They should, however, be 4 and 12, respectively, which leaves the total unchanged.

drying conditions. So far, approximately 500 natural constituents of Cannabis sativa L. have been identified, of which 70 are cannabinoids, the remainder terpenes, hydrocarbons, sugars, proteins, enzymes, flavonoids, vitamins, etc., as shown in Table 1.2.
Among the 70 known cannabis cannabinoids, the most important in terms of pharmacological relevance are tetrahydrocannabinol (THC), cannabidiol (CBD) and cannabinol (CBN) [Figure 1.1] 8.

![Figure 1.1: Structure of THC, CBD, CBN](image)

Other cannabinoid classes include cannabigerol (CBG), cannabichromene (CBC), cannabicyclol (CBL), cannabielsoin (CBE), cannabinodiol (CBND), cannabitriol (CBT) and other miscellaneous compounds. CBGs are inactive at cannabinoid receptors as compared to $\Delta^9$-THC, 2 but they exhibit antibacterial activity against gram positive bacteria. 9 CBD constitutes approximately 40% of the cannabinoids in cannabis organic extracts. 10 Medically, CBD has shown anticonvulsive, anti-inflammatory, and anti-anxiety effects 11 and is under investigation as an atypical antipsychotic to treat schizophrenia. 12

THC is the primary psychoactive component of cannabis, and its structure was elucidated by Gaoni, Mechoulam, et al. in 1964. 13 It exists in two isomeric forms known as $\Delta^9$-THC and $\Delta^8$-THC depending upon the position of the double bond in the C-ring, as shown in Figure 1.2. 14
Δ⁸- and Δ⁹-THC are virtually equivalent as to cannabinoid (CB) receptor affinity and pharmacological activity, but Δ⁸-THC has the advantage of being the chemically more stable isomer. The psychotropic effect of THC depends strictly on stereochemical factors. Natural Δ⁹-THC and Δ⁸-THC extracted from the cannabis exist in the (-)-(6aR, 10aR) configuration. However, its opposite enantiomer, (+)-(6aS, 10aS) has been synthesized in the laboratory\(^15\) and found to be inactive at CB receptors, confirming that Δ⁹-THC is active at cannabinoid receptors only in its (-) equatorial form, and not in the (+) axial form.

1.2 Cannabinoid Receptors

The quest to understand the molecular basis of the psychotropic effect of Δ⁹-THC led to the identification and characterization of CB receptors. The cannabinoid receptors are membrane-bound receptors which belong to a superfamily of G-protein coupled receptors (GPCRs). To date, two CB receptors, CB1 and CB2, have been isolated, cloned, and expressed. CB1 was cloned by Matsuda et al.\(^16\) and provided the initial definitive proof for the existence of CB receptors. Shortly thereafter, another CB receptor was cloned by Munro et al.\(^17\) and was
designated CB2. Recently it has been found that cannabinoids can activate another GPCR receptor\textsuperscript{18} GPR55 and it is currently under debate whether to classify GPR55 as CB3 receptor. CB1 receptors are primarily found in the CNS, and in various peripheral organs like lungs, kidney and liver, whereas CB2 receptors are expressed mainly in immune cells with high concentrations in spleen. Howlett et al.\textsuperscript{19} provided initial proof for the interaction between cannabinoids and CB receptors. She found that cannabinoids activate negatively coupled GPCRs (G\textsubscript{i/o}), thereby inhibiting the activity of adenyl cyclase (AC) and reducing levels of cellular cAMP. Cannabinoids also interact with other GPCRs and ion channels apart from CB1 and CB2 receptors.

Both CB1 and CB2 receptors are negatively coupled GPCRs; however, under certain conditions, CB1 and CB2 receptors can couple to G-protein G\textsubscript{s} and G\textsubscript{q} subtypes. The interaction of cannabinoids with negatively-coupled CB1 receptors leads to the inhibition of adenyl cyclase,\textsuperscript{20} the activation of mitogen-activated protein kinases (MAPK), the inhibition of certain voltage gated calcium channels and the activation of G-protein-linked inward rectifying potassium channels.\textsuperscript{21} The cumulative effect of activation of CB1 receptors by $\Delta^9$-THC leads to the feeling of euphoria and in some people a feeling of depression is also observed. Recent findings show that, frequent activation of CB receptors by regular cannabis use results in neurological changes, which may lead to cannabis addiction/ dependence, however, the exact mechanism is still under investigation.\textsuperscript{22}

CB1 and CB2 receptors exhibit 44\% sequence homology over the entire amino acid sequence\textsuperscript{17} and 68\% sequence homology between CB1 and CB2 receptors between their transmembrane region.\textsuperscript{23}
In addition to the cannabis sativa derived phytocannabinoids (e.g., THC), endocannabinoids (produced within the body, e.g., AEA and 2-AG) and synthetic cannabinoids (synthesized in the laboratory, e.g., HU 210) also bind to CB receptors and induce pharmacological responses. In addition, DiMarzo et al.\textsuperscript{24} provided evidences for the interaction and activation of well characterized vanilloid receptor-type 1 (TRPV1) by anandamide (AEA) and others have shown that endocannabinoids also interact with potassium channels, nicotinic receptors and various subtypes of 5-hydroxytryptamine (5-HT) receptors.\textsuperscript{25}

1.3 Endocannabinoid System (ECS)

The ECS is comprised of CB receptors, endogenous signaling ligands (endocannabinoids) that bind to CB receptors and enzymes that synthesize and degrade endocannabinoids. The isolation, cloning and expression of cannabinoid receptors in the early 1990s suggest the presence of endogenous analogs that act as ligands to these receptors. The quest to identify the endocannabinoids that bind to CB receptors led to the identification of two key endocannabinoids, i.e., arachidonylthanolamine (AEA) also known as anandamide (derived from a Sanskrit word ‘ananda’ meaning ‘bliss’) and 2-arachidonylglycerol (2-AG). Pharmacological studies suggest the role of ECS signaling in various physiological processes including neuromodulation,\textsuperscript{26} synaptic plasticity,\textsuperscript{27} appetite modulation,\textsuperscript{28} and immunomodulation.\textsuperscript{29} The ECS is also found to play a key role in the physiological and pathophysiological processes like inflammation, neuroprotection, multiple sclerosis, cancer palliation and analgesia\textsuperscript{30}. Recent studies indicate the existence of other endocannabinoids such
as virdhamine, noladin diethyl ether, N-arachidonoylserine, arachidonoyldepamine, and
docosatetraenoylethanol amide.\textsuperscript{31}

AEA, an arachidonic acid derivative, was isolated and characterized by Devane et al.\textsuperscript{32} in 1992. Biologically, it is synthesized within the lipid bilayer from \( N \)-acylphosphatidylethanolamine (NAPE) via multiple enzymatic pathways involving phospholipase A2, phospholipase C and NAPE-phospholipase D (PLD).\textsuperscript{33} AEA is produced at very low levels and exhibits a short half-life due to the rapid degradation by fatty acid amide hydrolase (FAAH), which breaks it into arachadonic acid and ethanolamine, [Figure 1.3]. AEA’s antinociceptive and anxiolytic effect resemble those of \( \Delta^9 \)-THC.

\textbf{Figure 1.3:} Endocannabinoid system: Biosynthesis and Inactivation of anandamide and 2-AG

2-AG, an ester of arachidonic acid and glycerol, was first isolated and characterized by Mechoulam et al. in 1995 from canine gut.\textsuperscript{34} As compared to AEA, 2-AG is present in brain in
relatively high levels and is synthesized by the hydrolysis of diacylglycerol (DAG) by DAG-lipase (DGL). Recent studies also suggest the enzymatic formation of 2-AG by the action of phospholipase C (PLC). 2-AG’s pharmacological responses include neuroprotection, cardioprotection and antiproliferation. 2-AG is hydrolyzed by the membrane bound enzyme monoacyl glycerol lipase (MGL). Endocannabinoid hydrolysis by enzymes (FAAH and MGL) inactivates these messengers and, therefore, modulates a number of neurobehavioral processes in mammals, such as, pain, sleep, feeding and locomotion.

1.4 Effects of Cannabis

Several countries and some states within USA have legalized medicinal cannabis to treat nausea, pain and to alleviate the symptoms of some chronic illness. The psychotropic effects of cannabis are primarily due to THC and tetrahydrocannabivarin (THCV, a CB antagonist present in low concentrations), although other non-psychoactive constituents of cannabis like CBD and CBN also modulate physiological functions. There is documented clinical evidence confirming that CBD regulates the psychoactive effect of THC. Marijuana with a higher CBD: THC ratio is less likely to cause anxiety than marijuana with a lower CBD: THC ratio. CBD also inactivates a particular isoform of cytochrome P450 (cyp450) necessary for THC metabolism, thereby indirectly increasing the concentration of THC. It is due to the inherent characteristics of cannabinoids of being highly lipophilic, they exhibit depot effect, which means that they become sequestered within the fatty tissue and diffuse slowly into the blood stream, making for a very long and unpredictable half-life.
It is found that major physiological functions of the body are affected by cannabis use. Prominent effects of cannabis use include anxiety reduction, sedation, analgesia and appetite stimulation. Interestingly, its acute toxicity is very low and so far no death has been reported, making it a safe addictive drug as compared to alcohol, opiates and benzodiazepines. The median lethal dose (LD$_{50}$) of THC is 1270 mg/kg for male rats and 730 mg/kg for female rate after oral consumption.$^{38}$ Following are the acute/chronic effects of cannabis usage:

**Neurological Effects**

The behavioral effects produced by cannabinoids are mainly due to the activation of CB1 receptors present in the brain. CB1 receptors are highly expressed in certain brain regions like basal ganglia (controls motor coordination and learning), cerebellum (motor control and cognitive functions like attention, language), hippocampus (controls the learning, memory and stress control), and nucleus accumbens (called the reward center of brain).$^{39}$ A very low dose of 2.5 mg cannabis in cigarette form induces a feeling of intoxication, decreased anxiety, depression and tension within minutes after smoking and reaches a plateau within 2-3 h of smoking. However, THC can also produce severe anxiety, panic attacks and psychosis depending on the dose. Hallucinations are very common with high doses of cannabis. Other related effects include increase sensitivity to visual and audio stimuli.

**Effect on Appetite**

The hyperphagia caused by use of cannabis has been well recognized. Recently, it has been demonstrated clinically that the higher the cannabis CBD content relative to THC, the weaker the hyperphagic response.$^{40}$
Chronic Use of Cannabis

It is well documented that the chronic cannabis users remain impaired even when they were not intoxicated. These impairments include decreased attention, inability to analyze complex information, and memory deficits. These effects can last for months or longer. Cannabis withdrawal syndrome has also been demonstrated in various preclinical and clinical studies. Withdrawal symptoms including restlessness, aggression, anorexia and muscle tremors are similar to those of alcohol, opiates and benzodiazepines, but their severity is not as severe as with other addictive drugs. Cannabis also affects the cardiovascular (e.g., tachycardia and vasodilation) and respiratory (e.g. bronchitis) systems, and is an immunosuppressant.

1.5 Cannabinoid-Based Drugs on the Market

The early 20th century witnessed the usage of cannabis as a medication for various indications varying from pain, rabies and eating disorders. However, medicinal cannabis use declined due to its high abuse potential and the availability of alternative novel therapies. The late 20th century witnessed three consecutive events: the regulatory approval of Δ⁹-THC in the United States; isolation and cloning of CB receptors; and identification of endocannabinoids. These events caused a resurgence of interest in the development of cannabinoid-based medications, which led to the development of Nabilone (synthetic analog of Δ⁹-THC) and Sativex (THC: CBD, 1:1 ratio in the form of oral mucosal spray) for indications like nausea and vomiting associated with chemotherapy, appetite stimulation for acquired immunodeficiency syndrome (AIDS) patients, and neuropathic pain in multiple sclerosis (MS) patients. Rimonabant, a CB1 receptor inverse agonist approved for obesity in certain countries outside the United States was recently
withdrawn from the market due to severe adverse effects such as depression and increased incidences of suicidal tendencies. Currently three marketed cannabinoid are:

**Dronabinol** (Marinol®) is the first synthetic Δ⁹-THC, approved by the United States Food and Drug Administration (USFDA) in 1985 for the treatment of nausea and vomiting in cancer patients undergoing chemotherapy, and as an appetite stimulant for anorexic patients. Dronabinol is currently under investigation as a treatment for cannabis dependence/addiction. It exerts its pharmacological response by acting as a partial agonist at CB1 receptors in brain and CB2 receptors in the immune system. However, Marinol is still not a widely accepted medication due to variable and poor absorption, poor tolerability and unpredictable pharmacokinetic (PK) and pharmacodynamic (PD) characteristics.⁷

**Nabilone** is a synthetic cannabinoid approved in 2006 by USFDA as an antiemetic for patients who are not responsive to conventional anti-emetics and as adjunct therapy for neuropathic pain. Its side effects include drowsiness and dizziness. In the US, its marketing was slowed because of its classification as a schedule 2 drug by the US Drug Enforcement Agency (DEA) in 1998.⁷

**Sativex** is a 1:1 mixture of THC and CBD in the form of oromucosal spray. It was developed by GW Pharmaceuticals and was approved in Canada in 2005 for MS patients to alleviate neuropathic pain, spasticity and overactive bladder. Sativex has shown positive clinical trial results, patients experiencing relief from neuropathic pain,⁴⁴ spasticity,⁴⁵ muscle spasm,⁴⁵ and bladder-related symptoms.⁴⁶ In 2010, Sativex was approved in UK as a prescription medication for the treatment of MS, and it is expected to get regulatory approval in Germany, France, Italy and Spain by the end of this year.⁴⁷
1.6 Clinical Viability and PK/PD of Cannabinoids

The decline in the use and development of cannabis/cannabinoid-based therapies in mid-1900s can be attributed to its erratic PK/PD profile and abuse potential. The erratic PK/PD profile of cannabinoid-based therapies could be due to many factors. The first and foremost is the high lipophilicity of THC ($\text{cLog p} = 7.23$) which causes its sequestration in adipose tissue (depot effect) and from which it slowly diffuses into the bloodstream to exert its effect, resulting in an unpredictable and long half-life. The second reason is the variation in the concentration of THC in marijuana. The content of THC and other cannabinoids varied considerably among marijuana samples confiscated during 1980-1996. The last reason is due to the lack of sufficient information regarding basic pharmacokinetic/pharmacodynamic parameters among different routes of administration (oral, sublingual, rectal, inhalation). All these routes exhibit different onsets of action and systemic bioavailabilities. $\Delta^9$-THC is available as pharmaceutical preparation as Marinol® (also known as Dronabinol®). According to the German pharmaceutical monograph, Marinol® contains at least 95% of $\Delta^9$-THC, 2% of $\Delta^8$-THC, and 3% of other substances like cannabinol (CBN) and cannabidiol (CBD). $\Delta^9$-THC is highly sensitive to heat and light and undergoes oxidation to CBN on storage, resulting in reduced THC content. For these reasons, $\Delta^9$-THC preparations should be stored in amber containers, tightly packed at low temperatures. $\Delta^9$-THC is also sensitive to acidic conditions and undergoes rapid degradation at pH $\sim$ 1. Its half life is assumed to be 1 h at stomach pH ($\sim$ 1.0).

Absorption, Distribution, Metabolism and Excretion (ADME) Profile of $\Delta^9$-THC

The ADME profile of $\Delta^9$-THC after oral consumption is slow and erratic. Other cannabinoids (e.g., HU 211), CBD and CBN exhibit a ADME profile similar to $\Delta^9$-THC. The ADME
profile of cannabinoids is similar both in male and female humans.\textsuperscript{57} THC is absorbed by lungs and intestines after oral ingestion, followed by high concentrations in extracellular water, where it gets bound to plasma proteins. It is also sequestered in fat tissues from where it is released slowly into the blood stream. THC can be metabolized by microsomal enzymes or be excreted via the kidneys. Free THC reaches the site of action, where it activates the CB receptors and induces cannabinergic effects.

The common routes for ingesting Δ\textsubscript{9}-THC include following:\textsuperscript{48}

1. Inhalation (i.e., cannabis cigarette smoking)
2. Oral route (in the form of Dronabinol\textsuperscript{®} capsules or baked foods)
3. Rectal route (in the form of suppositories, tried in some patients)
4. Mucosal sprays (e.g., Sativex\textsuperscript{®})
5. Ophthalmic route to reduce intraocular pressure and aerosols are under investigations.

The ADME parameters of Δ\textsubscript{9}-THC are discussed in details below:

**Absorption:**

The absorption profile of cannabinoids (primarily Δ\textsubscript{9}-THC) depends on various factors like the route of administration, the smoking technique, and the variation in the THC content of the marijuana smoked. Efficient smoking technique can increase the absorption and availability of THC, depending upon the depth of inhalation, frequency and duration of puff and holding of breath.\textsuperscript{48} It is estimated that approximately 30% of THC gets destroyed by pyrolysis during marijuana smoking.\textsuperscript{48} The peak plasma THC concentration is reached within minutes (3-10 min)
after inhalation followed by rapid decline,\textsuperscript{57-58} whereas it takes 1-2 h after oral THC consumption to attain peak plasma THC concentrations.\textsuperscript{57-58}

Systemic THC bioavailability varies from 2-56\% following inhalation and depends on smoking technique.\textsuperscript{48} THC was also found in measurable concentrations among passive cannabis smokers.\textsuperscript{59} The absorption of THC after oral ingestion exhibits an erratic and unpredictable profile, with peak plasma concentration varying from 1-2 h\textsuperscript{58} to 4 h\textsuperscript{60} and in some cases there was more than one peak of plasma concentration.\textsuperscript{58}

The peak plasma concentration of THC after a single oral dose of 15 mg also shows inter-subject variation from 1-4 hours.\textsuperscript{61} This results in unpredictable duration of action after oral THC consumptions leading to variable efficacy among subjects.

**Distribution:**

The erratic distribution phase of THC following the absorption phase is due to high lipophilicity and high tissue (especially adipose tissue) sequestration.\textsuperscript{62} THC exhibits high plasma protein binding, and therefore a very low fraction is available for pharmacological activity. Some 90\% of the THC in blood is found in plasma, the remaining 10\% in red blood cells (RBCs). Virtually all (95-98\%) of the THC in plasma is bound to plasma proteins, especially lipoproteins. 11-OH-THC, the metabolite of THC, is more tightly bound to plasma proteins, and only 1\% is available in free form for pharmacological activity.\textsuperscript{63} Being highly lipophilic, THC has a large apparent volume of distribution (V\textsubscript{d}), i.e., approximately (10 L/kg).\textsuperscript{64} *In vivo* models show that it gets sequestered in highly vascularized tissues like liver, lungs, heart, kidneys, spleen, muscle, mammary gland, and pituitary gland, in contrast to fatty tissues and brain (e.g., less than 1\% of administered THC reaches brain).\textsuperscript{48, 65}
The exact form and quantity of THC in fatty tissue, i.e., whether it is in the free form or conjugated with the lipophilic molecules of adipose tissues, triglycerides, is under debate. Studies suggest that the metabolite of THC, 11-OH THC, may be complexed with triglycerides. This conjugation leads to the enhanced lipophilic character resulting in longer residence in the fatty tissue and slow diffusion into the blood stream. The longer residence in the adipose tissue, the slow diffusion into the blood stream and the production of active metabolite are the prime reasons for the clinically unfavorable characteristics of THC i.e., its unpredictable duration of action and variable efficacy and detoxification.66

**Metabolism**

Liver is the predominant site of THC metabolism and different metabolites are observed, depending on the route of administration. THC is metabolized by cyp 450 iso-enzymes, and the metabolic rate is species dependent. For example, in rats more than 80% THC is metabolized within 5 minutes,67 at a 3-fold slower rate than in mice. CYP2CP is key metabolizing enzyme responsible for the allylic oxidation at C9 position of THC.68 CYP2CP, it is considered to be the same enzyme responsible for the drug-drug interactions involving THC. Lipophilic compounds have to undergo multiple structural modifications including phase-1 modification and phase-2 conjugation reactions to be excreted from the body. Monohydroxylated compounds are the major metabolites of THC69 with C-11 being the prime site for oxidation [Figure 1.4].

THC on allylic oxidation generates the 11-hydroxy analog, which upon further oxidation generates THC-COOH, which may be conjugated with glucuronic acid, making it sufficiently polar to be excreted from the body. In humans, THC undergoes allylic oxidation, epoxidation,
aliphatic oxidation, decarboxylation and conjugation. Apart from liver, THC also undergoes metabolism in heart and lungs.

**Figure 1.4**: Main metabolic pathways of Δ⁹-THC. *Figure taken from Journal of Cannabis Therapeutics 2003, 3, 3-51*

**Elimination**

THC exhibits a slow elimination rate, primarily due to the depot effect. As soon as the THC gets metabolized by microsomal enzymes, its polar metabolites starts to get excreted from the body, in response to this THC which is sequestered in the fat tissues starts to diffuse slowly into the blood stream and thereby continues to maintain its pharmacological response. So, it is difficult to calculate a true elimination THC half life. Wall et al. reported that THC has a half life of 25-36 h, 11-OH-THC has a half life of 12-36 and THC-COOH has a half life of 25-55 h in males and females. Studies reveal that THC gets excreted primarily as its metabolites in urine (20-35%), feces (65-80%) and unchanged (< 5%) after oral ingestion. The pharmacokinetic profile of other cannabinoids, especially CBD, CBN and Nabilone, in terms of half life, plasma concentration and metabolic routes resembles that of THC. Cannabidiol is found to inactivate cyp 450 oxidative systems, responsible for metabolism of THC. The inhibition of cyp450
pathway (which metabolizes THC) by CBD is one of the reasons for prolonged recreational usage of THC.\textsuperscript{75}

Nabilone, the synthetic 9-keto analog of THC, is the result of extensive structure activity relationship of various cannabinoids and is available in US and Europe for more than two decades for the treatment of nausea and vomiting associated with chemotherapy. Nabilone after oral ingestion reaches peak plasma concentration within 1-4 h.\textsuperscript{76} Its metabolite and distribution profiles and plasma protein binding in humans have yet to be determined. However, one of the metabolites in plasma was characterized as isomeric carbinol which has a longer half life than nabilone.\textsuperscript{77} 91\% of Nabilone is excreted within 7 days, 23\% in urine and 67\% in feces.\textsuperscript{76}

To address the erratic PK/PD profile of orally administered THC-based medications (Dronabinol and Nabilone), other route of administration are being pursued. This led to the development of Sativex (1:1 mixture of THC : CBD) in sublingual formulation with an improved pharmacokinetic profile.\textsuperscript{48} Currently, other routes of administration (rectal or transdermal) to improve Sativex bioavailability and the duration of action are under investigation. However, there is still a need for a clinically viable, THC based medications with good oral bioavailability, predictable duration of action, and consistent efficacy and detoxification.

1.7 Need for Novel THC-Based Therapies

As already discussed above, THC-based therapies like Dronabinol (Marinol\textsuperscript{®}) and Nabilone suffer from undesirable pharmacological properties including poor bioavailability, unpredictable onset/ offset of action and detoxification. The high lipophilicity of THC leads to a large volume
of distribution ($V_d$), sequestration in adipose tissue (“depot effect”), and high (> 97%) plasma protein binding\textsuperscript{78} making THC a less attractive clinical medication. The clear medical need for the novel cannabinoid-based medications as described below, has encouraged us to pursue the design and development of novel cannabinoid ‘agonist-based’ medications with improved ‘druggability’, i.e., improved oral availability, a predictable time course of action, and controllable detoxification. These novel compounds may find application in following indications:

**Addiction:**

Even with clinically accepted therapeutics for atleast certain substance-abuse disorders, drug addiction is a persistent medical problem. The addiction potential of drugs varies from substance to substance and also from individual to individual. For example, drugs like codeine or alcohol need more prolonged exposure than heroin or cocaine to elicit addiction. Similarly, people with psychological/ emotional co-morbidities are more susceptible to addiction than psychologically stable individuals.\textsuperscript{79}

According to the World Drug Report (2008), cannabis remains the most widely used illicit drug in the world, with 3.9% of the global population (166 million people) aged between 15-64 years having used cannabis in 2006, followed by amphetamines 0.6% (24.7 million) and heroin 0.4% (12 million people). The frequent activation of the cannabinoid receptors (particularly CB1) by regular cannabis use results in pathological neuroadaptive mechanisms, which may lead to cannabis addiction/ dependence, the exact mechanism of which remains under investigation.\textsuperscript{22} The number of patients who need treatment for cannabis dependence is much higher than those who seek treatment for other illicit substances, but the proportion of those who are actually under
treatment for cannabis dependence is much less as compared to other illicit drugs. This may be due to the perception that cannabis is relatively a safe drug without dependence liability. However, clinical evidence highlights the severity of the withdrawal syndrome following discontinuation of chronic cannabis use by at least some abusers. Although the time course of emotional and behavioral symptoms may resemble those of other illicit drugs, the potential severity of these symptoms supports the view that cannabis addiction is an unmet medical need meriting clinical attention. The current scarcity of treatment options and the high relapse rates associated with them are aversive to patients seeking treatment.

One of the key strategies to combat cannabis dependence/addiction is to administer low oral doses of Δ⁹-THC (Dronabinol®). A daily oral dose of 30 mg Δ⁹-THC/day reduced withdrawal discomfort, whereas 90 mg/day was even more effective with minimal adverse effects. Currently, Dronabinol is undergoing phase-II clinical trials for the treatment of marijuana addiction. Also, it is under phase-II clinical investigation as adjunct therapy with naltraxone to treat opioid dependence. The novel cannabinoid analogs being designed and synthesized in this project can be used as candidate prototypes with clinical potential as medicines for drug addiction/dependence, including the treatment of cannabis addiction, opioid dependence and amphetamine addiction.

Pain:

Pain is defined as “an unpleasant sensory or emotional experience associated with actual or potential tissue damage”. Pain is primarily of two types, nociceptive and neuropathic. Nociceptive pain involves nociceptors which detect mechanical, chemical and thermal changes and carry signals to brain via spinal cord. Neuropathic pain is caused by nervous-system damage
either centrally or peripherally or both due to disease or trauma. Cannabinoids (Nabilone) offer better treatment option for symptomatic relief from neuropathic pain, as most of the conventional pharmacotherapies to treat this condition, like tricyclic antidepressants (TCAs), anticonvulsants and opioid analgesics are often refractory. Neuropathic pain, resulting from nerve injury or toxic insults, affects the 3-8% of individuals worldwide, is often refractory to conventional pharmacotherapies which include Gabapentin (anticonvulsant), 5% Lidocane, opioid analgesics, tramadol hydrochloride, and tricyclic depressants. All of these treatment options are associated with significant adverse effects. Gabapentin offers significant benefit in reducing neuropathic pain, but it is associated with somnolence, dizziness, balance and walking difficulties, as well as cognitive problems in elderly patients. Opioid analgesics offer significant improvement of pain management, but their use is limited due to adverse effects like constipation, sedation, nausea and cognitive impairment. Tramadol, apart from common adverse effects like dizziness, nausea, constipation, increase the risk of seizures in predisposal patients. Tricyclic antidepressants can lead to increased risk of suicidal tendencies due to overdose; also they should be taken with caution for patients who have history of cardiovascular disease, glaucoma, or urinary retention.

A lack of safe and efficacious treatment options necessitates the development of a novel analgesic to manage neuropathic pain. Cannabinoids have shown promising results in managing neuropathic pain in animal models for traumatic nerve injury, toxic insults and based on successful clinical trials, Nabilone was approved for symptomatic pain relief associated with neuropathic pain in humans. However Nabilone therapy suffers from drawbacks such as unpredictable time course of action, variable efficacy and detoxification necessitating the need to improve its pharmacokinetic profile by designing and synthesizing novel analogs with controlled
detoxification and improved druggability. The novel cannabinoid analogs being synthesized under this project may find therapeutic utility in the treatment of pain.

Glaucoma:

Glaucoma is the leading cause of blindness in the United States and the second leading cause of blindness in the world. It is characterized by increased intraocular pressure (IOP) which further damages optical nerve at its point of attachment to retina. This leads to narrowing of visual field and may result in blindness. Although several drugs are available for the treatment of glaucoma with the focus on reducing IOP, most have limited application due to undesirable side effects and development of tolerance. Additionally, in most cases multiple drug regimens are required for controlling the IOP of glaucoma patients. Smoking of cannabis has been long known to have beneficial effects on glaucoma patients. The key psychoactive constituent of cannabis, Δ⁹-THC, has been shown to decrease IOP in glaucoma patients. The undesirable CNS side effects associated with Δ⁹-THC combined with its unpredictable pharmacokinetic and pharmacodynamic profile have hindered its utility for such treatments. The recent discovery of the presence of retinal cannabinoid receptors has opened new avenues for the treatment of glaucoma with cannabinoid agonists.

In addition to the above-mentioned conditions, novel cannabinoids synthesized here may find potential therapeutic use can be used in numerous other conditions including cancer, stroke, osteoporosis, multiple sclerosis, neurodegenerative diseases, inflammatory diseases, Huntington’s disease, canavan disease, spinal muscular atrophy, prion disease, nausea and vomiting, spasticity, chemotherapy induced nausea and vomiting (CINV), human
immunodeficiency virus (HIV), wasting syndrome, cachexia, dementia, anorexia affective disorders, tourette syndrome.
Chapter 2: Objective and Specific Aims

2.1 Long-Term Objective

The long-term objective of this dissertation is to design and synthesize novel cannabinergic analogs as candidate therapeutics, devoid of existing drawbacks of Dronabinol® and Nabilone®, i.e., unpredictable duration of action, poor bioavailability, and variable efficacy and detoxification.

2.2 Rational Drug Design

A ‘controlled inactivation approach’ has been used to design the novel cannabinergic analogs with improved druggability. This approach integrates two drug discovery approaches, i.e., ‘soft drug design’ and ‘modulation of polarity’ within the key pharmacophoric sites of DMH-Δ8-THC to achieve the desired pharmacological effect. ‘Soft drugs design’ is aimed to generate safer drugs with improved therapeutic index via predictable metabolic biotransformation. These drugs are expected to undergo metabolic inactivation in a prescribed manner after exhibiting the desired therapeutic effect. Various marketed drugs like esmolol, remifentanil and loteprednol etabonate are the result of successful incorporation of this concept. It is also demonstrated within our group (Bergman et al. unpublished work) that the depot effect exhibited by the lipophilic compound can be reduced by increasing its polarity. Unlike lipophilic drugs, polar drugs tend to undergo rapid diffusion from fatty tissue to the blood stream and produce the pharmacological effect. Soft drugs are distinct from prodrugs, which are inactive compounds whose design generally addresses concerns such as membrane permeability, water solubility and other physicochemical properties like too rapid elimination or difficulty in formulation. A
Prodrugs may offer a pharmacologically better response, optimum delivery at the site of action and easier formulation design, leading to an improved bioavailability than the parent drug.87 On the other hand, a soft drug is a bioactive compound with a metabolically labile feature within its key pharmacophoric site. Soft drugs are expected to produce a desired pharmacological response, and predictable metabolic biotransformation and the production of inactive metabolites.

Our rational design of novel cannabinergic analogs incorporates an ester group, hydrolyzable by esterases, within the key pharmacophoric sites of DMH-Δ⁸-THC in such a way that the resulting carboxylic acid metabolite, formed after enzymatic hydrolysis has no (or minimal) activity at CB receptors [Figure 2.1].
2.3 Specific Aims

The long term aim of my dissertation is to improve the druggability of existing THC-based medications by controlling the metabolic biotransformation through enzymatic (esterase) inactivation. These novel analogs should also display the desired pharmacological profile of a cannabinergic ligand. We designed and synthesized novel cannabinoid agonists that display, in vivo, predictable duration of action, good oral bioavailability and controlled detoxification as compared to current cannabinoid-based therapies (i.e., Dronabinol®, Nabilone®). The specific aims were:

1) **Design and Synthesize DMH-Δ⁸-THC Analogs with an Ester group at C2’ Position**

Dimethylheptyl (DMH)-Δ⁸-THC was selected as a starting template for the structure activity relationship (SAR) as an initial approach to a lead compound with desired pharmacological activity. DMH-Δ⁸-THC is the optimized analog of Δ⁹-THC and exhibits high binding affinities ($K_d$ values, CB1: 0.83 nM, CB2: 0.49 nM) at both principal CB receptors as compared to Δ⁹-THC (CB1: 40 nM, CB2: 39.5 nM) [Figure 2.2].

![Figure 2.2: Cannabinoid receptor binding affinities of Δ⁸-THC and DMH-Δ⁸-THC](image-url)
Earlier work in our lab (Jing, Thakur et al., unpublished results) suggested that (DMH)-Δ⁸-THC analogs with an ester group at C1’ position (i.e., analogs lacking the geminal dimethyl group at C1’ position) show a significant loss of binding affinity ($Ki > 500 \text{ nM}$) towards CB receptors. So, our objective was to study the effect of an ester group at C2’ position (keeping the C1’ geminal dimethyl group) on binding to CB receptors.

Analogs with acceptable CB receptor binding affinity data ($Ki < 30 \text{ nM}$) would then be tested for \textit{in vitro} metabolic stability towards esterases. We also planned to functionalize the terminal carbon of the side chain with various functional groups, e.g., bromo, cyano, imidazole, morpholine and azide, to modulate the compound’s polarity and CNS permeability.

Additionally, it was planned to explore the pharmacophoric limits of side-chain length required for optimum activity at CB receptors. The objective was to generate a lead compound with high affinity ($Ki < 30 \text{ nM}$) towards CB receptors, predictable \textit{in vitro} metabolic stability, and the desired functional activity (indexed as changes in cAMP and β-arrestin) to carry forward for further profiling in \textit{in vivo} studies.

The key representative analog with ester group at C2’ position is depicted in Figure 2.3.

*Figure 2.3:* Representative example of DMH-Δ⁸-THC analog with ester group at C2’ position.
2) Design and synthesize analogs with facilitated enzymatic hydrolysis

Published data suggested that a geminal dimethyl group alpha to the ester functionality may provide significant resistance against enzymatic hydrolysis by esterases.\textsuperscript{90} If a similar undesired outcome to be encountered with our designed analogs (Specific Aim 1), it was planned to synthesize a subsequent generation of analogs more easily hydrolyzed by esterases without compromising the binding affinity and pharmacological activity at cannabinoid receptors. Our design of next-generation of analogs incorporated following modifications:

a) **Reverse Ester Analog**: Moving the ester group away from geminal dimethyl group from C2\textsuperscript{'} position to C3\textsuperscript{'} position (reverse ester) was an attractive approach to facilitate the enzymatic hydrolysis by the esterases. The ester group in these analogs would appear to be sufficiently exposed to undergo facile enzymatic inactivation by esterases. The key representative example of the reverse ester analog is shown in Figure 2.4.

![Figure 2.4: Reverse ester analog](image)

b) **Δ\textsuperscript{8}-THC ester Analog Lacking Geminal Dimethyl group at C1\textsuperscript{'} Position and C1\textsuperscript{'}-Methyl Substituted Δ\textsuperscript{8}-THC Ester Analog**: Removal of the geminal dimethyl group at C1\textsuperscript{'} position [Figure 2.5] or the incorporation of monomethyl at C1\textsuperscript{'} position in place of the geminal dimethyl group [Figure 2.6] represented alternative approach to facilitate compound enzymatic hydrolysis by esterases. Since these analogs were sterically less
hindered at C1’ position as compared to those with the geminal dimethyl group, they were expected to undergo more rapid enzymatic hydrolysis \textit{in vitro} and \textit{in vivo}. Incorporation of the monomethyl at C1’ position generates a new chiral center [stereochemistry at 6a and 10a \((R, R)\) is locked] at C1’, producing a mixture of diastereoisomers. It was planned to separate the diastereomers chemically and profile each \textit{in vitro} and \textit{in vivo}.

![Figure 2.6: C1'-methyl substituted ester analog of Δ^8-THC](image)

### 3) Ester Analogs with Improved Polarity to Reduce the Depot Effect

![Figure 2.7: Chemical structure of AM 411 and AM 4054](image)
Earlier studies in our group (Bergman et al., unpublished work) suggested that depot effect inherent to THC-based medications can be reduced by increasing the polarity of the compound. AM 4054 (potent CB1 agonist developed by our group) is a polar analog of AM 411 (another CB1 agonist developed by our group) [Figure 2.7]. AM 4054 was synthesized by reducing the C-ring and replacing the C9 methyl group in AM 411 by the hydroxymethyl group (β-conformation). *In vivo* studies (Bergman et al., unpublished work) showed that AM 4054 at a dose of 0.03 mg/kg had a rapid recovery from operant behavior in monkeys as compared to AM 411 at a dose of 1.0 mg/kg. Keeping these results in mind, following three approaches were used to modulate the depot effect by increasing compound polarity. It was thus planned to incorporate polar hydroxy group within strategic position of DMH-Δ⁸-THC ester analog [Figure 2.8], as summarized below:

a) Hexahydro hydroxymethyl (β-conformation) ester analog [structure 1 in Figure 2.8]

b) 11-hydroxy ester analog [structure 2 in Figure 2.8]

c) 9-hydroxy ester analog, both α and β epimers [structure 3 in Figure 2.8]

*Figure 2.8:* Structures of analogs for improved polarity
4) Ester group in Other Key Pharmacophoric Sites of DMH-Δ⁸-THC

We also aimed to incorporate an ester group at different pharmacophoric sites of DMH-Δ⁸-THC and study the pharmacological activity of the respective analogs. The other key pharmacophoric sites (apart from the side chain) chosen for modification were the geminal dimethyl group at C1’ position and the C-ring. These modifications employed the β-lactone at C1’ position and the lactone in the C-ring. The key representative examples of β-lactone at C1’ position and the lactone in the C-ring are shown in Figure 2.9 and Figure 2.10 respectively.

![Figure 2.9 β-lactone at C1’ position](image1)

![Figure 2.10: Lactone in the C-Ring](image2)

5) Design and Synthesize Ester Analogs with CB2 Selectivity

Activation of CB2 receptors can elicit therapeutic immuno-modulatory effects in animals. Targeting selectively the CB2 receptor is a primary goal for treating neuropathic pain. The design of novel CB2 selective THC analogs should be such that they exhibit no pharmacological activity at central CB1 receptor, thereby eliminating the risk of central CB1 mediated psychotropic effects. Biaryl analogs and analogs with short side chains tend to bind preferentially to CB2 receptors rather than to CB1 receptors. Therefore, we planned to synthesize CB2 selective biaryl analogs with shorter ester side chains and characterize their pharmacological profile in vitro and in vivo.
6) Analogs with Thioester and Amide group at C2′ Position, Analogs with Cyclobutyl at C1′ Position and Analogs with reduced cLogP and increased tPSA

To extend our SAR and have a more detailed understanding of the enzymatic inactivation, it was planned to incorporate the thioester and the amide group at C2′ position and profile the analogs in vitro and in vivo. The hydrolysis rate of the amide group by amidases is relatively slow as compared to the thioester group, which undergoes rapid hydrolysis by ubiquitous esterases. From a therapeutic perspective, it would be interesting to generate analogs with widely varying durations of action. Studies within our group suggested that cyclobutyl at C1′ position of DMH-Δ^8-THC imparts selectivity towards CB1 receptors. In order to obtain a CB1 selective cannabinergic analog with controlled detoxification, it was planned to incorporate cyclobutyl group at C1′ position of DMH-Δ^8-THC (keeping an ester group at C2′ position) and profile in vitro and in vivo. All the analogs designed so far in this dissertation had cLogP values ranging from 4.8 to 6.5, which was relatively on higher side as compared to the cLogP values of the most of marketed drugs, i.e., they do not follow Lipinski’s ‘rule of 5’. Although there are multiple parameters that need to be considered while improving the PK/PD profile of a compound in vivo, at this stage we planned to focus on cLogP and tPSA only as an initial effort. Therefore it was planned to synthesize analogs with cLogP values from 2.5-3.5 to improve druggability.

7) In vitro and in vivo screening of novel cannabinergic analogs to identify a lead compound for advanced preclinical profiling

In vitro: Analogs would be initially characterized biochemically by determining their in vitro affinities for the two primary cannabinoid (CB1 and CB2) receptors followed by in vitro metabolic stability towards plasma esterases. In vitro affinities would be determined by using
standard competitive radiometric binding assays and forebrain synaptosomal membrane from the rat brain (for CB1 receptors) and HEK293 cell membranes (recombinant human CB2 receptors). Displacement of tritiated CP-55,940 (standard) from these membranes by novel analogs will be used to determine binding affinity (IC\textsubscript{50} values) for the novel analogs.

Plasma stability will be determined by incubating the test analog and its metabolite in mouse plasma. Sample of the mixture taken at various endpoints will be analyzed using HPLC.

Analogs with acceptable CB-receptor binding affinities (Ki < 30 nM) would be profiled externally for their functional activity using two well-accepted functional biochemical assays i.e., β-arrestin assay (at DUKE University) and cAMP assays (at CEREP Labs). We expected to identify novel cannabinergic analogs with favorable agonist affinity towards CB receptor, predictable in vitro half-life, and good functional activity. Novel compounds with these characteristics would be considered “successful analogs” for the purpose of additional profiling in vivo.

**In vivo:** Initial in vivo profiling of the successful analogs would be conducted using two well defined rodent assays (hypothermia and analgesia) for cannabinergic activity. The first assay determines the ability of test compound to act as a CB1 agonist and induce hypothermia, the second determines the ability of the test compound to activate cannabinergic signaling and thereby reduce nociceptive pain. The details of both assays are discussed in preliminary studies below.

Data from rodent studies will enable the identification of a lead compound with desired pharmacological activity to be studied further for advanced preclinical studies.
Chapter 3: Synthesis of Novel Cannabinergic Analogs with Controlled Detoxification

3.1 Synthesis of Novel Cannabinergic Analogs with an Ester group at C2’ position

The retrosynthetic analysis for the synthesis of novel cannabinergic analogs with ester group at C2’ position required the esterification of tricyclic carboxylic acid intermediate 4 with alkyl halides in the presence of sodium bicarbonate. The experimental Schemes for the synthesis of tricyclic carboxylic acid intermediate is summarized in Scheme 3.1, whereas, the detailed experimental scheme for the synthesis of novel cannabinergic analogs with ester group at C2’ position are summarized in Schemes 3.2 and 3.3.

**Scheme 3.1: Synthesis of tricyclic carboxylic acid intermediate 4**

**Chemistry**

The synthesis of tricyclic carboxylic acid intermediate 4 is accomplished in 4 steps [Scheme 3.1]. Sequential deprotonation of (3,5-dimethoxyphenyl)acetonitrile with sodium hydride and geminal dimethylation using iodomethane gave intermediate 1 in 95% yield. This reaction furnished the product in higher yield than a previously reported method (69% yield) which involved the
Scheme 3.2: Synthesis of (-)-Δ^2-THC analogs with an ester group at C2' position
Scheme 3.3: (-)-Δ8-THC analogs with polar groups at terminal carbon of side chain
nucleophilic aromatic substitution (S$_{N}$Ar) of 1-fluoro-3,5-dimethoxybenzene by isobutyronitrile anion. Hydrolysis of the resulting nitrile group under basic conditions$^{92,94}$ gave carboxylic acid intermediate \textit{2} in 93% yield. Subsequent cleavage of the aryl methyl ether groups using boron tribromide in CH$_2$Cl$_2$ afforded \textit{3} in 85% yield.$^{95}$ Condensation of resulting resorcinol \textit{3} with (+)-cis/trans-\textit{p}-mentha-2,8-dien-1-ol in the presence of catalytic amounts of \textit{p}-toluenesulfonic acid (\textit{p}-TSA)$^{96}$ afforded desired tricyclic carboxylic acid intermediate \textit{4} in 32% yield.

The tricyclic carboxylic acid intermediate \textit{4} (AM7408) was alkylated with various alkyl halides under our optimized microwave (MW) conditions (NaHCO$_3$, DMF, 165°C, 12 min) to give the desired analogs with an ester group at C2’ position as shown in Scheme 3.2. Nucleophilic substitution of the terminal bromo group by various nucleophiles, e.g., sodium cyanide, imidazole, morpholine and azide, gave the respective cannabinergic analogs carrying the ester group at C2’ position as shown in Scheme 3.3.

### 3.2 Reverse Ester Δ$^8$-THC Analog, C1’-Methyl Substituted Δ$^8$-THC Ester Analog and Δ$^8$-THC Ester Analog Lacking Gemial Dimethyl at C1’ Position

The recognition of the fact that geminal dimethyl group alpha to the ester group provides extensive stability to the molecule towards enzymatic inactivation by esterase, led us to design and synthesize a second generation cannabinergic analogs with reduced steric hindrance at C1’ position. The first approach used in this regard is to move the ester group away from geminal dimethyl group from the C2’ to C3’ position (reverse ester) to enable rapid enzymatic inactivation by esterases as compared to the analog with geminal dimethyl group alpha to the ester group.
The second approach is to replace the geminal dimethyl group at C1’ position by monomethyl group, which offers relatively less steric hindrance towards enzymatic inactivation by esterases than geminal dimethyl group. The last approach being used to facilitate the enzymatic inactivation by esterase is to remove the geminal dimethyl group at C1’ position and study its pharmacological profile *in vitro* and *in vivo*.

### 3.2.1 Reverse Ester Analog

![Scheme 3.4: Synthesis of (-)-DMH-Δ⁸-THC reverse ester analog](image)
**Chemistry:**

The key step in the synthesis of reverse ester analog 23 was a Mitsunobu reaction involving intermediate 22 and valeric acid. Alcohol 22 was synthesized in four steps starting from nitrile 1. The reduction of nitrile 1 using diisobutylaluminium hydride (DIBAL-H)\(^97\) in anhydrous CH\(_2\)Cl\(_2\) gave aldehyde 19 in quantitative yield. Treatment of 19 with sodium borohydride\(^98\) in methanol gave alcohol 20 in excellent yield. This was followed by the cleavage of the aryl methyl ether groups using boron tribromide in anhydrous CH\(_2\)Cl\(_2\)\(^95\) to give resorcinol 21 in 48% yield.

Condensation of 21 with (+)-cis/trans-\(\text{p-mentha-2,8-dien-1-ol}\) using our optimized MW conditions gave alcohol 22 in 30% yield. Mitsunobu reaction\(^99\) on alcohol 22, using valeric acid, triphenyl phosphine (TPP) and diethylazodicarboxylate (DEAD) in dry THF, led to the desired reverse ester analog 23 in 41% yield [ Scheme 3.4].

### 3.2.2 C1’-Methyl Substituted Ester Analog

An alternative approach to reduce the steric hindrance imposed by the geminal dimethyl group at C1’ involves the deletion of one methyl group. This modification generates a new chiral center at C1’ and since the stereochemistry at 6a and 10a positions is locked (\(R, R\)), a mixture of diastereomers is produced. It was planned to separate the mixture of diastereomers using preparative LCMS and study their pharmacological profile individually.
3.2.2.1 C1′-Methyl Substituted Ester Analog (Mixture of Diastereoisomers)

Synthesis of the C1′ methyl substituted ester analog is summarized in Scheme 3.5. The key step i.e., methylation of (3,5-dimethoxyphenyl)acetonitrile using NaH, MeI and DMF, was carried out at low temperature to suppress the formation of the geminal dimethylated product. Thus at -78°C the desired monomethylated product was formed almost exclusively, while at room temperature the geminal dimethyl product was also produced (45% yield) along with the monomethylated product.

Scheme 3.5: Synthesis of C1′ methyl substituted (−)-Δ⁸-THC ester analog

Chemistry

Alkylation of commercially available (3,5-dimethoxyphenyl)acetonitrile at -78°C using iodomethane and sodium hydride in dry DMF¹⁰⁰ gave desired mono-methylated intermediate 24
in 75% yield [Scheme 3.5]. Hydrolysis of the nitrile 24 using NaOH in n-BuOH/H2O under reflux afforded desired carboxylic acid intermediate 25 in quantitative yield. Cleavage of the aryl methyl ether groups using boron tribromide gave resorcinol 26, which was condensed with (+)-cis/trans-p-mentha-2,8-dien-1-ol in the presence of catalytic amounts of p-TSA to give tricyclic carboxylic acid intermediate 27 in 40% yield. Microwave irradiation of 27 and butylbromide using our optimized conditions gave the desired C1′-methyl substituted ester analog 28 in 80% yield as mixture of diastereomers.

Various attempts to separate the mixture of diastereomers using preparative LCMS were unsuccessful. So, an alternative, asymmetric chiral synthetic approach was used to synthesize the diastereomers separately using using Evans chiral auxiliary (substituted oxazolidinone) as described below.

### 3.2.2.2 C1′-Methyl Substituted Ester Analog [C1′, 6a, 10a (R,R,R) Diastereomer]

**Chemistry**

The synthesis of the desired diastereomer was accomplished in 7 steps as summarized in Scheme 3.6. Acylation of the commercially available (3,5-dimethoxyphenyl)acetic acid by adding intermittently, 1.5 M stock solution of thionyl chloride in 1-H benzotriazole gave acyl chloride 29 in 92% yield. The resulting acyl chloride 29 was converted to chiral imide 30 using Evan’s chiral auxiliary (R)-4-benzyl-oxazolidin-2-one. The resulting chiral imide 30 was alkylated using sodium hexamethyldisilylamine and methyl iodide to furnish 31 in good yield. As expected, the major diastereomer (9:1) was produced by the alkylation of the less shielded face of chiral auxiliary. Subsequent hydrolysis of the purified alkylated imide under mild basic
conditions (LiOH, THF: H₂O) gave 32. The cleavage of aryl methyl ether groups of 32 under standard boron tribromide conditions afforded resorcinol 33, which was condensed with (+)-cis/trans-\(\text{p}\)-mentha-2,8-dien-1-ol in the presence of catalytic amounts of \(p\)-TSA to give 34. The resulting tricyclic carboxylic acid moiety 34 was irradiated using bromobutane and sodium bicarbonate to give the desired diastereomer 35 in 67% yield.

\[
\begin{align*}
\text{OMe} & \quad \text{MeO} \\
\text{Me} & \quad \text{COOH} \\
\text{Me} & \quad \text{COCl} \\
\text{OMe} & \quad \text{MeO} \\
\text{OMe} & \quad \text{COOH}
\end{align*}
\]

\[
\begin{align*}
\text{SOCl}_2, 1\text{H-Benztriazole} & \quad \text{CH}_2\text{Cl}_2, 15 \text{ min} \\
\text{BuLi, } & \quad \text{HO}, -30^\circ\text{C}, \text{THF} \\
\text{LiOH, THF: H}_2\text{O (50:50)} & \quad 0^\circ\text{C}, 2 \text{ h} \\
\text{NaHCO}_3, & \quad 165^\circ\text{C}, 12 \text{ min}
\end{align*}
\]

**Scheme 3.6**: Synthesis of C1′-methyl substituted ester analog [C1′,6a,10a (R,R,R) Diastereomer]
3.2.2.3 C1'-Methyl Substituted Ester Analog [C1’, 6a, 10a (S,R,R) Diastereomer]

![Chemical Structures]

Scheme 3.7: Synthesis of C1'-methyl substituted ester analog [C1’,6a,10a (S,R,R) Diastereomer]

Chemistry

The synthesis of the desired diastereomer was analogous to the synthesis of 35 except in this case, a different chiral auxiliary was used. The detailed experimental scheme is summarized in Scheme 3.7. Chiral imide 36 was synthesized from acyl chloride 29 using evan’s chiral auxiliary, (S)-4-isopropyl-oxazolidin-2-one. The resulting chiral imide 36 was alkylated using reaction conditions analogous to the synthesis of 31 [Refer Scheme 3.6] to give 37. As expected in this
case, the major diastereomer (9:1) was produced by the alkylation of the less shielded face of chiral auxillary. Subsequent synthetic steps i.e., hydrolysis of the purified alkylated imide 37, the cleavage of the aryl methyl ether groups of 38 and coupling with (+)-cis/trans-p-mentha-2,8-dien-1-ol\textsuperscript{96} to give 40 were analogous to the already optimized conditions used for the synthesis of 35. The resulting intermediate 40 was irradiated using bromobutane and sodium bicarbonate to give the desired diastereomer 41.

### 3.2.3 Analogs Lacking Geminal Dimethyl group at C1′ Position

![Scheme 3.8: Synthesis of (-)-\(\Delta^8\)-THC analogs lacking geminal dimethyl group at C1′ position](image)

**Scheme 3.8**: Synthesis of (-)-\(\Delta^8\)-THC analogs lacking geminal dimethyl group at C1′ position
Chemistry

The synthesis of analogs without geminal dimethyl group at C1’ position is summarized in Scheme 3.8. Hydrolysis of (3,5-dimethoxyphenyl)acetonitrile under aqueous basic conditions\textsuperscript{92} gave intermediate 42 in 88% yield. Cleavage of the aryl methyl ether groups using boron tribromide\textsuperscript{95} led to the resorcinol 43 in 88% yield. Condensation of the resulting resorcinol 43 with \(+\)-cis/trans-\(p\)-mentha-2,8-dien-1-ol in the presence of catalytic amounts of \(p\)-TSA\textsuperscript{96} gave 44 in 40% yield. The tricyclic carboxylic acid intermediate 44 was irradiated using 1,4-dibromobutane and sodium bicarbonate to give 45 in 59% yield. Irradiating 44 with bromobutane using our optimized conditions gave 47 in 52% yield. The terminal bromo functionality of 45 was converted to the nitrile 46 using sodium cyanide in DMSO.

3.3 11-Hydroxy hexahydro, 11-Hydroxy and 9-Hydroxy Ester Analogs of \((-\Delta^8\)-THC

The polar analogs were expected to undergo rapid diffusion through lipid bilayer to the blood stream and undergo enzymatic inactivation after exhibiting desired pharmacological profile. The chemistry of first polar analog in this series i.e., 11-hydroxy hexahydro ester analog is described below.

3.3.1 Hexahydro Ester Analog with Hydroxymethyl group (\(\beta\)-conformation) at C9 Position

The erratic PK profile of cannabinoids can be attributed to its high lipophilicity as well as to the formation of the pharmacologically active metabolite, 11-OH-THC (formed by the allylic oxidation of \(\Delta^9\)-THC at C9 position). Theoretically, the PK profile of cannabinoids can be

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improved by various methods. One such method involves the blockade of the formation of 11-hydroxy analog by preventing the allylic oxidation of THC. This can be achieved by reducing the C-ring of Δ⁹-THC. Secondly, published literature indicated that the hydroxy methyl group at C9 position is more potent in vivo as β-epimer than α-epimer¹⁰⁴, so it was planned to synthesize hehahydro ester analog with hydroxymethyl group at C9 position in the β-conformation.

Scheme 3.9: Synthesis of Hexahydro-9β-hydroxymethyl analog of DMH-Δ⁸-THC
Chemistry

The synthesis of hexahydro ester analog is summarized in Scheme 3.9. The first step involved the alkylation of 3 with n-bromobutane utilizing our optimized conditions to give 48 in quantitative yield. Coupling of 48 with a mixture of nopinone diacetates (prepared according to Archer et al.)\textsuperscript{105} in the presence of catalytic amounts of p-TSA gave norpinanone 49 in 53\% yield. Subsequent enclosure of dibenzo[\textit{b,d}]pyran was initiated via catalytic amounts of TMSOTf to generate the ketone 50 in good yield. Treatment of 50 with methoxymethyl triphenyl phosphonium chloride in benzene produced a mixture of enol ethers 51 which were hydrolyzed using wet trichloroacetic acid to give aldehyde (mixture of \(\alpha\) and \(\beta\) epimers) 52. Epimerization using potassium carbonate in methanol gave 53 in 78\% yield. Sodium borohydride reduction of aldehyde 53 led to the desired hexahydro analog 54 in 72\% yield.

3.3.2 11-Hydroxy Ester Analog of DMH-\Delta^8-THC

Chemistry

The proposed synthetic route for the synthesis of 11-hydroxy ester analog involved the lewis acid catalyzed condensation of resorcinol 3 with [(1\textit{R}, 5\textit{S})-4-hydroxy-6,6-dimethylbicyclo[3.1.1]hept-2-en-2-yl] methyl pivalate (prepared according to Liddle, et al.)\textsuperscript{106} to give the pivalate protected tricyclic carboxylic acid as shown in Scheme 3.10. However, several attempts to couple 3 with the pivalate ester terpene were unsuccessful.\textsuperscript{106} This was hypothesized initially to be due to the presence of the strong electron withdrawing carboxylic acid group in 3, which may be deactivating the aromatic ring to prevent the coupling. However, this hypothesis may seem incorrect because carboxylic acid was connected to the aromatic ring via quaternary
carbon. Therefore, it was unlikely that carboxylic acid may cause the deactivation of the aromatic ring. However, various approaches were used to reduce the electron withdrawing effect of carboxylic acid and attempted to couple with pivalate ester terpene. One such approach involved the formation of dithiolane\textsuperscript{107} at C1’ position in place of carboxylic acid, in which case, the coupling was successful, but deprotection of the dithiolane ring was ineffective. An
alternative approach employed for the synthesis of 11-hydroxy ester analog involved the allylic oxidation of 4 using selenium dioxide, which proceeded via ene reaction to give 55 [Scheme 3.10]. Sodium borohydride reduction of the aldehyde 55 in methanol gave 56 in 42% yield. The tricyclic carboxylic acid intermediate 56 was irradiated with bromobutane and 1-bromobutane-4-imidazole to give the desired 11-hydroxy ester analogs 57 and 58 respectively. Although this synthetic route led to desired 11-hydroxy ester analogs but it was unattractive due to the low yielding allylic oxidation of 4 (11% yield) to form 55.

The overall yield of the desired 11-hydroxy ester analog was improved utilizing an alternative approach. Under this approach, carboxylic acid 3 was esterified and then condensation with the pivalate ester terpene106 was attempted. Interestingly, this reaction proved to be successful and gave 60 in high 40% yield [Scheme 3.11]. The methyl and pivalate ester groups were hydrolyzed in one step, using strong basic conditions (NaOH) under reflux, to give the intermediate 56a. The resulting tricyclic acid intermediate 56a was irradiated with bromobutane and sodium bicarbonate to give desired 11-hydroxy ester analog 57 in 47% yield.

Scheme 3.11: Modified experimental Scheme for the synthesis of 11-hydroxy analog
3.3.3 9-Hydroxy Ester Analog (α and β epimers) of DMH-Δ⁸-THC

Chemistry

The proposed synthetic route for the synthesis of 9-hydroxy ester analog involved the condensation of resorcinol acid 3 with the mixture of nopinone diacetates using catalytic amounts of p-TSA. However, several attempts to couple 3 with the mixture of nopinone diacetates were unsuccessful. We again opted to reduce the electron withdrawing effect of carboxylic acid intermediate and then try to condense with mixture of nopinone diacetates. In order to reduce the electron withdrawing effect of carboxylic acid following two approaches were used:

1: Carboxylic acid moiety was transformed into oxazoline
2: Carboxylic acid moiety was converted into methyl ester

The carboxylic acid moiety of 3 was transformed to oxazoline and then the condensation with mixture of diacetates was attempted. Interestingly, although the condensation reaction was initiated but the complete conversion of starting material to the product was never achieved. However, while transforming carboxylic acid to 2-oxazolines, novel methodology using microwave was developed. This method offers various benefits as compared to existing ones and was published in Tetrahedron Letters 50 (2009), 5780-5782 as described below.

Various methods are available to transform carboxylic acid to oxazoline. One of the earlier methods have used conventional heating of equimolar concentrations of carboxylic acids and 2-aminoethanol at 200°C followed by the dehydration of N-acyl-2-aminoethanols using phosphorous pentoxide to generate oxazolines. Thionyl chloride and other reagents have also
<table>
<thead>
<tr>
<th>Entry</th>
<th>Acid</th>
<th>Oxazoline Product</th>
<th>Reaction Conditions</th>
<th>% Yield</th>
</tr>
</thead>
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<td>170°C, 15 min</td>
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</tr>
<tr>
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</tr>
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</tr>
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<td>170°C, 15 min</td>
<td>83</td>
</tr>
<tr>
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</tr>
<tr>
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</tr>
<tr>
<td>7</td>
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<td>170°C, 15 min</td>
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</tr>
<tr>
<td>8</td>
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<td>170°C, 15 min</td>
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</tr>
<tr>
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<td>78</td>
</tr>
<tr>
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<td>COOH</td>
<td><img src="image12" alt="Oxazoline Product" /></td>
<td>170°C, 25 min</td>
<td>78</td>
</tr>
</tbody>
</table>
been used for this transformation\textsuperscript{110}. A recent method used 2-chloro-4,6-dimethoxy-1,3,5-triazine and \(N\)-methylmorpholine to generate a complex (1 equivalent), which upon treatment with carboxylic acids and 3 equivalents of 2-amino-2-methyl-1-propanol at room temperature, gave the corresponding oxazolines.\textsuperscript{111} More recently, Kangani and Kelley\textsuperscript{112} reported using bis(2-methoxyethyl)aminosulfur trifluoride (Deoxo-Fluor, 2.2 equivalents) with carboxylic acids in the one pot synthesis of amides from secondary amines or 2-oxazolines from 1.8 equivalents of 2-amino-2-methyl-1-propanol at 0°C. This method reportedly worked better than the triazine complex\textsuperscript{111} coupling for aliphatic and benzoic acids. We were unable to prepare the corresponding 2-oxazoline of 3,5-dimethoxy phenylacetic acid by this method, however, we developed an efficient method which is a variation of the direct synthesis\textsuperscript{113} of 2-oxazolines from carboxylic acids using microwave.

We transformed various carboxylic acids into their corresponding 2-substituted 4,4-dimethyl-2-oxazolines with microwave heating using 2-amino-2-methyl-1-propanol [Table 3.1]. Various carboxylic acids and microwave heating conditions were studied for this transformation. Microwave heating in closed vessels led to intermediate amide formation, but no cyclization. However, by performing the reaction in an open vessel mode, which allows for the dehydration of the amide intermediates, the 2-oxazoline products were prepared in moderate to high yield. Open vessel mode microwave heating of carboxylic acids in excess 2-amino-2-methyl-1-propanol without solvent at 150°C for 5 min led to intermediate amides with very little oxazoline formation. The optimized reaction conditions required heating at 170°C for 15 min, except for Entries 9, 10, and 12 which required 25 min and Entry 11 that required 40 min for oxazoline formation. Longer heating tends to result in more decomposition byproducts. Yield of 2-phenyl (Entry 1, 85%; \textit{lit.}\textsuperscript{111} 78%; \textit{lit.}\textsuperscript{112} 97%; \textit{lit.}\textsuperscript{113a} 90%) and aliphatic (Entry 11, 73%; \textit{lit.}\textsuperscript{111} 82%;
lit.\textsuperscript{112} 99\%; lit.\textsuperscript{113a} 84\%) 2-oxazolines were comparable to the mild methods using coupling and dehydrating reagents or zinc oxide assisted microwave conditions for these thermally stable carboxylic acids. In addition to aliphatic and aromatic carboxylic acids, Entries 3, 4, and 12 demonstrate the application of this open vessel methodology to unsaturated carboxylic acids. The method was also successfully applied to dicarboxylic acids as demonstrated by entries 9 and 10. We were unable to prepare the corresponding 2-oxazaole of 3,5-dimethoxyphenylacetic acid using Deoxo-Fluor, but obtained a 73\% isolated yield (Entry 6) using open vessel microwave heating with 6 equivalents of 2-amino-2-methyl-1-propanol at 170 °C for 15 min. A comparable 68\% isolated yield of this oxazoline was obtained from 3,5-dimethoxyphenylacetic acid using 2 equivalents of 2-aminoalcohol with longer heating (40 min) conditions, and when using 1 equivalent of 2-aminoalcohol was used, a 60\% isolated yield was obtained after 40 min. Thus, using an excess of 2-aminoalcohol, when possible, results in higher conversion to 2-oxazoline, less amide intermediate and other byproduct impurity, and less product decomposition. So, in conclusion, this method proved to be simple and efficient for the conversion of carboxylic acids into 2-oxazolines in good yield. Unlike some of the previously reported methods, our microwave open vessel technique requires only the reactants (solvent-free), relatively short reaction times, and simple chromatographic purifications.

**Typical Procedure**

The 3,5-dimethoxyphenyl acetic acid (500 mg, 2.55 mmol) was mixed with 2-methyl-2-aminopropanol (1.36 g, 15.3 mmol, 6 equivalents) in a CEM microwave vessel. The resulting mixture was irradiated using the open vessel mode at 170 °C for 15 min. The reaction mixture was quenched with water (4 mL) and extracted with ethyl acetate (3 x 10 mL). The combined organic extracts were dried (MgSO\textsubscript{4}), and concentrated by rotoevaporation under \textit{vacuo} to give
the crude product, which was then chromatographed on silica gel using a Biotage SP2 eluting with acetone/hexane (0% to 30% acetone gradient) to give the corresponding 2-(3,5-dimethoxyphenylmethyl)-2-oxazoline (464 mg, 1.86 mmol, 73% yield, Entry 6) as a faint yellow liquid product that was homogeneous by TLC (40:60 acetone/hexane, \( R_f \) 0.65).

NMR data

**Entry 1**: 1H NMR (500 MHz, chloroform-\( d \)) \( \delta \) 1.38 (s, 6H), 4.10 (s, 2H), 7.39 (dd, \( J = 8.0 \), 7.5 Hz, 2H), 7.46 (tt, \( J = 7.5 \), 1.5 Hz, 1H), 7.94 (dd, \( J = 8.0 \) Hz, 1.5 Hz, 2H) Liquid

**Entry 2**: 1H NMR (500 MHz, chloroform-\( d \)) \( \delta \) 1.43 (s, 6H), 4.15 (s, 2H), 7.25-7.38 (m, 2H), 7.62-7.68 (m, 2H) Liquid

**Entry 3**: 1H NMR (500 MHz, chloroform-\( d \)) \( \delta \) 1.34 (s, 6H), 4.03 (s, 2H), 6.58 (d, \( J = 16.5 \) Hz, 2H), 7.28 (d, \( J = 16.5 \) Hz, 1H), 7.32 (d, \( J = 8.5 \) Hz, 2H), 7.49 (d, \( J = 8.5 \) Hz, 2H). Melting point: 86 °C

**Entry 4**: 1H NMR (500 MHz, chloroform-\( d \)) \( \delta \) 1.34 (s, 6H), 4.03 (s, 2H), 6.62 (dd, \( J = 16.0 \), 1.5 Hz, 2H), 7.31-7.37 (m, 4H), 7.47 (d, \( J = 8.0 \) Hz, 2H) Liquid

**Entry 5**: 1H NMR (500 MHz, chloroform-\( d \)) \( \delta \) 1.37 (s, 6H), 2.37 (s, 3H), 4.07 (s, 2H), 7.19 (d, \( J = 7.5 \) Hz, 2H), 7.82 (br d, \( J = 7.5 \) Hz, 2H). Melting Point: 45 °C

**Entry 6**: 1H NMR (500 MHz, chloroform-\( d \)) \( \delta \) 1.28 (s, 6H), 3.52 (s, 2H), 3.76 (s, 6H), 3.91 (s, 2H), 6.34 (t, \( J = 2.2 \) Hz, 1H), 6.46 (d, \( J = 2.2 \) Hz, 2H). Liquid

**Entry 7**: 1H NMR (500 MHz, chloroform-\( d \)) \( \delta \) 1.38 (s, 6H), 3.81 (s, 6H), 4.09 (s, 2H), 6.56 (t, \( J = 2.5 \) Hz, 1H), 7.09 (d, \( J = 2.5 \) Hz, 2H). Liquid
**Entry 8:** 1H NMR (500 MHz, chloroform-\textit{d}) $\delta$ 1.26 (s, 6H), 3.60 (s, 2H), 3.88 (s, 2H), 7.28 (tt, $J$ = 7.5, 1.5 Hz, 1H), 7.34-7.39 (m, 4H), 7.50-7.55 (m, 4H). Melting point: 60 °C

**Entry 9:** 1H NMR (500 MHz, chloroform-\textit{d}) $\delta$ 1.26 (s, 12H), 1.22-1.35 (m, 16H), 1.61 (quintet, $J$ = 7.5 Hz, 4H), 2.23 (t, $J$ = 7.5 Hz, 4H), 3.86 (s, 4H). Liquid

**Entry 10:** 1H NMR (500 MHz, methanol-\textit{d}4) $\delta$ 1.25 (s, 12H), 2.40 (s, 4H), 3.56 (s, 4H). Melting Point: 142 °C

**Entry 11:** 1H NMR (500 MHz, chloroform-\textit{d}) $\delta$ 0.88 (t, $J$ = 6.5 Hz, 3H), 1.22-1.38 (s, 34H), 1.62 (quintet, $J$ = 7.5 Hz, 2H), 2.24 (t, $J$ = 7.5 Hz, 2H), 3.91 (s, 2H). Liquid

**Entry 12:** 1H NMR (500 MHz, chloroform-\textit{d}) $\delta$ 0.86 (t, $J$ = 6.5 Hz, 3H), 1.24 (s, 6H), 1.25-1.36 (m, 6H), 1.62 (quintet, $J$ = 7.5 Hz, 2H), 2.05 (dt, $J$ = 7.1, 7.1 Hz, 2H), 2.15 (dt, $J$ = 7.2, 7.2 Hz, 2H), 2.23 (t, $J$ = 7.4 Hz, 2H), 2.76-2.84 (m, 6H), 3.86 (s, 2H), 5.30-5.41 (m, 8H). Liquid

The alternative approach already used in earlier section [Section 3.3.2] to decrease the electron withdrawing effect of carboxylic acid intermediate 3, involved the transformation of the carboxylic acid moiety into methyl ester as shown in Scheme 3.12. The resulting methyl ester resorcinol 59 was attempted to condense with a mixture of nopinone diacetates. Interestingly, the condensation reaction proceeded smoothly in the presence of catalytic amounts of $p$-TSA 105, 108 to generate norpinanone 61 in 52% yield. The next step involved the enclosure of the dibenzo-\textit{[b,d]}pyran ring of 61 using standard TMSOTf conditions.114 The ring enclosure reaction gave primarily the desired product 62 along with small traces of unreacted and inseparable 61. It was planned to proceed for the next hydrolysis step without further purification and then separate the mixture. Therefore, the mixture of 61 and 62 was used as such for the next hydrolysis reaction
Scheme 3.12: Synthesis of 9-hydroxy ester analog of DMH-\(\Delta^8\)-THC
using sodium hydroxide in THF: water (50:50) to give 63 in 43% yield. Irradiating 63 and sodium bicarbonate in DMF using our optimized conditions afforded ketone 64 in 68% yield. Sodium borohydride reduction of ketone 64 in methanol gave corresponding equatorial hydroxyl analog 65, along with traces of separable axial isomer 68. K-selectride reduction of 63 in THF led to corresponding axial hydroxyl intermediate 67, which was irradiated under our optimized microwave conditions using sodium bicarbonate and bromobutane to give desired axial 9-hydroxy ester analog 68. Sodium borohydride reduction of 63 in methanol gave 66 in desired $\beta$-conformation.108

### 3.4 $\beta$-Lactone at C1’ Position and Lactone in the C-Ring of DMH-$\Delta^8$-THC

Extensive SAR have been generated by modifying the B-Ring and the C-Ring of DMH-$\Delta^8$-THC keeping ester group at C2’ position in the side chain. Additionally, the pharmacophoric limits of side chain length have also been explored by synthesizing the short chain as well as the long chain ester analogs with bulky substitutions like imidazole and morpholine at terminal carbon of side chain. In pursuit to have a more detailed understanding of the tolerance and pharmacological relevance of the ester group within DMH-$\Delta^8$-THC we have extended our SAR by incorporating the ester group in other key pharmacophoric sites. It is important to reemphasize that the metabolite so formed after enzymatic inactivation should have no (or minimal) activity at CB receptors. Two key pharmacophoric sites of DMH-$\Delta^8$-THC which were identified for modification include:

1: $\beta$-lactone at C1’ position of DMH-$\Delta^8$-THC

2: Lactone in the C-ring
3.4.1 β-Lactone at C1’ Position of DMH-Δ^8-THC in place of the Geminal Dimethyl group

Geminal dimethyl group at the C1’ position of DMH-Δ^8-THC has been shown to play a pivotal role in enhancing the affinity and pharmacological activity at CB receptors. Earlier work in our group investigated various cyclic substitutions such as cyclopropyl, cyclobutyl, cyclopentyl and cyclohexyl at C1’ position in place of the geminal dimethyl group.\(^{97a}\) It was found that cyclopropyl and cyclopentyl group at C1’ position are optimal pharmacophores for both the CB receptors while the cyclobutyl group at C1’ interacts selectively with CB1 receptors and not with CB2 receptors.\(^{97a}\)

In line with our rational drug design, β-lactone at C1’ position of the side chain of DMH-Δ^8-THC was introduced. The analog design was based on the rationale that the compound should retain high affinity and pharmacological activity at CB receptors and undergo enzymatic inactivation by esterases in a predictable manner generating inactive metabolites.

**Chemistry**

The detailed experimental scheme for the synthesis of β-lactone analog is summarized in Scheme 3.13. The first step in the synthesis of β-lactone at C1’ involved the synthesis of the phenone 69 from 3, 5-dimethoxybenzonitrile using commercially available Grignard reagent, hexyl magnesium bromide, in the presence of copper bromide.\(^{97b, 107, 115}\) Boron trifluoride diethyl etherate-catalyzed reaction of phenone 69 with 1,2-ethane dithiol\(^{107}\) provided 70 in quantitative yield. Cleavage of the aryl methyl ether groups\(^{95}\) led to resorcinol 71 which was condensed with (+)-cis/trans-p-mentha-2,8-dien-1-ol\(^{96}\) in the presence of catalytic amounts of p-TSA to give 72. Thiolane deprotection using silver nitrate in ethanol\(^{107}\) gave intermediate 73; followed by the protection of the free phenolic group with tert-butyl dimethyl chlorosilane (TBDMS-Cl)\(^{116}\) to
produce ketone 74 in quantitative yield. The transformation of the ketone 74 to the desired β-lactone analog was the challenging and the key step. Various synthetic methodologies have been employed for this transformation. A one step method by Danheiser et al. involved the addition of thiol ester enolates to ketone 74 to yield aldolate, which undergoes spontaneous cyclization to produce the β-lactone. A model reaction was performed on cyclohexanone using conditions optimized by Danheiser et al., to transform the ketone group of cyclohexanone to β-lactone.
Interestingly, the model reaction proceeded smoothly; however, various attempts to transform ketone 74 to β-lactone using similar conditions were unsuccessful.

Another published procedure by Vostrikov et al.\textsuperscript{118} first forms a β-hydroxy ester of the ketone or aldehyde using lithium derivatives of ethyl acetate. Subsequent hydrolysis of the resulting ester leads to a β-hydroxyl carboxylic acid, which can be cyclized using oxalyl chloride to produce β-lactone.

However, performing a classical Reformatsky reaction\textsuperscript{119} on 74 proceeded smoothly to give β-hydroxy ester 75 in 55% yield. Hydrolysis of 75 under reflux using sodium hydroxide in THF/H\textsubscript{2}O\textsuperscript{118} gave β-hydroxy carboxylic acid 76 in good yield. (Under these conditions the TBDMS group also was hydrolyzed). Subsequent cyclization utilizing N-phenyl-bis(trifluoromethane sulfonimide)\textsuperscript{120} as a dehydrating agent led to the desired β-lactone 77 as shown in Scheme 3.13.

### 3.4.2 Lactone in the C-ring of DMH-Δ\textsuperscript{8}-THC

Disruption of the tricyclic core of DMH-Δ\textsuperscript{8}-THC may result in diminished affinity and pharmacological activity at CB receptors. An ester group can be incorporated strategically within the C-ring in such a manner that, the compound retains high affinity and pharmacological activity at CB receptors and undergoes enzymatic hydrolysis by esterases. Accordingly, the carboxylic acid metabolite, formed after enzymatic inactivation, should have no (or minimal) activity at CB receptors. It was interesting to first evaluate the tolerability of 7-membered lactones at CB receptors; and based on in vitro CB receptor affinity and the metabolic stability, these analogs could be carried forward for in vivo studies (hypothermia and analgesia).
Chemistry

The synthesis of novel analogs containing a lactone in the C-Ring is summarized in Scheme 3.14. The key step in the synthesis of C-Ring lactone was the Baeyer-Villiger (B V) oxidation on

Scheme 3.14: Synthesis of DMH-Δ⁸-THC analogs with lactone in C-Ring
Nabilone 79 was synthesized in two steps starting with the condensation of the commercially available dimethylheptyl resorcinol with a mixture of nopinone diacetates in the presence of catalytic amounts of p-TSA to give norpinanone 78 in 72% yield. Subsequent cyclization using TMSOTf as a lewis acid proceeded smoothly to give nabilone 79.

The next step was to perform the B V Oxidation on symmetrical ketone 79 to give the mixture of regioisomers. However, various attempts to perform the Baeyer-Villiger oxidation on 79 was not successful, probably due to the presence of free phenolic group. The free phenolic group was protected with various protecting groups such as; TBDMS-Cl, benzyl and acetate group, and B V oxidation reaction was performed on them individually. It was found that the reaction did not proceed with TBDMS-Cl or benzyl protected intermediates under standard conditions mentioned below:

1. condition: meta chloroperoxybenzoic acid (mCPBA), 0°C, CH₂Cl₂
2. condition: H₂O₂ (35% w/v in H₂O), trifluoroacetic anhydride, 0°C
3. condition: O₂, Benzaldehyde, carbon tetrachloride, 20-40 °C
4. condition: Na₂CO₃/ CF₃COOH, 0 °C

However, B V oxidation reaction proceeded smoothly when acetyl protected intermediate 80 was treated with mCPBA in CH₂Cl₂ at room temperature to give a mixture of inseparable regioisomers 81. Subsequent deprotection of 81 using mild basic conditions resulted in lactone ring opening, offering a mixture of carboxylic acid intermediates 82 and 83 which were separated using column chromatography. Cyclization of individual carboxylic acid
intermediates 82 and 83 using methanesulfonic acid gave the desired lactone regioisomers 84 and 85 respectively.

3.5 DMH-$\Delta^8$-THC Analogs with Amide and Thioester at C2′ Position

The enzymatic inactivation of amide group by amidases is relatively slow as compared to hydrolysis of ester group by esterases. Conversely, thioesters are hydrolyzed by ubiquitous esterases more rapidly as compared to an ester group.\textsuperscript{125} Utilizing this concept, amide and thioester groups were incorporated at C2′ position of DMH-$\Delta^8$-THC.

\begin{center}
\textbf{Scheme 3.15}: Synthesis of DMH-$\Delta^8$-THC analogs with amide and thioester groups at C2′ position
\end{center}
Chemistry

The synthesis of amide and thioester analogs is summarized in Scheme 3.15. Deoxo-Fluor (bis(2-methoxyethyl)aminosulfur trifluoride)\textsuperscript{126} was employed as a thermally stable amide coupling agent for the transformation of the carboxylic acid 4 into the desired amide 86 in 75\% yield. Acylation of intermediate 4 to acid chloride 87 using the reported procedure by Chaudhari et al.\textsuperscript{127} proceeded smoothly. Subsequent, coupling with propanethiol in pyridine gave the desired thioester analog 88 in 43\% yield [Scheme 3.15].

3.6 Biaryl Analogs with Ester group at C2’ Position

Opening of the pyran ring of $\Delta^9$-THC produces cannabidiol, a bicyclic cannabinoid devoid of many psychoactive properties produced by $\Delta^9$-THC or $\Delta^8$-THC.\textsuperscript{128} Mounting evidences are available which indicated that replacement of the terpenyl moiety of the bicyclic cannabinoid template (a) [Figure 3.1] with a substituted planar ring system, template (b), [Figure 3.1] resulted in enhanced CB2 receptor affinity and selectivity. The first such report of compounds with the biphenyl template (b) as CB2 selective analogs was disclosed by investigators at the Merck Frosst Laboratory\textsuperscript{129}. Interestingly, certain templates e.g., HU 308 [Figure 3.1], discovered by Mechoulam et al., are also known to exhibit CB2 selectivity (approx 5000 fold)\textsuperscript{130} and are under evaluation as a potent analgesics.

With the goal of designing and synthesizing CB2 selective compounds with controlled detoxification and which are devoid of undesirable psychotropic side effects, novel bicyclic compounds were pursued as described below.
Figure 3.1: Novel bicyclic cannabinoids

**Chemistry**

Retrosynthetic analysis of desired bicyclic cannabinergic analogs with controlled detoxification required the coupling of commercially available phenyl boronic acids with aryl halides using Suzuki coupling conditions.

Depicted in Scheme 3.16, synthesis of aryl bromide 89, starts from commercially available 4-bromo-3,5-dihydroxybenzoic acid. Borane reduction in THF afforded alcohol 89 in quantitative yield. Although the conversion of 89 to the benzyl chloride has been reported, a more convenient and higher yielding approach was adopted. This involved refluxing a mixture of 89 and triphenyl phosphine in dry carbon tetrachloride for two hours to give 90 in moderate yield. Treating a solution of 90 in dimethylsulfoxide with sodium cyanide gave benzyl nitrile 91 in 74% yield. Sequential deprotonation of 91 using sodium hydride and geminal dimethylation using iodomethane in DMF afforded dimethyl nitrile 92 in 74% yield.
Scheme 3.16: Synthesis of Biaryl analogs with ester group in side chain

Subsequent hydrolysis of the nitrile group using sodium hydroxide in aqueous n-butanol under reflux afforded carboxylic acid intermediate 93, which was alkylated with iodoethane to give 94 in 83% yield. Suzuki coupling of commercially available 3-cyanophenyl boronic acid with 94 produced biphenyl intermediate 95 in good yield. The cleavage of the aryl methyl ether groups using standard boron tribromide conditions gave desired biaryl analog with an ester group in side chain.
In pursuit to extend our SAR of biaryl analogs with ester group in the side chain, dimethyl substituted biaryl ester and dochloro biaryl ester analogs were also pursued. The synthesis of such analogs is summarized in Scheme 3.17. Suzuki coupling of 94 with 3,5-dimethylphenyl boronic acid gave 97, followed by the cleavage of the aryl methyl ether groups under controlled...
conditions gave a mixture of dihydroxy analog 98 and mono hydroxy analog 99, which were separated using column chromatography. Suzuki coupling of 94 and 3,5-dichlorophenyl boronic acid gave 100 in 54% yield. Subsequent cleavage of the aryl methyl ether groups under controlled conditions gave a mixture of dihydroxy analog 101 and mono hydroxy analog 102. The mixture was successfully separated using column chromatography.

3.7 Cyclobutyl at C1’ Position in place of DMH-Δ^8-THC

Chemistry

The synthesis of the desired DMH-Δ^8-THC analogs with cyclobutyl group at C1’ position is summarized in Scheme 3.18. The retrosynthetic analysis for the synthesis of desired cyclobutyl DMH-Δ^8-THC analogs required the synthesis of common tricyclic intermediate 106, which was synthesized in 4 steps starting from commercially available (3,5-dimethoxyphenyl)acetonitrile. Sequential deprotonation using KHMDS and alkylation with dibromopropane gave intermediate 103 with cyclobutyl at C1’ position. Subsequent hydrolysis of the nitrile group gave carboxylic acid intermediate 104. Cleavage of the aryl methyl ether groups gave resorcinol 105 which, upon condensation with (+)-cis/trans-p-mentha-2,8-dien-1-ol in the presence of catalytic amounts of p-TSA, afforded desired tricyclic acid 106 in 47% yield. Irradiating 106 using dibromobutane and sodium bicarbonate gave 107 with bromo functionality at the terminal carbon of the side chain. The bromo functionality was transformed to cyano analog 108 using sodium cyanide in DMSO, while bromo group of 107 was transformed to azide 109 using tetra-n-butylammonium azide in CH2Cl2. Irradiating 106 using dibromobutane under or optimized conditions afforded 110 in 67% yield.
Scheme 3.18: Synthesis of novel analogs with cyclobutyl at C2’ position
3.8 Analogs with Reduced cLogP and Increased tPSA

The designed analogs with improved molecular features like cLogP: 2.5-4.0 and tPSA ranging from 70-100 were designed and synthesized as shown in Figure 3.2 and Scheme 3.19 respectively.

![Chemical structures with cLogP and tPSA values](image)

**Figure 3.2:** Novel analogs with reduced cLogP and increased tPSA

**Chemistry**

The synthesis of THC analogs with improved Pk/Pd profile is summarized in Scheme 3.19. Alkylation of carboxylic acid intermediate 63 using dibromopropane and potassium carbonate
in DMF\textsuperscript{134} gave \textit{111}. Subsequent nucleophilic displacement of bromo group by cyanide anion in DMSO using sodium cyanide led to \textit{112} in 67% yield. Carbonyl reduction of \textit{112} using sodium borohydride in methanol afforded desired analog \textit{113}. Similarly, displacement of the bromo group of \textit{111} by imidazole using potassium carbonate in DMSO afforded \textit{114} in 84% yield. Carbonyl reduction of \textit{49} using sodium borohydride in methanol at room temperature gave \textit{115} in 91% yield.

\begin{center}
\textbf{Scheme 3.19:} Synthesis of novel THC analogs with reduced cLogP and increased \textit{t}PSA
\end{center}
Chapter 4: Experimental Section

Modern synthetic chemistry skills and advanced instrumentation were used to synthesize the novel $\Delta^8$-THC analogs. Final products were routinely obtained through multistep synthesis, which involved purification and characterization of intermediates at every synthetic step. All reagents and solvents were purchased from Aldrich Chemical Co., unless otherwise specified, and used without further purification. All anhydrous reactions were performed under a static argon or nitrogen atmosphere in flame-dried glassware using scrupulously dry solvents. Flash column chromatography employed silica gel 60 (230-400 mesh). All compounds were demonstrated to be homogeneous by analytical TLC on precoated silica gel TLC plates (Merck, 60 F245 on glass, layer thickness 250 $\mu$m), and chromatograms were visualized by phosphomolybdic acid staining. Melting points were determined on a micromelting point apparatus. IR spectra were recorded on a Perkin-Elmer Spectrum one FT-IR spectrometer. NMR spectra was recorded in CDCl$_3$, unless otherwise stated, on a Varian Mercury-300 (1H at 300MHz, 13C at 75MHz) or on a BrukerDMX-500 (1H at 500MHz, 13C at 125MHz) and chemical shifts were reported in units of $\delta$ relative to internal TMS. Multiplicities are indicated as br (broadened), s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), and coupling constants ($J$) are reported in hertz (Hz).

Low and high-resolution mass spectra were performed in School of Chemical Sciences, University of Illinois at Urbana; Champaign. Compound purity was determined by elemental analysis and HPLC. Elemental analyses (CHN) were obtained in Robertson Microlit Laboratory, Madison, NJ, and HPLC purity were performed within the CDD biochemistry group (JodiAnne Wood).
The instruments used for synthesis of novel compounds and intermediates includes mass spectrometers, liquid chromatographs, biotage purification systems, nuclear magnetic resonance (NMR) spectrometers, infrared (IR) spectrometers and polarimeter and microwave systems as described below.

**Mass spectrometry (MS)** was used to measure the molecular mass of intermediates and target compounds. **Micro mass ZQ, Waters 2996** photodiode array detector, **Waters 2424** ELS detector, **Waters 515** HPLC pumps, **Waters 2525** binary gradient module and mass spectrometry instrument from PE SCIEX as **API 150 MCA**. The instrument can be used for preparative purification to remove the impurities from the final compound, which are difficult to remove by conventional techniques.

**Compound purification:** Conventional purification techniques was used as required include silica-based column chromatography. More advanced purification approaches were used for complex mixtures, which involved automated Biotage purification systems (**Model # 09491**).

**Nuclear Magnetic Resonance (NMR):** Chromatographic compound purification was followed by chemical confirmation of the desired product with NMR spectroscopy (400 Hz and 700 Hz), a selective and nondestructive technique.

**Infrared Spectroscopy (IR)** is another valuable tool to determine the structural information about the intermediates and molecules being synthesized. IR were taken from advanced, Fourier transformation (FT) based IR system from Perkin Elmer (**Spectrum one FT-IR spectrometer**).

**Polarimeter:** A **Digipol – 781-TDV** automated polarimeter from Rudolph Instruments was used to distinguish between different stereoisomers.
**Microwave:** Microwave is used in organic chemistry as a valuable tool to facilitate synthetic routes less amenable to conventional heating, thereby offering improved reaction yield, decreased reaction times, and solvent free conditions in some cases. CDD is equipped with 2 advanced microwave systems, **CEM Discover®** and **Biotage Initiator™ 2.0.**

The experimental procedure for the synthesis of intermediates and products are described below:

2-(3, 5-Dimethoxyphenyl)-2-methylpropanenitrile (1). To a stirred suspension of sodium hydride (6.7 g, 169.2 mmol) in dry DMF (40 mL) at 0°C under an argon atmosphere was added dropwise a solution of (3,5-dimethoxyphenyl)acetonitrile (10.0 g, 56.2 mmol) and iodomethane (10.5 mL, 169.2 mmol) in dry DMF (40 mL). The reaction mixture was stirred for 15 min at 0°C, warmed to room temperature and stirred for 2 h. The reaction mixture was quenched with saturated aqueous NH₄Cl solution and diluted with diethyl ether. The organic layer was separated and the aqueous layer was extracted with diethyl ether (3 x 20 mL). The combined organic layer was washed with water and brine, dried (MgSO₄) and concentrated in vacuo. The residue was chromatographed on silica gel (25% ethyl acetate in hexane) to give 1 as colorless oil (11.01 g, 95% yield).

**1H NMR (500 MHz, chloroform-d) δ ppm** 1.71 (s, 6 H, -C(CH₃)₂-) 3.82 (s, 6 H, 2 x OCH₃) 6.40 (t, J = 2.5 Hz, 1 H, ArH) 6.61 (d, J = 2.0 Hz, 2 H, ArH).

2-(3, 5-Dimethoxyphenyl)-2-methylpropanoic acid (2). Sodium hydroxide (1.9 g, 48.7 mmol) was added to a stirred mixture of 1 (4.0 g, 19.4 mmol) in 3 mL of n-butanol: water (2:1) mixture and the resulting mixture was refluxed for 4 h. The reaction mixture was cooled to room temperature and excess of n-Butanol was removed under reduced pressure. The residue was acidified with dropwise addition of 2 N HCl and diluted with diethyl ether. The organic layer was separated and the aqueous layer was extracted with diethyl ether (3 x 20 mL). The combined
organic layer was washed with water and brine, dried (MgSO₄) and concentrated under vacuo. The residue was chromatographed on silica gel (30% diethyl ether in hexane) to give 2 (3.7 g, 93% yield) as a white solid. MP 99 °C; IR (neat): 2932, 1694, 1595 cm⁻¹; ¹H NMR (500 MHz, chloroform-d) δ ppm 1.57 (s, 6 H, -C(CH₃)₂-) 3.79 (s, 6 H, 2 x OCH₃) 6.36 (t, J = 2.0 Hz, 1 H, ArH) 6.54 (d, J = 2.5 Hz, 2 H, ArH).

2-(3, 5-Dihydroxyphenyl)-2-methylpropanoic acid (3). To a stirred solution of 2 (680 mg, 3.0 mmol) in dry CH₂Cl₂ (25 mL) at -78°C, under an argon atmosphere, was added boron tribromide (10.6 mL, 10.6 mmol, 1M solution in CH₂Cl₂). Following the addition, the reaction mixture was gradually warmed to room temperature and stirred for 3 h. Unreacted boron tribromide was destroyed by the addition of methanol and ice at 0°C. The resulting mixture was warmed to room temperature, and volatiles were removed under vacuo. The residue was diluted with diethyl ether and washed with saturated aqueous NaHCO₃ solution, water and brine. The organic layer was dried (MgSO₄), filtered, and concentrated under vacuo. The residue was chromatographed on silica gel (40% diethyl ether in hexane) to give 3 (3.2 g, 85% yield) as white solid. MP 176 °C; IR (solid): 3180, 1688, 1601 cm⁻¹; ¹H NMR (500 MHz, methanol-d₄) δ ppm 1.48 (s, 6 H, -C(CH₃)₂-) 6.15 (t, J = 2.5 Hz, 1 H, ArH) 6.33 (d, J = 2.5 Hz, 2 H, ArH); HRMS calcd for C₁₀H₁₃O₄ 197.0814, found 197.0806.

2-[(6aS,10aR)-6a,7,10,10a-Tetrahydro-1-hydroxy-6,6,9-trimethyl-6H-benzo[c]chromen-3-yl]-2-methylpropanoic acid (4). To a stirred solution of 3 (200 mg, 1.0 mmol) and (+)-cis/trans-p-mentha-2,8-dien-1-ol (170 mg, 1.1 mmol) in CHCl₃ (10 mL) was added p-TSA (38 mg, 0.2 mmol). The resulting mixture was refluxed for 6 h. The reaction mixture was cooled to room temperature and diluted with water (3 mL) and CHCl₃ (5 mL). The organic layer was separated and the aqueous layer was extracted with CHCl₃ (3 x 10 mL). The combined organic layer was
washed with water and brine, dried (MgSO₄) and concentrated under vacuo. The residue was chromatographed on silica gel (20% diethyl ether in hexane) to give 4 (110 mg, 32% yield) as light yellow gum. IR (CHCl₃): 2971, 2920, 1699 cm⁻¹; ¹H NMR (500 MHz, chloroform-d) δ ppm 1.09 (s, 3 H, 6α-CH₃) 1.37 (s, 3 H, 6β-CH₃) 1.49 (s, 3H, 1’ CH₃) 1.51 (s, 3H, 1’ CH₃) 1.68 (s, 3 H, 9-CH₃) 1.74 - 1.89 (m, 3H, 10β-H, 7β-H, 6a-H) 2.10 -2.16 (m, 1 H, 7α-H) 2.70 (td, J = 11.0, 4.5 Hz, 1 H) 3.19 (dd, J = 16.5, 4.5 Hz, 1 H, 10α-H), 5.42 (d, J = 4.5 Hz, 1 H, 8-H) 6.29 (d, J = 2.0 Hz, 1 H, ArH) 6.45 (d, J = 2.0 Hz, 1H, ArH); HRMS calcd for C₂₀H₂₇O₄ 331.1909, found 331.1901; HPLC Purity: 95%.

Synthesis of compound 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18

Typical Procedure for the esterification of tricyclic carboxylic acid intermediate 4 in MW:

A mixture of alkyl halide (1.5 equivalents), 4 (1 equivalents) and sodium bicarbonate (1.5 equivalents) in DMF (2 mL) was heated at 165°C for 12 min using microwave irradiation. The reaction mixture was cooled to room temperature and diluted with water and ethyl acetate. The organic layer was separated and the aqueous layer was extracted with ethyl acetate (3 x 5 mL). The combined organic layer was washed with water and brine, dried (MgSO₄) and concentrated under vacuo. The residue was chromatographed on silica gel to give pure product.

4-Bromobutyl-2-[(6aS,10aR)-6a,7,10,10a-tetrahydro-1-hydroxy-6,6,9-trimethyl-6H-benzo chromen-3-yl]-2-methylpropanoate (5). (% yield 20); Light yellow gum, IR (CHCl₃): 3402, 2970, 2917, 1727 cm⁻¹; ¹H NMR (500 MHz, chloroform-d) δ ppm 1.1 (s, 3H, 6α-CH₃) 1.38 (s, 3H, 6β-CH₃) 1.68- 1.86 (m, 10H, especially s at δ 1.70 3H, 9-CH₃) 1.51 (s, 6H, -C(CH₃)₂-) 2.1-2.2 (m,1H) 2.69 (dt, J = 11.0, 4.5Hz, 1H, 10a-H) 3.20 (dd, J = 16.5, 4.5 Hz, 1H, 10α-H) 3.32 (t, J = 6.5, 2H, 7’-CH₂) 4.09 (t, J = 6.5, 2H, 4’-CH₂) 5.18 (s, 1H, OH). 5.42 (d, J = 4.5, 1H, 8-H)
Butyl-2-[(6aS, 10aR)-6a, 7, 10, 10a-tetrahydro-1-hydroxy-6, 6, 9-trimethyl-6H-benzo[c]chromen-3-yl]-2-methylpropanoate (6). (% yield 68); Light yellow gum; IR (CHCl₃): 3413, 2962, 2932, 1728, 1702 cm⁻¹; ^1H NMR (500 MHz, chloroform-d) δ ppm 0.87 (t, J = 7.32 Hz, 3 H, 7′-CH₃) 1.10 (s, 3 H, 6α-CH₃) 1.23 - 1.32 (m, 2 H) 1.39 (s, 3 H, 6β-CH₃) 1.51 (s, 6 H, -C(CH₃)₂-) 1.53 - 1.58 (m, 2 H) 1.69 (s, 3 H, 9-CH₃) 1.76 - 1.89 (m, 3 H, 10β-H, 7β-H, 6a-H) 2.11 - 2.20 (m, 1 H, 7α-H ) 2.70 (td, J = 11.0, 4.5 Hz, 1 H, 10aH) 3.21 (dd, J = 16.0, 5.0 Hz, 1 H, 10α-H) 4.07 (t, J = 6.35 Hz, 2 H, 8′-CH₂) 5.11 (s, 1H, OH) 5.24 (s, 1H, ArH) 5.43 (d, J = 4.5 Hz, 1 H, 8-H) 6.26 (s, 1 H, ArH) 6.43 (d, J = 2.0 Hz, 1H, ArH); HRMS calcd for C₂₄H₃₄O₄ 386.2457, found 386.2460.

5-Bromopentyl-2-[(6aS,10aR)-6a,7,10,10a-tetrahydro-1-hydroxy-6,6,9-trimethyl-6H-benzo[c]chromen-3-yl]-2-methylpropanoate (7). (% yield 47); Light yellow gum; IR (CHCl₃): 3407, 2973, 2930, 1727, 1702 cm⁻¹; ^1H NMR (500 MHz, chloroform-d) δ ppm 1.10 (s, 3 H, 6α-H) 1.19-1.26 (m, 2 H) 1.39 (s, 3 H, 6β-H) 1.52 (s, 6 H, -C(CH₃)₂-) 1.62 - 1.75 (m, 5 H) 1.76 - 1.90 (m, 3 H, 10β-H, 7β-H, 6a-H) 1.96 - 2.06 (m, 2 H) 2.15 (d, J = 15.0 Hz, 1 H, 7α-H) 2.70 (td, J = 10.5, 4.5 Hz, 1 H, 10a-H) 3.20 (dd, J = 16.0, 4.5 Hz, 1 H, 10α-H) 4.07 (t, J = 6.35 Hz, 2 H, 8′-CH₂) 4.91 - 5.01 (m, 2 H, 4′-CH₂) 5.44 (br s, 1 H, OH) 5.67 - 5.81 (m, 1 H, 8-H) 6.25 (s, 1 H, ArH) 6.44 (s, 1H, ArH); HRMS calcd for C₂₅H₃₅O₄Br 478.1718, found 478.1715.

4-(1H-Imidazol-1-yl) butyl 2-[(6aR,10aR)-6a,7,10,10a-tetrahydro-1-hydroxy-6,6,9-trimethyl-6H-benzo[c]chromen-3-yl]-2-methylpropanoate (8). (% yield 47); Light yellow solid, MP 74°C; IR (CHCl₃): 2971, 2931, 1727 cm⁻¹; ^1H NMR (500 MHz, chloroform-d) δ ppm
1.09 (s, 3 H, 6α-CH3) 1.38 (s, 3 H, 6β-CH3) 1.47 (d, J = 9.0 Hz, 6 H, -C(CH3)2-) 1.69 (s, 3 H, 9-CH3) 1.70 - 1.82 (m, 7 H) 2.10 - 2.16 (m, 1 H, 7α-H) 2.72 (td, J = 11.0, 5.0 Hz, 1 H, 10a-H) 3.39 (dd, J = 16.5, 4.0 Hz, 1 H, 10α-H) 3.88 (td, J = 6.5, 2.5 Hz, 2 H, 7′CH2) 3.96 (t, J = 5.0 Hz, 2 H, 4′-CH2) 5.42 (d, J = 3.5 Hz, 1 H, 8-H) 6.23 (d, J = 2.0 Hz, 1 H, ArH) 6.36 (d, J = 2 Hz, 1 H, ArH) 6.86 (s, 1 H) 7.10 (s, 1 H) 7.50 (s, 1H); HRMS calcd for C27H36O4N2 452.26751, found 452.26659.

3-Bromopropyl-2-[(6aR, 10aR)-6a, 7, 10, 10a-tetrahydro-1-hydroxy-6, 6, 9-trimethyl-6H-benzo[c]chromen-3-yl]-2-methylpropanoate (9). (% yield 51); ¹H NMR (500 MHz, chloroform-d) δ ppm 1.10 (s, 3H, 6α-CH3) 1.38 (s, 3H, 6β-CH3) 1.51 (s, 6H, -C(CH3)2-) 1.69 (s, 3H, 9-CH3) 1.75 - 1.89 (m, 3H, 10β-H, 7β-H, 6a-H) 2.08 (t, J = 6.50 Hz, 2H, 6′-CH2) 2.12 - 2.19 (m, 1H, 7α-H) 2.70 (td, J = 10.50, 4.50 Hz, 1H, 10a-H) 3.08- 3.38 (m, 3H) 4.19 (t, J = 6.5 Hz, 2H, 4′-CH2) 5.42 (d, J = 5.0 Hz, 1H, 8-H) 5.76 (s, 1H, OH) 6.27 (d, J = 2.0 Hz, 1H, ArH) 6.41 (d, J = 1.5 Hz, 1H, ArH).

Methyl-2-[(6aR, 10aR)-6a, 7, 10, 10a-tetrahydro-1-hydroxy-6,6,9-trimethyl-6H-benzo[c]chromen-3-yl]-2-methylpropanoate (10). (% yield 64); ¹H NMR (500 MHz, chloroform-d) δ ppm 1.10 (s, 3H, 6α-CH3) 1.38 (s, 3H, 6β-CH3) 1.50 (s, 6H, -C(CH3)2-) 1.69 (s, 3H, 9-CH3) 1.76 - 1.86 (m, 3H, 10β-H, 7β-H, 6a-H) 2.09 - 2.21 (m, 1H, 7α-H) 2.70 (td, J = 11.0, 5.0 Hz, 1H, 10a-H) 3.23 (dd, J = 15.5, 4.5 Hz, 1H, 10α-H) 3.66 (s, 3 H, 4′-CH3) 5.43 (d, J = 4.5 Hz, 1H, 8-H) 5.76 (s, 1H, OH) 6.27 (d, J = 2.0 Hz, 1H, ArH) 6.42 (d, J = 2.0 Hz, 1H, ArH); HRMS calcd for C21H28O4 344.1988, found 344.1992.

Ethyl-2-[(6aR,10aR)-6a,7,10,10a-tetrahydro-1-hydroxy-6,6,9-trimethyl-6H-benzo[c]chromen-3-yl]-2-methylpropanoate (11). (% yield 87); ¹H NMR (500 MHz, chloroform-d) δ
ppm 1.11 (s, 3H, 6α-H) 1.20 (t, J = 6.5 Hz, 3H, 5′-CH₃) 1.39 (s, 3H, 6β-H) 1.51 (s, 6H, -C(CH₃)₂-) 1.69 (s, 3H, 9-CH₃) 1.75 - 1.89 (m, 3H, 10β-H, 7β-H, 6a-H) 2.10 - 2.18 (m, 1H, 7α-H) 2.70 (td, J = 11.0, 4.50 Hz, 1H, 10a-H) 3.23 (dd, J = 16.0, 4.50 Hz, 1H, 10α-H) 4.11 (q, J = 14.5, 7.0 Hz, 2H, 4′-CH₂) 5.43 (d, J = 5.0 Hz, 1H, 8-H) 5.74 (s, 1H, OH) 6.27 (d, J = 1.0 Hz, 1H, ArH) 6.41 (d, J = 1.0 Hz, 1H, ArH); HRMS calcd for C₂₂H₃₀O₄ 358.2144, found 358.2145.

2-Bromoethyl-2-[(6aR,10aR)-6a,7,10,10a-tetrahydro-1-hydroxy-6,6,9-trimethyl-6H-benzo[c]chromen-3-yl]-2-methylpropanoate (12). (% yield 48); ¹H NMR (500 MHz, chloroform-d) δ ppm 1.10 (s, 3H, 6α-H) 1.38 (s, 3H, 6β-H) 1.53 (s, 6H, -C(CH₃)₂-) 1.69 (s, 3H, 9-CH₃) 1.75 - 1.86 (m, 3H, 10β-H, 7β-H, 6a-H) 2.12 - 2.19 (m, 1H, 7α-H) 2.69 (td, J = 11.0, 4.50 Hz, 1H, 10a-H) 3.21 (dd, J = 16.0, 4.50 Hz, 1H, 10α-H) 3.45 (t, J = 6.0 Hz, 2H, 5′-CH₂) 4.36 (t, J = 6.0 Hz, 2H, 4′-CH₂) 5.04 (s, 1H, OH) 5.43 (d, J = 4.0 Hz, 1H, 8-H) 6.27 (d, J = 1.5 Hz, 1H, ArH) 6.42 (d, J = 1.0 Hz, 1H, ArH); HRMS calcd for C₂₂H₂₉O₄Br 436.1249, found 436.1250.

4-Cyanobutyl-2-[(6aS,10aR)-6a,7,10,10a-tetrahydro-1-hydroxy-6,6,9-trimethyl-6H-benzo[c]chromen-3-yl]-2-methylpropanoate (13). A solution of 5 (20 mg, 0.04 mmol) and sodium cyanide (21 mg, 0.4 mmol) in dry DMSO (3 mL) was stirred at 50°C for 3 h. The reaction mixture was cooled to room temperature and diluted with water and ethyl acetate. The organic layer was separated and the aqueous layer was extracted with ethyl acetate (3 x 2 mL). The combined organic layer was washed with water and brine, dried (MgSO₄) and concentrated in vacuo. The residue was chromatographed on silica gel (20% ethyl acetate in hexane) to give 13 (9.5 mg, 56% yield) as light yellow gum. IR (CHCl₃): 3404, 2973, 2933, 1726, 1705 cm⁻¹; ¹H NMR (500 MHz, chloroform-d) δ ppm 1.1 (s, 3H, 6α-H) 1.38 (s, 3H, 6β-H) 1.50 (s, 3H, 1′-CH₃) 1.51 (s, 3H, 1′-CH₃) 1.59- 1.90 (m, 7H) 1.69 (s, 3H, 9′-CH₃) 2.12- 2.15 (m, 1H, 7α-H) 2.27 (t, J
= 7.0Hz ,2H, 7′-CH₂) 2.70 (dt, J =11.0, 5.0 Hz, 1H, 10α-H) 3.20 (dd, J = 16.0, 3.5 Hz, 1H, 10α-H) 4.11 (t, J = 6.5, 2H, 4′-CH₂) 5.26 (s, 1H, OH) 5.42 (d, J = 5.0, 1H, 8-H) 6.26 (d, J = 2.0, 1H, ArH) 6.41 (d, J = 2.0, 1H, ArH); HRMS calcd for C₂₅H₃₄NO₄ 412.2488, found 412.2480.

4-Morpholinobutyl 2-[(6aR,10aR)-1-hydroxy-6,6,9-trimethyl-6a,7,10,10a-tetrahydro-6H-benzo[c]chromen-3-yl]-2-methylpropanoate (14). To a stirred solution of 5 (170 mg, 0.3 mmol) and triethylamine (0.15 mL, 1.0 mmol) in dry CH₃CN (15 mL) at room temperature under an argon atmosphere was added morpholine (0.21 mL, 3.6 mmol). The resulting solution was stirred at that temperature for 24 h and diluted with water and ethyl acetate. The organic layer was separated and the aqueous layer was extracted with ethyl acetate (3 x 5 mL). The combined organic layer was washed with water and brine, dried (MgSO₄) and concentrated in vacuo. The residue was chromatographed on silica gel (48% acetone in hexane) to give 14 (140 mg, 81% yield) as light yellow gum. ¹H NMR (500 MHz, chloroform-d) δ ppm 1.10 (s, 3 H, 6α-H ) 1.37 (s, 3 H, 6β-H) 1.49 (d, J = 7.5 Hz, 6 H, -C(CH₃)₂-) 1.56- 1.66 (m, 4H) 1.68 (s, 3 H, 9-CH₃) 1.75 - 1.82 (m, 3 H, 10β-H, 7β-H, 6a-H) 2.10- 2.18 (m, 1 H, 7α-H) 2.33- 2.41 (m, 4H) 2.69 (td, J = 4.5, 11.0 Hz, 1H, 10a-H) 3.32 (dd, J = 4.5, 16.0 Hz, 1H, 10α-H) 3.73- 3.82 (m, 6H) 4.10- 4.18 (m, 2H, 4′-CH₂) 5.42 (d, J = 4.0 Hz, 1H, 8-H) 6.13 (d, J = 2.0 Hz, 1H, ArH) 6.38 (d, J = 1.5 Hz, 1H, ArH); HRMS calcd for C₂₅H₃₄NO₄ 412.2488, found 412.2480.

3-Cyanopropyl-2-[(6aR,10aR)-6a,7,10,10a-tetrahydro-1-hydroxy-6,6,9-trimethyl-6H-benzo[c]chromen-3-yl]-2-methylpropanoate (15). The synthesis was carried out as described for 13 using sodium cyanide (95.5 mg, 1.9 mmol) and 9 (110 mg, 0.2 mmol) in DMSO (6 mL) to give 15 (95 mg, 98% yield) as light yellow gum. ¹H NMR (500 MHz, chloroform-d) δ ppm 1.10 (s, 3H, 6α-H ) 1.38 (s, 3H, 6β-H) 1.51 (s, 6H, -C(CH₃)₂-) 1.69 (s, 3H, 9-CH₃) 1.75 - 1.87 (m, 1H, 10β-H, 7β-H, 6a-H) 1.89 - 1.96 (m, 2H, 5′-CH₂) 2.10 - 2.18 (m, 1H, 7α-H) 2.21 (t, J = 7.50 Hz,
2H, 6′-CH₂) 2.70 (dt, J = 11.0, 5.0 Hz, 1H, 10a-H) 3.23 (dd, J = 16.0, 4.0 Hz, 1H, 10α-H) 4.13-
4.18 (m, 2H, 4′-CH₂) 5.42 (d, J = 4.0 Hz, 1H, 8-H) 5.82 (s, 1H, OH) 6.29 (d, J = 2.0 Hz, 1H,
ArH) 6.40 (d, J = 2.0 Hz, 1H, ArH); HRMS calcd for C₂₄H₃₁NO₄ 397.2253, found 397.2254.

3-Azidopropyl-2-[(6αR,10αR)6a,7,10a-tetrahydro-1-hydroxy-6,6,9-trimethyl-6H-
benzo[c]chromen-3-yl]-2-methylpropanoate (16). To the stirred solution of 9 (40 mg, 0.09
mmol) in dry CH₂Cl₂ (5 mL) was added tetra-n-butylammonium azide (254 mg, 0.9 mmol). The
resulting mixture was heated at 40°C for 48 h. The reaction mixture was warmed to room
temperature and diluted with water and ethyl acetate. The organic layer was separated and the
aqueous layer was extracted with ethyl acetate (3 x 3 mL). The combined organic layer was
washed with water and brine, dried (MgSO₄) and concentrated in vacuo. The residue was
chromatographed on silica gel (17% ethyl acetate in hexane) to give 16 (25 mg, 70% yield) as
light yellow gum.¹H NMR (500 MHz, chloroform-d) δ ppm 1.10 (s, 3H, 6α-H ) 1.38 (s, 3H, 6β-
H) 1.51 (s, 6H, -C(CH₃)₂-) 1.69 (s, 3H, 9-CH₃) 1.80 - 1.86 (m, 5H, especially p at 5′-CH₂) 2.09 -
2.21 (m, 1H, 7α-H) 2.71 (td, J = 10.50, 4.50 Hz, 1H, 10a-H) 3.14-3.30 (m, 4H, especially t at
3.18 for 6′-CH₂) 4.13 - 4.18 (t, J = 6Hz, 2H, 4′-CH₂) 5.19 (s, 1H, OH) 5.43 (d, J = 4.50 Hz, 1H,
8-CH₂) 6.25 (d, J = 2.0 Hz, 1H, ArH) 6.42 (d, J = 2.0 Hz, 1H, ArH); HRMS calcd for
C₂₃H₃₁N₃O₄ 413.2315, found 413.2317; IR (CHCl₃): 3409, 2968, 2928, 2097, 1728, 1707 cm⁻¹.

3-(1H-Imidazol-1-yl)propyl-2-[(6αR,10αR)-6a,7,10a-tetrahydro-1-hydroxy-6,6,9-
trimethyl-6H-benzo[c]chromen-3-yl]-2-methylpropanoate (17). Imidazole (147 mg, 2.1
mmol) was added to a stirred suspension of 9 (196.0 mg, 0.4 mmol) and potassium carbonate
(594 mg, 4.3 mmol) in DMSO (5 mL). The resulting mixture was stirred at room temperature for
14 h and diluted with water and ethyl acetate. The organic layer was separated and the aqueous
layer was extracted with ethyl acetate (3 x 2 mL). The combined organic layer was washed with
water and brine, dried (MgSO₄) and concentrated in vacuo. The residue was chromatographed on silica gel (50% acetone in hexane) to give 17 (78 mg, 41% yield) as light yellow gum. ¹H NMR (500 MHz, chloroform-d) δ ppm 1.07 (s, 3H, 6α-H) 1.37 (s, 3H, 6β-H) 1.49 (d, J = 4.0 Hz, 6H, -C(CH₃)₂-) 1.69 (s, 3H, 9-CH₃) 1.72 - 1.87 (m, 3H, 10β-H, 7β-H, 6a-H) 1.92 - 2.01 (m, 2H) 2.08-2.18 (m, 1 H, 7α-H) 2.73 (td, J = 11.5, 4.5 Hz, 1H, 10a-H) 3.42 (dd, J = 17.0, 4.0 Hz, 1H, 8-CH₂) 6.32 (d, J = 2.0 Hz, 1H, ArH) 6.40 (d, J = 2.0 Hz, 1H, ArH) 6.64 (s, 1H) 7.04 (s, 1 H) 7.33 (s, 1H); HRMS calcd for C₂₆H₃₄N₂O₄ 438.2518, found 438.2518.

3-Morpholinopropyl 2-[(6αR,10aR)-1-hydroxy-6,6,9-trimethyl-6α,7,10,10a-tetrahydro-6H-benzo[c]chromen-3-yl]-2-methylpropanoate (18). The synthesis was carried out as described for 14 using 9 (265 mg, 0.6 mmol), triethylamine (0.25 mL, 1.7 mmol) and morpholine (0.3 mL, 5.8 mmol) in dry CH₃CN (15 mL) to give 18 (298 mg, 78% yield). IR (neat): 2970, 2161, 1726, 1417 cm⁻¹; ¹H NMR (500 MHz, chloroform-d) δ ppm 1.10 (s, 3H, 6α-H) 1.37 (s, 3H, 6β-H) 1.49 (d, J = 5.5 Hz, 6H, -C(CH₃)₂-) 1.67 (s, 3H, 9-CH₃) 1.75- 1.83 (m, 5H) 2.11- 2.18 (m, 1 H, 7α-H) 2.24- 2.36 (m, 2H) 2.41 (br, s, 4H) 2.68 (td, J = 4.5, 11.0 Hz, 1H, 10a-H) 3.26 (dd, J = 4.5, 16.5 Hz, 1H, 10α-H) 3.71 (t, J = 4.5 Hz, 4H) 4.12 (m, 2H, 4'-CH₂) 5.41 (d, J = 4.0 Hz, 1H, 8-H) 6.23 (d, J = 2.0 Hz, 1H, ArH) 6.38 (d, J = 2.5 Hz, 1H, ArH); HRMS calcd for C₂₇H₃₉NO₅ 457.2828, found 457.2829.

2-(3, 5-Dimethoxyphenyl)-2-methylpropanal (19). To a stirred solution of 1 (3.0 g, 14.6 mmol) in dry CH₂Cl₂ (50 mL) at -78°C under an argon atmosphere was added dropwise DIBAL-H (37 mL, 37 mmol, 1 M solution in CH₂Cl₂). The reaction mixture was warmed to room temperature and stirred for 1 h. The reaction mixture was quenched with 10% solution of potassium sodium tartrate and diluted with CH₂Cl₂. The organic layer was separated and the aqueous layer was
extracted with CH₂Cl₂ (3 x 20 mL). The combined organic layer was washed with water and brine, dried (MgSO₄) and concentrated under vacuo. The residue was chromatographed on silica gel (20% ethyl acetate in hexane) to give 19 (2.8 g, 94%) as a colorless oil. ¹H NMR (500 MHz, chloroform-δd) δ ppm 1.44 (s, 6H, -C(CH₃)₂-) 3.80 (s, 6H, 2 x OCH₃) 6.40 (t, J = 2.5 Hz, 1H, ArH) 6.40 - 6.42 (m, 2H, ArH) 9.47 (s, 1H, CHO); IR (neat): 3524, 2970, 1724, 1592, 1151 cm⁻¹.

2-(3,5-Dimethoxyphenyl)-2-methylpropan-1-ol (20). To a stirred solution of 19 (1.7 g, 8.4 mmol) in methanol (40 mL) at room temperature was added in small portions sodium borohydride (1.4 g, 37.8 mmol). The resulting mixture was stirred at the same temperature for 1 h, quenched with 1N HCl and diluted with ethyl acetate. The organic layer was separated and the aqueous layer was extracted with ethyl acetate (3 x 10 mL). The combined organic layer was washed with water and brine, dried (MgSO₄) and concentrated under vacuo. The residue was chromatographed on silica gel (20% ethyl acetate in hexane) to give 20 (1.6 g, 94%) as a colorless oil. IR (neat): 3432, 2960, 1592, 1151 cm⁻¹; ¹H NMR (500 MHz, chloroform-δd) δ ppm 1.30 (s, 6H, -C(CH₃)₂-) 3.58 (s, 2H, CH₂) 3.79 (s, 6H, 2 x OCH₃) 6.34 (t, J = 1.5 Hz, 1H, ArH) 6.53 (d, J = 2.0 Hz, 2H, ArH).

5-(1-Hydroxy-2-methylpropan-2-yl)benzene-1, 3-diol (21). The synthesis was carried out as described for 3 using 20 (400 mg, 2.0 mmol) and boron tribromide (6.6 mL, 6.6 mmol, IM solution in CH₂Cl₂) in dry CH₂Cl₂ to give 21 (165 mg, 48%) as light yellow oil. ¹H NMR (500 MHz, chloroform-δd) δ ppm 1.28 (s, 6H, -C(CH₃)₂-) 3.56 (d, J = 6.0 Hz, 2H, CH₂) 4.84 (s, 2 x OH) 6.22 (t, J = 2.0 Hz, 1H, ArH) 6.43 (d, J = 2.5 Hz, 2H, ArH); HRMS calcd for C₁₂H₁₃O₃ 205.0865, found 205.0845.
(6aR, 10aR)-3-(1-Hydroxy-2-methylpropan-2-yl)-6, 6, 9-trimethyl-6a,7,10,10a-tetrahydro-6H-benzo[c]chromen-1-ol (22). To a stirred solution of 21 (165 mg, 0.9 mmol) and (+)-cis/trans-p-mentha-2,8-dien-1-ol (166 mg, 1.0 mmol) in CHCl₃ (3 mL) was added p-TSA (52 mg, 0.3 mmol). The resulting mixture was irradiated in MW at 150°C for 10 min. The reaction mixture was cooled to room temperature and diluted with water and CHCl₃. The organic layer was separated and the aqueous layer was extracted with CHCl₃ (3 x 3 mL). The combined organic layer was washed with water and brine, dried (MgSO₄) and concentrated under vacuo. The residue was chromatographed on silica gel (15% ethyl acetate in hexane) to give 22 (80 mg, 30%) as light orange colored solid. MP 92°C; IR (CHCl₃): 3340, 2967, 1415 cm⁻¹; ¹H NMR (500 MHz, chloroform-d) δ ppm 1.10 (s, 3H, 6α-CH₃) 1.24 (s, 6H, -C(CH₃)₂-) 1.38 (s, 3H, 6β-CH₃) 1.7 (s, 3H, 9-CH₃) 1.76 - 1.88 (m, 4H) 2.11-2.19 (m, 1H, 7α-H) 2.71 (td, J = 4.5, 10.5 Hz, 1H, 10a-H) 3.19 (dd, J = 3.5, 15.5 Hz, 1H, 10α-H) 3.54 (s, 2H, CH₂) 5.43 (d, J = 4.5 Hz, 1H, 8-H) 6.28 (d, J = 1.5 Hz, 1H, ArH) 6.43 (d, J = 1.5 Hz, 1H, ArH).

2-[(6aR, 10aR)-6a, 7, 10, 10a-Tetrahydro-1-hydroxy-6, 6, 9-trimethyl-6H-benzo[c]chromen-3-yl]-2-methyl propylpentanoate (23). Triphenyl phosphine (223 mg, 0.9 mmol) was added to the stirred solution of 13 (180 mg, 0.6 mmol) and valeric acid (1.0 mL, 0.8 mmol) in dry THF (10 mL). The resulting mixture was cooled to 0°C and DEAD (0.15 mL, 0.8 mmol) was added dropwise. The reaction mixture was warmed to room temperature and stirred for 20 h. The mixture was quenched with 1N HCl and diluted with ethyl acetate. The organic layer was separated and the aqueous layer was extracted with ethyl acetate (3 x 10 mL). The combined organic layer was washed with water and brine, dried (MgSO₄) and concentrated under vacuo. The residue was chromatographed on silica gel (25% ethyl acetate in hexane) to give 23 (93 mg, 41%) as light yellow gum. ¹H NMR (500 MHz, chloroform-d) δ ppm 0.88 (t, J = 7.5 Hz, 3H, 8'-
CH₃) 1.10 (s, 3H, 6α-CH₃) 1.18-1.22 (m, 2H, 7′-CH₂) 1.28 (s, 6H, -C(CH₃)₂-) 1.38 (s, 3H, 6β-
CH₃) 1.50-1.58 (m, 2H, 6′-CH₂) 1.70 (s, 3H, 9-CH₃) 1.7-1.89 (m, 3H, 10β-H, 7β-H, 6a-H) 2.10-
2.20 (m, 1H, 7α-H) 2.28 (t, J = 7.5 Hz, 2H, 5′-CH₂) 2.70 (dt, J = 5.0, 11.5 Hz, 1H, 10a-H) 3.20
(dd, J = 5.0, 15.0 Hz, 1H, 10α-H) 4.07 (s, 2H, 2′-CH₂) 4.94 (s, 1H, OH) 5.43 (d, J = 5Hz, 1H,
8-H) 6.27 (d, J = 2.0 Hz, 1H, ArH) 6.42 (d, J = 2.0 Hz, 1H, ArH); HRMS calcd for C₂₅H₃₆O₄
400.2614, found 400.2611; IR (CHCl₃): 3389, 2968, 2159, 1709, 1416, 907 cm⁻¹.

2-(3,5-Dimethoxyphenyl)propanenitrile (24). A solution of (3,5-dimethoxyphenyl)acetonitrile
(5.0 gm, 28.2 mmol) and iodomethane (6.0 g, 42.4 mmol) in dry DMF (30 mL) was added at -
78°C to the suspension of sodium hydride (1.4 g, 34 mmol, 60% dispersion in oil) in dry DMF
(50 mL). The resulting mixture was warmed to room temperature and stirred for 2 h. The
reaction mixture was quenched with saturated NH₄Cl solution and diluted with ethyl acetate. The
organic layer was separated and the aqueous layer was extracted with ethyl acetate (3 x 20 mL).
The combined organic layer was washed with water and brine, dried (MgSO₄) and concentrated
in vacuo. The residue was chromatographed on silica gel (20% Ethyl acetate in hexane) to give
24 (4.0 g, 75% yield) as colourless oil. ¹H NMR (500 MHz, chloroform-d) δ ppm 1.63 (d, J =
7.5 Hz, 3H, 1 x CH₃) 3.81 (s, 6H, 2 x OCH₃) 3.84 (q, J = 7.0, 14.5 Hz, 1H, benzylic H) 6.41 (t,
J = 2.5 Hz, 1H, ArH) 6.54 (d, J = 2.5 Hz, 2H, ArH); IR (neat): 2940, 2242, 1595, 1151 cm⁻¹.

2-(3,5-Dimethoxyphenyl)propanoic acid (25). Sodium hydroxide (1.2 g, 30.0 mmol) was
added to the stirred mixture of 24 (2.3 g, 12.0 mmol) in 3 mL of n-Butanol: H₂O (2:1). The
resulting mixture was refluxed for 8 h. The reaction mixture was cooled to room temperature and
the excess of n-butanol was removed under reduced pressure. The residue was acidified using 1N
HCl and diluted with ethyl acetate. The organic layer was separated and the aqueous layer was
extracted with ethyl acetate (3 x 20 mL). The combined organic layer was washed with water
and brine, dried (MgSO₄) and concentrated under vacuo. The residue was chromatographed on silica gel (85% ethyl acetate in hexane) to give 25 (2.3 g, 92% yield) as colourless oil. \(^1\)H NMR (500 MHz, chloroform-\(d\)) \(\delta\) ppm 1.49 (d, \(J = 7.5\) Hz, 3H, 1 x CH₃) 3.66 (q, \(J = 7.0, 14.5\) Hz, 1H, benzylic H) 3.77 (s, 6H, 2 x OCH₃) 6.37 (t, \(J = 2.5\) Hz, 1H, ArH) 6.47 (d, \(J = 2.0\) Hz, 2H, ArH); IR (neat): 2938, 1699, 1593 cm\(^{-1}\).

2-(3,5-Dihydroxyphenyl)propanoic acid (26). The synthesis was carried out as described for 3 using boron tribromide (38.4 mL, 38.4 mmol, 1 M solution in CH₂Cl₂) and 25 (2.3 g, 11 mmol) in dry CH₂Cl₂ (30 mL) to give 26 (1.5 g, 78% yield). \(^1\)H NMR (500 MHz, chloroform-\(d\)) \(\delta\) ppm 1.40 (d, \(J = 7.5\) Hz, 3H, 1 x CH₃) 3.56 (q, \(J = 6.5, 14.0\) Hz, 1H, benzylic H) 6.20 (t, \(J = 2.0\) Hz, 1H, ArH) 6.30 (d, \(J = 2.5\) Hz, 2H, ArH); IR (neat): 3363, 1702, 1597, 1259 cm\(^{-1}\).

2-[(6a\(^R\), 10a\(^R\))-1-Hydroxy-6, 6, 9-trimethyl-6\(^a\), 7, 10, 10a-tetrahydro-6\(^H\)-benzo[c]chromen-3-yl]propanoic acid (27). The synthesis was carried out as described for 4 using p-TSA (138 mg, 0.7 mmol), 26 (660 mg, 3.6 mmol) and (+)-cis/trans-p-mentha-2,8-dien-1-ol (609 mg, 4.0 mmol) in CHCl₃ (30 mL) to give 27 as a mixture of diastereomers 27e₁ and 27e₂. \(^1\)H NMR (500 MHz, chloroform-\(d\)) \(\delta\) ppm 1.08 (s, 6H, 6α-CH₃, 27e₁, 27e₂) 1.36 (s, 6H, 6β-H, 27e₁, 27e₂), 1.39-1.45 (m, 6H, -CH-(CH₃)-, 27e₁, 27e₂) 1.68 (s, 6H, 9-CH₃, 27e₁, 27e₂) 1.71 -1.91 (m, 6H, 27e₁, 27e₂) 2.14 (br d, \(J = 14.5\) Hz, 2H, 27e₁, 27e₂) 2.69 (td, \(J = 4.0, 10.5\) Hz, 2H, 27e₁, 27e₂) 3.24 (d and d overlapping, \(J = 19.5\) Hz, 2H, 27e₁, 27e₂) 3.55 (q and q overlapping, \(J = 6.5, 14.0\) Hz, 2H, 1'H, 27e₁, 27e₂) 5.41 (br d, \(J = 4.0\) Hz, 2H, 8-H, 27e₁, 27e₂) 6.30 (d and d overlapping, \(J = 2.0\) Hz, 2H, ArH, 27e₁, 27e₂) 6.35 (d and d overlapping, \(J = 2.0\) Hz, 2H, ArH, 27e₁, 27e₂); HRMS calcd for C₁₉H₂₄O₄ 316.1675, found 316.1675.
Butyl 2-[(6aR, 10aR)-1-hydroxy-6, 6, 9-trimethyl-6a, 7, 10, 10a-tetrahydro-6H-benzo[c]chromen-3-yl]propanoate (28). The synthesis was carried out as described for 6 using sodium bicarbonate (55 mg 0.6 mmol), 27 (175 mg, 0.5 mmol) and bromobutane (114 mg, 0.8 mmol) in DMF (2 mL) to give 28 as a mixture of diastereomers 28e1 and 28e2 (165 mg, 80% yield) as light yellow gum. IR (neat): 3398, 2961, 1732, 1708, 1182 cm\(^{-1}\), \(^1\)H NMR (500 MHz, chloroform-\(d\)) \(\delta\) ppm 0.874 (t, \(J = 7.5\) Hz, 3H, 7'-CH\(_3\), 28e1) 0.877 (t, \(J = 7.5\) Hz, 3H, 7'-CH\(_3\), 28e2) 1.09 (s, 6H, 6\(\alpha\)-CH\(_3\), 28e1, 28e2) 1.25-1.35 (m, 4H, 6'-CH\(_2\), 28e1, 28e2) 1.37 (s, 6H, 6\(\beta\)-CH\(_3\), 28e1, 28e2), 1.43 (d, \(J = 1.5\) Hz, 3H, -CH-(CH\(_2\)-)) 1.44 (d, \(J = 1.5\) Hz, 3H, -CH(CH\(_3\))-) 1.52 (p, \(J = 7.0, 14.5\) Hz, 4H, 5'-CH\(_2\), 28e1, 28e2) 1.68 (s, 6H, 9-CH\(_3\), 28e1, 28e2) 1.75 -1.89 (m, 6H) 2.12 (br d, \(J = 14.5\) Hz, 2H, 28e1, 28e2) 2.69 (td, \(J = 4.0, 10.5\) Hz, 2H, 28e1, 28e2) 3.24 (d and d overlapping, \(J = 19.5\) Hz, 2H, 28e1, 28e2) 3.5-3.59 (m, 2H, 1'H, 28e1, 28e2) 4.01-4.12 (m, 4H, 4'-CH\(_2\), 28e1, 28e2) 5.42 (br d, \(J = 4.0\) Hz, 2H, 8-H, 28e1, 28e2) 5.74 (br, s, 2H, OH, 28e1, 28e2) 6.30 (d and d overlapping, \(J = 2.0\) Hz, 2H, ArH, 28e1, 28e2) 6.35 (d and d overlapping, \(J = 2.0\) Hz, 2H, ArH, 28e1, 28e2); HRMS calcd for C\(_{23}\)H\(_{32}\)O\(_4\) 372.2301, found 372.2305.

2-(3,5-Dimethoxyphenyl)acetyl chloride (29). To the stirred solution of (3,5-dimethoxyphenyl)acetic acid (1.0 g, 5.0 mmol) in dry CH\(_2\)Cl\(_2\) (40 mL) was added intermittently 4.2 mL of 1.5 M stock solution [thionyl chloride (5.4 mL) and benzotriazole (9.0 g) in 50 mL CH\(_2\)Cl\(_2\)]. The reaction mixture was filtered through celite after stirred at room temperature for 20 min and diluted with CH\(_2\)Cl\(_2\). The organic layer was washed with dilute 1N HCl, water and brine, dried (MgSO\(_4\)) and concentrated under \textit{vacuo} to give 29 (1.3 g) as crude product which was used as such for next reaction without purification.

(R)-4-Benzyl-3-(2-(3,5-dimethoxyphenyl)acetyl)oxazolidin-2-one (30). To the stirred solution of (R)-4-benzyl oxazolidin-2-one (740mg, 4.2 mmol) in dry THF (10 mL) at -30°C was added...
dropwise n-butyl lithium (2.6 mL, 4.2 mmol, 1.6 M solution in hexane). The resulting mixture was stirred at same temperature for 30 min. To the stirred mixture was added a solution of 29 (900 mg, 4.6 mmol) in dry THF (3 mL). The reaction mixture was warmed to room temperature and stirred for 4 h. The reaction mixture was quenched with sodium bisulfite and diluted with ethyl acetate. The organic layer was separated and the aqueous layer was extracted with ethyl acetate (3 x 10 mL). The combined organic layer was washed with water and brine, dried (MgSO₄) and concentrated under vacuo. The residue was chromatographed on silica gel (20% acetone in hexane) to give 30 (980 mg, 66% yield). ¹H NMR (500 MHz, chloroform-d) δ ppm 2.76 (dd, J = 9.5, 14.0 Hz, 1H) 3.24 (dd, J = 3.0, 13.5 Hz, 1H) 3.77 (s, 6H) 4.10 - 4.19 (m, 2H) 4.26 (q, J = 15.5, 42.0 Hz, 2H) 4.64 - 4.70 (m, 1H) 6.39 (t, J = 2.5 Hz, 1H) 6.5 (d, J = 2.0 Hz, 2H) 7.13 (dd, J = 1.5, 3.5 Hz, 2H) 7.21 - 7.31 (m, 3H).

(R)-4-Benzyl-3-[((R)-2-(3,5-dimethoxyphenyl)propanoyl]oxazolidin-2-one (31). To the stirred solution of 30 (980 mg, 2.8 mmol) in dry THF (20 mL) at -78 °C was added dropwise sodium hexamethyldisilylamide (3.0 mL, 3.0 mmol, 1M in THF) over a period of 5 min. After stirred for 1 h at -78°C, iodomethane (1.0 mL, 14 mmol) was added. The mixture was stirred for 1 h at -78°C, allowed to warm up to -30°C and stirred for 1 h. The reaction mixture was quenched with acetic acid (30 mL) in diethyl ether (40 mL) and filtered over a pad of celite. The filterate was concentrated in vacuo and the crude product was chromatographed on silica gel (15% ethyl acetate in hexane) to give 31 (640 mg, 63% yield) as viscous oil. ¹H NMR (500 MHz, chloroform-d) δ ppm 1.53 (d, J = 7.0 Hz, 3H) 2.8 (dd, J = 10.0, 13.5 Hz, 1H) 3.33 (dd, J = 3.0, 13.5 Hz, 1H) 3.76 (s, 6H) 4.0 - 4.11 (m, 2H) 4.54 - 4.62 (m, 1H) 5.06 (q, J = 7.5, 14.0 Hz, 1H) 6.34 (t, J = 2.0 Hz, 1H) 6.53 (d, J = 2.5 Hz, 2H) 7.21 (d, J = 7.5 Hz, 2H) 7.26 (t, J = 7.5 Hz, 1H) 7.32 (t, J = 8.0 Hz, 2H); HRMS calcd for C₂₁H₂₄N₀₅ 370.1654, found 370.1669.
(R)-2-(3,5-Dimethoxyphenyl)propanoic acid (32). A mixture of alkylated oxazolidinone 3 (100 mg, 0.3 mmol) and lithium hydroxide (19 mg, 0.8 mmol) in THF (2.5 mL): H₂O (2.5 mL) was stirred at 0°C for 2 h. The reaction mixture was warmed to room temperature and the solvent was removed in vacuo. The residue was acidified (concentrated HCl), until pH 1 and extracted with CH₂Cl₂. The organic layer was separated and the aqueous layer was extracted with CH₂Cl₂ (3 x 3 mL). The combined organic layer was washed with water and brine, dried (MgSO₄) and concentrated under vacuo. The residue was chromatographed on silica gel (66% ethyl acetate in hexane) to give 32 (28 mg, 50% yield) as a colourless oil. ¹H NMR (500 MHz, chloroform-d) δ ppm 1.47 (d, J = 7.5 Hz, 3H) 3.66 (q, J = 14.5, 7.5 Hz, 1H) 3.77 (s, 6H) 6.37 (t, J = 2.5 Hz, 1H) 6.47 (d, J = 2.0 Hz, 2H).

(R)-2-(3,5-Dihydroxyphenyl)propanoic acid (33). The synthesis was carried out as described for 3 using boron tribromide (4.8 mL, 4.8 mmol, 1M solution in CH₂Cl₂) and 32 (288 mg, 1.4 mmol) in dry CH₂Cl₂ (20 mL) to give 33 (180 mg, 73% yield). ¹H NMR (500 MHz, chloroform-d) δ ppm 1.38 (d, J = 7.0 Hz, 3H) 3.53 (q, J = 7.0, 14.5 Hz, 1H) 6.18 (t, J = 7.0, 14.5 Hz, 1H) 6.29 (d, J = 2.0 Hz, 2H).

(R)-2-[(6aR,10aR)-1-Hydroxy-6,6,9-trimethyl-6a,7,10,10a-tetrahydro-6H-benzo[c]chromen-3-yl]propanoic acid (34). The synthesis was carried out as described for 4 using p-TSA (38 mg, 0.2 mmol), 67 (180 mg, 1.0 mmol) and (+)-cis/trans-p-mentha-2,8-dien-1-ol (166 mg, 1.0 mmol) in CHCl₃ (15 mL) to give 34 (120 mg, 40% yield). HRMS calcd for C₁₉H₂₄O₄ 316.1675, found 316.1676.
(Note: The product could not be isolated in pure form, because of very little amounts of overlapping impurities. The reaction mixture was carried forward as such for the next reaction without further purification).

(R)-Butyl 2-[(6aR,10aR)-1-hydroxy-6,6,9-trimethyl-6a,7,10,10a-tetrahydro-6H-benzoc]
chronen-3-yl]propanoate (35). The synthesis was carried out as described for 6 using bromobutane (49 mg, 0.4 mmol), 34 (58 mg, 0.2 mmol) and sodium bicarbonate (19 mg, 0.2 mmol) in DMF (2 mL) to give 35 (46 mg, 67% yield) as a light yellow gum. $^1$H NMR (500 MHz, chloroform-d) $\delta$ ppm 0.87 (t, $J = 7.5$ Hz, 3H) 1.09 (s, 3H) 1.29 (q, $J = 8.5$ Hz, 15.5 Hz, 2H) 1.37 (s, 3H) 1.44 (dd, $J = 3.0, 6.5$ Hz, 3H) 1.57 (p, $J = 7.0, 14.0$ Hz, 2H) 1.68 (s, 3H) 1.75-1.88 (m, 3H) 2.11 (d, $J = 15.5$ Hz, 1H) 2.69 (dt, $J = 4.5, 10.5$ Hz, 1H) 3.24 (d, $J = 19.5$ Hz, 1H) 3.55 (q, $J = 7.5$ Hz, 14.5 Hz, 1H) 4.01 - 4.17 (m, 2H) 4.41 – 4.46 (m, 1H) 6.34 (s, 1H); HRMS calcd for C$_{23}$H$_{32}$O$_4$ 372.2301, found 372.2304; HPLC Purity 100%.

(S)-3-(2-(3,5-Dimethoxyphenyl)acetyl)-4-isopropyloxazolidin-2-one (36). The synthesis was carried out as described for 30 using (S)-4-isopropyl-oxazolidin-2-one (1.7 g, 13.3 mmol), n-butyl lithium (8.3 mL, 13.3 mmol, 1.6 M solution in hexane) and 29 (2.6 g, 12.1 mmol) in dry THF (45 mL) to give 36 (4.72 g, 65% yield). $^1$H NMR (500 MHz, chloroform-d) $\delta$ ppm 0.89 (dd, $J = 7.0, 37.5$ Hz, 6H) 2.3-2.4 (m, 1H) 3.76 (s, 6H) 4.13 - 4.22 (m, 2H) 4.27 (q, $J = 14.5, 23.5$ Hz, 2H) 4.41 – 4.46 (m, 1H) 6.36 (t, $J = 2.5$ Hz, 1H) 6.47 (d, $J = 2.0$ Hz, 2H).

(S)-3-[(S)-2-(3,5-Dimethoxyphenyl)propanoyl]-4-isopropyloxazolidin-2-one (37). The synthesis was carried out as described for 31 using 36 (4.63 g, 15.06 mmol), NaHMDS (16.57 mL, 16.57 mmol, 1M in THF) and iodomethane (4.7 mL, 75.3 mmol) in THF to give 37 (4.0 g, 82% yield) as viscous oil. $^1$H NMR (500 MHz, chloroform-d) $\delta$ ppm 0.9 (dd, $J = 6.5, 8.0$ Hz,
(S)-2-(3,5-Dimethoxyphenyl)propanoic acid (38). The synthesis was carried out as described for 32 using 37 (3.72 g, 11.53 mmol), lithium hydroxide (831 mg, 34.7 mmol) in THF (40 mL): H2O (40 mL) to give 38 (2.21 g, 90% yield). 1H NMR (500 MHz, chloroform-d) δ ppm 1.47 (d, J = 7.0 Hz, 3H) 3.66 (q, J = 10.0, 15.0 Hz, 1H) 3.75 (s, 6H) 6.36 (t, J = 2.5 Hz, 1H) 6.47 (d, J = 2.5 Hz, 2H) 11.91 ( s, 1H).

(S)-2-(3,5-Dihydroxyphenyl)propanoic acid (39). The synthesis was carried out as described for 33 using boron tribromide (32.46 mL, 32.46 mmol, 1M solution in CH2Cl2), 38 (1.95 g, 9.27 mmol) in dry CH2Cl2 (80 mL) to give 39 (1.45 g, 90% yield). 1H NMR (500 MHz, methanol-d) δ ppm 1.38 (d, J = 7.5 Hz, 3H) 3.54 (q, J = 7.5, 14.5 Hz, 1H) 6.17 (t, J = 2.0 Hz, 1H) 6.28 (d, J = 2.5 Hz, 2H).

(S)-2-[(6aR,10aR)-1-Hydroxy-6,6,9-trimethyl-6a,7,10,10a-tetrahydro-6H-benzo[c]chromen-3-yl]propanoic acid (40). The synthesis was carried out as described for 34 using p-TSA (50 mg, 0.27 mmol), 39 (250 mg, 1.37 mmol) and (+)-cis/trans-p-mentha-2,8-dien-1-ol (230 mg, 1.5 mmol) in CHCl3 to give 40 (137 mg, 32% yield). HRMS calcd for C19H24O4 316.1675, found 31.1670.

(Note : The product could not be purified due to the presence of little amounts of overlapping impurity. The reaction mixture was carried forward as such for next reaction without further purification).

(S)-Butyl 2-[(6aR,10aR)-1-hydroxy-6,6,9-trimethyl-6a,7,10,10a-tetrahydro-6H-benzo[c]chromen-3-yl]propanoate (41). The synthesis was carried out as described for 35 using...
bromobutane (213 mg, 1.56 mmol), 40 (250 mg, 0.78 mmol) and sodium bicarbonate (80 mg, 0.94 mmol) in DMF to give 41 (95 mg, 33% yield). IR (neat): 3401, 2962, 1707, 1182 cm⁻¹; ¹H NMR (500 MHz, chloroform-d) δ ppm 0.87 (t, J = 7.5 Hz, 3H) 1.09 (s, 3H) 1.28 - 1.59 (m, 4H) 1.37 (s, 3H) 1.44 (d, J = 6.5Hz, 3H) 1.54 - 1.59 (m, 2H) 1.69 (s, 3H) 1.76 - 1.85 (m, 3H) 2.10 - 2.18 (m, 1H) 2.69 (dt, J = 4.0, 10.5 Hz, 1H) 3.21 (dd, J = 4.0, 16.0 Hz, 1H) 3.55 (q, J = 7.0, 14.0 Hz, 1H) 4.05- 4.12 (m, 2H) 5.23 (s, 1H) 5.42 (d, J = 4.5 Hz, 1H) 6.27 (d, J = 2.0 Hz, 1H) 6.36 (d, J = 1.5 Hz, 1H); HRMS calcd for C₂₃H₃₄O₄ 372.2301, found 372.2299.

2-(3,5-Dimethoxyphenyl)acetic acid (42). The synthesis was carried out as described for 2 using sodium hydroxide (3.2 g, 81.17 mmol) and 3,5-dimethoxyphenylacetic acid (5.7 g, 28.01 mmol) in 5 mL of n-butanol: water (2:1) mixture to give 42 (5.6 g, 88% yield) as colorless oil. ¹H NMR (500 MHz, chloroform-d) δ ppm 3.57 (s, 2H, benzylic CH₂) 3.77 (s, 6H, 2 x OCH₃) 6.38 (t, J = 2.0 Hz, 1H, ArH) 6.43 (d, J = 2.5 Hz, 2H, ArH).

2-(3,5-Dihydroxyphenyl)acetic acid (43). The synthesis was carried out as described for 3 using 42 (350 mg, 1.78 mmol) and boron tribromide (6.24 mL, 6.24 mmol, IM solution in CH₂Cl₂) in dry CH₂Cl₂ (15 mL) to give 43 (260 mg, 88% yield). ¹H NMR (500 MHz, methanol-d) δ ppm 3.4 (s, 2H, benzylic CH₂) 6.18 (t, J = 2.0 Hz, 1H, ArH) 6.21 (d, J = 2.0 Hz, 2H, ArH).

2-[(6α,10α)-6a,7,10,10a-Tetrahydro-1-hydroxy-6,6,9-trimethyl-6H-benzo[c]chromen-3-yl]acetic acid (44). The synthesis was carried out as described for 4 using p-TSA (230 mg, 1.18 mmol), 43 (1.0 g, 5.94 mmol) and (+)-cis/trans-p-mentha-2,8-dien-1-ol (1.0 g, 6.54 mmol) in CHCl₃ to give 44 (650 mg, 40% yield) as a light yellow oil. ¹H NMR (500 MHz, chloroform-d) δ ppm 1.06 (s, 3H, 6α-CH₃) 1.36 (s, 3H, 6β-CH₃) 1.67 (s, 3H, 9-CH₃) 1.73-1.85 (m, 3H, 10β-H, 7β-H, 6α-H) 2.08- 2.18 (m, 1H, 7α-H) 2.67 (td, J = 4.5, 11.0 Hz, 1H, 10α-H) 3.19 (dd, J =
4.5, 16 Hz, 1H, 10α-H) 3.44 (s, 2H, benzylic CH₂) 5.41 (d, J = 3.5 Hz, 1H, 8-H) 6.18 (d, J = 1.5 Hz, 1H, ArH) 6.33 (d, J = 1.5 Hz, 1H, ArH); HRMS calcd for C₁₈H₂₂O₄ 302.1518, found 302.1522; HPLC Purity 97.8%.

4-Bromobutyl-2-[(6aR,10αR)-6a,7,10,10a-tetrahydro-1-hydroxy-6,6,9-trimethyl-6H-benzo [c]chromen-3-yl]acetate (45). The synthesis was carried out as described for 5 using 44 (175 mg, 0.57 mmol), sodium bicarbonate (75 mg, 0.86 mmol) and dibromobutane (315 mg, 1.44 mmol) in DMF to give 45 (159 mg, 59% yield). IR (CHCl₃): 3406, 2967, 1712, 1430 cm⁻¹; ¹H NMR (500 MHz, chloroform-d) δ ppm 1.10 (s, 3H, 6α-CH₃) 1.36 (s, 3H, 6β-CH₃) 1.70 (s, 3H, 9-CH₃) 1.74 - 1.86 (m, 4H) 1.86 - 1.96 (m, 3H) 2.09 - 2.22 (m, 1H, 7α-H) 2.70 (td, J = 11.0, 4.5 Hz, 1H, 10a-H) 3.18 (dd, J = 4.5, 15 Hz, 1H, 10α-H) 3.40 (t, J = 6.5 Hz, 2H, 4′-CH₂) 3.46 (s, 2H, benzylic H) 4.14 (t, J = 5.5 Hz, 2H, 4′-CH₂) 5.24 (s, 1H, OH) 5.43 (d, J = 5.0 Hz, 1H, 8-H) 6.25 (s, 1H, ArH) 6.33 (s, 1H, ArH); HRMS calcd for C₂₂H₂₉O₄Br 436.1249, found 436.1252.

4-Cyanobutyl-2-[(6aR,10αR)-6a,7,10,10a-tetrahydro-1-hydroxy-6,6,9-trimethyl-6H-benzo [c]chromen-3-yl]acetate (46). The synthesis was carried out as described for 13 using 45 (80 mg, 0.178 mmol) and sodium cyanide (90 mg, 1.78 mmol) in DMSO (5 mL) to give 46 (50 mg, 68% yield) as a light yellow less gum. IR (neat): 3405, 2925, 2248, 1732 cm⁻¹; ¹H NMR (500 MHz, chloroform-d) δ ppm 1.09 (s, 3H, 6α-CH₃) 1.37 (s, 3H, 6β-CH₃) 1.64 - 1.87 (m, 10H) 2.09 - 2.20 (m, 1H, 7α-H) 2.36 (t, J = 7.0 Hz, 2H, 7′-CH₂) 2.70 (td, J = 11.0, 4.5 Hz, 1H, 10a-H) 3.22 (dd, J = 16.0, 4.5 Hz, 1H, 10α-H) 3.47 (s, 2H, benzylic CH₂) 4.14 (t, J = 6.0 Hz, 2H, 4′-CH₂) 5.42 (d, J = 4.0 Hz, 1H, 8-H) 5.72 (s, 1H, OH) 6.27 (d, J = 2.0 Hz, 1H, ArH) 6.31 (d, J = 2.0 Hz, 1H, ArH); HRMS calcd for C₂₃H₂₉NO₄ 383.2096, found 383.2091. HPLC Purity: 94.5%.
Butyl-2-[(6aR,10aR)-6a,7,10a-tetrahydro-1-hydroxy-6,9-trimethyl-6H-benzo[c]
chromen-3-yl]acetate (47). The synthesis was carried out as described for 6 using 44 (130 mg, 0.42 mmol), sodium bicarbonate (77 mg, 0.85 mmol) and bromobutane (43 mg, 1.05 mmol) in DMF (1.5 mL) to give 47 (80 mg, 52% yield) as light yellow oil. $^1$H NMR (500 MHz, chloroform- $d$) $\delta$ ppm 0.91 (t, $J = 7.5$ Hz, 3H, 7$'$-CH$_3$) 1.08 (s, 3H, 6$\alpha$-CH$_3$) 1.38 (s, 3H, 6$\beta$-CH$_3$) 1.30-1.42 (m, 5H, especially at $\delta$ 1.37, s, 3H) 1.57 - 1.65 (m, 2H) 1.70 (s, 3H, 9-CH$_3$) 1.73 - 1.89 (m, 3H, 10$\beta$-H, 7$\beta$-H, 6aH) 2.10 - 2.20 (m, 1H, 7$\alpha$-H) 2.70 (td, $J = 11.0$, 4.5 Hz, 1H, 10aH) 3.20 (dd, $J = 16.0$, 4.5 Hz, 1H, 10$\alpha$-H) 3.46 (s, 2H, benzylic CH$_2$) 4.09 (t, $J = 6.5$ Hz, 2H, 4$'$-CH$_2$) 5.4 (s, 1H, OH) 5.42 (d, $J = 5.0$ Hz, 1H, 8-H) 6.25 (d, $J = 1.5$ Hz, 1H, ArH) 6.32 (d, $J = 1.5$ Hz, 1H, ArH); HRMS calcd for C$_{22}$H$_{30}$O$_4$ 358.2144, found 358.2143; HPLC Purity 98.9%.

Butyl 2-(3,5-dihydroxyphenyl)-2-methylpropanoate (48). The synthesis was carried out as described for 6 using bromobutane (1.7 g, 12.22 mmol), 3 (1.6 g, 8.15 mmol) and sodium bicarbonate (825 mg, 9.78 mmol) in DMF (1.5 mL) to give 48 (1.9 g, 93% yield). $^1$H NMR (500 MHz, chloroform- $d$) $\delta$ ppm 0.85 (t, $J = 7.5$ Hz, 3H) 1.27 (h, $J = 8.0$, 15.0 Hz, 2H) 1.49 - 1.58 (m, 8H) 4.05 (t, $J = 7.0$ Hz, 2H) 6.25 (t, $J = 2.5$ Hz, 1H) 6.42 (d, $J = 2.0$ Hz, 2H) 6.66 (s, 2H).

Butyl 2-(4-[(1R,2S,5R)-6,6-dimethyl-4-oxobicyclo[3.1.1]heptan-2-yl]-3,5-dihydroxyphenyl)-2-methylpropanoate (49). p-TSA (829 mg, 4.35 mmol) was added to the solution of 48 (1.0 g, 3.96 mmol) and mixture of diacetates (1.2 g, 4.95 mmol) in CHCl$_3$ (20 mL). The resulting mixture was stirred at room temperature under dark for 5 days to ensure the complete conversion of starting material to the product. The reaction mixture was diluted with ethyl acetate and washed sequentially with water, saturated aqueous NaHCO$_3$ and brine. The organic phase was dried over MgSO$_4$ and the solvent was removed under vacuo. The residue was chromatographed on silica gel (20% ethyl acetate in hexane) to give 49 (810 mg, 53% yield). $^1$H NMR (500 MHz,
chloroform-$d$) $\delta$ ppm 0.84 (t, $J = 7.5$ Hz, 3H) 0.98 (s, 3H) 1.26 (q, $J = 7.5$, 15.0 Hz, 2H) 1.33 (s, 3H) 1.45 (s, 6H) 1.54 (p, $J = 6.5$, 14.5 Hz, 2H) 2.25 (t, $J = 5.0$ Hz, 1H) 2.42- 2.63 (m, 4H) 3.63 (dd, $J = 7.5$, 19.0 Hz, 1H) 3.89 (t, $J = 7.5$ Hz, 1H) 4.05 (t, $J = 7.0$ Hz, 2H) 6.35 (s, 2H) 7.13 (s, 2H); HRMS calcd for C$_{23}$H$_{33}$O$_5$ 389.2328 found 389.2333; IR (CHCl$_3$): 3361, 2959, 1727, 1683, 1421, 1265 cm$^{-1}$; HPLC Purity: 100%.

Butyl 2-[(6a$R$,10a$S$)-1-hydroxy-6,6-dimethyl-9-oxo-6a,7,8,9,10,10a-hexahydro-6$H$-benzo[c]chromen-3-yl]-2-methylpropanoate (50). TMSOTf (1.8 mL, 0.3 M solution in CH$_3$NO$_2$, 0.52 mmol) was added at 0°C under an argon atmosphere to the stirred solution of 49 (680 mg, 1.75 mmol) in anhydrous CH$_2$Cl$_2$: CH$_3$NO$_2$ (3:1 mixture, 20 mL). Stirred was continued for 3 h while the temperature was allowed to rise to 25°C. The reaction mixture was quenched with saturated aqueous NaHCO$_3$ solution and diluted with ethyl acetate. The organic layer was separated and the aqueous layer was extracted with ethyl acetate (3 x 5 mL). The combined organic layer was collected, washed with water and brine, dried (MgSO$_4$) and concentrated under vacuo. The residue was chromatographed on silica gel (15% acetone in hexane) to give 50 (480 mg, 70% yield). $^1$H NMR (500 MHz, chloroform-$d$) $\delta$ ppm 0.86 (t, $J = 7.5$ Hz, 3H) 1.11 (s, 3H) 1.20 - 1.34 (m, 2H) 1.47 (s, 3H) 1.50 (s, 6H) 1.51- 1.57 (m, 2H) 1.97 (dt, $J = 12.00$, 3.0 Hz, 1H) 2.08 – 2.21 (m, 2H) 2.42- 2.52 (m, 1H) 2.59-2.68 (m, 1H) 2.89 (dt, $J = 3.5$, 13.0 Hz, 1H) 4.06 (dt, $J = 2.0$, 6.5 Hz, 2H) 6.38 (q, $J = 1.5$, 16 Hz, 2H) 6.36 (d, $J = 1.5$ Hz, 1H) 6.39 (d, $J = 1.5$Hz, 1H) 7.37 (s, 1H).

Butyl 2-[(6a$R$,10a$S$)-1-hydroxy-9-(methoxymethylene)-6,6-dimethyl-6a,7,8,9,10,10a-hexahydro-6$H$-benzo[c]chromen-3-yl]-2-methylpropanoate (51). (Methoxymethyl)triphenylphosphonium chloride (1.9 g, 5.53 mmol) was suspended in 30 mL of dry benzene. Sodium tert-amylate (610 mg, 5.53 mmol) was added, and the reaction mixture was stirred for 5 min at 25°C.
Intermediate **50** (430 mg, 11 mmol) was dissolved in minimum amount of dry benzene and
transfered to the solution of ylide via cannula. The reaction mixture was stirred at room
temperature for 8 h. The reaction mixture was quenched with saturated aqueous NH₄Cl solution,
diluted with ethyl acetate. The organic layer was separated and the aqueous layer was extracted
with ethyl acetate (3 x 20 mL). The combined organic layer was washed with water and brine,
dried (MgSO₄) and concentrated under *vacuo*. The residue was chromatographed on silica gel
(20% acetone in hexane) to give **51** (110 mg, 48% yield). ¹H NMR (500 MHz, chloroform-*)δ ppm 0.84 (t, *J* = 7.5 Hz, 3H) 1.05 (s, 3H) 1.09 (dt, *J* = 4.5, 13.5 Hz, 1H) 1.25 (q, *J* = 7.5, 15.0
Hz, 2H) 1.39 (s, 3H) 1.50 (s, 6H) 1.50- 1.68 (m, 5H) 1.78 (dt, *J* = 5.5, 14.0 Hz, 1H) 1.85 -1.92
(m, 1H) 2.41 (dt, *J* = 3.5, 11.0 Hz, 1H) 2.93 (dt, *J* = 2.0 Hz, 1H) 3.5 (dd, *J* = 2.0 Hz, 13.5 Hz,
1H) 3.56 (s, 3H) 4.05 (t, *J* = 7.0 Hz, 2H) 5.91 (s, 1H) 6.22 (br,s,1H) 6.28 ( d, *J* = 2.0 Hz, 1H)
6.39 (d, *J* = 2.0 Hz, 1H); HRMS calcd for C₂₅H₃₆O₅ 416.2563, found 416.2562.

**Butyl 2-[(6a*R*,10a*S*)-9-formyl-1-hydroxy-6,6-dimethyl-6a,7,8,9,10,10a-hexahydro-6H-
benzo[c]chromen-3-yl]-2-methylpropanoate (52).** To the stirred solution of **51** (100 mg, 0.24
mmol) in CHCl₃ (5 mL) was added wet trichloroacetic acid (330 mg, 1.92 mmol). The resulting
mixture was stirred at room temperature for 3 h. The reaction mixture was quenched with
saturated aqueous NaHCO₃ solution and diluted with CH₂Cl₂. The organic layer was separated
and the aqueous layer was extracted with CH₂Cl₂ (3 x 3mL). The combined organic layer was
washed with water and brine, dried (MgSO₄) and concentrated under *vacuo*. The residue was
chromatographed on silica gel (20% acetone in hexane) to give mixture of epimers **52** (90 mg,
93% yield) which was used in next reaction as such for epimerization without purification.

**Butyl 2-[(6a*R*,9R,10a*S*)-9-formyl-1-hydroxy-6,6-dimethyl-6a,7,8,9,10,10a-hexahydro-6H-
benzo[c]chromen-3-yl]-2-methylpropanoate (53).** To the stirred solution of **52** (90 mg, 0.22
mmol) in methanol was added potassium carbonate (220 mg, 1.56 mmol). The resulting mixture was stirred at room temperature overnight. The reaction mixture was filtered, washed with water and brine, dried (MgSO₄) and concentrated under vacuo. The residue was chromatographed on silica gel (15% acetone in hexane) to give 53 (70 mg, 78% yield). ¹H NMR (500 MHz, chloroform-d) δ ppm 0.84 (t, J = 7.5 Hz, 3H) 1.08 (s, 3H) 1.20 - 1.28 (m, 4H) 1.39 (s, 3H) 1.48 (s, 6H) 1.49- 1.57 (m, 6H) 1.78 (br, s, 1H) 1.96 – 2.01 (m, 2H) 2.12 (d, J = 12.5 Hz, 1H) 2.44-2.6 (m, 2H) 3.54 (d, J = 12.5 Hz, 1H) 4.05 (t, J = 6.5 Hz, 2H) 6.28 (d, J = 2.0 Hz, 1H) 6.29 (br, s, 1H) 6.40 (d, J = 2.0 Hz, 1H) 9.65 (s, 1H); HRMS calcd for C₂₄H₃₄O₅ 402.2406, found 402.2401.

**Butyl-2-[(6aR,9R,10aS)-1-hydroxy-9-(hydroxymethyl)-6,6-dimethyl-6a,7,8,9,10,10a-hexahydro-6H-benzo[c]chromen-3-yl]-2-methylpropanoate (54).** Sodium borohydride (40 mg, 1.04 mmol) was added in small portions to the stirred solution of 53 (70 mg, 0.17 mmol) in methanol at room temperature. The reaction mixture was stirred at same temperature for 1 h. The reaction mixture was quenched with 1N HCl and diluted with ethyl acetate. The organic layer was separated and the aqueous layer was extracted with ethyl acetate (3 x 2 mL). The combined organic layer was washed with water and brine, dried (MgSO₄) and concentrated under vacuo. The residue was chromatographed on silica gel (15% acetone in hexane) to give 54 (50 mg, 72% yield). ¹H NMR (500 MHz, chloroform-d) δ ppm 0.72 (q, J = 12.0, 24.0 Hz, 1H) 0.84 (t, J = 7.0 Hz, 3H) 1.0 (s, 3H) 1.05 - 1.15 (m, 2H) 1.26 (p, J = 7.5, 15.0 Hz, 2H) 1.35 (s, 3H) 1.47 (d, J = 6.0 Hz, 6H) 1.53 (p, J = 6.5, 14.0 Hz, 12H) 1.77 (br, s, 1H) 1.89 (m, 2H) 2.44 (dt, J = 2.5, 10.5 Hz, 1H) 2.73 (br, s, 1H) 3.32 (d, J = 12.5 Hz, 1H) 3.49 (m, 2H) 4.04 (r, J = 6.5 Hz, 2H) 6.27 (d, J = 2.0 Hz, 1H) 6.35 (d, J = 2.0 Hz, 1H) 7.24 (br, s, 1H); HRMS calcd for C₂₄H₃₆O₅ 404.2563, found 404.2559; IR (CHCl₃): 3379, 2971, 1727, 1703, 1141 cm⁻¹.
2-[(6aR,10aR)-9-Formyl-6a,7,10,10a-tetrahydro-1-hydroxy-6,6-dimethyl-6H-benzo[c]chromen-3-yl]-2-methyl propanoic acid (55). Selenium dioxide (100 mg, 0.9 mmol) was added to a solution of 4 (150 mg, 0.45 mmol) in acetic acid (1.5 mL). The resulting mixture was irradiated in MW at 130°C for 15 min. The reaction mixture was cooled to room temperature and diluted with ethyl acetate. The organic layer was separated and the aqueous layer was extracted with ethyl acetate (3 x 2 mL). The combined organic layer was washed with water and brine, dried (MgSO₄) and concentrated under vacuo to get the crude product which was chromatographed on silica gel (20% ethyl acetate in hexane) to give 55 (19 mg, 11% yield) as a light yellow oil. ¹H NMR (500 MHz, chloroform-d) δ ppm 1.14 (s, 3H, 6α-CH₃) 1.42 (s, 3H, 6β-CH₃) 1.50 (s, 3H, CH₃) 1.51 (s, 3H, CH₃) 1.91-1.77 (m, 3H, 10β-H, 7β-H, 6α-H) 2.61-2.52 (m, 1H, 7α-H) 2.65 (td, J = 4.5, 11.0 Hz, 1H, 10a-H) 3.90-3.84 (m, 1H, 10α-H) 6.32 (d, J = 2.0 Hz, 1H, H-2) 6.45 (d, J = 2.0 Hz, 1H, H-4) 6.85-6.86 (m, 1H, 8-H) 9.48 (s, 1H, 9-CHO).

2-[(6aR, 10aR)-6a,7,10,10a-Tetrahydro-1-hydroxy-9-(hydroxymethyl)-6,6-dimethyl-6H-benzo[c]chromen-3-yl]-2-methylpropanoic acid (56). To the stirred solution of 55 (75 mg, 0.21 mmol) in aqueous methanol (5 mL) was added in small portions sodium borohydride (40 mg, 0.97 mmol). The resulting mixture was stirred for 1 h at room temperature. The reaction mixture was quenched with 1N HCl and diluted with ethyl acetate. The organic layer was separated and the aqueous layer was extracted with ethyl acetate (3 x 2 mL). The combined organic layer was washed with water and brine, dried (MgSO₄) and concentrated under vacuo. The residue was chromatographed on silica gel (40% ethyl acetate in hexane) to give 56 (30 mg, 42% yield) as light yellow gum. ¹H NMR (500 MHz, chloroform-d) δ ppm 0.90 (s, 3H, 6α-CH₃) 1.23-1.52 (m, 10H, especially s, 3H, 6β-H) 1.75-1.83 (m, 3H, 10β-H, 7β-H, 6α-H) 2.16-2.22 (m, 1H, 7α-H) 2.62-2.71 (m, 1H, 10aH) 3.49-3.58 (m, 1H, 10α-H) 4.05 (dd, J = 12.5, 31 Hz, 2H,
C11- CH₂) 5.71 (s, 1H, 8-H) 6.34 (s, 1H, ArH) 6.44 (s, 1H, ArH); HRMS calcd for C₂₀H₂₆O₅ 346.1780, found 346.1780. HPLC Purity: 100%.

2-[(6aR, 10aR)-6a,7,10,10a-Tetrahydro-1-hydroxy-9-(hydroxymethyl)-6,6-dimethyl-6H-benzo[c]chromen-3-yl]-2-methylpropanoic acid (56a). Sodium hydroxide (70 mg, 1.79 mmol) was added to the stirred solution of 60 (200 mg, 0.44 mmol) in THF: H₂O (50:50 mixture, 10 mL). The resulting mixture was refluxed for 12 h and then warmed to room temperature. The reaction mixture was quenched with 1N HCl and diluted with ethyl acetate. The organic layer was separated and the aqueous layer was extracted with ethyl acetate (3 x 3 mL). The combined organic layer was washed with water and brine, dried (MgSO₄) and concentrated under vacuo. The residue was chromatographed on silica gel (50% acetone in hexane) to give 56a (110 mg, 70% yield) as light yellow gum. \(^1\)H NMR (500 MHz, chloroform-d) δ ppm 0.90 (s, 3H, 6α-CH₃) 1.23-1.52 (m, 10H, especially s, 3H, 6β-H) 1.75- 1.83 (m, 3H, 10β-H, 7β-H, 6aH) 2.16- 2.22 (m, 1H, 7α-H) 2.62-2.71 (m, 1H, 10aH) 3.49- 3.58 ( m, 1H, 10α-H) 4.05 ( dd, J = 12.5, 31 Hz, 2H, C11- CH₂) 5.71 (s, 1H, 8-H) 6.34 (s, 1H, ArH) 6.44 (s, 1H, ArH); HRMS calcd for C₂₀H₂₆O₅ 346.1780, found 346.1780. HPLC Purity: 100%.

Butyl-2-[(6aR, 10aR)-6a, 7, 10, 10a-tetrahydro-1-hydroxy-9-(hydroxymethyl)-6,6-dimethyl-6H-benzo[c]chromen-3-yl]-2-methylpropanoate (57). The synthesis was carried out as described for 6 using bromobutane (70 mg, 0.49 mmol), 56 (50 mg, 0.14 mmol) and sodium bicarbonate (20 mg, 0.23 mmol) in DMF (1.5 mL) to give 57 (30 mg, 48% yield) as light yellow gum. IR (CHCl₃): 3292, 2931, 1727, 1703, 1619, 1578, 1417, 1263, 1156, 836 cm⁻¹; \(^1\)HNMR (500 MHz, chloroform-d) δ ppm 0.87 (t, J = 7.5 Hz, 3H, 7'-CH₃) 1.11 (s, 3H, 6α-CH₃) 1.24 - 1.32 (m, 3H) 1.40 (s, 3H, 6β-CH₃) 1.51 (s, 6H, -C(CH₃)₂-) 1.53 - 1.62 (m, 4H) 1.78 - 1.94 (m, 3H) 2.24 (m as d, J = 15.0 Hz, 1H) 2.71 (td, J = 11.0, 4.5 Hz, 1H) 3.41 (dd, J = 16.0, 4.0Hz, 1H)
4.02 - 4.09 (m, 4H, 11-CH₂ + 4'-CH₂) 5.43 (s, 1H, OH) 5.75 (br d, J = 5.0 Hz, 1H, H-8) 6.26 (d, J = 2.0 Hz, 1H, ArH) 6.43 (d, J = 2.0 Hz, 1H, ArH); HRMS calcd for C₂₄H₃₄O₅ 402.2406, found 402.2407; Anal. Calcd for C₂₄H₃₄O₅: C, 71.61; H, 8.51%. Found: C, 70.26; H, 7.91%.

HPLC Purity: 100%.

**Butyl-2-(6aR,10aR)-6a,7,10,10a-tetrahydro-1-hydroxy-9-(hydroxymethyl)-6,6-dimethyl-6H-4-(1H-imidazol-1-yl) (58).** The synthesis was carried out as described for 6 using bromobutane-4-imidazole (215 mg, 1.05 mmol), 56 (200 mg, 0.60 mmol) and sodium bicarbonate (80 mg, 0.90 mmol) in DMF (1.5 mL) to give 58 (55 mg, 20% yield) as light yellow gum. ¹H NMR (500 MHz, chloroform-d) δ ppm 1.09 (s, 3H, 6α-CH₃) 1.36 (s, 3H, 6β-CH₃) 1.45-1.56 (m, 8H, especially s-CH₃ and s-CH₃) 1.6-1.87 (m, 5H) 2.15-2.24 (m, 1H, 7α-H) 2.71 (td, J = 4.0, 11.5 Hz, 1H, 10α-H) 3.58 (d, J = 17.0 Hz, 1H, 10α-H) 3.74 (t, J = 6.5Hz, 2H) 3.92-4.09 (m, 4H) 5.67 (br s, 1H, 8-H) 6.31 (s, 1H, ArH) 6.36 (s, 1H, ArH) 6.82 (s, 1H) 7.06 (s, 1H) 7.47 (s, 1H); HRMS calcd for C₂₇H₃₇N₂O₅ 469.2702, found 469.2707.

**Methyl 2-(3,5-dihydroxyphenyl)-2-methylpropanoate (59).** The synthesis was carried out as described for 6 using iodomethane (900 mg, 6.35 mmol), 3 (500 mg, 2.54 mmol) and sodium bicarbonate (320 mg, 3.82 mmol) in DMF (2.0 mL) to give 59 (350 mg, 66% yield) as colorless oil. ¹H NMR (500 MHz, chloroform-d) δ ppm 1.47 (s, 6H, -C(CH₃)₂-) 3.61 (s, 3H, 1 x CH₃) 6.25 (t, J = 2.5 Hz, 1H, ArH) 6.41 (d, J = 2.0 Hz, 2H, ArH) 6.90 (s, 2H, 2 x OH).

**[(6aR,10aR)-1-Hydroxy-3-(1-methoxy-2-methyl-1-oxopropan-2-yl)-6,6-dimethyl-6a,7,10,10a-tetrahydro-6H-benzo[c]chromen-9-yl]methyl pivalate (60).** Borontrifluoride diethyl etherate (0.50 mL, 3.7 mmol) was added at -20°C under an argon atmosphere to a solution of 59 (130 mg, 0.61 mmol) and pivalate ester terpene (170 mg, 0.68 mmol) in dry CH₂Cl₂ (10 mL). The reaction
mixture was allowed to warm up to room temperature and stirred for 1 h. The reaction mixture was quenched with saturated aqueous NaHCO₃ solution and diluted with CH₂Cl₂. The organic layer was separated and the aqueous layer was extracted with CH₂Cl₂ (3 x 5 mL). The combined organic layer was washed with water and brine, dried (MgSO₄) and concentrated under vacuo. The residue was chromatographed on silica gel (15% ethyl acetate in hexane) to give 60 (110 mg, 40% yield) as light yellow gum.

(Note: The product had inseparable overlapping impurities, so the product was used as such for the next reaction without further purification).

Methyl 2-(3,5-dihydroxy-4-((1R,2R,5S)-6,6-dimethyl-4-ketobicyclo[3.1.1]heptan-2-yl)phenyl)-2-methylpropanoate (61). To a stirred solution of 59 (350 mg, 1.66 mmol) and mixture of nopinone diacetates (695 mg, 2.91 mmol) in CHCl₃ (11 mL) was added p-TSA monohydrate (350 mg, 1.82 mmol). The resulting mixture was stirred at room temperature under dark for 5 days to ensure the complete conversion of the starting material to product. The reaction mixture was diluted with ethyl acetate and washed subsequently with water, saturated aqueous NaHCO₃, and brine. The organic phase was dried (MgSO₄) and concentrated under vacuo. The crude product was chromatographed on silica gel (18% ethyl acetate in hexane) to give 61 (280 mg, 52% yield) as a white amorphous powder. IR (CHCl₃): 3402, 2977, 2530, 1709, 1682, 580, 1269, 980 cm⁻¹; ¹H NMR (500 MHz, methanol-d₄) δ ppm 0.97 (s, 3H, 6-CH₃) 1.37 (s, 3H, 6-CH₃) 1.49 (s, 6H, -C(CH₃)₂-) 2.19 (t, J = 5.50 Hz, 1H, 5-H) 2.39 - 2.53 (m, 3H, 1-H, 7α-H, 7β-H) 2.58 - 2.64 (m, 1H, 3β-H) 3.65 (s, 3H, 4'-CH₂) 3.72 (dd, J = 18.5, 7.0 Hz, 1H, 3α-H) 4.02 (t, J = 8.0 Hz, 1H, 4-H) 6.31 (s, 2H, ArH);
Methyl-2-[(6aR,10aR)-6a,7,8,9,10,10a-hexahydro-1-hydroxy-6,6-dimethyl-9-oxo-6H-benzo[c]chromen-3-yl]-2-methylpropanoate (62). The synthesis was carried out as described for 50 using TMSOTf (0.65 mL, 0.19 mmol, 0.3 M solution in CH₃NO₂) and 61 (220 mg, 0.63 mmol) in anhydrous CH₂Cl₂: CH₃NO₂ (3:1 mixture, 12 mL) to give 62 (120 mg, 54% yield) and small traces of inseparable 61. This mixture was used as such for next reaction without further purification. HRMS calcd for C₂₀H₂₇O₅ 347.1858, found 347.1857.

2-[(6aR,10aR)-6a,7,8,9,10a-hexahydro-1-hydroxy-6,6-dimethyl-9-oxo-6H-benzo[c]chromen-3-yl]-2-methylpropanoic acid (63). Sodium hydroxide (30 mg, 0.69 mmol) was added to the stirred solution of 62 (120 mg, 0.34 mmol) in 1:1 mixture of THF: water (8 mL). The reaction mixture was stirred at room temperature for 8 h. The reaction mixture was quenched with 1N HCl and diluted with ethyl acetate. The organic layer was separated and the aqueous layer was extracted with ethyl acetate (3 x 3 mL). The combined organic layer was washed with water and brine, dried (MgSO₄) and concentrated under vacuo. The crude product was chromatographed on silica gel (48% acetone in hexane) to give 63 (50 mg, 43% yield) as a white solid. MP 154°C; IR (solid): 3340, 1694, 1601 cm⁻¹; ¹H NMR (500 MHz, methanol-d₄) δ ppm 1.15 (s, 3H, 6α-CH₃) 1.46 (s, 3H, 6β-CH₃) 1.48-1.57 (m, 7H, 7ax-H, 2x CH₃ at 1°C 1.94 (td, J = 3.0, 12.0 Hz, 1H, 6a-H) 2.06 - 2.22 (m, 2H, 10ax-H, 7eq-H) 2.48 - 2.54 (m, 2H, 8eq-H, 8-ax-H) 2.79 - 2.87 (m, 1H, 10a-H) 3.87 (dd, J = 15.0, 3.50 Hz, 1H, 10eq-H) 6.32 (d, J = 2.0, 1H, ArH) 6.37 (d, J = 2.0 Hz, 1H, ArH); HRMS calcd for C₁₉H₂₅O₅ 333.1702, found 333.1700. HPLC purity: 95.8%.

Butyl-2-[(6aR,10aR)-6a,7,8,9,10a-hexahydro-1-hydroxy-6,6-dimethyl-9-oxo-6H-benzo[c]chromen-3-yl]-2-methylpropanoate (64). The synthesis was carried out as described for 6 using 63 (50 mg, 0.15 mmol), sodium bicarbonate (20 mg, 0.22 mmol) and bromobutane
(55 mg, 0.40 mmol) in DMF (1.5 mL) to give 64 (40 mg, 68% yield) as light yellow gum. IR (CHCl₃): 3356, 2958, 1697, 1419 cm⁻¹; ¹H NMR (500 MHz, chloroform-­d) δ ppm 0.86 (t, J = 7.5 Hz, 3H, 7-CH₃) 1.11 (s, 3H, 6α-CH₃) 1.20 - 1.34 (m, 2H) 1.47 (s, 3H, 6β-H) 1.50 (s, 6H, -C(CH₃)_2-) 1.51- 1.57 (m, 3H) 1.97 (dt, J = 12.0, 3.0 Hz, 1H) 2.08 – 2.21 (m, 2H) 2.42- 2.52 (m, 1H) 2.59-2.68 (m, 1H) 2.89 (dt, J = 3.5, 13.0 Hz, 1H) 4.06 (dt, J = 2.0, 6.5 Hz, 3H) 6.36 (d, J = 1.5 Hz, 1H, ArH) 6.39 (d, J = 1.5Hz, 1H, ArH) 7.37 (s, 1H); HRMS calcd for C₂₃H₃₂O₅ 388.2250, found 388.2246; Anal. Calcd for C₂₃H₃₂O₅: C, 71.11; H, 8.30%. Found: C, 70.94; H, 8.02%. HPLC Purity: 98.3%.

Butyl-2-[(6aR,9R,10aR)-6a,7,8,9,10,10a-hexahydro-1,9-dihydroxy-6,6-dimethyl-6H-benzo[c]chromen-3-yl]-2-methylpropanoate (65). To the stirred solution of 64 (40 mg, 0.09 mmol) in methanol (3 mL) at -78°C was added in small portions sodium borohydride (4.0 mg, 0.09 mmol). The resulting mixture was stirred at same temperature for 2 h. The reaction mixture was quenched with 2 N HCl and diluted with ethyl acetate. The organic layer was separated and the aqueous layer was extracted with ethyl acetate (3 x 2 mL). The combined organic layer was washed with water and brine, dried (MgSO₄) and concentrated under vacuo. The crude product was chromatographed on silica gel (24% acetone in hexane) to give 65 (30 mg, 84% yield) as light yellow gum. IR (CHCl₃): 3329, 2971, 1727, 1704, 1142 cm⁻¹; ¹H NMR (500 MHz, chloroform-­d) δ ppm 0.85 (t, J = 7.5 Hz, 3H, 7’-CH₃) 1.05 (s, 3H, 6α-H) 1.13- 1.17 (m, 2H, 7ax-H, 10ax-H) 1.29 (sextet, J = 7.0, 14.5, 19.5 Hz, 2H, 6’-CH₂) 1.38 (s, 3H, 6β-H) 1.40-1.47 (m, 1H, 8ax-H) 1.48 (s, 6H, -C(CH₃)_2-) 1.50-1.56 (m, 3H) 1.63 (br s, 1H, OH) 1.89- 1.92 (m, 1H, 7eq-H) 2.16 (m as br d, J = 11.0 Hz, 1H, 8eq-H) 2.47 (dt, J = 2.5, 11.5 Hz, 1H, 10a-H) 3.48-3.54 ( m, 1H, 10eq-H) 3.86 (ddddd, J = 16.0, 15.0, 4.5, 4.0Hz, 1H, 9ax-H) 4.04 ( t, J = 6.5 Hz,
2H, 4’-CH2) 6.08 (br s, 1H, OH) 6.22 (d, J = 2.0 Hz, 1H, ArH) 6.38 (d, J = 1.5 Hz, 1H, ArH); HRMS calcd for C_{23}H_{34}O_{5} 390.2406, found 390.2408; HPLC Purity: 100%.

2-[(6a{R},9R,10aR)-6a,7,8,9,10,10a-hexahydro-1,9-dihydroxy-6,6-dimethyl-6H-benzo[c]chromen-3-yl]-2-methylpropanoic acid (66). The synthesis was carried out as described for 65 using 63 (55 mg, 0.16 mmol) and sodium borohydride (25 mg, 0.66 mmol) in methanol (6 mL) to give 66 (45 mg, 81% yield) as a light yellow gum. \(^1\)H NMR (500 MHz, methanol-\(d_4\)) \(\delta\) ppm 0.92 - 1.00 (m, 2H, 7ax-H, 10ax-H) 1.05 (s, 3H, 6\(\alpha\)-H) 1.14 - 1.25 (m, 1H, 8ax-H) 1.36 (s, 3H, 6\(\beta\)-H) 1.39 -1.45 (m, 1H, 6a-H) 1.47 (s, 6H, -C(CH_3)_2-) 1.87 - 1.94 (m, 1H, 7eq-H) 2.13 (br m as d, \(J = 12.0\) Hz, 1H, 8eq-H) 2.46 (td, \(J = 11.5, 2.5\) Hz, 1H, 10a-H) 3.50 - 3.58 (m, 1H, 10eq-H) 3.74 (dddd, \(J = 16.0, 15.5, 4.5, 4.0\)Hz, 1H, 9ax-H) 6.27 (d, \(J = 2.0\) Hz, 1H, ArH) 6.35 (d, \(J = 2.0\) Hz, 1H, ArH); HRMS calcd for C_{19}H_{26}O_{5} 334.1780, found 334.1777; HPLC Purity: 98.9%.

2-[(6a{R},9S,10aR)-6a,7,8,9,10,10a-hexahydro-1,9-dihydroxy-6,6-dimethyl-6H-benzo[c]chromen-3-yl]-2-methylpropanoic acid (67). K-selectride (0.15 mL, 0.48 mmol) was added under an argon atmosphere to the solution of 63 (40 mg, 0.12 mmol) in dry THF (6 mL). The resulting mixture was stirred at room temperature for 2 h. The reaction mixture was quenched with 1N HCl and diluted with ethyl acetate. The organic layer was separated and the aqueous layer was extracted with ethyl acetate (3 x 3 mL). The combined organic layer was washed with water and brine, dried (MgSO_4) and concentrated under \textit{vacuo}. The crude product was chromatographed on silica gel (38% acetone in hexane) to give 67 (40 mg, 95% yield). \(^1\)H NMR (500 MHz, methanol-\(d_4\)) \(\delta\) ppm 1.09 (s, 3H, 6\(\alpha\)--CH_3) 1.16 - 1.24 (m, 1H) 1.36 (s, 3H, 6\(\beta\)--CH_3) 1.46-1.71 (m, 10H, especially s at 1.47 for 6H for -C(CH_3)_2-) 1.91 - 1.96 (m, 1H, 8eq-H) 2.94 (dt, \(J = 11.5, 3.0\) Hz, 1H, 10a-H) 3.36- 3.44 (m, 1H, 10eq-H) 4.13 (t, \(J = 3.0\)Hz, 1H, 9eq-H)
Butyl-2-[(6aR,9S,10aR)-6a,7,8,9,10,10a-hexahydro-1,9-dihydroxy-6,6-dimethyl-6H-
benzo[c]chromen-3-yl]-2-methylpropanoate (68). The synthesis was carried out as described
for 6 using 67 (40 mg, 0.10 mmol), sodium bicarbonate (15 mg, 0.16 mmol) and bromobutane
(35 mg, 0.25 mmol) in DMF (1.5 mL) to give 68 (18 mg, 42% yield) as light yellow gum. IR
(CHCl₃): 3357, 2926, 1706, 1140 cm⁻¹; ¹H NMR (500 MHz, chloroform-d) δ ppm 0.87 (t, J =
7.5 Hz, 3H, 7′-CH₃) 1.07 (s, 3H, 6α-CH₃) 1.25 - 1.39 (m, 5H) 1.39 (s, 3H, 6β-CH₃) 1.44 (s, 1H,
OH) 1.49 - 1.60 (m, 9H, especially s at 1.51, 6H) 1.62 - 1.71 (m, 1H) 1.96 (d, J = 13.5 Hz, 1H,
8eq-H) 2.96 (t, J = 11.0 Hz, 1H, 10a-H) 3.24 (dd, J = 14.0, 2.5 Hz, 1H, 10eq-H) 4.07 (t, J = 6.0
Hz, 2H, 4′-CH₂) 4.28 (bs s, 1H, 9eq-H) 6.31 (d, J = 2.0 Hz, 1H, ArH) 6.41 (d, J = 1.5 Hz, 1H,
ArH); HRMS calcd for C₂₃H₃₄O₅ 390.2406, found 390.2408.

1-(3,5-Dimethoxyphenyl)heptan-1-one (69). Hexylmagnesium bromide (25 mL, 49.02 mmol,
2M solution in diethyl ether) was transferred under an argon atmosphere to a single neck flask
containing 3,5-dimethoxybenzonitrile (5.0 g, 30.64 mmol) in dry THF (50 mL). To the resulting
mixture was added copper bromide (440 mg, 3.06 mmol). The resulting reaction mixture was
refluxed for 2 h, quenched with 1N HCl and diluted with ethyl acetate. The organic layer was
separated and the aqueous layer was extracted with ethyl acetate (3 x 20 mL). The combined
organic layer was washed with water and brine and dried (MgSO₄) and concentrated in vacuo.
The residue was chromatographed on silicagel (12% acetone in hexane) to give 69 (7.05 g, 92% 
yield). ¹H NMR (500 MHz, chloroform-d) δ ppm 0.88 (t, J = 7.5 Hz, 3H) 1.28 - 1.38 (m, 6H)
1.69 (p, J = 7.5, 15.0 Hz, 2H) 2.88 (t, J = 7.5 Hz, 2H) 3.79 (s, 6H) 6.60 (d, J = 2.5 Hz, 1H,
ArH) 7.0 (d, J = 2.5 Hz, 2H, ArH).
2-(3,5-Dimethoxyphenyl)-2-hexyl-1,3-dithiolane (70). To a stirred solution of 69 (2.5 g, 9.98 mmol) and ethane dithiol (2.0 mL, 24.96 mmol) in dry CH$_2$Cl$_2$ (40 mL) at 0°C was added boron trifluoride diethyl etherate (0.25 mL, 1.99 mmol). The reaction mixture was stirred at that temperature for 3 h and at completion, saturated aqueous NaHCO$_3$ solution was added. The resulting mixture was diluted with ethyl acetate. The organic layer was separated and the aqueous layer was extracted with CH$_2$Cl$_2$ (3 x 15 mL). The combined organic layer was collected, washed with water and brine, dried (MgSO$_4$) and concentrated under vacuo. The residue 70 (3.2 g, 96% yield) was of sufficient purity and used in the next step as such without further purification. $^1$H NMR (500 MHz, Chloroform-d) δ ppm  0.83 (t, $J = 7.0$ Hz, 3H) 1.6- 1.9 (m, 6H)  2.31 (t, $J = 8.0$ Hz, 2H) 2.75 (dd, $J = 3.0$, 4.5 Hz, 2H) 3.19 -3.27 (m, 2H) 3.32- 3.4 (m, 2H) 3.80 (s, 6H) 6.33 (t, $J = 2.5$ Hz, 1H) 6.87 (d, $J = 2.0$ Hz, 2H).

5-(2-Hexyl-1,3-dithiolan-2-yl)benzene-1,3-diol (71). The synthesis was carried out analogous to the preparation of 3 using boron tribromide (2.7 mL, 27.95 mmol, neat solution) and 70 (3.3 g, 9.98 mmol) in dry CH$_2$Cl$_2$ (80 mL) to give crude 71 (2.9 g, 95%) which was of sufficient purity and used in the next reaction as such without further purification.

(6aR,10aR)-3-(2-Hexyl-1,3-dithiolan-2-yl)-6,6,9-trimethyl-6a,7,10,10a-tetrahydro-6H-benzo[c]chromen-1-ol (72) The synthesis was carried out as described for 4 using p-TSA (150 mg, 0.80 mmol), 71 (1.2 g, 4.02 mmol) and (+)-cis/trans-p-mentha-2,8-dien-1-ol (675 mg, 4.42 mmol) in CHCl$_3$ (40 mL) to give crude product 72, which was of sufficient purity and used in the next step as such without further purification.

1-(6aR,10aR)-1-Hydroxy-6,6,9-trimethyl-6a,7,10a-tetrahydro-6H-benzo[c]chromen-3-yl)heptan-1-one (73). A solution of silver nitrate (1.4 g, 8.32 mmol) in water (0.5 mL) was
added to the solution of 72 (1.2 g, 2.77 mmol) in 90% ethanol (50 mL) at room temperature. The resulting mixture was stirred for 3 h. At completion the precipitates were removed and washed with ethyl acetate, water and brine, dried (MgSO₄). Solvent was evaporated under vacuo to get crude product which was chromatographed on silicagel (15% acetone in hexane) to give 73 (800 mg, 80% yield). IR (CHCl₃): 2956, 2920, 1685, 1566, 1415, 1092 cm⁻¹.

(Note: NMR shows the presence of small traces of inseparable starting material along with desired product)

1-(6aR,10aR)-1-(Tert-butyldimethylsilyloxy)-6,6,9-trimethyl-6a,7,10,10a-tetrahydro-6H-benzo[c]chromen-3-yl)heptan-1-one (74). Imidazole (1.6 g, 23.84 mmol) was added to the stirred solution of 73 (850 mg, 2.38 mmol) and TBDMS-Cl (1.8 g, 11.9 mmol) in dry DMF (5 mL) at room temperature. The resulting mixture was stirred for 24 h, quenched with water and diluted with ethyl acetate. The organic layer was separated and the aqueous layer was extracted with ethyl acetate (3 x 20 mL). The combined organic extract was washed with water and brine, dried (MgSO₄) and concentrated under vacuo. The residue was chromatographed on silica gel (5% acetone in hexane) to give 74 (1.0 g, 93% yield). ¹H NMR (500 MHz, chloroform-d) δ ppm 0.18 (s, 3H, Si-CH₃) 0.32 (s, 3H, Si-CH₃) 0.89 (t, J = 7.0 Hz, 3H, 7′-CH₃) 1.02 (s, 9H, 3 x CH₃ of t-butyl of TBDMS) 1.09 (s, 3H, 6α-CH₃) 1.30-1.38 (m, 4H) 1.40 (s, 3H, 6β-CH₃) 1.68 -1.89 (m, 7H) 2.16 – 2.19 (m, 1H, 7α-H) 2.65 (td, J = 4.0, 10.5 Hz, 1H, 10a-H) 2.87 (t, J = 8.0 Hz, 2H, 2′-CH₂) 3.27 (dd, J = 4.5, 17.5 Hz, 1H, 10α-H) 5.42 (br d, J = 4.0 Hz, 1H, 8-H) 7.0 (d, J = 2.0 Hz, 1H, ArH) 7.06 (d, J = 1.5 Hz, 1H, ArH).

Ethyl-3-[(6aR,10aR)-1-(Tert-butyldimethylsilyloxy)-6,6,9-trimethyl-6a,7,10,10a-tetrahydro-6H-benzo[c]chromen-3-yl]-3-hydroxynonanoate (75). Activated zinc dust (180 mg, 2.78
mmol) was added under an argon atmosphere to the stirred solution of ketone 74 (660 mg, 1.39 mmol) and ethyl bromo acetoacetate (0.3 mL, 2.78 mmol) in dry THF (15 mL). The reaction mixture was gently warmed to 60°C in order to start the strongly exothermic reaction. Thereafter stirring was continued for an additional 4 h without heating. The reaction mixture was poured into ice and diluted with ethyl acetate. The organic layer was separated and the aqueous layer was extracted with ethyl acetate (3 x 5 mL). The combined organic layer was washed with water and brine, dried (MgSO₄) and concentrated under vacuo. The residue was chromatographed on silica gel (6% acetone in hexane) to give 75 (590 mg, 75.7% yield) as mixture of diastereomers 75e₁ and 75e₂. ¹H NMR (500 MHz, chloroform-d) δ ppm 0.10 (s, 3H, Si-CH₃, 75e₁) 0.11 (s, 3H, Si-CH₃, 75e₂) 0.26 (s, 6H, Si-CH₃, 75e₁, 75e₂) 0.82 (t and t overlapping, J = 6.5 Hz, 6H, 7'-CH₃, 75e₁, 75e₂) 0.99 (br s, 18H, 6 x CH₃, 75e₁, 75e₂) 1.04 (s, 3H, 6α-H, 75e₁) 1.05 (s, 3H, 6α-CH₃, 75e₂) 1.08-1.25 (q, J = 7.0, 14.5 Hz, 8H, 75e₁, 75e₂) 1.15-1.30 (m, 18H, 75e₁, 75e₂) 1.36 (s, 6H, 6β-CH₃, 75e₁, 75e₂) 1.68 (s, 6H, 9-CH₃, 75e₁, 75e₂) 1.73-1.89 (m, 6H, 75e₁, 75e₂) 2.1-2.19 (m, 2H, 7α-H, 75e₁, 75e₂) 2.56 (dt, J = 4.0, 11.0 Hz, 2H, 10a-H, 75e₁, 75e₂) 2.72 (ddd, J = 8.0, 16.0, 23.5 Hz, 4H, 75e₁, 75e₂) 3.19-3.29 (br d, J = 16.5 Hz, 2H, 10a-H, 75e₁, 75e₂) 3.95-4.12 (m, 4H, 75e₁, 75e₂) 5.40 (br d, J = 4.5 Hz, 2H, 8-H, 75e₁, 75e₂) 6.40-6.48 (m, 4H, ArH, 75e₁, 75e₂); IR (CHCl₃): 3500, 2930, 1714, 1415, 1184 cm⁻¹.

3-Hydroxy-3-[(6aR,10aR)-1-hydroxy-6,6,9-trimethyl-6a,7,10,10a-tetrahydro-6H-benzo[c]chromen-3-yl]nonanoic acid (76). The synthesis was carried out analogous to the preparation of 56a using sodium hydroxide (50 mg, 1.14 mmol) and 75 (160 mg, 0.28 mmol) in 50:50 mixture of THF: water (10 mL) to give 76 (90 mg, 75% yield) as light yellow gum as a mixture of diastereomers 76e₁ and 76e₂. ¹H NMR (500 MHz, chloroform-d) δ ppm 0.82 (t and t overlapping, J = 6.5 Hz, 6H, 7'-CH₃, 76e₁, 76e₂) 1.08 (s, 3H, 6α-CH₃, 76e₁) 1.09 (s, 3H, 6α-H,
76e₂) 1.14 - 1.29 (m, 18H, 76e₁, 76e₂) 1.37 (s, 6H, 6β-CH₃, 76e₁, 76e₂) 1.64-1.75 (m, 10H, especially s at 1.69 for 9-CH₃, 76e₁, 76e₂) 1.76-1.89 (m, 6H, 75e₁, 75e₂) 2.10-2.17 (m, 2H, 7α-H, 76e₁, 76e₂) 2.69 (td, J = 4.5, 11.0 Hz, 2H, 10a-H, 76e₁, 76e₂) 2.80 (dd and dd overlapping, J = 3.0, 16.0 Hz, 4H, 76e₁, 76e₂) 3.20 (d and d overlapping, J = 4.0, 16.5 Hz, 2H, 10α-H, 76e₁, 76e₂) 5.42 (d, J = 4.5 Hz, 2H, 8-H, 76e₁, 76e₂) 6.31 (br, s, 2H, ArH, 76e₁, 76e₂) 6.44 (d and d overlapping, J = 1.5 Hz, 1H, ArH); IR (CHCl₃): 3375, 2928, 1708, 1418, 1032 cm⁻¹.

4-Hexyl-4-[(6aR,10aR)-1-hydroxy-6,6,9-trimethyl-6a,7,10,10a-tetrahydro-6H-benzo[c]chromen-3-yl] oxetan-2-one (77). 1,1,1-trifluoro-N-phenyl-N-(trifluoromethylsulfonyl) methanesulfonamide (90 mg, 0.24 mmol) was added to the stirred solution of 91 (70 mg, 0.16 mmol) and triethylamine (47 µL, 0.33 mmol) in dry CH₂Cl₂ (3 mL) at 0°C and the resulting mixture was stirred for 1 h. The reaction mixture was warmed to room temperature and stirred over night. The reaction mixture was quenched with saturated NaHCO₃ solution and diluted with ethyl acetate. The organic phase was separated and the aqueous layer was extracted with ethyl acetate (3 x 5 mL). The combined organic layer was washed with water and brine, dried (MgSO₄) and concentrated under vacuo. The residue was chromatographed on silicagel (24% acetone in hexane) to give desired analog 77 (15 mg, 18% yield) as light yellow gum as mixture of diastereomers 77e₁ and 77e₂. ¹H NMR (500 MHz, Chloroform-d) δ ppm 0.82 (t, J = 8.0 Hz, 6H, 7'-CH₃, 77e₁, 77e₂) 1.09 (s, 6H, 6α-CH₃, 77e₁, 77e₂) 1.13 - 1.29 (m, 20H, 77e₁, 77e₂) 1.38 (s, 3H, 6β-CH₃) 1.7 (s, 6H, 9-CH₃, 77e₁, 77e₂) 1.76-1.91 (m, 6H, 77e₁, 77e₂) 2.25-2.35 (m, 2H, 7α-H, 77e₁, 77e₂) 2.69 (td, J = 4.5, 11.0 Hz, 2H, 10a-H, 77e₁, 77e₂) 3.20 (d, J = 18.0 Hz, 2H, 10α-H, 77e₁, 77e₂) 3.21-3.47 (m, 4H, 77e₁, 77e₂) 5.42 (d, J = 4.0 Hz, 2H, 77e₁, 77e₂) 6.24 (d and d overlapping, J = 1.5 Hz, 2H, ArH, 77e₁, 77e₂) 6.36 (d and d overlapping, J = 1.5 Hz, 2H, ArH, 77e₁, 77e₂).
(1\text{R},4\text{R},5\text{R})-4-(2,6-Dihydroxy-4-(2-methyloctan-2-yl)phenyl)-6,6-dimethylbicyclo[3.1.1]
heptan-2-one (78). The synthesis was carried out analogous to the preparation of 49 using
mixture of nopinone diacetates (730 mg, 3.04 mmol), \textit{p}-TSA ( 530 mg, 2.78 mmol) and
dimethylheptyl resorcinol (600 mg, 2.53 mmol) in CHCl\textsubscript{3} (15 mL) to give 78 (675 mg, 72% yield). \textsuperscript{1}H NMR (500 MHz, chloroform-\textit{d}) \(\delta\) ppm 0.84 (t, \(J = 7.0\) Hz, 3H, 7\text{′}-CH\textsubscript{3}) 0.99 (s, 3H, 6\text{α}-CH\textsubscript{3}) 1.00 - 1.10 (m, 2H) 1.16 - 1.27 (m,12H, especially s at 1.19, 6H, -CH(CH\textsubscript{3})\textsubscript{2}-) 1.36 (s, 3H, 6\text{β}-CH\textsubscript{3}) 1.46- 1.51 (m, 2H) 2.31 (t, \(J = 5.5, 1H, 5\)-H) 2.43- 2.56 (m, 2H, 7\text{α}-H, 7\text{β}-H) 2.57- 2.68 (m, 2H, 3\text{β}-H, 1-H) 3.52 (q, \(J = 7.5, 19.0\) Hz, 1H, 3\text{α}-H) 3.94 (t, \(J = 7.5\) Hz, 1H, 4-H) 5.12 (s, 2H, 2 x OH) 6.28 (s, 2H, ArH).

(6\text{aR},10\text{aR})-1-Hydroxy-6,6-dimethyl-3-(2-methyloctan-2-yl)-7,8,10,10a-tetrahydro-6\textit{H}-
benzo[c]chromen-9 (6\text{aH})-one (79). The synthesis was carried out as described for 50 using 78
(675.0 mg, 1.81 mmol), TMSOTf (1.9 mL, 0.3 M solution in CH\textsubscript{3}NO\textsubscript{2}, 0.54 mmol) in anhydrous
CH\textsubscript{2}Cl\textsubscript{2}: CH\textsubscript{3}NO\textsubscript{2}(3:1 mixture, 20 mL) to give 79 (490 mg, 73% yield). \textsuperscript{1}H NMR (500 MHz,
chloroform-\textit{d}) \(\delta\) ppm 0.83 (t, \(J = 6.5\) Hz, 3H, 7\text{′}-CH\textsubscript{3}) 1.01 - 1.08 (m, 2H) 1.13 (s, 3H, 6\text{α}-CH\textsubscript{3})
1.15- 1.26 (m, 12H, especially s at 1.20, 6H, -CH(CH\textsubscript{3})\textsubscript{2}-) 1.44- 1.59 (m, 6H, especially s at 1.48, 3H, 6\text{β}-CH\textsubscript{3}) 1.98 (td, \(J = 2.5, 12.0\) Hz, 1H, 6a-H) 2.11- 2.25 (m, 2H, 10ax-H, 7eq-H) 2.45 –
2.58 (m, 1H, 8ax-H) 2.60- 2.70 (m, 1H, 8eq-H) 2.90 (td, \(J = 3.0, 12.5\) Hz, 1H, 10a-H) 4.13-4.21
(m, 1H, 10eq-H) 6.35 (d, \(J = 1.5\) Hz, 1H, ArH) 6.38 (d, \(J = 1.0\) Hz, 1H, ArH) 7.64 (s, 1H, OH).

(6\text{aR},10\text{aR})-6,6-(Dimethyl-3-(2-methyloctan-2-yl)-9-oxo-6a,7,8,9,10,10a-hexahydro-6\textit{H}-
benzo[c]chromen-1-yl)acetate (80). The mixture of acetic anhydride (30 mg, 0.26 mmol), 79
(50 mg, 0.13 mmol) and pyridine (50 mg, 0.65 mmol) in dry CH\textsubscript{2}Cl\textsubscript{2}(5 mL) was stirred at room
temperature for 3 h. The mixture was then poured onto the crushed ice and extracted with ethyl
acetate (3 x 2 mL). The combined organic layer was washed with 1N HCl (5 x 5 mL), water and
brine, dried (MgSO₄) and concentrated under vacuo. The crude product was chromatographed on silica gel (9% acetone in hexane) to give 80 (50 mg, 90% yield). ¹H NMR (500 MHz, chloroform- dissolved) δ ppm: 0.84 (t, J = 7.0 Hz, 3H, 7′-CH₃) 1.0 - 1.09 (m, 2H) 1.12 (s, 3H, 6α-CH₃) 1.16- 1.28 (m, 12H, especially s at 1.20, 6H, -CH(CH₃)₂-) 1.44- 1.54 (m, 6H, especially s at 1.48, 3H, 6β-CH₃) 1.98 (td, J = 2.5 , 12.0 Hz, 1H, 6α-H) 2.11- 2.25 (m, 2H, 10αx-H, 7eq-H) 2.32 (s, 3H, OAc) 2.37-2.76 (m, 3H, 8ax-H, 8eq-H, 10α-H) 3.24-3.31 (m, 1H, 10eq-H) 6.52 (d, J = 1.5 Hz, 1H, ArH) 6.70 (d, J = 1.0 Hz, 1H, ArH).

(5αR,11bR)-6,6-(Dimethyl-9-(2-methyloctan-2-yl)-3-oxo-3,4,5,5a,6,11b-hexahydro-1H-oxepino[4,3-c]chromen-11-yl)acetate (81). To the stirred solution of 80 (30 mg, 0.07 mmol) in dry CH₂Cl₂ (5 mL) at 0°C was added dropwise a solution of mCPBA (65 mg, 0.36 mmol) in dry CH₂Cl₂ (2mL). The reaction mixture was warmed to room temperature after stirred at 0°C for 1 h and stirred for additional 12 h. The reaction mixture was quenched with saturated sodium bicarbonate solution and diluted with CH₂Cl₂. The organic layer was separated and the aqueous layer was extracted with CH₂Cl₂ (3 x 2 mL). The combined organic layer was washed with water and brine, dried (MgSO₄) and concentrated under vacuo. The residue was chromatographed on silica gel (10% acetone in hexane) to give 81 (30 mg, 95% yield). IR (CHCl₃): 2928, 1798, 1734, 1185 cm⁻¹.

(Note: The product is mixture of inseparable regio-isomers)

2-[(3R,4R)-5-Hydroxy-3-(2-hydroxyethyl)-2,2-dimethyl-7-(2-methyloctan-2-yl)chroman-4-yl]acetic acid (82).

3-[(3R,4R)-5-Hydroxy-4-(hydroxymethyl)-2,2-dimethyl-7-(2-methyloctan-2-yl)chroman-3-yl]propanoic acid (83).
The reaction was carried out as described for 32 using lithium hydroxide (23 mg, 0.55 mmole) and 81 (30 mg, 0.07 mmol) in 50:50 mixture of THF : water (5 mL) to give mixture of separable isomers 82 (15 mg, 50% yield) and 83 (9 mg, 35% yield).

NMR of intermediate 82:

Could not be isolated in very pure form, carried forward for cyclization without further purification.

NMR, HRMS and HPLC purity of intermediate 83:

\(^1\)H NMR (500 MHz, chloroform-\(d\)) \(\delta\) ppm 0.83 (t, \(J = 6.5\) Hz, 3H) 1.0 - 1.10 (m, 2H) 1.15- 1.28 (m, 16H) 1.33 (s, 3H) 1.47- 1.59 (m, 4H) 1.64- 2.0 (m, 6H) 2.4-2.58 (m, 2H) 2.80 (m, 1H) 3.82 – 3.93 (m, 2H) 6.35 (d, \(J = 2.0\) Hz, 1H) 6.47 (d, \(J = 1.0\) Hz, 1H); HRMS calcd for C\(_{24}\)H\(_{39}\)O\(_5\) 407.2797, found 407.2801; HPLC Purity: 95.8%.

\((5aR,11bR)-11\)-Hydroxy-6,6-dimethyl-9-(2-methyloctan-2-yl)-4,5,5a,6-tetrahydro-1H-oxepino[4,5-c]chromen-2(11bH)-one (84). Methanesulfonic acid (9.0 mg, 0.08 mmol) was added to the stirred solution of 82 (12 mg, 0.03 mmol) and dimethylaminopyridine (10 mg, 0.08 mmol) in toluene (5 mL). The resulting mixture was stirred at room temperature for 1 h and then diluted with ethyl acetate. The organic layer was separated and washed with 2M NaOH. The organic layer was separated and the aqueous layer was extracted (3 x 1 mL) with ethyl acetate. The combined organic layer was washed with water and brine, dried (MgSO\(_4\)) and concentrated under vacuo. The residue was chromatographed on silica gel (26% acetone in hexane) to give 84 (5 mg, 43% yield). \(^1\)H NMR (500 MHz, chloroform-\(d\)) \(\delta\) ppm 0.84 (t, \(J = 6.5\) Hz, 3H) 1.0 - 1.10 (m, 2H) 1.16- 1.28 (m, 17H) 1.47 (s, 3H) 1.49- 1.6 (m, 2H) 1.72- 1.79 (m, 1H) 2.29 (t, \(J = 15.0\) Hz, 1H) 2.81 (ddd, \(J = 4, 11.5\) Hz, 1H) 3.06 (dd, \(J = 4.0, 15.5\) Hz, 1H) 3.66- 3.79 (m, 2H) 6.58
(5aR,11bR)-11-Hydroxy-6,6-dimethyl-9-(2-methyloctan-2-yl)-4,5,5a,6-tetrahydro-1H-oxepino[4,3-c]chromen-3(11bH)-one (85). The synthesis was carried out as described for 84 using methanesulfonic acid (9.0 mg, 0.08 mmol), 83 (12 mg, 0.03 mmol) and DMAP (11 mg, 0.08 mmol in toluene (5 mL) to give 85 (9 mg, 78% yield). $^1$H NMR (500 MHz, Chloroform-$d$) $\delta$ ppm 0.86 (t, $J = 6.5$ Hz, 3H) 1.03 - 1.10 (m, 5H) 1.18- 1.30 (m, 14H) 1.42 (s, 3H) 1.48- 1.54 (m, 1H) 1.95 (dt, $J = 3.0$, 12.5 Hz, 1H) 2.10-2.16 (m, IH) 2.70- 2.90 (m, 3H) 4.40 (q, $J = 7.5$, 12.0 Hz, 1H) 4.94 (br,s, 1H) 5.31 (dd, $J = 1.5$, 12.0 Hz, 1H) 6.28 (d, $J = 2.0$ Hz, 1H) 6.41 9d, $J = 2.0$ Hz, 1H); HPLC Purity: 100%; IR (CHCl$_3$): 3320, 2929, 1713, 1418 cm$^{-1}$; IR (CHCl$_3$): 3380, 2927, 2856, 1711, 1622, 1418, 1043 cm$^{-1}$.

2-[(6aR,10aR)-6a,7,10,10a-Tetrahydro-1-hydroxy-6,6,9-trimethyl-6H-benzo[c]chromen-3-yl]-2-methyl-N-pentylpropanamide (86). Carboxylic acid intermediate 4 (200 mg, 0.60 mmol), was dissolved in CH$_2$Cl$_2$ (3 mL) under an argon atmosphere and cooled to 0°C. To the cooled solution, triethylamine (0.12 mL, 0.90 mmol) was added followed by the drop wise addition of bis(2-methoxyethyl) aminosulfur trifluoride (0.13 mL, 0.72 mmol). After stirred for 15 min, a solution of amylamine (80 mg, 0.90 mmol) in dry CH$_2$Cl$_2$ (6 mL) was added. The resulting mixture was stirred at 0°C for additional 15 min and then allowed to warm to room temperature. Stirring was continued for 2 h at room temperature and then diluted with CH$_2$Cl$_2$ and saturated aqueous sodium bicarbonate solution. The organic layer was separated and the aqueous layer was extracted with ethyl acetate (3 x 5 mL). The combined organic layer was washed with water and brine, dried (MgSO$_4$) and concentrated under vacuo. The residue was chromatographed on silica gel (12% ethyl acetate in hexane) to give 86 (170 mg, 75% yield) as light yellow gum. $^1$H NMR
(500 MHz, chloroform-\textit{d}) $\delta$ ppm 0.81 (t, $J = 7.0$ Hz, 3H, 7$'$-CH$_3$) 1.08-1.16 (m, 5H, especially 1.14, s, 6$\alpha$-CH$_3$) 1.20 (quintet, $J = 7.0$ Hz, 2H) 1.35 (quintet, $J = 7.5$ Hz, 2H) 1.40 (s, 3H, 6$\beta$-CH$_3$) 1.49 (s, 6H, -C(CH$_3$)$_2$-) 1.70 (s, 3H, 9-CH$_3$) 1.76-1.90 (m, 3H) 2.14 (br d, $J = 13.5$ Hz, 1H, 7$\alpha$-H) 2.74 (td, $J = 4.5$, 10.5Hz, 1H, 10a-H) 3.13 (q, $J = 7.0$, 13.0 Hz, 2H, 4$'$-CH$_2$) 3.40 (dd, $J = 13.5$, 1.5Hz, 1H, 8-H) 6.25 (d, $J = 2.0$ Hz, 1H, ArH) 6.41 (d, $J = 1.5$ Hz, 1H, ArH) 8.53 (s, 1H, NH); HRMS calcd for C$_{25}$H$_{38}$O$_3$N 400.2852, found 400.2858; IR (neat): 3274, 2930, 1642 cm$^{-1}$.

2-[(6$\alpha$R, 10aR)-6a,7,10,10a-Tetrahydro-1-hydroxy-6,6,9-trimethyl-6$\text{H}$-benzo[c]chromen-3-yl]-2-methylpropanoyl chloride (87). The synthesis was carried out as described for 29 using 4 (100 mg, 0.30 mmol) and 1.5 M stock solution thionyl chloride and benzotriazole in CH$_2$Cl$_2$ (20 mL) to give 87 (90 mg, 86% yield) as crude product which was used as such in the next reaction without further purification.

S-Propyl-2-[(6$\alpha$R,10aR)-6a,7,10,10a-tetrahydro-1-hydroxy-6,6,9-trimethyl-6$\text{H}$-benzo[c]chromen-3-yl]-2-methylpropanethioate (88). Pyridine (1mL) was added under an argon atmosphere to the stirred solution of 87 (70 mg, 0.20 mmol) and propanethiol (22 $\mu$L, 0.24 mmol) in dry CH$_2$Cl$_2$ (5 mL). The resulting mixture was stirred at room temperature for 3 h and diluted with water (2 mL) and ethyl acetate (5 mL). The organic layer was separated and the aqueous layer was extracted with ethyl acetate (3 x 3 mL). The combined organic layer was washed with water and brine, dried (MgSO$_4$) and concentrated under vacuo. The residue was chromatographed on silica gel (16% ethyl acetate in hexane) to give 88 (50 mg, 66% yield).$^1$H NMR (500 MHz, Chloroform-\textit{d}) $\delta$ ppm 0.93 (t, $J = 7.5$ Hz, 3H, 6$'$-CH$_2$) 1.10 (s, 3H, 6$\alpha$-CH$_3$) 1.39 (s, 3H, 6$\beta$-CH$_3$) 1.55 (s, 6H, -C(CH$_3$)$_2$-) 1.52- 1.60 (m, 2H) 1.70 (s, 3H, 9-CH$_3$) 1.76 - 1.91 (m, 3H, 10$\beta$-H, 7$\beta$-H, 6a-H) 2.11 - 2.18 (m, 1H, 7$\alpha$-H) 2.71 (td, $J = 10.5$, 4.5 Hz, 1H, 10a-H)
2.79 (td, $J = 7.5, 2.5$ Hz, 2H, 4′-CH$_2$) 3.16 - 3.26 (m, 1H, 10α-H) 5.03 (s, 1H, OH) 5.43 (d, $J = 4.5$ Hz, 1H, 8-H) 6.25 (d, $J = 2.0$ Hz, 1H, ArH) 6.46 (d, $J = 2.0$ Hz, 1H, ArH); HRMS calcd for C$_{23}$H$_{33}$O$_3$S 389.2150, found 389.2161; IR (CHCl$_3$): 3434, 2926, 1678, 1417 cm$^{-1}$.

**2-(4-Bromo-3,5-dimethoxyphenyl)acetonitrile (91).** To a stirred suspension of sodium cyanide (8.9 g, 180.77 mmol) in DMSO (50 mL) at room temperature was added a solution of 90 (4.8 g,
18.07 mmol) in DMSO (40 mL) over a period of 10 min. The reaction mixture was stirred vigorously overnight and then diluted by ice, saturated aqueous NaCl solution and ethyl acetate. The organic layer was separated and the aqueous phase was extracted with ethyl acetate (3 x 20 mL). The combined organic layer was washed with water and brine, dried (MgSO₄) and concentrated under vacuo. The residue was chromatographed on silica gel (20% ethyl acetate in hexane) to give 91 (3.4 g, 74% yield). ¹H NMR (500 MHz, chloroform-d) δ ppm 3.73 (s, 2H, benzylic CH₂) 3.91 (s, 6H, 2 x OCH₃) 6.52 (s, 2H, ArH); IR (neat): 2946, 2244, 1587, 1122 cm⁻¹.

**2-(4-Bromo-3,5-dimethoxyphenyl)-2-methylpropanenitrile (92).** The synthesis was carried out analogous to the preparation of 1 using sodium hydride (1.5 g, 37.5 mmol), 91 (3.2 g, 12.5 mmol) and iodomethane (2.34 mL, 37.5 mmol) in dry DMF (40 mL) to give 92 (2.6 g, 74% yield). ¹H NMR (500 MHz, chloroform-d) δ ppm 1.74 (s, 6H, -C(CH₃)₂-) 3.93 (s, 6H, 2 x OCH₃) 6.66 (s, 2H, ArH); IR (neat): 2985, 2233, 1584, 1120 cm⁻¹.

**2-(4-Bromo-3,5-dimethoxyphenyl)-2-methylpropanoic acid (93).** Sodium hydroxide (10 mL, 35% solution) was added to the stirred solution of 92 (1.0 g, 3.5 mmol) in ethanol: methanol mixture (80:20, 10 mL). The resulting solution was refluxed for 10 h. The reaction mixture was acidified with 2N HCl and diluted with diethyl ether. The organic layer was separated and the aqueous layer was extracted with diethyl ether (3 x 20 mL). The combined organic layers were collected, washed with water and brine, dried (MgSO₄), concentrated under vacuo. The crude was chromatographed on silica gel (50% ethyl acetate in hexane) to give 93 (830 mg, 78% yield); ¹H NMR (500 MHz, chloroform-d) δ ppm 1.61 (s, 6H, -C(CH₃)₂-) 3.89 (s, 6H, 2 x OCH₃) 6.60 (s, 2H, ArH); IR (CHCl₃): 2981, 1699, 1584, 1120 cm⁻¹.
Ethyl 2-(4-bromo-3,5-dimethoxyphenyl)-2-methylpropanoate (94). The mixture of ethyl iodide (1.7 mL, 21.04 mmol), 93 (800 mg, 2.63 mmol) and sodium bicarbonate (670 mg, 7.91 mmol) in DMF (2.0 mL) was irradiated in microwave 165°C for 12 min. The reaction mixture was brought to room temperature and diluted with water and ethyl acetate. The organic layer was separated and the aqueous layer was extracted with ethyl acetate (3 x 10 mL). The combined organic layer was washed with water and brine, dried (MgSO₄) and concentrated under vacuo. The crude product was chromatographed on silicagel (8% ethyl acetate in hexane) to give 94 (1.52 g, 83% yield). ¹H NMR (500 MHz, chloroform-d) δ ppm 1.2 (t, J = 7.0 Hz, 3H) 1.57 (s, 6H, -C(CH₃)₂-) 3.89 (s, 6H, 2 x OCH₃) 4.14 (q, J = 6.0, 12.5 Hz, 2H) 6.55 (s, 2H, ArH); HRMS calcd for C₁₄H₂₀O₄Br 331.0545, found 331.0543; IR (neat): 2971, 1722, 1581, 832 cm⁻¹.

Ethyl 2-(3′-cyano-2,6-dimethoxybiphenyl-4-yl)-2-methylpropanoate (95). 3-cyanophenyl boronic acid (270 mg, 1.81 mmol) was added to the stirred solution of 94 (500 mg, 1.5 mmol) in 4.0 mL of DME and the resulting mixture was degassed for 10 min. Water (0.5 mL) was added to the resulting mixture and the reaction mixture was further degassed for 10 min. To the resulting mixture was added barium hydroxide (709 mg, 2.25 mmol), tetrakis(triphenylphosphine)palladium (350 mg, 0.3 mmol) and the reaction mixture was again degassed for additional 10 min. The degassed reaction mixture was irradiated in microwave for 12 min at 160 °C. The reaction mixture was cooled to room temperature and diluted with water and diethyl ether. The organic layer was separated and the aqueous layer was extracted with diethyl ether (3 x 10 mL). The combined organic layer was washed with water and brine, dried (MgSO₄) and concentrated under vacuo. The crude product was chromatographed on silica gel (10% ethyl acetate in hexane) to give 95 (250 mg, 47% yield). ¹H NMR (500 MHz, chloroform-d) δ ppm 1.25 (t, J = 7.0 Hz, 3H) 1.62 (s, 6H, -C(CH₃)₂-) 3.74 (s, 6H, 2 x OCH₃) 4.18 (q, J = 117
7.0 Hz, 2H) 6.62 (s, 2H, ArH) 7.46 (t, \(J = 8.0\) Hz, 1H) 7.57 (tt, \(J = 1.5, 7.5\) Hz, 2H) 7.65 (t, \(J = 1.5\) Hz, 1H); HRMS calcd for C\(_{21}\)H\(_{24}\)NO\(_4\) 354.1705, found 354.1717; IR (CHCl\(_3\)): 2979, 2225, 1726, 1124 cm\(^{-1}\).

**Ethyl 2-(3’-cyano-2,6-dihydroxybiphenyl-4-yl)-2-methylpropanoate (96).** The synthesis was carried out as described for 3 using 95 (35 mg, 0.098 mmol), boron tribromide (0.6 mL, 0.58 mmol, 1M solution in CH\(_2\)Cl\(_2\)) in dry CH\(_2\)Cl\(_2\) (4 mL) to give 96 (8 mg, 31% yield). \(^1\)H NMR (500 MHz, chloroform-\(d\)) \(\delta\) ppm 1.23 (t, \(J = 7.0\) Hz, 3H) 1.78 (s, 6H, \(-C(CH_3)\_2\)-) 4.15 (q, \(J = 7.0, 14.5\) Hz, 2H) 6.11 (s, 2H, 2 x OH) 6.54 (s, 2H, ArH) 7.56 (t, \(J = 7.5\) Hz, 1H) 7.65 (m, 1H) 7.72 (m, 1H) 7.77 (s, 1H); HRMS calcd for C\(_{19}\)H\(_{20}\)NO\(_4\) 326.1392, found 326.1388; IR (CHCl\(_3\)): 3395, 2983, 2230, 1736 cm\(^{-1}\).

**Ethyl 2-(2,6-dimethoxy-3’',5’'-dimethylbiphenyl-4-yl)-2-methylpropanoate (97).** The synthesis was carried out as described for 95 using (3,5-dimethylphenyl)boronic acid (240 mg, 1.5 mmol), 94 (260 mg, 0.78 mmol), water (0.5 mL), barium hydroxide (400 mg, 1.24 mmol) and tetrakis(triphenylphosphine)palladium (180 mg, 0.15 mmol) in DME (3 mL) to give 97 (200 mg, 71% yield). \(^1\)H NMR (500 MHz, chloroform-\(d\)) \(\delta\) ppm 1.23 (t, \(J = 7.0\) Hz, 3H) 1.61 (s, 6H, \(-C(CH_3)\_2\)-) 2.32 (s, 6H, 2 x Ar-CH\(_3\)) 3.71 (s, 6H, 2 x OCH\(_3\)) 4.17 (q, \(J = 7.0, 14.5\) Hz, 2H) 6.60 (s, 2H, ArH) 6.93 (s, 3H, ArH); HRMS calcd for C\(_{22}\)H\(_{29}\)O\(_4\) 357.2066, found 357.2065; IR (CHCl\(_3\)): 2974, 1725, 1120 cm\(^{-1}\).

**Ethyl 2-(2,6-dihydroxy-3’',5’'-dimethylbiphenyl-4-yl)-2-methylpropanoate (98).** The synthesis was carried out as described for 96 using 97 (160 mg, 0.45 mmol) and boron tribromide (1.8 mL, 1.79 mmol) in CH\(_2\)Cl\(_2\) (10 mL) to give the crude mixture of 98 and 99 which was separated
using column chromatography (15% acetone in hexane) to give pure product 98 (75 mg, 51% yield) and 99 (42 mg, 28% yield).

**Compound 98**

$^1$H NMR (500 MHz, chloroform-$d$) δ ppm (98) 1.23 (t, $J = 7.0$ Hz, 3H) 1.55 (s, 6H, -C(CH$_3$)$_2$-)
2.36 (s, 6H, 2 x Ar-CH$_3$) 4.16 (q, $J = 7.0$, 14.5 Hz, 2H) 4.98 (s, 2H, 2 x OH) 6.56 (s, 2H, ArH)
7.0 (s, 2H, ArH) 7.09 (s, 1H, ArH); HRMS calcd for C$_{20}$H$_{25}$O$_4$ 329.1753, found 329.1745; IR (CHCl$_3$): 3385, 2975, 1714, 1145 cm$^{-1}$;

**Compound 99**

$^1$H NMR (500 MHz, chloroform-$d$) δ ppm (99) 1.23 (t, $J = 7.5$ Hz, 3H) 1.58 (s, 6H, -C(CH$_3$)$_2$-)
2.35 (s, 6H, 2 x Ar-CH$_3$) 3.72 (s, 3H, Ar-OCH$_3$) 4.17 (q, $J = 7.5$, 14.5 Hz, 2H) 5.08 (s, 1H, OH)
6.49 (d, $J = 1.5$ Hz, 1H, ArH) 6.65 (d, $J = 1.5$ Hz, 1H, ArH) 6.96 (s, 2H, ArH) 7.02 (s, 1H, ArH); HRMS calcd for C$_{21}$H$_{27}$O$_4$ 343.1909, found 343.1898: IR (CHCl$_3$): 3436, 2923, 1725, 1145 cm$^{-1}$.

**Ethyl 2-(3′,5′-dichloro-2,6-dimethoxybiphenyl-4-yl)-2-methylpropanoate (100).** The synthesis was carried out as described for 95 using (3,5-dichlorophenyl)boronic acid (250 mg, 1.29 mmol), 94 (215 mg, 0.64 mmol), water (0.5 mL), barium hydroxide (325 mg, 1.02 mmol) and tetrakis(triphenylphosphine)palladium (150 mg, 0.12 mmol) in DME (3 mL) to give 100 (140 mg, 54% yield). $^1$H NMR (500 MHz, chloroform-$d$) δ ppm 1.23 (t, $J = 7.0$ Hz, 3H) 1.61 (s, 6H, -C(CH$_3$)$_2$-)
3.73 (s, 6H, 2 x OCH$_3$) 4.16 (q, $J = 6.5$, 14.0 Hz, 2H) 6.59 (s, 2H, ArH) 7.22 (d,
$J = 1.5$ Hz, 2H, ArH) 7.27 (t, $J = 1.5$ Hz, 1H, ArH); HRMS calcd for $C_{20}H_{23}O_4\ Cl_2$ 397.0973, found 397.0961; IR (CHCl$_3$): 2976, 1725, 1121 cm$^{-1}$.

**Ethyl 2-(3',5'-dichloro-2,6-dihydroxybiphenyl-4-yl)-2-methylpropanoate (101).** The synthesis was carried out as described for 96 using 100 (140 mg, 0.35 mmol) and boron tribromide (1.0 mL, 1.05 mmol) in CH$_2$Cl$_2$ (10 mL) to give the crude mixture of 101 and 102 which was separated using column chromatography (16% acetone in hexane) to give pure product 101 (38 mg, 29% yield) and 102 (43 mg, 31% yield).

**Compound 101:**

$^1$H NMR (500 MHz, chloroform-$d$) δ ppm (101) 1.22 (t, $J = 6.5$ Hz, 3H) 1.53 (s, 6H, -C(CH$_3$)$_2$-) 4.13 (q, $J = 6.0$, 14.0 Hz , 2H) 6.48 (s, 2H, ArH) 7.32- 7.38 (m, 3H, ArH) 7.36 (m, 1H); HRMS calcd for $C_{19}H_{21}O_4\ Cl_2$ 383.0817, found 383.0809; IR (CHCl$_3$): 3361, 2977, 1695, 1037 cm$^{-1}$.

**Compound 102:**

$^1$H NMR (500 MHz, chloroform-$d$) δ ppm (102) 1.23 (t, $J = 7.5$ Hz, 3H) 1.57 (s, 6H, -C(CH$_3$)$_2$-) 3.73 (s, 3H, Ar-OCH$_3$) 4.17 (q, $J = 7.0$, 14.0 Hz, 2H) 6.50 (s, 1H, ArH) 6.61 (d, $J = 1.0$ Hz, 1H, ArH) 7.26 (d, $J = 2.0$ Hz, 2H, ArH) 7.36 (s, 1H, ArH); HRMS calcd for $C_{18}H_{19}O_4\ Cl_2$ 369.0660, found 369.0659; IR (CHCl$_3$): 3407, 2928, 1726, 1701, 1101 cm$^{-1}$.

**1-(3,5-Dimethoxyphenyl)cyclobutanecarbonitrile (103).** To the stirred solution of (3,5-dimethoxyphenyl)acetonitrile (5.0 g, 28.21 mmol) in dry THF (90 mL) at -16 °C under an argon atmosphere was added KHMDS (17 g, 84.64 mmol). The reaction mixture was stirred at the same temperature for 5 min and then a solution of dibromopropane (3.2 mL, 31.03 mmol) in dry THF (3 mL) was added dropwise. Following the addition, the reaction was stirred for 2 h at -16
0°C and quenched by the addition of saturated aqueous NH₄Cl and diluted with ethyl acetate. The organic layer was separated, and the aqueous phase was extracted with ethyl acetate (3 x 20 mL). The combined organic layer was washed with water and brine, dried (MgSO₄) and concentrated under vacuo. The crude product was chromatographed on silica gel (12% acetone in hexane) to give 103 (1.8 g, 30% yield). ¹H NMR (500 MHz, chloroform-d) δ ppm 2.0- 2.09 (m, 1H) 2.32 – 2.44 (m, 1H) 2.55- 2.64 (m, 2H) 2.72 -2.79 (m, 2H) 3.78 (s, 6H) 6.38 (t, J = 2.0 Hz, 1H) 6.53 (d, J = 2.0 Hz, 2H).

1-(3,5-Dimethoxyphenyl)cyclobutanecarboxylic acid (104). The synthesis was carried out as described for 2 using sodium hydroxide (350 mg, 8.72 mmol), 103 (760 mg, 3.49 mmol) in aqueous n-butanol to give 104 (730 mg, 88% yield). ¹H NMR (500 MHz, chloroform-d) δ ppm 1.80- 1.95 (m, 1H) 2.05- 2.15 (m, 1H) 2.54 (q, J = 9.0, 20.5 Hz, 2H) 2.80 -2.90 (m, 2H) 3.82 (s, 6H) 6.40 (t, J = 2.5 Hz, 1H) 6.5 (d, J = 1.5 Hz, 2H) 12.16 (s, 1H); HRMS calcd for C₁₃H₁₇O₄ 237.1127, found 237.1121.

1-(3,5-Dihydroxyphenyl)cyclobutanecarboxylic acid (105). The synthesis was carried out as described the 3 using boron tribromide (12 mL, 11.85 mmol, 1.0 M solution in CH₂Cl₂) and 104 (700 mg, 2.96 mmol) in dry CH₂Cl₂ to give 105 (590 mg, 95%). ¹H NMR (500 MHz, methanol-d) δ ppm 1.8- 1.9 (m, 1H) 1.92-2.06 (m, 1H) 2.39- 2.49 (m, 2H) 2.70 -2.77 (m, 2H) 6.15 (t, J = 2.5 Hz, 1H) 6.28 (d, J = 2.0 Hz, 2H); HRMS calcd for C₁₁H₁₃O₄ 209.0814, found 209.0806.

1-[(6aR,10aR)-1-Hydroxy-6,6,9-trimethyl-6a,7,10,10a-tetrahydro-6H-benzo[c]chromen-3-yl]cyclobutanecarboxylic acid (106). The synthesis was carried out analogous to the preparation of 4 using p-TSA (120 mg, 0.62 mmol), 105 (650 mg, 3.12 mmol) and (+)-cis/trans-p-mentha-2,8-dien-1-ol (520 mg, 3.43 mmol) in CHCl₃ (15 mL) to give 106 (500 mg,
47% yield). $^1$H NMR (500 MHz, chloroform-$d$) $\delta$ ppm 1.11 (s, 3H) 1.39 (s, 3H) 1.7 (s, 3H) 1.76-1.88 (m, 4H) 1.92-2.25 (m, 1H) 2.16 (dd, $J = 4.5, 12.5$ Hz, 1H) 2.36-2.49 (m, 2H) 2.68-2.78 (m, 3H) 3.21 (dd, $J = 4.5, 16.0$ Hz, 1H) 5.43 (d, $J = 4.5$ Hz, 1H) 6.23 (d, $J = 2.0$ Hz, 1H) 6.42 (d, $J = 1.5$ Hz, 1H); HRMS calcd for C$_{21}$H$_{27}$O$_4$, 343.1909, found 343.1897; HPLC Purity 100%

4-Bromobutyl 1-[(6a$R$,10a$R$)-1-hydroxy-6,6,9-trimethyl-6a,7,10,10a-tetrahydro-6H-benzo[c]chromen-3-yl]cyclobutanecarboxylate (107). The synthesis was carried out as described for 7 using dibromobutane (140 mg, 0.63 mmol), 106 (145.0 mg, 0.42 mmol) and sodium bicarbonate (40 mg, 0.46 mmol) in DMF (1.5 mL) to give 107 (45 mg, 23% yield). $^1$H NMR (500 MHz, chloroform-$d$) $\delta$ ppm 1.10 (s, 3H) 1.38 (s, 3H) 1.69 (s, 3H) 1.71-1.97 (m, 9H) 2.14 (dd, $J = 4.5, 11.5$ Hz, 1H) 2.45 (p, $J = 10.0, 19.5$ Hz, 2H) 2.66-2.77 (m, 3H) 324 (dd, $J = 4.0, 15.5$ Hz, 1H) 3.31 (t, $J = 6.5$ Hz, 2H) 4.10 (dt, $J = 1.5, 6.0$ Hz, 2H) 5.42 (d, $J = 4.5$ Hz, 1H) 5.85 (s, 1H) 6.24 (d, $J = 2.0$ Hz, 1H) 6.39 (d, $J = 2.0$ Hz, 1H); HRMS calcd for C$_{25}$H$_{34}$O$_4$Br 477.1640, found 477.1636; HPLC purity 97.3%

4-Cyanobutyl 1-[(6a$R$,10a$R$)-1-hydroxy-6,6,9-trimethyl-6a,7,10,10a-tetrahydro-6H-benzo[c]chromen-3-yl]cyclobutanecarboxylate (108). The synthesis was carried out as described for 13 using 107 (180 mg, 0.36 mmol) and sodium cyanide (150 mg, 2.93 mmol) in dry DMSO (10 mL) to give 108 (100 mg, 62% yield). $^1$H NMR (500 MHz, chloroform-$d$) $\delta$ ppm 1.09 (s, 3H) 1.38 (s, 3H) 1.54 -1.63 (m, 2H) 1.69 (s, 3H) 1.70- 1.99 (m, 8H) 2.11- 2.18 (m, 1H) 2.26 (t, $J = 7.0$ Hz, 2H) 2.41- 2.50 (m, 2H) 2.67-2.79 (m, 3H) 3.26 (dd, $J = 5.0, 16.5$ Hz, 1H) 4.11 (t, $J = 6.0$ Hz, 2H) 5.42 (d, $J = 4.0$ Hz, 1H) 5.94 (s, 1H) 6.26 (d, $J = 2.0$ Hz, 1H) 6.37 (d, $J = 1.5$ Hz, 1H); HRMS calcd for C$_{26}$H$_{34}$O$_4$N 424.2488, found 424.2491; IR (CHCl$_3$): 3400, 2970, 2250, 1723, 1703 cm$^{-1}$; HPLC Purity 98.3%
4-Azidobutyl 1-[(6aR,10aR)-1-hydroxy-6,6,9-trimethyl-6a,7,10,10a-tetrahydro-6H-benzo[c]chromen-3-yl]cyclobutane carboxylate (109). To the stirred solution of 108 (65.0 mg, 0.13 mmol) in dry CH₂Cl₂ (10 mL) under an argon atmosphere was added tetra-n-butylammonium azide (390 mg, 1.36 mmol). The resulting mixture was stirred at 40°C for 48 h and diluted with water and ethyl acetate. The organic layer was separated and the aqueous layer was extracted with CH₂Cl₂ (3 x 3 mL). The combined organic layer was washed with water and brine, dried (MgSO₄) and concentrated under vacuo. The crude product was chromatographed on silica gel (18% acetone in hexane) to give 109 (50 mg, 81% yield). ¹H NMR (500 MHz, chloroform-d) δ ppm 1.10 (s, 3H) 1.38 (s, 3H) 1.49 -1.56 (m, 2H) 1.63-1.69 (m, 3H) 1.7 (s, 3H) 1.77- 1.98 (m, 4H) 2.12- 2.19 (m,1H) 2.45 (t, J = 9.0 Hz, 2H) 2.65-2.78 (m, 3H) 3.21 (t, J = 7.0 Hz, 3H) 4.09 (td, J = 1.5, 6.5 Hz, 2H) 5.35 (br,s, 1H) 5.43 (d, J = 4.0 Hz, 1H ) 6.21 (d, J = 1.5 Hz, 1H) 6.39 (d, J = 1.5 Hz, 1H); HRMS calcd for C₂₅H₃₄O₄N₃ 440.2549, found 440.2536; IR (CHCl₃): 3417, 2098, 1721, 1702 cm⁻¹.

Butyl 1-[(6aR,10aR)-1-hydroxy-6,6,9-trimethyl-6a,7,10,10a-tetrahydro-6H-benzo[c]chromen-3-yl]cyclobutane carboxylate (110). The synthesis was carried out as described for 6 using bromobutane (89 mg, 0.65 mmol), 106 (90 mg, 0.26 mmol) and sodium bicarbonate (40 mg, 0.47 mmol) in DMF (1.5 mL) to give 110 (70 mg, 67% yield). ¹H NMR (500 MHz, chloroform-d) δ ppm 0.85 (t, J = 7.5 Hz, 3H) 1.09 (s, 3H) 1.26 (m, 2H) 1.38 (s, 3H) 1.54 (m, 2H) 1.69 (s, 3H) 1.75- 1.99 (m,5H) 2.15 (dd, J = 4.5, 11.5 Hz, 1H) 2.45 (p, J = 9.5, 19.0 Hz, 2H) 2.65-2.79 (m, 3H) 3.21 (dd, J = 4.0, 16.5 Hz, 1H) 4.03-4.10 (m, 2H) 5.42 (d, J = 4.5 Hz, 1H ) 6.01 (s,1H) 6.26 (d, J = 1.5 Hz, 1H) 6.39 (d, J = 2.0 Hz, 1H); HRMS calcd for C₂₅H₃₅O₄ 399.2535, found 399.2536; HPLC purity 100%.
3-Bromopropyl 2-[(6aR,10aR)-1-hydroxy-6,6-dimethyl-9-oxo-6a,7,8,9,10,10a-hexahydro-6H-benzo[c]chromen-3-yl]-2-methylpropanoate (111). Potassium carbonate (100 mg, 0.72 mmol) was added to the stirred solution of 63 (200 mg, 0.60 mmol) and dibromopropane (145 mg, 0.72 mmol) in dry DMF (5 mL) under argon at room temperature. The resulting mixture was stirred at same temperature for 12 h and then quenched with 1 N HCl and diluted with ethyl acetate. The organic layer was separated and the aqueous layer was extracted with ethyl acetate (3 x 10 mL). The combined organic layer was washed with water and brine, dried (MgSO₄) and concentrated under vacuo to get crude product which was then chromatographed on silica gel (26% acetone in hexane) to give 111 (130 mg, 48% yield) as light yellow gum. ¹H NMR (500 MHz, chloroform-d) δ ppm 1.14 (s, 3H) 1.49 (s, 3H) 1.52-1.58 (m, 1H) 1.53 (s, 6H) 1.99 (dt, J = 2.5, 12.0 Hz, 1H) 2.0-2.22 (m, 4H) 2.45-2.55 (m, 1H) 2.61-2.70 (m, 1H) 2.92 (dt, J = 3.5, 7.5 Hz, 1H) 3.27 (dt, J = 2.0, 6.5 Hz, 2H) 4.09-4.15 (m, 1H) 4.16-4.24 (m, 2H) 6.39 (s, 2H) 7.80 (br, s, 1H); HRMS calcd for C₂₂H₃₀O₅Br 453.1277, found 453.1280; IR (CHCl₃): 3294, 2974, 1726, 1695, 1417, 1138 cm⁻¹.

3-Cyanopropyl 2-[(6aR,10aR)-1-hydroxy-6,6-dimethyl-9-oxo-6a,7,8,9,10,10a-hexahydro-6H-benzo[c]chromen-3-yl]-2-methylpropanoate (112). The synthesis was carried out as described for 13 using sodium cyanide (140 mg, 2.91 mmol), 111 (130 mg, 0.29 mmol) in dry DMSO (5 mL) to give 112 (80 mg, 67% yield) as light yellow gum. ¹H NMR (500 MHz, chloroform-d) δ ppm 1.12 (s, 3H) 1.48 (s, 3H) 1.52 (d, J = 2.5 Hz, 6H) 1.82-2.02 (m, 4H) 2.10-2.20 (m, 2H) 2.23 (t, J = 6.5 Hz, 2H) 2.45-2.54 (m, 1H) 2.60 (m, 1H) 2.89 (dt, J = 3.5, 13.0 Hz, 1H), 4.03-4.09 (m, 1H) 4.16 (t, J = 5.5 Hz, 2H) 6.37 (d, J = 1.5 Hz, 1H) 6.39 (d, J = 2.0 Hz, 1H) 7.65 (br, s, 1H); HRMS calcd for C₂₃H₃₀O₅N 400.2124, found 400.2130; IR (CHCl₃): 3431, 3236, 2970, 1718, 1696, 1417, 1260 cm⁻¹.
3-Cyanopropyl 2-[(6aR,9R,10aR)-1,9-dihydroxy-6,6-dimethyl-6a,7,8,9,10,10a-hexahydro-6H-benzo[c]chromen-3-yl]-2-methylpropanoate (113). The synthesis was carried out as described for 65 using sodium borohydride (30 mg, 0.75 mmol), 112 (60 mg, 0.15 mmol) in methanol (5 mL) to give 113 (35 mg, 58% yield) as light yellow gum. IR (CHCl₃): 3428, 2934, 1729, 1417, 1140 cm⁻¹; ¹H NMR (500 MHz, chloroform-d) δ ppm 1.06 (s, 3H) 1.38 (s, 3H) 1.50 (d, J = 3.0 Hz, 6H) 1.74 (br, s, 2H) 1.85-1.96 (m, 3H) 2.14-2.16 (m, 1H) 2.19 (t, J = 6.5 Hz, 2H) 2.28 (br, s, 1H) 2.48 (dt, J = 6.5, 11.0 Hz, 1H) 3.54-3.61 (m, 1H) 3.84-3.91 (m, 1H) 4.15 (t, J = 6.0 Hz, 2H) 6.22 (d, J = 1.0 Hz, 1H) 6.35 (d, J = 1.0 Hz, 1H) 6.88 (br, s, 1H); HRMS calcd for C₂₃H₃₂O₅N 402.2280, found 402.2289; HPLC purity: 98.4%.

3-(1H-Imidazol-1-yl)propyl 2-[(6aR,10aR)-1-hydroxy-6,6-dimethyl-9-oxo-6a,7,8,9,10,10a-hexahydro-6H-benzo[c]chromen-3-yl]-2-methylpropanoate (114). The synthesis was carried out as described for 17 using potassium carbonate (1.2 g, 8.25 mmol), 111 (250 mg, 0.60 mmol) and imidazole (300 mg, 4.41 mmol) in dry DMF (6 mL) to give 114 (205 mg, 84% yield) as light yellow gum. IR (CHCl₃): 3099, 2975, 1721, 1702, 1415, 1141 cm⁻¹; ¹H NMR (500 MHz, chloroform-d) δ ppm 1.08 (s, 3H) 1.46 (s, 3H) 1.49 (d, J = 6.0 Hz, 6H) 1.50-1.54 (m, 1H) 1.90-2.0 (m, 3H) 2.02 -2.16 (m, 2H) 2.35-2.48 (m, 1H) 2.54 -2.6 (m, 1H) 2.90 (dt, J = 3.5, 7.5 Hz, 1H) 3.76 (m, 2H) 3.85 -4.0 (m, 2H) 4.0- 4.1 (m, 1H) 6.40 (d, J = 2.0 Hz, 1H) 6.41 (d, J = 2.0 Hz, 1H) 6.68 (s, 1H) 6.98 (s, 1H) 7.10 (s, 1H) 7.30 (s, 1H); HRMS calcd for C₂₅H₃₃O₅N₂ 441.2389, found 441.2383; HPLC Purity: 99.6%.

Butyl 2-(3,5-dihydroxy-4-[(1R,2R,4S,5R)-4-hydroxy-6,6-dimethylbicyclo[3.1.1]heptan-2-yl]phenyl]-2-methylpropanoate (115). The synthesis was carried out as described for 65 using sodium borohydride (25 mg, 0.61 mmol) and 49 (60 mg, 0.15 mmol) in methanol (5 mL) to give 115 (54 mg, 91% yield) as light yellow gum. ¹H NMR (500 MHz, chloroform-d) δ ppm 0.85 (t, J
= 7.5 Hz, 3H) 1.22-1.29 (m, 8H) 1.48 (s, 6H) 1.50-1.57 (m, 2H) 1.71 (d, J = 9.5 Hz, 1H) 1.98-2.21 (m, 6H) 2.70-2.79 (m, 1H) 3.96 (t, J = 8.5 Hz, 1H) 4.07 (t, J = 7.0 Hz, 2H) 4.46-4.5 (m, 1H) 6.11 (s, 2H) 6.31 (s, 2H); HRMS calcd for C_{23}H_{34}O_{5} 390.2406, found 390.2409; IR (CHCl\textsubscript{3}): 3393, 2930, 1701, 1617, 1420, 1009 cm\textsuperscript{-1}; HPLC Purity: 100%.
Chapter 5: Pharmacological Evaluation of Novel Cannabinergic Analogs with Controlled Detoxification

The novel synthesized cannabinergic analogs were screened pharmacologically (by CDD Biochemistry group and CDD Collaborators as mentioned below) using following assays:

1: *In vitro* screening

- Cannabinoid receptor binding assays (CDD Biochemistry group)
- Plasma stability (Dr JodiAnne Wood)
- $\beta$-arrestin assay (Dr Larry Barak, Duke university, NC)
- Cyclic adenosine monophosphate (cAMP) assay (CEREP)

2: *In vivo* assays (Dr Carol Paronis)

- Hypothermia
- Antinociception test (Tail flick test)

*In vitro* Screening

Novel analogs were initially characterized biochemically by determining their *in vitro* affinities for the two primary cannabinoid receptors followed by evaluation of their *in vitro* metabolic stability towards plasma esterases (mouse). *In vitro* affinities were determined using competitive radioanalog binding assays, with forebrain synaptosomal membrane from rat brain (CB1) and HEK293 cell membranes (for CB2 receptors). Displacement of the tritiated CP-55,940
(standard) form these membranes by novel analogs were used to determine the binding affinity (IC$_{50}$ values).

Plasma stability was determined by incubating the test analog and its proposed metabolite in mouse plasma. Compounds and their proposed metabolites were diluted (100 µM) in mouse plasma or acetonitrile and incubated at 37°C. At various time points, samples were taken, diluted 1:2 in acetonitrile and centrifuged to precipitate plasma proteins. The resulting supernatant was analyzed by HPLC. Chromatographic separations were achieved by using a Supelco Discovery C18 (4.6 x 250 mm) column on a Waters Alliance HPLC system. Mobile phase consisted of acetonitrile (A) and a mixture of 60% water (acidified with 8.5% o-phosphoric acid) and 40% acetonitrile (B). Gradient elution was started with 5% A, transitioned to 95% A and held for five minutes before returning to starting conditions; the flow rate was 1 mL/min. UV detection was used at each compound’s maximal absorbance.

The in vitro and in vivo potency of compounds depends on several factors. A nanomolar binding affinity of a compound indicate high affinity of the compound towards the target receptors, but does not itself give functional information i.e., whether it activates the downstream signaling pathways and with what degree of selectivity.

Cannabinoids binding to CB receptors can influence various signaling pathways including adenyl cyclase, arrestin, mitogen-activated protein kinases (MAPK), certain voltage gated calcium channels and G-protein-linked inward rectifying potassium channels. The key ligands ($Ki < 30$ nM) were initially screened for two well established functional assays i.e., β-arrestin (DUKE University) and cAMP (CEREP). Since this effort represented screening of a large number of compounds, it was most practical to obtain initially two- point data for cAMP from
CEREP Laboratories as a percentage of the control agonist response for agonist mode and a percentage inhibition of the control agonist response for antagonist mode.

**cAMP Assay**

Compounds were tested for agonist and antagonist activity at both the hCB1 and hCB2 receptors stably expressed in Chinese hamster ovary cells (CHO) cells.

**Agonist activity:**

The 7.5x10^3 cells/well, suspended in HBSS buffer complemented with 20 mM HEPES (pH 7.4), were distributed in microplates in the presence of either the reference agonist (hCB1: CP 55940, hCB2: WIN 55212-2) at 100 nM or various concentrations of the test compound. Thereafter, the adenylyl cyclase activator NKH 477 was added at a final concentration of 3 μM. Following 10 min incubation at 37°C, the cells were lysed, and the fluorescence acceptor (D2-labeled cAMP) and fluorescence donor (anti-cAMP antibody labeled with europium cryptate) were added. After 60 min at room temperature, the fluorescence transfer was measured at λ_{ex} = 337 nm and λ_{em} = 620 and 665 nm using a microplate reader (Rubystar, BMG). The cellular cAMP concentration was determined by dividing the signal measured at 665 nm by that measured at 620 nm (ratio). The results were expressed as a percent of the control response to 100 nM of the reference agonist.

**Antagonist mode:**

The 7.5x10^3 cells/well, suspended in HBSS buffer complemented with 20 mM HEPES (pH 7.4), were distributed in microplates and preincubated for 5 min at room temperature in the presence of either of the reference antagonist (hCB1: AM 281; hCB2: AM 630) or various concentrations of test compound. Thereafter, the reference agonist (hCB1: CP55940, hCB2: WIN 55212-2) and
the adenylyl cyclase activator NKH 477 were added. Following 10 min incubation at 37°C, the cells were lysed and processed for fluorescent cAMP assay, as above. The results were expressed as a percent inhibition of the control response of the reference agonists.

Analogs with CB receptor binding affinities less than 30 nM, predictable plasma half-lives, and favorable functional activity were considered “successful analogs” to be studied further in vivo to identify the lead compound with most favorable and predictable agonist efficacy and detoxification.

**β-Arrestin**

U2OS cells were stably transfected with green fluorescent protein-tagged beta-arrestin (β-arrestin GFP). Human CB1 (CB1-U2OS) or CB2 (CB2-U2OS) receptors were used to trace β-arrestin intracellular translocation upon potential agonist/antagonist treatment. For agonist assay, cells were treated with $10^{-4} \sim 10^{-10}$ M test compound for 45 min at 37°C. For antagonist assay, cells were treated with $10^{-4} \sim 10^{-10}$ M test compound for 20 min, and then 1µM or 0.1 µM concentration of WIN55212 was added to cells expressing CB1 or CB2, respectively. Cells were incubated for another 45 min at 37°C. The cells were fixed with paraformaldehyde (PFA), and confocal fluorescent images were taken for data analysis. Results were summarized from two experiments.

**In vivo Screening**

The successful analogs resulting from in vitro screening were profiled in vivo using a well-accepted rodent assay for cannabinergic (i.e., central CB1 receptor) activity, hypothermia. The hypothermia test determines the ability of a test compound to act as a central CB1 agonist and
decrease body temperature. A dose range from 0.01 mg/kg to 3 mg/kg was examined subcutaneously (s.c.), initially based upon the in vitro CB-receptor agonist potency, so as to be able to characterize compound potency in vivo and onset/offset of action. The hypothermia test involves monitoring of rat core temperature with a thermistor probe for up to 6 h. Drugs were initially dissolved in a solution of 20% ethanol, 20% Alkamuls, and 60% saline and further diluted with saline. Injections were administered subcutaneous (s.c.) in a volume of 1 mL/kg. Two temperature values recorded prior to the drug injection were averaged to obtain a single baseline temperature. Temperature recorded after drug injection was expressed as change from baseline. Group means and SEM were calculated, and time- or dose-effect functions were analyzed using standard ANOVA or paired t-test procedures. Wherever appropriate, ANOVA was followed by Bonferroni’s post-hoc test or by Dunnet’s multiple comparison t-test. In all cases statistical significance was set at p < 0.05.

Analogs demonstrating the desired in vivo hypothermic activity were tested in another well-accepted rodent assay for antinociception (Tail flick test). This test measures spinal nociception as sensitivity of the animal to increasing temperature and indicate the ability of a test compound to activate cannabinergic signaling in vivo and thereby reduce nociceptive pain at the pharmacologically relevant doses. In the test the distal third of the rat’s tail was exposed to a heat source, and the time the animal takes to move its tail away from the heat source was measured. The response was expressed as a percentage of the maximum possible effect (% MPE). Dose-effect functions were constructed using the maximum effect recorded in each rat at a given dose of drug.

The pharmacological evaluations of novel cannabinergic analogs with controlled detoxification are summarized below.
5.1 Cannabinergic Analogs with an Ester group at C2’ Position of DMH-Δ^8-THC

The cannabinoid receptor affinities (Ki) of the novel DMH-Δ^8-THC analogs with ester group at C2’ position and functional activity are shown in Table 5.1 and Table 5.2 respectively. The *in vivo* potency (rat hypothermia) of these analogs is shown in Figures 5.1, 5.2 and 5.3.

**Observation and Results**

The synthesized novel cannabinergic analogs with ester group at C2’ position showed low nanomolar binding affinities (Ki < 15 nM) towards CB receptors, except for compounds 8 (AM 7420), 10 (AM 7431) and 14 (AM 4801), which bind to CB receptors with reduced affinities [Table 5.1]. Compound 4 (AM 7408), the metabolite formed after enzymatic hydrolysis of novel ester analogs, showed no affinity towards CB receptors.

The key representative ester analogs with desired CB receptor affinities were further profiled for *in vitro* plasma (mouse) stability to obtain the half life (t1/2) and evaluating their functional activity (cAMP and β-arrestin assays). The data indicated that compounds 5 (AM 7407), 8 (AM 7420), 10 (AM 7431), 13 (AM 7409), 14 (AM 4801), 17 (AM 7428) and 18 (AM 7488) were hydrolyzed (t1/2) by mouse plasma esterases within 15 min, 40 min, 60 min, 68 min, 120 min, 200 min and 240 min, respectively. The cAMP data indicated that compound 13 (AM 7409) at 10 nM concentration produced 96% and 78% of control agonist response at CB1 and CB2 receptors respectively, whereas at 1 μM concentration it produced 99% and 94% of control agonist response at CB1 and CB2 receptors respectively [Refer Table 5.2]. However, in β-arrestin assay, compound 13 (AM 7409) induced arrestin recruitment at CB2 receptors with an EC_{50} of 20 nM, whereas at CB1 receptors it inhibited the agonist induced arrestin recruitment.
analogs, such as compound 17 (AM7428) and compound 11 (AM 7433), showed functional activity similar to compound 13 (AM 7409) in both cAMP and β-arrestin assays. However,

Table 5.1: Affinities ($K_i$), cLogP, tPSA and $t_{1/2}$ of DMH-$\Delta^8$-THC analogs with ester functionality at C2' position

<table>
<thead>
<tr>
<th>Cpd #</th>
<th>AM #</th>
<th>rCB1 (nM)</th>
<th>mCB2 (nM)</th>
<th>hCB2 (nM)</th>
<th>cLogP</th>
<th>TPSA</th>
<th>$t_{1/2}$ (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>AM 7408</td>
<td>&gt;10,000</td>
<td>&gt;10,000</td>
<td>&gt;10,000</td>
<td>4.1</td>
<td>66.7</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>AM 7407</td>
<td>1.20</td>
<td>0.86</td>
<td>0.80</td>
<td>5.8</td>
<td>55.7</td>
<td>15</td>
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<tr>
<td>6</td>
<td>AM 7410</td>
<td>0.66</td>
<td>0.87</td>
<td>0.60</td>
<td>6.5</td>
<td>55.7</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>AM 7411</td>
<td>1.20</td>
<td>1.1</td>
<td>0.58</td>
<td>6.9</td>
<td>55.7</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>AM 7420</td>
<td>29.13</td>
<td>8.20</td>
<td>7.74</td>
<td>5.3</td>
<td>71.7</td>
<td>120</td>
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<tr>
<td>9</td>
<td>AM 7427</td>
<td>0.80</td>
<td>1.14</td>
<td>0.66</td>
<td>6.0</td>
<td>55.7</td>
<td></td>
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<tr>
<td>10</td>
<td>AM 7431</td>
<td>83.14</td>
<td>36.86</td>
<td>34.52</td>
<td>5.0</td>
<td>55.7</td>
<td>60</td>
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<tr>
<td>11</td>
<td>AM 7433</td>
<td>2.75</td>
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<td>1.50</td>
<td>5.5</td>
<td>55.7</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>AM 7434</td>
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<td>3.20</td>
<td>2.90</td>
<td>5.7</td>
<td>55.7</td>
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<tr>
<td>13</td>
<td>AM 7409</td>
<td>0.75</td>
<td>0.97</td>
<td>0.86</td>
<td>5.1</td>
<td>79.5</td>
<td>40</td>
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<tr>
<td>14</td>
<td>AM 4801</td>
<td>33.85</td>
<td>71.8</td>
<td>39.3</td>
<td>5.5</td>
<td>68.2</td>
<td>68</td>
</tr>
<tr>
<td>15</td>
<td>AM 7438</td>
<td>0.50</td>
<td>0.76</td>
<td>1.41</td>
<td>4.9</td>
<td>79.5</td>
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</tr>
<tr>
<td>16</td>
<td>AM 7439</td>
<td>1.02</td>
<td>0.76</td>
<td>0.88</td>
<td>6.4</td>
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<tr>
<td>17</td>
<td>AM 7428</td>
<td>3.42</td>
<td>2.07</td>
<td>1.28</td>
<td>5.2</td>
<td>71.3</td>
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<td>18</td>
<td>AM 7488</td>
<td>15.29</td>
<td>10.46</td>
<td>8.71</td>
<td>5.5</td>
<td>68.2</td>
<td>200</td>
</tr>
</tbody>
</table>
Table 5.2: Functional data (cAMP and β-arrestin) of DMH-Δ8-THC analogs with ester group at C2’ position (* % of control agonist response, ** % inhibition of the control agonist response, X no response)

<table>
<thead>
<tr>
<th>AM #</th>
<th>cAMP (Agonist*)</th>
<th>β-arrestin (EC₅₀)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CAMP (nM)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CB1</td>
<td>CB2</td>
</tr>
<tr>
<td>7409</td>
<td>10 nM</td>
<td>96</td>
</tr>
<tr>
<td></td>
<td>1 µM</td>
<td>99</td>
</tr>
<tr>
<td>7428</td>
<td>10 nM</td>
<td>84</td>
</tr>
<tr>
<td></td>
<td>1 µM</td>
<td>97</td>
</tr>
<tr>
<td>7433</td>
<td>10 nM</td>
<td>61</td>
</tr>
<tr>
<td></td>
<td>1 µM</td>
<td>94</td>
</tr>
<tr>
<td>7488</td>
<td>10 nM</td>
<td>66</td>
</tr>
<tr>
<td></td>
<td>1 µM</td>
<td>92</td>
</tr>
<tr>
<td>4801</td>
<td>10 nM</td>
<td>-32</td>
</tr>
<tr>
<td></td>
<td>1 µM</td>
<td>90</td>
</tr>
</tbody>
</table>

compound 18 (AM 7488) at 1 µM concentration in cAMP assays produced 92 % of the control agonist response at CB1 receptors, whereas, at CB2 receptors it acted as an antagonist (118 %
inhibition of the control agonist response). In the \( \beta \)-arrestin assay, compound 18 (AM 7488) inhibited the arrestin recruitment at both the CB1 and CB2 receptors with an EC\(_{50}\) of 810 nM and 6.6 nM respectively [Table 5.2]. The functional profile of compound 14 (AM 4801) was found to be similar to 18 (AM 7488). The successful ligands (\( Ki < 30 \text{nM} \), predictable \textit{in vitro} half-life and functionally potent ligand) were profiled for \textit{in vivo} evaluation.

![Figure 5.1](image1.png)

**Figure 5.1:** \textit{In vivo} evaluation (rat hypothermia) of AM 7408, AM 7488, AM 7428, AM 7420

![Figure 5.2](image2.png)

**Figure 5.2:** \textit{In vivo} evaluation (hypothermia) of AM 7407, AM 7409, AM 7433

The \textit{In vivo} data (rat hypothermia) indicated that the metabolite of this series of analogs (ester group at C2' position of DMH-\( \Delta^8 \)-THC) i.e., compound 4 (AM 7408) produced no hypothermia in rats upto a dose of 1.0 mg/kg [Refer Figure 5.1]. Interestingly, parent compounds such as 8
(AM 7420), 17 (AM 7428) and 18 (AM 7488) also produced no hypothermic response up to a
dose of 3.0 mg/kg as shown in Figure 5.1.

Compounds 5 (AM 7407) and 13 (AM 7409) produced a 4-5°C drop in body temperature at a
dose of 0.3 mg/kg, whereas compound 11 (AM 7433) produced similar hypothermic response at
a dose of 3.0 mg/kg as shown in Figure 5.2. Although, compounds such as 5 (AM 7407) and 13
(AM 7409) produced a potent hypothermic response in vivo, but they were not inactivated within
6 h of the study duration as shown in Figure 5.3.

Figure 5.3: In vivo evaluation (hypothermia) of AM 4054, AM 7407, AM 7433, AM 7409

5.2 Pharmacological Evaluation of Analogs Designed to Facilitate Enzymatic Hydrolysis

5.2.1 Reverse Ester DMH-Δ⁸-THC Analog

The CB receptor binding affinity of the reverse ester analog 23 (AM 7416) is shown in Table
5.3. The in vivo data (rat hypothermia) of the reverse ester analog 23 (AM 7416) is shown in
Figure 5.4.
**Table 5.3**: Affinities \((K_i)\) towards CB receptors, \(c\text{LogP}\) and \(tPSA\) of reverse ester analog

<table>
<thead>
<tr>
<th>Cpd #</th>
<th>AM #</th>
<th>rCB1 (nM)</th>
<th>mCB2 (nM)</th>
<th>hCB2 (nM)</th>
<th>cLogP</th>
<th>tPSA</th>
<th>(t_{1/2}) (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>22</td>
<td>AM 7417</td>
<td>447</td>
<td>&gt;1,000</td>
<td>&gt;1,000</td>
<td>4.6</td>
<td>49.6</td>
<td>-</td>
</tr>
<tr>
<td>23</td>
<td>AM 7416</td>
<td>0.5</td>
<td>1.2</td>
<td>1.0</td>
<td>7.1</td>
<td>55.7</td>
<td>10</td>
</tr>
</tbody>
</table>

**Observation and Results**

![Graph showing body temperature changes over time](image)

**Figure 5.4**: *In vivo* evaluation (Hypothermia) of reverse ester analog

The CB receptor binding affinity data [Table 5.3] indicated that compound 23 (AM 7416) bind to both the CB1 and CB2 receptors with low nanomolar affinity, whereas its metabolite, 22 (AM 7417) showed no affinity toward either of CB receptors. Compound 23 (AM 7416) has a high \(c\text{LogP}\) of 7.1 and \(tPSA\) of 55.7 and underwent enzymatic inactivation *in vitro* by mouse plasma esterases with a half-life \((t_{1/2})\) of 10 min. *In vivo* data indicated that compound 23 (AM 7416) produced a decrease in core body temperature in rats in a dose dependent manner. A dose of 1.0 mg/kg produced 2-3°C drop in body temperature, whereas a dose of 3.0 mg/kg produced a 4°C
drop in body temperature [Figure 5.4]. However, compound 23 (AM 7416) was not inactivated in vivo within 6 h of study duration.

### 5.2.2 Pharmacological Evaluation of C1′-Methyl Substituted Ester Analog

The binding affinity towards CB receptors and functional activity data for C1′-methyl substituted ester analogs are shown in Table 5.4 and Table 5.5, respectively. The in vivo (rat hypothermia) data of C1′-methyl substituted ester analogs are shown are Figure 5.5.

#### Observation and Results

**Table 5.4:** Affinities ($K_i$), cLogP, rPSA and $t_{1/2}$ of C1′-methyl substituted ester analog

<table>
<thead>
<tr>
<th>Cpd #</th>
<th>AM #</th>
<th>rCB1 (nM)</th>
<th>mCB2 (nM)</th>
<th>hCB2 (nM)</th>
<th>$K_i$ (nM)</th>
<th>cLogP</th>
<th>rPSA</th>
<th>$t_{1/2}$ (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>27</td>
<td>AM 7458</td>
<td>&gt;10,000</td>
<td>&gt;10,000</td>
<td>&gt;10,000</td>
<td>3.4</td>
<td>66.7</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>AM 7459</td>
<td>0.7</td>
<td>1.2</td>
<td>1.0</td>
<td>4.9</td>
<td>55.7</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>34</td>
<td>AM 7461</td>
<td>&gt;10,000</td>
<td>&gt;10,000</td>
<td>&gt;10,000</td>
<td>3.4</td>
<td>66.7</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>35</td>
<td>AM 7462</td>
<td>0.9</td>
<td>1.4</td>
<td>1.4</td>
<td>4.9</td>
<td>55.7</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>AM 7464</td>
<td>&gt;10,000</td>
<td>&gt;10,000</td>
<td>&gt;10,000</td>
<td>3.4</td>
<td>66.7</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>41</td>
<td>AM 7463</td>
<td>2.4</td>
<td>1.3</td>
<td>1.5</td>
<td>4.9</td>
<td>55.7</td>
<td>6</td>
<td></td>
</tr>
</tbody>
</table>

The C1′-methyl substituted ester analogs bind to both the CB receptors with low nanomolar ($K_i < 2.5$ nM) affinity. However, the respective metabolites showed no affinity towards either of CB
receptors. *In vitro* plasma stability data indicated that both the diastereomers i.e., compound \(35\) (AM 7462) and \(41\) (AM 7463) were readily hydrolyzed by mouse esterases with a half life of 7 min and 6 min respectively.

The cAMP data indicated that compound \(35\) (AM 7462) showed 92% and 65% of the control agonist response at CB1 and CB2 receptors at 1 µM concentration respectively. Also, compound \(35\) (AM 7462) inhibited the arrestin recruitment at CB1 receptors with an EC\(_{50}\) of 31nM [Refer Table 5.5]. The other diastereomer i.e., compound \(41\) (AM 7463) showed 99% and 90% of control agonist response at CB1 and CB2 receptors, respectively, at 1 µM test concentration. In β-arrestin assays, compound \(41\) (AM 7463) induced arrestin recruitment at CB2 receptors with an EC\(_{50}\) of 41nM, whereas at CB1 receptors it inhibited the agonist induced arrestin recruitment with an EC\(_{50}\) of 33 nM as shown in Table 5.5.

Table 5.5: Functional data (cAMP and β-arrestin) of C1'-methyl substituted ester analog (* % of control agonist response, ** % inhibition of the control agonist response, X no response)
In vivo data showed that compound 41 (AM 7463) significantly decreased the core body temperature in rats with a 3-4°C drop in body temperature at a dose of 1.0 mg/kg or 3.0 mg/kg. At a dose of 0.3 mg/kg compound 41 (AM 7463) produced small but significant decrease in body temperature and rats recovered from hypothermia within 6 h of study duration. Conversely, compound 35 (AM7462) produced no hypothermic response in rats up to a dose of 3 mg/kg [Refer Figure 5.5].

![Graph showing temperature changes over time for different doses of AM 7463 and AM 7462.]

**Figure 5.5:** *In vivo* evaluation of C1’-methyl substituted ester analogs

### 5.2.3 Pharmacological Evaluation of Analogs Lacking Geminal Dimethyl group at C1’ Position

The CB receptor affinity of novel analogs without geminal dimethyl group at CB receptors and functional activity data are shown in Table 5.6 and Table 5.7, respectively. The *in vivo* activity (rat hypothermia) of ester analog without germinal dimethyl group is shown in Figure 5.6.
Table 5.6: Affinities towards CB receptors ($K_i$), cLogP and $t_{PSA}$ of ester analogs lacking geminal dimethyl group

<table>
<thead>
<tr>
<th>Cpd #</th>
<th>AM #</th>
<th>Binding affinity ($K_i$)</th>
<th></th>
<th></th>
<th>cLogP</th>
<th>TPSA</th>
<th>$t_{1/2}$ (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>44</td>
<td>AM 7425</td>
<td>&gt;10,000</td>
<td>&gt;10,000</td>
<td>&gt;10,000</td>
<td>3.8</td>
<td>66.7</td>
<td>-</td>
</tr>
<tr>
<td>45</td>
<td>AM 7424</td>
<td>2.2</td>
<td>11.6</td>
<td>7.1</td>
<td>5.7</td>
<td>55.7</td>
<td>3</td>
</tr>
<tr>
<td>46</td>
<td>AM 7425</td>
<td>14.3</td>
<td>2.1</td>
<td>1.2</td>
<td>4.2</td>
<td>79.5</td>
<td>4.8</td>
</tr>
<tr>
<td>47</td>
<td>AM 7423</td>
<td>40.3</td>
<td>27.0</td>
<td>8.2</td>
<td>5.8</td>
<td>55.7</td>
<td>5</td>
</tr>
</tbody>
</table>

Table 5.7: Functional data (cAMP) of ester analog lacking geminal dimethyl group (* % of control agonist response, ** % inhibition of the control agonist response)

<table>
<thead>
<tr>
<th>AM #</th>
<th>cAMP</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Agonist*</td>
<td>Antagonist**</td>
<td>CB1</td>
<td>CB2</td>
<td>CB1</td>
<td>CB2</td>
<td></td>
</tr>
<tr>
<td>7425</td>
<td>10 nM</td>
<td>53</td>
<td>38</td>
<td>3</td>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 µM</td>
<td>70</td>
<td>47</td>
<td>6</td>
<td>34</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Observation and Results

CB receptor binding affinity data indicated that compounds 45 (AM 7424) and 46 (AM 7425) exhibited high affinity ($K_i < 15$ nM) towards CB receptors whereas compound 47 (AM7423) showed reduced affinity towards CB receptors, especially at the CB1 receptor [Table 5.6].
Compound 44 (AM 7426), the metabolite formed by enzymatic hydrolysis by esterases, showed no affinity towards CB receptors. *In vitro* metabolic stability data indicated that parent compounds 45 (AM 7424), 46 (AM 7425) and 47 (AM7423) were hydrolyzed in less than 5 min ($t_{1/2}$) by mouse plasma esterases. The cAMP assay results showed that compound 46 (AM 7425), at 1µM concentration, exhibited 70% and 47% of control agonist response at CB1 and CB2 receptors, respectively [Table 5.7]. *In vivo* data indicated that compound 46 (AM 7425) produced small and delayed hypothermic response at a dose of 3.0 mg/kg, with the significant effect appearing after 2.5 h, as shown in Figure 5.6.

![Figure 5.6: In vivo evaluation (Hypothermia) of ester analog lacking geminal dimethyl group](image)

5.3 Pharmacological Evaluation of Novel Cannabinergic Analogs with Increased Polarity

5.3.1 Pharmacological Results of Hexahydro Analog with Hydroxymethyl ($\beta$-conformation) group at C9 Position of DMH-$\Delta^8$-THC

The *in vitro* binding affinity and functional data of hexahydro ester analog are shown in Table 5.8 and Table 5.9, respectively, and the *in vivo* response in the hypothermia test is shown in Figure 5.7.
Observation and Results

Compound 54 (AM 7499) exhibited low nanomolar binding affinity (Ki < 7.0 nM) at both CB1 and CB2 receptors [Table 5.8]. The cAMP data indicated that compound 54 (AM 7499) showed 100% of control agonist response at 10 nM concentration at either of the CB receptors [Table 5.9]. Compound 54 (AM 7499) also induced arrestin recruitment at both CB receptors, with an EC50 of 20 pM at CB1 receptor and 72 pM at CB2 [Table 5.9].

Table 5.8: Affinities (Ki) toward CB receptors, cLogP and tPSA of hexahedral analog

<table>
<thead>
<tr>
<th>Cpd #</th>
<th>AM #</th>
<th>Binding affinity (Ki)</th>
<th>cLogP</th>
<th>TPSA</th>
<th>t1/2 (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>rCB1 (nM)</td>
<td>mCB2 (nM)</td>
<td>hCB2 (nM)</td>
<td></td>
</tr>
<tr>
<td>54</td>
<td>AM 7499</td>
<td>2.4</td>
<td>4.3</td>
<td>6.5</td>
<td>4.9</td>
</tr>
</tbody>
</table>

Table 5.9: Functional data (cAMP and β-arrestin) of hexahydro ester (* % of control agonist response, ** % inhibition of the control agonist response, X no response)

<table>
<thead>
<tr>
<th>AM #</th>
<th>cAMP</th>
<th>β-arrestin (EC50)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Agonist*</td>
<td>Antagonist**</td>
</tr>
<tr>
<td></td>
<td>CB1</td>
<td>CB2</td>
</tr>
<tr>
<td>7499</td>
<td>10 nM</td>
<td>97</td>
</tr>
<tr>
<td></td>
<td>1 μM</td>
<td>97</td>
</tr>
</tbody>
</table>
The *in vitro* metabolic stability of this compound remains to be determined. *In vivo* data indicated that compound 54 (AM 7499) produced a decrease in core body temperature in a dose dependent manner as shown in Figure 5.7. A dose of 0.1 mg/kg produced 3-4°C drop in temperature whereas a dose of 0.3 mg/kg produced a 5-6 degree drop in body temperature in rats. It was found that rats recovered from hypothermia within 6 h of study duration at a dose of 0.1 mg/kg. Compound 54 (AM 7499) was found to be more potent than AM 4054 (potent CB1 agonist developed by CDD) at both the doses of 0.1 mg/kg and 0.3 mg/kg [Refer Figure 5.7].

![Figure 5.7: In vivo evaluation (Hypothermia) of Hexahydro ester analog](image)

**5.3.2 Pharmacological Evaluation of 11-Hydroxy Ester Analogs**

The binding affinity towards CB receptors and functional activity of 11-hydroxy ester analog are shown in Table 5.10 and Table 5.11, respectively. The *in vivo* data of 11-hydroxy ester analog in rat hypothermia and tail flick test are shown in Figure 5.8.
Table 5.10: Affinities ($K_i$), $c$LogP, $t$PSA and $t_{1/2}$ of 11-hydroxy ester analog

<table>
<thead>
<tr>
<th>Cpd #</th>
<th>AM #</th>
<th>rCB1 (nM)</th>
<th>mCB2 (nM)</th>
<th>hCB2 (nM)</th>
<th>$c$LogP</th>
<th>TPSA</th>
<th>$t_{1/2}$ (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>56</td>
<td>AM 7419</td>
<td>&gt;10,000</td>
<td>&gt;10,000</td>
<td>&gt;10,000</td>
<td>3.0</td>
<td>86.9</td>
<td>-</td>
</tr>
<tr>
<td>57</td>
<td>AM 7418</td>
<td>0.60</td>
<td>1.5</td>
<td>0.80</td>
<td>4.5</td>
<td>75.9</td>
<td>5</td>
</tr>
<tr>
<td>58</td>
<td>AM 7421</td>
<td>25.3</td>
<td>11.6</td>
<td>8.7</td>
<td>3.2</td>
<td>91.5</td>
<td>120</td>
</tr>
</tbody>
</table>

Table 5.11: Functional data (cAMP and $\beta$-arrestin) of 11-hydroxy analog (* % of control agonist response, ** % inhibition of the control agonist response, X: no response)

<table>
<thead>
<tr>
<th>AM #</th>
<th>cAMP</th>
<th>$\beta$-arrestin (EC$_{50}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Agonist*</td>
<td>Antagonist**</td>
</tr>
<tr>
<td></td>
<td>CB1</td>
<td>CB2</td>
</tr>
<tr>
<td>7418</td>
<td>10 nM</td>
<td>98</td>
</tr>
<tr>
<td></td>
<td>1 µM</td>
<td>99</td>
</tr>
</tbody>
</table>

Observations and Results

Compound 57 (AM 7418) bind to both the CB receptors with high binding affinity ($K_i < 1$ nM), as shown in Table 5.10. Compound 58 (AM 7421) exhibited reduced affinity, especially at CB1 receptors, whereas, the metabolite i.e., compound 56 (AM 7419) showed no affinity towards CB receptors. In vitro plasma stability data suggested that compound 57 (AM 7418) was hydrolyzed
**Hypothermia**

![Graph showing temperature changes over time for different treatments](image)

**Analgesia (tail flick)**

![Graph showing dose response](image)

*Figure 5.8: In vivo evaluation (Hypothermia and Analgesia) of 11-hydroxy ester analog*

In mouse plasma with a half-life ($t_{1/2}$) of 5 min, whereas compound 58 (AM 7421) was stable in mouse plasma upto 2 h. The cAMP assay data indicated that compound 57 (AM 7418) at a 10 nM concentration, showed 98% and 99% of control agonist response at CB1 and CB2 receptors, respectively. Compound 57 (AM 7418) also induced arrestin recruitment at both the CB1 and CB2 receptors with equally high EC$_{50}$ of 0.1 nM at both the receptors [Table 5.11].
*In vivo* data indicated that compound 57 (AM 7418) produced a potent 5°C drop in body temperature at a dose of 0.3 mg/kg in rats, whereas, at a dose 0.1 mg/kg compound 57 (AM 7418) decreased the core body temperature by 2-3 degrees and rats recovered from hypothermia within 5 h as shown in Figure 5.8. Compound 57 (AM 7418) was equipotent (peak effects) to AM 4054 at both the doses of 0.1 mg/kg and 0.3 mg/kg. Similar potent and predictable effects were seen in tail flick test. Compound 57 (AM 7418) showed MPE at a dose of 1.0 mg/kg and no *in vivo* inactivation was seen at this dose. However, a dose of 0.1 mg/kg it produced a significant analgesic effect and to have the similar analgesic effect morphine was administered at a dose of 10 mg/kg (100-fold higher dose).

### 5.3.3 Pharmacological Evaluation of 9-Hydroxy Ester Analogs

The *in vitro* binding affinity and functional activity of 9-hydroxy ester analogs is shown in Table 5.12 and Table 5.13, respectively. The *in vivo* activity of 9-hydroxy ester analog is shown in Figure 5.9.

#### Observation and Results

*In vitro* binding affinity data showed that compounds 65 (AM 7446) and 68 (AM 7456) bind to both CB receptors with high affinity ($K_i < 7 \text{ nM}$), whereas their respective metabolite i.e., compounds 66 (AM 7454) and 67 (AM 7455) exhibited no affinity towards cannabinoid receptors [Table 5.12]. *In vitro* plasma stability data showed that compounds 65 (AM 7446) and 68 (AM 7456) were metabolized by mouse plasma esterases with a $t_{1/2}$ of 3 min and 8 min respectively. The cAMP data indicated that compound 65 (AM 7446) exhibited 90% and 107% of control agonist response at a 10 nM concentration at CB1 and CB2 receptors, respectively.
In the arrestin assay, compound 68 (AM 7446) induced arrestin recruitment with an EC\textsubscript{50} of 46 pM and 41 pM at CB1 and CB2 receptors, respectively [Table 5.13].

Table 5.12: Affinities (K\textit{i}) towards CB receptors, cLogP and TPSA of 9-hydroxy ester analogs

<table>
<thead>
<tr>
<th>Cpd #</th>
<th>AM #</th>
<th>CB1 (nM)</th>
<th>CB2 (nM)</th>
<th>CB2 (nM)</th>
<th>cLogP</th>
<th>TPSA</th>
<th>t\textsubscript{1/2} (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>65</td>
<td>AM 7446</td>
<td>0.1</td>
<td>0.2</td>
<td>0.2</td>
<td>4.5</td>
<td>75.9</td>
<td>3</td>
</tr>
<tr>
<td>66</td>
<td>AM 7454</td>
<td>&gt;10,000</td>
<td>&gt;10,000</td>
<td>&gt;10,000</td>
<td>3.0</td>
<td>86.9</td>
<td>-</td>
</tr>
<tr>
<td>67</td>
<td>AM 7455</td>
<td>&gt;10,000</td>
<td>&gt;10,000</td>
<td>&gt;10,000</td>
<td>3.0</td>
<td>86.9</td>
<td>-</td>
</tr>
<tr>
<td>68</td>
<td>AM 7456</td>
<td>3.1</td>
<td>6.6</td>
<td>4.5</td>
<td>4.5</td>
<td>75.9</td>
<td>8</td>
</tr>
</tbody>
</table>

Table 5.13: Functional data (cAMP and \(\beta\)-arrestin) of 9-hydroxy analog (\(\beta\)-epimer) (* % of control agonist response, ** % inhibition of the control agonist response, X no response)

<table>
<thead>
<tr>
<th>AM #</th>
<th>cAMP</th>
<th>(\beta)-arrestin (EC\textsubscript{50})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Agonist*</td>
</tr>
<tr>
<td>7446</td>
<td>10 nM</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>1 µM</td>
<td>85</td>
</tr>
</tbody>
</table>
In vivo (rat hypothermia) data indicated that compound 65 (AM 7446) decreased the core body temperature in a dose dependent manner with a 5°C drop in body temperature at a dose of 3.0 mg/kg, as shown in Figure 5.9. At a dose of 1.0 mg/kg it produced significant decrease in body temperature and rats recovered from hypothermia within 6 h of study duration. However, at a dose of 3 mg/kg, compound 65 (AM 7446) produced a large hypothermic effect but, no in vivo inactivation was seen within 6 h of study duration in rats at this dose [Refer Figure 5.9].

![Image of Figure 5.9: In vivo evaluation (Hypothermia) of 9-hydroxy ester analog](image)

5.4 Pharmacological Evaluation of Analogs with β-Lactone at C1’ Position and Lactone in the C-ring

The in vitro binding affinities towards CB receptors of β-lactone at C1’ position and lactone in the C-ring of DMH-∆⁸-THC are shown in Table 5.14.

Observation and Results

The CB receptor binding affinity data [Table 5.14] showed that compound 77 (AM 7487, β-lactone at C1’ position) bind to CB1 receptors with good affinity ($K_i = 15.9 \text{ nM}$) whereas its affinity towards CB2 receptors was reduced significantly ($K_i$: mCB2 = 40.7 nM and hCB = 55.5 nM).
Compound 76 (AM 7486), the metabolite so formed after enzymatic hydrolysis of compound 77 (AM 7487) by mouse plasma esterase exhibited no affinity towards CB receptors. On the other hand, CB receptor affinity data suggested that compound 84 (AM 4807, C-ring lactone) bind preferentially to CB1 receptors with binding affinity ($K_i$) of 99 nM, whereas its affinity towards CB2 receptors is much less ($K_i = 802.7$ nM). However, its metabolite, i.e., compound 88 (AM 4808) showed no affinity towards CB receptors. Interestingly, the other regioisomer i.e., compound 85 (AM 4809), bind to CB1 receptors with high affinity ($K_i = 4.6$ nM) as shown in Table 5.14. The metabolite of compound 85 (AM 4809) i.e., compound 83 (AM 4806), exhibited no any affinity towards CB receptors.

**Table 5.14:** Affinities towards CB receptors, cLogP and tPSA of $\beta$-lactone at C1’ position and C-Ring lactone

<table>
<thead>
<tr>
<th>Cpd #</th>
<th>AM #</th>
<th>rCB1 (nM)</th>
<th>mCB2 (nM)</th>
<th>hCB2 (nM)</th>
<th>cLogP</th>
<th>TPSA</th>
<th>t1/2 (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>76</td>
<td>AM 7486</td>
<td>600</td>
<td>&gt;1000</td>
<td>&gt;1000</td>
<td>4.7</td>
<td>86.7</td>
<td>-</td>
</tr>
<tr>
<td>77</td>
<td>AM 7487</td>
<td>15.9</td>
<td>40.7</td>
<td>55.5</td>
<td>5.2</td>
<td>55.7</td>
<td>-</td>
</tr>
<tr>
<td>82</td>
<td>AM 4808</td>
<td>1000</td>
<td>1000</td>
<td>1000</td>
<td>5.9</td>
<td>86.9</td>
<td>-</td>
</tr>
<tr>
<td>84</td>
<td>AM 4807</td>
<td>99</td>
<td>802.7</td>
<td>-</td>
<td>5.8</td>
<td>55.7</td>
<td>-</td>
</tr>
<tr>
<td>83</td>
<td>AM 4806</td>
<td>1000</td>
<td>1000</td>
<td>1000</td>
<td>5.9</td>
<td>86.9</td>
<td>-</td>
</tr>
<tr>
<td>85</td>
<td>AM 4809</td>
<td>4.6</td>
<td>792</td>
<td>51.8</td>
<td>5.8</td>
<td>55.7</td>
<td>14.6</td>
</tr>
</tbody>
</table>

*In vitro* metabolic stability data showed that compound 85 (AM 4809, C-ring lactone) was hydrolyzed in 14.6 min ($t_{1/2}$) by mouse plasma esterases. The *in vivo* potency determination and
other pharmacological evaluation of analogs with lactone at C1’ position and C-ring lactone are underway.

5.5 Pharmacological Evaluation of Analogs with Amide and Thioester group at C2’ Position

The *in vitro* binding affinity and functional data of analogs with amide and thioester group at C2’ position are shown in Table 5.15 and Table 5.16, respectively.

<table>
<thead>
<tr>
<th>Cpd #</th>
<th>AM #</th>
<th>rCB1 (nM)</th>
<th>mCB2 (nM)</th>
<th>hCB2 (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>86</td>
<td>AM 7422</td>
<td>6.3</td>
<td>9.2</td>
<td>2.8</td>
</tr>
<tr>
<td>88</td>
<td>AM 7430</td>
<td>0.5</td>
<td>0.8</td>
<td>0.7</td>
</tr>
</tbody>
</table>

**Observation and Results**

Compound 86 (AM 7422, amide at C2’ position) and compound 88 (AM 7430, thioester at C2’ position) exhibited high affinity toward cannabinoid receptors. The cAMP assay indicated that compound 86 (AM 7422) induced 101% and 62% of control agonist response at a high concentration of 1 µM at CB1 receptors and CB2 receptors, respectively, and inhibited arrestin recruitment at CB2 receptors with an EC50 of 55 nM [Table 5.16]. The *in vivo* evaluation of these analogs is in progress.
Table 5.16: Functional data (cAMP and β-arrestin) of analog with amide at C2′ position (* % of control agonist response, ** % inhibition of the control agonist response, X no response)

<table>
<thead>
<tr>
<th>AM #</th>
<th>cAMP</th>
<th>β-arrestin (EC₅₀)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Agonist*</td>
<td>Agonist (nM)</td>
</tr>
<tr>
<td></td>
<td>CB1</td>
<td>CB2</td>
</tr>
<tr>
<td>7422</td>
<td>10 nM</td>
<td>77 40</td>
</tr>
<tr>
<td></td>
<td>1 µM</td>
<td>101 62</td>
</tr>
</tbody>
</table>

5.6 Pharmacological Evaluation of Biaryl Analogs with Short Ester Side Chain

The in vitro binding affinity of biaryl analogs is shown in Table 5.17.

Observation and Results

Compound 95 (AM 7414) showed no affinity towards CB receptors, whereas compound 96 (AM 7415) exhibited mild affinity and selectivity (approximately 40-fold) towards hCB2 receptors. It was observed that replacement of methoxy group of compound 97 (AM 7472) by hydroxy groups [compound 98 (AM 7473)] led to enhanced affinity towards CB receptors, especially at CB2 receptor. Similarly, replacing methoxy groups of compound 100 (AM 7475) by hydroxy groups [compound 101 (AM 7477)] led to enhanced affinity towards CB2 receptors. All other analogs in biaryl series showed low affinity towards cannabinoid receptors.
Table 5.17: Affinities ($K_i$) of biaryl analogs towards cannabinoid receptors

<table>
<thead>
<tr>
<th>Cpd #</th>
<th>AM #</th>
<th>rCB1 (nM)</th>
<th>mCB2 (nM)</th>
<th>hCB2 (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>95</td>
<td>AM 7414</td>
<td>&gt;1000</td>
<td>&gt;1000</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>96</td>
<td>AM 7415</td>
<td>3664</td>
<td>-</td>
<td>94.8</td>
</tr>
<tr>
<td>97</td>
<td>AM 7472</td>
<td>&gt;1000</td>
<td>&gt;1000</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>98</td>
<td>AM 7473</td>
<td>941</td>
<td>36</td>
<td>48.8</td>
</tr>
<tr>
<td>99</td>
<td>AM 7474</td>
<td>1000</td>
<td>108</td>
<td>375</td>
</tr>
<tr>
<td>100</td>
<td>AM 7475</td>
<td>&gt;1000</td>
<td>&gt;1000</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>101</td>
<td>AM 7477</td>
<td>191</td>
<td>10.3</td>
<td>20.9</td>
</tr>
<tr>
<td>102</td>
<td>AM 7476</td>
<td>1000</td>
<td>1000</td>
<td>700</td>
</tr>
</tbody>
</table>

5.7 Pharmacological Evaluation of Analogs with Cyclobutyl at C1’ Position

The binding affinities at CB receptors and functional activity of novel analogs with cyclobutyl at C1’ position analogs are shown in Table 5.18 and Table 5.19, respectively. The in vivo data (rat hypothermia) of these analogs is shown in Figure 5.10.

Observation and Results

The CB receptor binding affinity data indicated that compounds 107 (AM 7469), 108 (AM 7470), 109 (AM 7471) and 110 (AM 7468) exhibited low nanomolar ($K_i < 4$ nM) binding affinity at both the CB receptors. The metabolite i.e., compound 106 (AM 7467) exhibited no
affinity towards CB receptors [Table 5.18]. In vitro plasma stability studies showed that compound 110 (AM 7468) had a half-life of 40 min, followed by compound 107 (AM 7469) and 108 (AM 7470) with half-lives of 50 min and 107 min respectively.

Table 5.18: Affinities (Ki), cLogP and tPSA of novel cannabinergic ester analogs with cyclobutyl group at C1’ position

<table>
<thead>
<tr>
<th>Cpd #</th>
<th>AM #</th>
<th>rCB1 (nM)</th>
<th>mCB2 (nM)</th>
<th>hCB2 (nM)</th>
<th>cLogP</th>
<th>TPSA</th>
<th>t1/2 (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>106</td>
<td>AM 7467</td>
<td>&gt;10,000</td>
<td>&gt;10,000</td>
<td>&gt;10,000</td>
<td>4.7</td>
<td>66.7</td>
<td>-</td>
</tr>
<tr>
<td>107</td>
<td>AM 7469</td>
<td>0.3</td>
<td>3.7</td>
<td>0.7</td>
<td>6.6</td>
<td>55.7</td>
<td>50</td>
</tr>
<tr>
<td>108</td>
<td>AM 7470</td>
<td>0.9</td>
<td>1.5</td>
<td>1.8</td>
<td>5.0</td>
<td>79.5</td>
<td>107</td>
</tr>
<tr>
<td>109</td>
<td>AM 7471</td>
<td>0.9</td>
<td>1.9</td>
<td>2.7</td>
<td>7.0</td>
<td>104.5</td>
<td>-</td>
</tr>
<tr>
<td>110</td>
<td>AM 7468</td>
<td>0.7</td>
<td>0.7</td>
<td>0.4</td>
<td>6.7</td>
<td>55.7</td>
<td>40</td>
</tr>
</tbody>
</table>

In the cAMP assays, compound 108 (AM 7470) showed 96% and 58% of control agonist response at 10 nM concentration at CB1 and CB2 receptors, as shown in Table 5.19. In the β-arrestin assay, compound 108 (AM 7470) induced arrestin recruitment at CB2 receptors with an EC50 of 0.08 nM, whereas at CB1 receptors this compound inhibited arrestin recruitment with an EC50 of 1.7 nM as shown in Table 5.19. In vivo (rat hypothermia) data indicated that the metabolite, i.e., compound 106 (AM 7467) showed no hypothermic response in vivo, whereas compound 110 (AM 7468) decreased core body temperature in a dose-dependent manner with a 4.5°C maximal drop in body temperature at a dose of 1 mg/kg. However, no in vivo functional inactivation was seen within 6 h of study duration [Figure 5.10].
Table 5.19: Functional data (cAMP and β-arrestin) of analog with cyclobutyl at C2’ position (* % of control agonist response, ** % inhibition of the control agonist response, X no response)

<table>
<thead>
<tr>
<th>AM #</th>
<th>cAMP</th>
<th>β-arrestin (EC50)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Agonist</td>
<td>Antagonist</td>
</tr>
<tr>
<td></td>
<td>CB1</td>
<td>CB2</td>
</tr>
<tr>
<td>7470</td>
<td>10 nM</td>
<td>96</td>
</tr>
<tr>
<td></td>
<td>1 μM</td>
<td>96</td>
</tr>
</tbody>
</table>

Figure 5.10: In vivo evaluation (Hypothermia) of novel analog with cyclobutyl group at C1’ position

5.8 Pharmacological Evaluation of Analogs with reduced cLogP and increased tPSA

The binding affinity towards CB receptors and functional data of novel analogs with reduced cLogP and increased tPSA are shown in Table 5.20 and Table 5.21, respectively.
Observation and Results

The CB receptor binding affinity data showed that compounds 112 (AM 4811) and 113 (AM 4812) bind to CB receptors with high affinity ($K_i < 4$ nM). Compound 114 (AM 4816) bind to CB1 receptor with high affinity ($K_i < 7.5$ nM) whereas its affinity towards CB2 receptors was reduced significantly. Compound 49 (AM 7495) bind to hCB2 receptors with high affinity ($K_i=3.9$ nM), while its affinity towards mCB2 and rCB1 was reduced significantly [$K_i = 52.9$ nM (mCB2), $K_i = 69.3$ nM (rCB1)]. Similar results were observed for compound 115 (AM 7496) which bind preferentially to hCB2 receptors ($K_i=2.2$ nM) with high affinity, whereas its affinity towards mCB2 and rCB1 was reduced significantly [$K_i=77.7$ nM (mCB2), $K_i=33.9$ nM (rCB1)].

Table 5.20: In vitro binding affinity ($K_i$), cLogP, tPSA of ester analogs with improved PK profile

<table>
<thead>
<tr>
<th>Cpd #</th>
<th>AM #</th>
<th>rCB1 (nM)</th>
<th>mCB2 (nM)</th>
<th>hCB2 (nM)</th>
<th>cLogP</th>
<th>TPSA</th>
<th>t1/2 (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>112</td>
<td>AM 4811</td>
<td>0.6</td>
<td>3.5</td>
<td>0.8</td>
<td>2.6</td>
<td>96.6</td>
<td>-</td>
</tr>
<tr>
<td>113</td>
<td>AM 4812</td>
<td>0.33</td>
<td>1.6</td>
<td>0.7</td>
<td>2.85</td>
<td>99.78</td>
<td>-</td>
</tr>
<tr>
<td>114</td>
<td>AM 4816</td>
<td>7.5</td>
<td>101.7</td>
<td>120.6</td>
<td>2.8</td>
<td>88.4</td>
<td>-</td>
</tr>
<tr>
<td>49</td>
<td>AM 7495</td>
<td>69.3</td>
<td>52.9</td>
<td>3.9</td>
<td>3.9</td>
<td>83.8</td>
<td>-</td>
</tr>
<tr>
<td>115</td>
<td>AM 7496</td>
<td>33.9</td>
<td>77.7</td>
<td>2.2</td>
<td>4.2</td>
<td>86.9</td>
<td>-</td>
</tr>
</tbody>
</table>

In vitro metabolic stability of these analogs is being determined. The cAMP data suggested that compound 49 (AM 7495) showed 39% and 104% of control agonist response at CB1 and CB2
receptors, whereas compound 115 (AM 7496) showed 89% and 104% of control agonist response at CB1 and CB2 receptors [Table 5.21]. The β-arrestin assay data indicated that compounds 49 (AM 7495) and 115 (AM 7496) induced arrestin recruitment at CB2 receptors with an EC50 of 80 pM and 44 pM respectively [Table 5.21].

Table 5.21: Functional data (cAMP and β-arrestin) of analog with improved pk profile (* % of control agonist response, ** % inhibition of the control agonist response, X no response)

<table>
<thead>
<tr>
<th>AM #</th>
<th>cAMP</th>
<th>β-arrestin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Agonist*</td>
<td>Antagonist**</td>
</tr>
<tr>
<td></td>
<td>CB1</td>
<td>CB2</td>
</tr>
<tr>
<td>7495</td>
<td>10 nM</td>
<td>-45</td>
</tr>
<tr>
<td></td>
<td>1 µM</td>
<td>39</td>
</tr>
<tr>
<td>7496</td>
<td>10 nM</td>
<td>-10</td>
</tr>
<tr>
<td></td>
<td>1 µM</td>
<td>89</td>
</tr>
</tbody>
</table>
Chapter 6: Discussion and Conclusion

A ‘controlled inactivation approach’ has been used in designing and synthesizing novel cannabinergic analogs with controlled detoxification. This approach integrates ‘soft drug’ principle and ‘modulation of polarity’ in rational drug design to generate leads with desired pharmacological response, i.e., improved efficacy and predictable duration of action.

An ester group, hydrolyzable by ubiquitous esterases, was incorporated within the key pharmacophoric sites of DMH-Δ⁸-THC at strategic positions in such a manner that the analogs exhibited desired pharmacological response at cannabinoid receptors and underwent enzymatic inactivation by plasma esterases in a predictable manner. Also, the metabolites so formed after enzymatic inactivation showed no (or minimal) affinity and activity at CB receptors.

Prior studies in our group (Jing et al., unpublished work) indicated that substituting the DMH side chain of DMH-Δ⁸-THC [Figure 6.1] by an ester side chain (ester group at C1’ position, i.e., compounds lacking germinal dimethyl group at C1’ position) resulted in significant loss in affinity towards both the primary CB receptors ($K_i \sim 500$ nM). However, in our designed analogs, it was found that moving the ester group to C2’ position and retaining the germinal dimethyl group at C1’ position [AM 7410, Figure 6.1] resulted in analogs with low nanomolar

Figure 6.1: Structure of Δ⁹-THC, DMH-Δ⁸-THC, AM 7410
binding affinity (similar to DMH-Δ⁸-THC) towards both CB receptors. This reconfirmed that
geminal dimethyl group at C1’ position is one of the key components of DMH-Δ⁸-THC binding
to cannabinoid receptors. Interestingly, the inability of the metabolite i.e., compound 4 (AM
7408) to bind and activate cannabinoid receptors validated one of the aspects of our rational drug
design, according to which, the carboxylic acid metabolites should have no (or minimal) activity
at CB receptors. The reduction in affinity towards CB receptors exhibited by compounds 8 (AM
7420), 10 (AM 7431) and 14 (AM 4801) suggested that short chain (4-carbon) or long chain (7-
carbon) with imidazole and morpholine in the terminal carbon of the side chain was not tolerated
at both CB receptors.

The difference in in vitro metabolic stability (t_{1/2}) exhibited by parent compounds with ester
group at C2’ position suggested that duration of action can be modulated by varying the polar
functional groups at the terminal carbon of the side chain of DMH-Δ⁸-THC. It can be inferred
from this finding that compound polarity may have a unique role in modulating compound
plasma stability and can aid in designing ultra short-acting or long-acting cannabinergic analogs.

Compounds with desired cannabinoid receptor affinity and predictable plasma stability were
profiled for functional activity studies (cAMP and β-arrestin assays). The functional potency of
novel compounds in the cAMP assay was obtained by measuring their effect on cellular cAMP
level upon binding to cannabinoid receptors.

The cAMP assay suggested that, compounds 13 (AM 7409), 11 (AM 7433) and 17 (AM 7428)
[Figure 6.2] are potent agonists at CB1 and CB2 receptors, whereas in β-arrestin assay these
compounds recruited arrestin only at CB2 receptors and not at CB1 receptors. In short, these
analogs bind to CB1 receptors with high affinity, reduced cAMP levels (potency similar to CP
55940) but no arrestin translocation was observed in $\beta$-arrestin assays, suggesting that the receptor desensitization mechanism may not be mediated by $\beta$-arrestin pathways. However, at CB2 receptors, these analogs showed potent functional activity and also induced arrestin translocation from cytoplasm to cell membranes, suggesting a potent agonist activity at CB2 receptors and receptor desensitization mechanism mediated by $\beta$-arrestin pathway.

![Figure 6.2: Structure of AM 7409, AM 7428, AM 7433](image)

The functional selectivity demonstrated by compounds 13 (AM 7409), 11 (AM 7433) and 17 (AM 7428) suggested that these analogs are biased towards activating specific downstream signaling cascade. Such functional response is observed frequently and is a common characteristic exhibited by certain ligands which bind to GPCRs. Recently, it has been found that ligands can activate arrestin signaling cascade upon GPCR binding, independent of G-protein activation.

The lack of functional activity at CB2 receptors, exhibited by compounds 18 (AM 7488) and 14 (AM 4801) [Figure 6.3] suggested that these compounds probably are antagonist at CB2 receptors. However, at CB1 receptors, compounds 18 (AM 7488) and 14 (AM 4801) showed high affinity and potent functional activity (cAMP assay) but no arrestin translocation was seen in $\beta$-arrestin assays, suggestive of biased agonist response. It was interesting to find that compounds with bulky substitutions such as imidazole or morpholine at the terminal carbon of the side chain,
i.e., compounds 8 (AM 7420), 17 (AM 7428) and 18 (AM 7488), showed no hypothermic response (rat hypothermia) in spite of exhibiting good affinity and functional activity at CB receptors. Since hypothermia is a centrally mediated cannabinergic effect, it was hypothesized that these compounds may be peripherally restricted analogs under the acute, one exposure experimental conditions employed in this research project.

![Figure 6.3: Structure of AM 7488, AM 4801](image)

Although, compounds 5 (AM 7407), 13 (AM 7409) and 11 (AM 7433) significantly decreased the core body temperature \textit{in vivo} but they were not inactivated within 6 h of study duration, so no esterase activity was speculated \textit{in vivo}. It was hypothesized that, the prolonged hypothermic effect exhibited by these analogs, may be due to the presence of geminal dimethyl group alpha to the ester group which may provide extensive stability to the molecule towards enzymatic hydrolysis by esterases, or it may also attributed to the depot effect due to their high lipophilicities. These concerns were resolved by synthesizing and profiling second generation analogs with reduced steric hindrance at C1’ position and by increasing their polarity to counter the depot effect.

Three key approaches used to facilitate the enzymatic hydrolysis of the ester group at C2’ position in the side chain of DMH-\(\Delta^8\)-THC by esterases were: reverse ester analog (moving ester group away from the geminal dimethyl group); replacing the geminal dimethyl group at C1’
position by the monomethyl group and removal of the geminal dimethyl group at C1’ position. In all three approaches ester group was sufficiently exposed to undergo rapid enzymatic inactivation by esterases in vitro and in vivo.

Figure 6.4: Structure of AM 7416

Pharmacological evaluation of the reverse ester analog [compound 23 (AM 7416), Figure 6.4] indicated that binding affinity at CB receptors (CB1 and CB2) was retained even by moving the ester group away from the geminal dimethyl group, from the C2’ to the C3’ position and it underwent enzymatic inactivation in vitro by mouse plasma esterases with a reduced half-life of 10 minutes. The rapid in vitro inactivation was expected and encouraging finding due to the increased exposure of the ester group, which would invite rapid enzymatic hydrolysis by esterases as compared to the ester analogs with geminal dimethyl group at C2’ position. However, in vitro results were not reflected in in vivo studies because, compound 23 (AM 7416) showed no inactivation in vivo within 6 h of study duration. This result was again attributed to the depot effect due to compound’s high lipophilicity (cLogP: 7.1), which may invite its sequestration within fatty tissue and slow diffusion therefrom into the blood stream, leading to a very long and unpredictable half-life.

The next set of analogs synthesized to facilitate the enzymatic hydrolysis were C1’-methyl substituted ester analogs of Δ⁸-THC. CB receptor binding affinity data suggested that this modification does not compromise the compound’s low nanomolar affinity towards both CB
receptors. As expected, these analogs underwent rapid metabolic inactivation \textit{in vitro} by mouse plasma esterases as compared to the analogs with geminal dimethyl group at C1′ position, because the monomethyl group offers relatively less steric hindrance towards enzymatic hydrolysis by esterases.

![Figure 6.5: Structure of AM 7463 and AM 7462](image)

The functional activity of compound 41 (AM 7463) [Figure 6.5] was concentration dependent in cAMP assay, i.e., increasing its concentration from 10 nM to 1µM, transformed it from a weak agonist to a potent agonist. However, in the \(\beta\)-arrestin assay compound 41 (AM 7463) exhibited receptor specific response, i.e., at CB2 receptors it induced arrestin translocation, suggestive of its agonist activity, whereas, at CB1 receptors it inhibited the agonist induced arrestin translocation from the cytoplasm to the cell membrane, indicated that it may be an antagonist at CB1 receptors. Interestingly, the other diastereomer, i.e., compound 35 (AM 7462), even at high 1µM concentration acted as a weak agonist at CB2 receptors in cAMP assays but it induced the arrestin translocation from cytoplasm to cell membrane for receptor internalization. It is worth mentioning that although we saw differences between compounds 35 (AM 7462) and 41 (AM 7463) in functional assays, but these were based only on two point data and it is difficult to make an immediate judgment until similar results are replicated in eight point assays. However, we were able to extrapolate \textit{in vitro} results to \textit{in vivo} results, which suggested that, although both the compounds 35 (AM 7462) and 41 (AM 7463) bind to CB receptors with high affinity and
showed functional activity, but only one diastereomer i.e., compound 41 (AM 7463) produced a significant decrease in core body temperature in rats, while the other diastereomer i.e., compound 35 (AM 7462) did not influence the core body temperature upto a dose of 3.0 mg/kg. In short, the pharmacological evaluation of C1’ methyl substituted ester analogs suggested that reducing the steric hindrance at C1’ position i.e., deletion of one of the methyl groups may aid in rapid inactivation in vivo. As seen with the compound 41 (AM 7463), which underwent inactivation in vivo within 6 h at a dose of 0.3 mg/kg after it produced significant hypothermic response.

The last modification to facilitate the enzymatic hydrolysis of the compounds by esterases involved the deletion of geminal dimethyl group at C1’ position in DMH-Δ8-THC ester analog. This modification led to the compounds with reduced affinity towards CB receptors (especially at CB1 receptor). These results were in line with well established finding that, geminal dimethyl group is one of the key pharmacophores of DMH-Δ8-THC. However, these compounds underwent, as expected, enzymatic inactivation by plasma esterases rapidly, with half-life of 5 min. The key analog i.e., compound 46 (AM 7425) was further profiled for functional and in vivo activities. It was found to be a functionally weak agonist at both CB receptors and induced a small and delayed hypothermic effect (significant effect occuring at 2.5 h post injection) at a dose of 3.0 mg/kg. Compound 44 (AM 7426), the primary metabolite after enzymatic hydrolysis of THC analogs without geminal dimethyl group, showed no affinity and activity at CB receptors validated one of aspects of our drug design.

The pharmacological evaluation of sterically less hindered analogs demonstrated that probably it is not the steric factors which controls the desired pharmacological profile in vivo ( inactivation within 6 h of study duration) but may be, it is the depot effect which is dominant and making
compounds to be sequestered within fat cells and allow slow and erratic diffusion into the blood stream. So, the next generation of analogs with increased polarity (reduce depot effect) were pursued. With this goal in mind, we incorporated polar hydroxy group at strategic positions within DMH-Δ⁸-THC, with an ester group at C2’ position, such that resulting compounds might retain high affinity and functional activity at CB receptors and also show metabolic inactivation \textit{in vivo} in a predictable manner. Three approaches were used which involved the incorporation of polar hydroxy group at strategic positions: hexahydro ester analog, 11-hydroxy ester analog and 9-hydroxy ester analog [Figure 6.6].

![Figure 6.6: Structure of AM 7499, AM 7418, AM 7446](image)

The first analog synthesized in this series of designed polar analogs was the hexahydro-9-hydroxymethyl-DMH-Δ⁸-THC ester analog [AM 7499, Figure 6.6]. CB-receptor binding affinity data indicated that compound 54 (AM 7499) retained high affinity at CB receptors even if the C-ring was reduced and the methyl group at C9 position was substituted with a hydroxy methyl (β-conformation) group in the C-ring. This compound was functionally potent agonist at both CB receptors (cAMP assay) and also induced arrestin recruitment at both CB1 and CB2 receptors. The potent \textit{in vitro} response was reflected in \textit{in vivo} results which suggested that compound 54 (AM 7499) is a potent CB1 agonist \textit{in vivo}, given its large hypothermic effect at 1.0 mg/kg. Even at a low dose of 0.1 mg/kg compound 54 (AM 7499) showed a large hypothermic effect and was
also inactivated within 6 h of study duration validating the key research objective. The successful and desired in vitro and in vivo pharmacological profile demonstrated by compound 54 (AM 7499) led us to designate this compound as a lead compound. Currently, this compound is under evaluation for its pharmacological effect in other studies in vivo (e.g., antinociception).

The next analog in the series of polar analogs was 11-hydroxy-DMH-Δ8-THC ester analog [AM 7418, Figure 6.6]. CB receptor binding affinity data suggested that hydroxyl group at C11 position was well tolerated at both the CB receptors. Interestingly, compound 57 (AM 7418) was hydrolyzed by mouse plasma esterases in vitro with a half-life of 5 min and demonstrated a potent functional profile at both CB receptors as indicated by cAMP and β-arrestin assays. Compound 57 (AM 7418), significantly decreased the core body temperature at the dose of 0.1 mg/kg and the animals recovered from hypothermia within 6 h of study duration, validating the key aim of our research project. Compound 57 (AM 7418) was a second lead compound along with compound 54 (AM 7499), which exhibited high affinity and functional activity at CB receptors and showed a predictable duration of action (5 h) at a dose of 0.1 mg/kg. Compound 57 (AM 7418) also showed predictable potent antinociceptive response in tail flick test and was found to be almost 100 times more potent than morphine. Compound 57 (AM 7418) was designated as second lead compound.

The last modification employed in this series of polar analogs, incorporated a polar hydroxy group at C9 position [AM 7446, Figure 6.6]. Receptor binding affinity data indicated that modifications in C-ring, i.e., incorporation of 9-hydroxy group (both epimers) at C9 position was well tolerated at both the CB receptors. Compound 65 (AM 7446) was found to be a high affinity ligand, exhibited predictable in vitro plasma stability of 3 min and showed predictable hypothermic response in vivo. The in vivo results were in conjuction with the functional data
(cAMP and β-arrestin) which suggested that compound 65 (AM 7446) acted as a potent agonist at both the CB receptors even at a low concentration of 10 nM and also induced arrestin recruitment at both CB receptors. At a dose of 1.0 mg/kg compound 65 (AM7446) induced significant hypothermic response and animals also recovered within 6 h from hypothermia. The metabolites, i.e., compounds 66 (AM 7454) and 67 (AM 7455) exhibited no pharmacological activity at either of the cannabinoid receptors both in vitro and in vivo, validating the rationale behind the design of novel cannabinergic analogs.

So, to summarize the series of polar DMH-Δ⁸-THC ester analogs, it was found that two CB agonists i.e., compound 54 (AM 7499) and compound 57 (AM 7418) showed very potent hypothermic response [Figure 6.7]. Compound 54 (AM 7499) produced a large hypothermic effect (7-8°C drop in body temperature) at a dose of 1.0 mg/kg and at a dose of 0.1 mg/kg it produced a significant hypothermic effect and rats also recovered from hypothermia within 6 h of study duration [Figure 5.7].

In order to obtain more detailed understanding of the SAR, we incorporated an ester group at two other key pharmacophoric sites of DMH-Δ⁸-THC, geminal dimethyl group at C1’ position and
the C-ring. The incorporation of an ester group at these pharmacophoric sites led to $\beta$-lactone analog [AM 7487, Figure 6.8] and the C-ring lactone analog [AM 7487, Figure 6.8].

![Figure 6.8: Structure of AM 7487, AM 4809](image)

Incorporation of $\beta$-lactone at C1’ position of DMH-$\Delta^8$-THC resulted in reduction in affinity towards CB2 receptors. It was interesting to note that its metabolite i.e., compound 76 (AM 7486) showed no affinity toward CB receptor, validating the rationale behind the design of these novel cannabinergic analogs. The in vitro plasma stability and in vivo potency studies of $\beta$-lactone at C1’ position analog remains to be determined.

Modifications in the C-ring i.e., incorporating lactone in the C-ring of DMH-$\Delta^8$-THC led to two regioisomers. Initial pharmacological evaluation suggested that only one regioisomer i.e., compound 85 (AM 4809) [Figure 6.8] showed high affinity and selectivity (10 folds) towards CB1 receptors, whereas the other regioisomer showed reduced affinity towards CB receptors. The functional activity and in vivo potency studies of C-ring lactone [compound 85, AM 4809] remains to be determined.

With the objective to design long-acting and ultra short-acting cannabinoids with controlled detoxification, amide group [AM 7422, Figure 6.7] and thioester group [AM 7430, Figure 6.9] was incorporated at C2’ position of DMH-$\Delta^8$-THC. Amide analog was expected to undergo relatively slow inactivation by amidases, whereas the thioester analog may undergo rapid
Inactivation by ubiquitous esterases. *In vitro* receptor binding affinity data indicated that incorporation of a thioester or amide group in place of an ester group at C2′ position in the side chain was well tolerated at both CB receptors.

![Figure 6.9: Structure of AM 7422 and AM 7430](image)

Functional data suggested that compound 86 (AM 7422) acted as a weak agonist at CB2 receptor, whereas at CB1 receptors it acted as an agonist at 1µM concentration. The pharmacological evaluations of these analogs in terms of *in vitro* plasma stability and *in vivo* potency are under progress.

A smaller part my research project was to design and synthesize CB2 selective cannabinergic ligands which would be devoid of psychotropic effects. Biaryl analogs with shorter side chain preferentially bind to CB2 receptors. With this goal, biaryl analogs with short ester side chain were synthesized and evaluated pharmacologically.

![Figure 6.10: Structure of AM 7415](image)
Initial CB receptor binding affinity data among series of biphenyl analogs indicated that, only one compound i.e., compound 96 (AM 7415) [Figure 6.10] showed approximately 40 times selectivity for CB2 receptors. The pharmacological evaluation in terms of *in vitro* plasma stability and *in vivo* potency studies of this analogs remains to be studied.

Our group (Spyros et al.) has demonstrated that incorporation of cyclobutyl group at C1’ position of DMH-Δ^8^-THC imparts selectivity towards CB1 receptor. Having this goal, we incorporated cyclbutyl group at C1’ position with ester group at C2’ position of DMH-Δ^8^-THC. It was found that, incorporation of a cyclobutyl group at C1’ position in place geminal dimethyl group at C1’ position retains the high affinity at both the CB receptors, but we saw no selectivity towards CB1 receptors. It was interesting to note that carboxylic acid metabolite of these analogs showed no affinity and activity towards cannabinoid receptors, which validated our drug design. These compounds showed potent hypothermic effect *in vivo* but no *in vivo* inactivation was seen in rats within 6 h of study duration. These *in vivo* results were speculated to be due to the depot effect.

In summary, chemistry and biological efforts resulted in the development of extensive SAR around the key pharmacophoric sites of DMH-Δ^8^-THC, which led to the development of two key potent agonists i.e., compound 57 (AM 7418) and compound 54 (AM 7499) with predictable duration of action.

Following conclusions can be drawn from our research efforts and are summarized in Figure 6.11:

1. Ester group at C1’ position of DMH-Δ^8^-THC is not tolerated at CB receptors, however, movement of the ester group from C1’ to C2’ position and introduction of the geminal
dimethyl group at C1′ position led to cannabinoids which exhibited high affinity and activity towards cannabinoid receptors.

2. The side chain length of DMH-Δ^8-THC analogs with ester group at C2′ position should be between 5-7 carbon for optimum activity.

3. Substitutions at terminal carbon of side chain of DMH-Δ^8-THC ester analogs such as, bromo, cyano and azide are well tolerated at cannabinoid receptors, whereas bulky substitutions like imidazole and morpholine at terminal carbon of long chain (7-carbon) are not tolerated at cannabinoid receptors.

4. Monomethyl and cyclobutyl at C1′ position of DMH-Δ^8-THC ester analogs retains high affinity towards cannabinoid receptors. However, incorporating β-lactone at C1′ positions makes the compound CB1 selective and removal of geminal dimethyl group results in reduced affinity at CB receptors especially at CB1 receptors.

5. Polar substitutions in the C-ring, especially substituting the methyl group at C9 position by hydroxy methyl group (keeping an ester group at C2′ position) leads to analogs with high affinity and increase potency in vitro and in vivo with a predictable duration of action (< 5 h) at a dose of 0.1 mg/kg in rats.

6. Incorporating an ester group in the C-ring generates a mixture of regioisomers. Initial CB receptor binding affinity data suggested that only one regioisomer i.e., AM 4809 showed high affinity and selectivity for rCB1 receptors.

7. Polar substitutions in the C-ring, especially substituting methyl group at C9 position by hydroxy methyl group (keeping an ester group at C2′ position) leads to analogs with high
affinity and increase potency \textit{in vitro} and \textit{in vivo} with a predictable duration of action (<5 h) at a dose of 0.1 mg/kg in rats.

\textbf{Figure 6.11: Summary of SAR}

In summary, our research efforts led to the development of two lead CB agonists i.e., AM 7418 and AM 7499 [Refer Figure 6.12] which showed potent response \textit{in vitro} and \textit{in vivo} assays and also underwent inactivation within 6 h of study duration in rats. Two other lead agonists i.e., AM 7428 and AM 7488 [Refer Figure 6.12] showed no hypothermic response \textit{in vivo} in spite of
high *in vitro* affinity and functional activity. It was hypothesized that these analogs may be peripherally restricted cannabinoid agonists and needs further evaluation in select *in vivo* models for peripheral activity. β-lactone at C1’ position of DMH-Δ^8^-THC and C-ring lactone are novel pharmacophores which have never been explored by research investigators and invites further exploration and pharmacological evaluations.

![Figure 6.12: structure of AM 7418, AM 7499, AM 7428, AM 7488](image)

In conclusion, clinically unfavorable characteristics of cannabis based therapies due to high lipophilicity (*depot effect*) can be improved by controlled-inactivation approach (CIA), which integrates two key principles i.e., ‘Soft drug’ design and ‘modulation of polarity’. An ester group at C2’ position is a new pharmacophore and improves affinity and activity at cannabinoid receptors. Interestingly, none of the carboxylic acid metabolites showed any affinity and activity at cannabinoid receptors, which validated an important aspect of controlled inactivation approach.
Chapter 7: Future Directions

Ongoing and future efforts include evaluating compounds 8 (AM 7420), 17 (AM 7428) 18 (AM 7488) for select \textit{in vivo} models to categorize these analogs as peripherally restricted analogs. These studies can be accomplished by our collaborator at University of Calgary, Canada by Dr Martin Storr. His laboratory is well equipped with resources to perform \textit{in vivo} evaluations for peripherally restricted analogs (colonic propulsion and GI motility).

In C1′-methyl substituted ester analogs, only one diastereomer i.e., compound 41 (AM 7463) significantly decreased the core body temperature in rats at a dose of 1.0 mg/kg, whereas the other diastereomer i.e., compound 35 (AM 7462) was found to be inactive \textit{in vivo} upto a dose of 3.0 mg/kg. We plan to repeat the \textit{in vivo} assay and validate this novel finding. We hypothesized that compound 35 (AM 7462) does not cross BBB to induce hypothermic response in \textit{in vivo}. In order to prove this, we plan to perform \textit{in vivo} brain permeability studies within CDD (Dr Jodi Wood). The results obtained from this study will define future course of action i.e., if we do not see high brain penetration of this compound we can perform further \textit{in vivo} studies to categorize compound 35 (AM 7462) as a peripherally restricted analog.

Compound 57 (AM 7418) and compound 54 (AM 7499) [Refer Figure 6.5] are potential leads resulting from our research project, we plan to further investigate the effect of substitutions at terminal carbon of the side chain such as bromo, cyano, imidazole and morpholine and study their pharmacological profile \textit{in vitro} and \textit{in vivo}. Currently we have understood that incorporation of imidazole or morpholine in the terminal carbon restricts compound’s ability to
induce hypothermia \textit{in vivo}. So, it would be interesting to see how compound 54 (AM 7499) will perform \textit{in vivo} once their terminal carbon has imidazole or morpholine groups.

Incorporating an ester group in the C-ring generates a 7-membered lactone and it was found in initial binding affinity data that, one of the regioisomers i.e. compound 85 (AM 4809) exhibits selectivity for rCB1 receptors. It would be interesting to synthesize a 6-membered lactone in the C-ring of DMH-\(\Delta^8\)-THC and study its effect and activity at CB receptors. 6-membered lactone [Figure 7.1] may be a potential lead analog of nabilone with short and controlled duration of action.

![Design of 6-membered lactone](image)

**Figure 7.1:** Design of 6-membered lactone

Compound 86 (AM 7422) incorporates amide group at C2’ position in place of an ester group. It is planned to evaluate this analog while amidases in place of esterases. Similarly, thioesters are knowns to be hydrolyzed rapidly than esterases as they undergo hydrolysis by ubiquitous esterases rapidly. DMH-\(\Delta^8\)-THC amide analog and DMH-\(\Delta^8\)-THC thioester analog may lead to be potential cannabinergic analogs with long-acting or ultra short-acting cannabinergic ligands with predictable duration of action.
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