BIARYL ETHER AND BIARYL ANALOGS AS CANNABINOID RECEPTOR LIGANDS

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Abstract:

Two cannabinoid receptors (CB1 and CB2) belonging to the superfamily of G-protein coupled receptors (GPCRs) play a vital role in multiple physiological functions. CB1 is found primarily in the central nervous system (CNS) and CB2 in the periphery. Those compounds that bind to these receptors, irrespective of their structure, are considered cannabinoids. It is because of the widespread receptor distribution and its potential therapeutics that this is an attractive receptor target.

It is the purpose of this work to explore the structural modifications of biaryl ether and biaryl analogs as cannabinoid receptor ligands. Chapter One introduces the relevant background information on the biology, structure and physiology of cannabinoid receptors, ligands, and their therapeutic potential. An overview of the current state of the cannabinoid field is presented.

Chapter Two describes the Structure Activity Relationship studies (SARs) exploring the biaryl ether structure of BAY-59-3074, a novel partial agonist of the CB1/2 receptors. Here the rationale and design of the project is presented. Chapter Three further expands on the SAR of BAY59-3074 by exploring the conformational requirements for CB receptor binding and activation. A series of novel constrained dibenzofuran and dibenzopyran analogs were synthesized using the strategy of conformational restriction as a tool for molecular modification and design. These two chemotypes provide new leads for the development of CB2-selective cannabinergics.

Chapters Four and Five expand further on key compounds identified in Chapter Two. Chapter Four explores the biphenyl structure as CB2 receptor ligands. Chapter Five examines biaryl analogs as inhibitors of the endogenous deactivating protein Fatty Acid Amidase Hydrolase (FAAH).

Lastly, Chapter Six evaluates future directions for continuing investigations into the biaryl ether and biaryl cannabinoid templates. Here we discuss the future of CB therapeutics without CNS effects and consider future work.

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In memory of my grandmother,

Hea Chhay Sauv
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<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AC</td>
<td>Adenylyl Cyclase</td>
</tr>
<tr>
<td>2-AG</td>
<td>2-Arachidonoylglycerol</td>
</tr>
<tr>
<td>AAI</td>
<td>Aminoalkylindole</td>
</tr>
<tr>
<td>ADP</td>
<td>Adenosine 5’-Diphosphate</td>
</tr>
<tr>
<td>AEA</td>
<td>N-Arachidonoylethanolamine (Anandamide)</td>
</tr>
<tr>
<td>AIBN</td>
<td>2,2-Azobis(2-methylpropionitrile)</td>
</tr>
<tr>
<td>AIDS</td>
<td>Acquired Immuno-Deficiency Syndrome</td>
</tr>
<tr>
<td>AT</td>
<td>Anandamide Transporter</td>
</tr>
<tr>
<td>ATFM</td>
<td>Arachidonyl Trifluoromethyl Ketone</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine 5’-Triphosphate</td>
</tr>
<tr>
<td>BBB</td>
<td>Blood-Brain Barrier</td>
</tr>
<tr>
<td>cAMP</td>
<td>Cyclic Adenosine 5’-Monophosphate</td>
</tr>
<tr>
<td>CB1</td>
<td>First Cannabinoid Receptor</td>
</tr>
<tr>
<td>CB2</td>
<td>Second Cannabinoid Receptor</td>
</tr>
<tr>
<td>CC</td>
<td>Classical Cannabinoid</td>
</tr>
<tr>
<td>CHO</td>
<td>Chinese Hamster Ovary</td>
</tr>
<tr>
<td>CNS</td>
<td>Central Nervous System</td>
</tr>
<tr>
<td>DCM</td>
<td>Dichloromethane</td>
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<tr>
<td>DMAP</td>
<td>4-Dimethylaminopyridine</td>
</tr>
<tr>
<td>DME</td>
<td>1,2-Dimethoxyethane</td>
</tr>
<tr>
<td>DMF</td>
<td>Dimethylformamide</td>
</tr>
<tr>
<td>DMH</td>
<td>1’,1’-Dimethylheptyl</td>
</tr>
<tr>
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<td>Full Name</td>
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<tr>
<td>--------------</td>
<td>------------------------------------------------</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic Acid</td>
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<tr>
<td>FAAH</td>
<td>Fatty Acid Amide Hydrolase</td>
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<tr>
<td>FAK</td>
<td>Focal Adhesion Kinase</td>
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<tr>
<td>GABA</td>
<td>Gama-Aminobutyric Acid</td>
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<td>GDP</td>
<td>Guanosine 5'-Diphosphate</td>
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<td>GTP</td>
<td>Guanosine 5'-Triphosphate</td>
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<td>GPCR</td>
<td>G-Protein Coupled Receptor</td>
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<tr>
<td>GTPγS</td>
<td>Guanosine 5'-O-(3-thiotriphosphate)</td>
</tr>
<tr>
<td>hCB2</td>
<td>Human CB2 receptor</td>
</tr>
<tr>
<td>HEK</td>
<td>Human Embryonic Kidney</td>
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<td>HPLC</td>
<td>High Performance Liquid Chromatography</td>
</tr>
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<td>HRMS</td>
<td>High Resolution Mass Spectroscopy</td>
</tr>
<tr>
<td>HTS</td>
<td>High Throughput Screening</td>
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<tr>
<td>IC</td>
<td>Inhibition Concentration</td>
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<tr>
<td>IFN-γ</td>
<td>Interferon-γ</td>
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<tr>
<td>IL</td>
<td>Interleukin</td>
</tr>
<tr>
<td>IOP</td>
<td>Intraocular Pressure</td>
</tr>
<tr>
<td>Ki</td>
<td>Affinity Constant</td>
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<tr>
<td>LAH</td>
<td>Lithium Aluminum Hydride</td>
</tr>
<tr>
<td>LDA</td>
<td>Lithium Diisopropylamide</td>
</tr>
<tr>
<td>MAPK</td>
<td>Mitogen-Activated Protein Kinase</td>
</tr>
<tr>
<td>MGL</td>
<td>Monoacylglycerol Lipase</td>
</tr>
<tr>
<td>MOM</td>
<td>Methoxylmethyl</td>
</tr>
<tr>
<td>Acronym</td>
<td>Definition</td>
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<tr>
<td>---------</td>
<td>----------------------------------</td>
</tr>
<tr>
<td>mRNA</td>
<td>Messenger Ribonucleic Acid</td>
</tr>
<tr>
<td>NBS</td>
<td>N-Bromosuccinimide</td>
</tr>
<tr>
<td>NCC</td>
<td>Non-Classical Cannabinoid</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear Magnetic Resonance</td>
</tr>
<tr>
<td>PKA</td>
<td>Protein Kinase A</td>
</tr>
<tr>
<td>PMSF</td>
<td>Phenylmethanesulfonyl Fluoride</td>
</tr>
<tr>
<td>PTX</td>
<td>Pertussis Toxin</td>
</tr>
<tr>
<td>SAH</td>
<td>Southern Aliphatic Hydroxyl</td>
</tr>
<tr>
<td>SAR</td>
<td>Structure Activity Relationship</td>
</tr>
<tr>
<td>TBAF</td>
<td>Tetrabutylammonium Fluoride</td>
</tr>
<tr>
<td>THC</td>
<td>Tetrahydrocannabinol</td>
</tr>
<tr>
<td>THF</td>
<td>Tetrahydrofuran</td>
</tr>
<tr>
<td>TLC</td>
<td>Thin Layer Chromatography</td>
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<td>TM</td>
<td>Transmembrane</td>
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CHAPTER ONE:
INTRODUCTION TO CANNABINOIDs; ENDOGENOUS, EXOGENOUS
AND THEIR CANNABINOID RECEPTORS
1.1 History of Cannabinoids

The plant Cannabis Sativa L. has been used for medicinal and recreational purposes for centuries. It is thought to be native to Central Asia, but over the years has migrated through China, India, the Middle East, South Africa, South America and finally the west. (Adams 1996). These cultures have cultivated Cannabis for their fiber, oil, medicine and recreational purposes. Uses can be found as early as 2700 BC in a compendium of Chinese medicines, Shen Nung Ben Ts’ao, as a treatment for rheumatic pains, feminine problems, constipation and many other ailments (Mechoulam R.; Gaoni 1967).

It was not until 1839 that Cannabis was introduced to western medicine by O’Shaughnessy (Snyder 1971). The O’Shaughnessy report detailed Cannabis use in India over a period of 900 years, as a safe medicine. It reported Cannabis use as a successful treatment for seizures, tetanus and rheumatism, but with some undesirable side effects. This was the beginning of Cannabis’ acceptance in England and other European countries.

In the past century Cannabis use has come under a lot of scrutiny in the United States. Cannabis use is the third most commonly abused drug, below alcohol and cigarettes. Because of its wide abuse the Marijuana Tax Act of 1937 was enacted in an effort to control its use (Gaoni 1964; Musto 1987). This act banned the use of cannabis recreationally and medicinally, going as far as its removal from the Pharmacopoeia in 1941. However in 1971 Dronabinol (Marinol) was approved for treatment of nausea in cancer patients.

1.2 Ingredients of Cannabis

The female plants of Cannabis Sativa contain over four hundred chemical compounds, sixty of which belong to the terpenophenolic class of compounds known as
cannabinoids. The main psychoactive ingredient of Cannabis, (-)-Δ⁹-tetrahydrocannabinol (Δ⁹THC) was discovered in 1964 by Rafael Machoulam (Gaoni 1964) and the absolute stereochemistry of (-)-Δ⁹THC was shown to be 6aR and 10aR (Mechoulam R.; Gaoni 1967). The (-)-trans-isomer is 6-100 times more potent than the (+)-trans-isomer. (Dewey 1970).

(-)-Δ⁹THC and its positional, nearly equipotent isomer, (-)-Δ⁸THC, are the major psychoactive ingredients. Other ingredients include cannabinol, cannabidiol, cannabichromene, and other analogs makeup the non-psychoactive constituents of cannabis (Figure 1.1).

Figure1.1: Representative constituents of Cannabis

1.3 Cannabinoid Receptors

The Cannabinoid receptors belong to the superfamily of guanine protein coupled receptors (GPCRs) (Bockaert 1999). There are two receptor subtypes; Cannabinoid Receptor 1 (CB1) and Cannabinoid Receptor 2 (CB2). (Di Marzo 2000; Breivogel 2001; Hajos 2002; Pertwee 2005; Pavlopoulos 2006) and evidence for a possible third cannabinoid receptor (CB3).
1.3.1 Discovery of Cannabinoid Receptors

Through the observations made by Martin et. al (Martin 2002), Howlett and co-workers found that the inhibition of adenylyl cyclase by cannabinoids would require a guanine nucleotide binding protein, Gi. They also studied the radioactive binding of cannabimimetic CP-55940 to rat brain P2 membranes and synaptosomes (Devane 1988). Their observations showed saturatable, competitive and stereoselective feature consistent with allosteric regulation of a G-protein receptor. Matsuda et. al. isolated DNA, SKR6 from a rat cerebral cortex cDNA library which are associated with cannabinoid binding sites. This was transfected into Chinese hamster ovary (CHO) cells resulting in a protein product with 473 amino acid residues that exhibit cannabinoid-like inhibition of adenylyl cyclase. This receptor was named Cannabinoid Receptor 1 (CB1) (Pertwee 1997). Human (Gerard 1991) and mouse (Chakrabarti 1995; Abood 1997) CB1 cannabinoid receptors were also cloned.

Rat CB1 and human CB1 receptors comprise of 472 amino acid residues and share 97.3% sequence homology. They differ only in 13 residues at the N-terminus that has no substantial effect on ligand binding and share 100% sequence homology in the trans-membrane regions. Therefore pharmacologically the rat and human CB1 are identical (Matsuda 1990; Gerard 1991; Bramblett 1995).

The cannabinoid receptor 2 (CB2) was discovered from the human promyelocytic cell line HL60. They identified a membrane-bound protein sharing 44% sequence homology and 68% homology in the transmembrane regions with CB1 receptor. This was named Cannabinoid Receptor 2 (CB2) (Munro 1993). The human CB2 is comprised of 360 amino acid residues, while the mCB2 has 347. They differ in 60 amino acids mostly in the C-termini and are most
similar in the transmembrane regions. The CB2 receptor displays only 81% homology between the human and rat (Griffin 2000) and 82% between the human and mouse (Shire 1996) sequences. The rat and mouse CB2 are more homologous sharing 93% amino acid identity.

While there are only two cannabinoid receptor subtypes that have been fully characterized there is evidence that other cannabinoid receptor types may exist. Shire et. al. reported an amino-terminal variant of CB1, CB1A, synthesized from the cDNA of a human lymphoblastoid cell line (Shire 1995). Evidence from Breivogel et.al further supports this. They found that WIN55212-2, an aminoalkylindole compound, can stimulate [$^{35}$S]GTP$\gamma$S binding in the brains of CB1 knockout mice and could not be antagonized by SR141716A, a potent CB1 antagonist. It was also found by Jarai et.al. that anandamide could induce mesenteric vasodilation in CB1 knockout mice and CB1/CB2 double knockout mice (Jari 1999). They also found that CB1A is shorter than CB1 by 61 amino acids, the first 28 amino acids in the amino terminal are different, and the CB1A isoform has more hydrophobic residues relatively rich in proline.

1.3.2 Structure of Cannabinoid Receptors

The Cannabinoid receptor 1 (CB1) and Cannabinoid receptor 2 (CB2) are members of the guanine protein coupled receptors (GPCRs) on their intracellular sides. They consist of seven hydrophobic domains which span the membrane seven times, forming helical bundles embedded in the cell membranes (Watson 1994). They differ from other GPCRs by lacking cystine residues in the second and third extracellular loops and lack a highly conserved proline residue in the transmembrane 5 (TM5) domain.

The CB1 is a larger protein than CB2, having 72 more amino acid residues in the N-terminal, 15 additional residues in the third intracellular loop, and 13 additional residues in the
C-terminal region (Matsuda 1997). Differences are found mostly in the N-terminus, intracellular loop 3, and C-terminus. In the CB1, the N-terminal is uncommonly long and highly conserved. Rinaldi-Carmona, M., et. al. showed that it also appears to play no major role in ligand recognition when the first 89 amino acid residues are deleted and has no effect on binding of CP-55940.

**Figure 1.2:** The amino acid sequence of mCB1 receptor, with the mutated residues highlighted in black circles

**Figure 1.3:** Schematic presentation of human CB2 receptor, with the mutated residues highlighted in red
Homology between the CB1 and CB2 receptors is 44%, but range from 33-82% in each transmembrane region (Matsuda 1997). In the TM1: 50%; TM2: 66%; TM3: 80%; TM4: 33%; TM5: 40%; TM6: 60%; and TM7: 82% (Matsuda 1997). The third intracellular loop and the C-terminus are considerably different in length and homology. It is thought that this may contribute to the different cellular responses of the CB receptors. These regions are involved with G-proteins (Howlett 1998; Abadji 1999; Mukhopadhyay 1999; Mukhopadhyay 2001) as well as receptor desensitization and internalization. (Garcia 1998).

CB1 and CB2 are membrane-bound proteins and therefore are extremely difficult to purify. Currently there is no high resolution crystal structure of the CB receptors, but only computational homology models of human CB1 (Mahmoudian 1997) from bacteriorhodopsin and human CB2 (Xie 2003) from bovine rhodopsin.

1.3.3 Distribution of Cannabinoid Receptors

Through autoradiographic studies the CB1 receptor has been identified to be widely distributed in the human body but mostly in the central nervous system (CNS) (Herkenham 1990; Glass 1997). A high density of CB1 receptors are found in the basal ganglia, particularly the putamen, which regulates motor function (Herkenham 1990); cerebellum, which coordinates body movements; the hippocampus, which is involved in learning memory and response to stress; and the cerebral cortex, which is concerned with the integration of higher cognitive functions. Activation of the central CB1 receptor has been associated with psychotropic effects including sedation, hypothermia, catalepsy, cognitive dysfunction, hallucinations and euphoria. This distribution of the CB1 is representative of the known effects
of cannabinoids. Also the presence of CB1 receptors in regions associated with mediating reward (ventromedial striatum and nucleus accumbens) suggests association with dopamine receptors.

The presence of CB1 receptors in the periphery are far less than found in the CNS, but are localized in nerve terminals of the heart, vas deferens, urinary bladder and small intestines (Pertwee 1997; Reggio 1997). They also can be found in the testis (Gerard 1991), retina (Staiker 1999) and sperm cells (Schuel 1999).

The CB2 receptor is predominately expressed in the cells and tissues of the immune system (Galiegue 1995; Pettit 1996; Schatz 1997). High abundances have been found in the tonsils, but also detected in the low levels in the human spleen, pancreas, bone marrow, uterus, lung and thymus (Galiegue 1995). The CB2 distribution in the immune system may explain the immunomodulating effects of cannabinoids.

**1.3.4 Signaling transduction of Cannabinoid Receptors**

Cannabinoid receptors signal biological responses through a complex network. These responses are mediated through G-proteins. When a GPCR is activated it undergoes a conformational change to signal the G protein, which consists of three subunits, α, β, and γ. The GDP bound to the G-protein α subunit is replaced with GTP, leading to the dissociation of the G-protein from the receptor releasing the α subunit and the βγ heterodimer, that regulate various downstream effectors. The CB1 and CB2 receptors are coupled to Gi proteins that inhibit adenylyl cyclase.
1.3.5 Active (R) and Inactive (R*) States of CB Receptors

It has been hypothesized that the cannabinoid receptors (CBRs) may exist in two states; inactive R state (estimated population: 70%) and active R* state (estimated population: 30%) (Kearn 1999). In the inactive R state, the receptors are uncoupled from the effector mechanisms and in the active R* state, the receptors are functionally coupled to the effector mechanisms. It is thought that the highly conserved CWXP in the GPCRs is the motif involved in this process. P6.50 has been identified as the flexible hinge point involved that forms a kink by which the trans-membrane helices may rotate during activation to transition between the R and R* states. This has led to the theory of full agonism, partial agonism, inverse agonism, neutral agonism and antagonism of the CBR. Pertwee and coworkers have also postulated that agonists increase the proportion of the receptors in the R* state, inverse agonists/antagonists increase the proportion of the receptors in the R state and neutral antagonists have no effect on the state of the receptor.

1.3.6 Discovery of Endocannabinoids
With the discovery of the cannabinoid receptors, scientists sought out the natural endogenous ligand of the cannabinoid receptor. In 1992, the lipid cannabinoid-like compound, anandamide named after the Sanskrit word ananda (inner bliss) was discovered (Devane 1992). Soon after, identification of another endocannabinoid, 2-arachidonoylglycerol (2-AG) was characterized (Sugira 1995).

The endocannabinoid system is composed of three components. (i) cannabinoid receptors, (ii) endocannabinoid transport system(s), and (iii) the hydrolytic enzymes that degrade the endogenous ligands (Karanian 2006).

1.3.7 Biosynthesis of Endocannabinoids

A key endocannabinoid, Anandamide (AEA) is synthesized in the cytosol of postsynaptic neurons in response to specific depolarizing stimuli and under condition of cellular damage. The biosynthesis of anandamide and other acylethanolamines is depicted in Figure 1.5, by a two-step process. One process involves the coupling of arachidonyl moiety with phosphorylcholine (PC) catalyzed by N-acyltransferase in a Ca^{2+}-dependent manner (Schmid, Schmid et al.), forming N-acylphosphatidylethanolamines (NAPEs) (Di Marzo, Fontana et al. 1994; Sugira, Kondo et al. 1996; Cadas, Di Tomaso et al. 1997). The second process involves NAPE-selective phospholipase D (NAPE-PLD) induced hydrolysis (Schmid, Schmid et al. 1990; Hansen, Lauritzen et al. 1998; Schmid, Schmid et al. 2002). The NAT-catalyzed reaction involves the transfer of an arachidonoyl group from the sn-1 position of phospholipids to the N-position of phosphatidyl ethanolamine.
Figure 1.5: Biosynthetic pathway of Endocannabinoids

The biosynthesis of 2-Arachidonoylglycerol (2-AG), another key endocannabinoid is illustrated in Figure 1.6. 2-AG plays major roles in several metabolic pathways. It is a precursor or product of phosphor-, di-, triglycerides and arachidonic acid.
1.4 Major classes of cannabinergic ligands

The compounds, synthetic or natural, that bind to the cannabinoid receptors are considered cannabinergic ligands. These cannabinergic ligands are divided into five major classes according to their structural features despite their biological action. These classes are classical cannabinoids (CC), non-classical cannabinoids (NCC), aminoalkyl indoles (AAI), biarylprazoles and endocannabinoids.

1.4.1 Classical cannabinoids (CC)
Classical Cannabinoids include the phytocannabinoids and other synthetic analogs that are structurally similar to $\Delta^9$Tetrahydrocannabinol ($\Delta^9$THC) which are the ABC tricylic compounds bearing a terpene moiety and benzopyran ring.

![Δ⁹THC](image)

**Figure 1.7**: Representative Classical Cannabinoid

This class of cannabinoids has been the most studied with extensive Structure Activity Relationship (SAR) studies, reviewed by Pertwee, Reggio and Makriyannis et al. In this class there has been three pharmacophores identified: phenolic hydroxyl (PH), lipophilic side chain (SC) and northern aliphatic hydroxyl (NAH). The PH and SC are critical for CB1 affinity. Replacement of the PH with a methoxy, hydrogen or fluorine atom greatly diminishes CB1 affinity and CB2 to a lesser extent (Thakur 2005; Thakur 2005; Pavlopoulos 2006)

The C-3 side chain is considered the most critical and most studied pharmacophore. $\Delta^9$-THC has an n-pentyl side chain, by shortening this side chain by two carbons, Razdan et. al. found that potency decreases by 75%. Therefore shortening the chain length reduces affinity. Increasing the side chain length to hexyl, heptyl, and octyl provides a systematic increase in affinity and potency. Branching at the C1' position was also explored with the synthesis of 1’-1’-dimethylpentyl $\Delta^9$-THC and 1’-1’-dimethyl heptyl showed significant increases in affinity for the CB1 receptor, compared to the non-branched. Analogue with longer chains were tested, but found that affinity decreases as side chain length was increased past the heptyl chain. Therefore the heptyl is considered the optimized chain length.
Other branching tried along the heptyl chain at the C-1' and C-2' (1'S,2'R isomer) position. This analogue showed high affinity for CB1. Also substitutions at the C-1', C-2', C-3', C-4' were explored and shows lower affinity as substitution moves down the side chain. The optimized side chain has been the 1',1'-dimethylheptyl side chain.

Substitution at the terminus of the side chain was explored by the addition of bulkier halogens, such as Bromine, resulting in high affinity for CB1. Incorporation of cyano and other groups was found to dramatically increase potency with little affect on binding affinity. Also shortening the side chain by 1 carbon, still retains high affinity for the CB1 receptor.

Substitution of cyclic ring systems at the C1' position has also been explored in the Δ8-THC analogs. Addition of cyclic groups significantly enhances the affinity at the CB receptors (Papahatjis 1998; Papahatjis, Nahmias et al. 2006; Papahatjis, Nahmias et al. 2007)

Also by the addition of a northern aliphatic group (NAH) at the C-9 position with a carbonyl group or hydroxyl group (Archer 1977) or at the C-11 position enhances the affinity for the CB receptors.

1.4.2 Non-classical cannabinoids (NCC)

The non-classical cannabinoids lack the pyran ring, but retain the bicyclic portion of the classical cannabinoids. They are characterized by AC or ACD cyclic system. They share key pharmacophores with the classical cannabinoid class such as the side chain, phenolic hydroxyl, northern aliphatic hydroxyl with the addition of a southern aliphatic hydroxyl (SAH) moiety. Pfizer developed CP-55940, a high affinity ligand for the cannabinoid receptors.
Figure 1.8: Representative non-classical cannabinoids, with arrows indicating northern aliphatic hydroxy (NAH), southern aliphatic hydroxy (SAH), side chain (SC), and phenolic hydroxy (PH)

Hybrid cannabinoids are classical cannabinoids with the addition of a southern aliphatic hydroxyl extended from the pyran ring. Examples are AM919 and AM938 (Tius, Hill et al. 1995; Tius 1995; Harrington, Stergiades et al. 2000). Equatorial C6β-hydroxypropyl analog has higher affinity than its α-axial epimer and analogs with double or triple bonds have improved affinity for the CB receptor.

Figure 1.9: Representative hybrid cannabinoids

This class also includes bicyclic and biphenyl cannabinoids. Bicyclics are shown to be CB2 selective and the abnormal cannabidiols show no affinity for the CB1 or CB2 receptors.
1.4.3 Aminoalkylindoles

The aminoalkylindoles (AAIs) are not structurally similar to the classical or non-classical cannabinoids and were initially developed as non-steroidal anti-inflammatory drugs (NSAIDs) (Bell 1991). SAR on this class of compounds suggests that the C1-aminoethyl heterocyclic group and the benzomorpholino ring are not necessary to maintain receptor affinity, while the C-3 aroyl group has a significant impact on receptor affinities (D31-33).

![Representative Aminoalkylindoles](image)

**Figure 1.9**: Representative Aminoalkylindoles

1.4.4 Biarylpyrazoles
The biarylpyrazoles are agonist/inverse agonists of the cannabinoid receptor. They were first discovered by Sanofi Recherche in 1994, with the discovery of SR141716A. SAR studies have shown that the 1- and 5- substituted phenyl moieties are required for CB1 binding affinity (Landsman 1997). In computational studies, analogs of SR141716A show that the N-1 aromatic moiety mimics the C-3 aliphatic side chain of classical cannabinoids, and the C-3 and C-5 substituents of biarylpyrazoles are key for inverse agonistic/antagonistic activity (Mukhopadhyay, 2000). It has been suggested that these ligands produce their inverse effects by competitive surmountable antagonism of CB1 receptors of endogenously released endocannabinoids or by inverse agonism resulting from negative modulation of activity of CB1.

![Figure 1.10: Representative Biarylpyrazoles](image)

1.4.5 Endocannabinoids

The endocannabinoids are the natural ligands of the cannabinoid receptors. Key endocannabinoids are anandamide (AEA) and 2-arachidonoylglycerol (2-AG).
Figure 1.11: N-Anandamide (AEA) 2-Arachidonoyl glycerol (2-AG)

Anandamide is a highly lipophilic compound with four non-conjugated cis-double bonds and n-pentyl tail, similar to the classical cannabinoids. 2-AG, shares many structural similarities but has a different polar head group.

SAR studies of the endocannabinoids have produced many high affinity ligand such as R-methanandamide (AM356) (Abadji 1994; Lin 1998). While there is no structural similarity between the endocannabinoids and cannabinoids, evidence suggests that the two classes bind to the CB receptors. (Martin 2002; Thakur 2005; Pavlopoulos 2006). The aliphatic segment occupies a similar site as the alky side chain of the classical cannabinoids. Substitution of the terminal end group with bromine or cyano group shows increased receptor affinity.

1.4.6 Other cannabigeric ligands
Other classes of cannabinoids that exhibit cannabinergic activity are shown in Figure 1.12. These compounds do not fall under the traditional classes of cannabinergic ligands and structures are very diverse. The biphenyl structure (A) was reported by Merck Frosst (Low 1988) and exhibits selectivity for the CB2 receptor. Diarylbenzofuran (B) compounds were developed by Eli Lilly (Glass 1997) and are reported as selective CB1 inverse agonists. Aventis Pharma reported diarylmethyleneazetidine (C) analogs as CB1 receptor antagonists. Recently Bayer Heathecare reported a potent CB1/CB2 agonist (D) and a CB1 partial agonist (E) (De Vry, Denzer et al. 2004; De Vry and Jentzsch 2004)

1.5 Therapeutic potentials
systems. Son, more restricted, usage adds the condition that the response is not the same as great an effect (even when applied in large amounts) as can a full agonist acting through the same receptors. The second, more restricted, usage adds the condition that the response is submaximal because not enough of the receptors occupied by the partial agonist convert to the active form. Their clinical usefulness is derived from their ability to enhance deficient systems while simultaneously blocking excessive activity (Foreman & Johansen, 2003).

Table 1.1: Summary of CB Receptor Pharmacology (Jonsson, Persson et al. 2006)

<table>
<thead>
<tr>
<th>Localization</th>
<th>CB1 receptor</th>
<th>CB2 receptor</th>
</tr>
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<tbody>
<tr>
<td>Function in the CNS</td>
<td>↓ Transmitter release</td>
<td>Role in inflammation</td>
</tr>
<tr>
<td>Signal transduction</td>
<td>↓ cAMP, ↑ K+ and ↑ Ca2+ conductances, MAPK activation</td>
<td>↓ cAMP, MAPK activation</td>
</tr>
<tr>
<td>Examples of selective agonists</td>
<td>ACEA</td>
<td>HU-308, GW-40583, AM1241, JWH-133</td>
</tr>
<tr>
<td>Examples of selective antagonists/inverse agonist</td>
<td>SR141716A, AM251, LY320135</td>
<td>AM630, SR144528</td>
</tr>
<tr>
<td>Compounds in drug development</td>
<td>Selective antagonists/inverse agonists for the treatment of obesity, smoking cessation</td>
<td>Selective agonists for the treatment of inflammatory and neuropathic pain</td>
</tr>
</tbody>
</table>

1.5.1 Partial Agonists

The term partial agonist has come to be used in two slightly different senses. The first is to refer to an agonist that in a particular tissue or organism, under specified conditions, cannot elicit as great an effect (even when applied in large amounts) as can a full agonist acting through the same receptors. The second, more restricted, usage adds the condition that the response is submaximal because not enough of the receptors occupied by the partial agonist convert to the active form. Their clinical usefulness is derived from their ability to enhance deficient systems while simultaneously blocking excessive activity (Foreman & Johansen, 2003). Figure 1.13 illustrates this concept.
Figure 1.13: Sample Dose-Response Curve of Full and Partial Agonist

Exposing a receptor to a high level of a partial agonist will ensure that there is a constant, weak level of activity, whether a normal agonist is present at high or low levels. In addition, it has been suggested that partial agonism prevents the adaptive regulatory mechanisms that frequently develop after repeated exposure to potent full agonists or antagonists.

Figure 1.14: Representative partial agonists (a) Buprenorphine (b) Varenicline (c) Buspirone
There are several drugs on the market today that are receptor partial agonists. Such examples are buprenorphine, varenicline, and buspirone (Figure 1.14). Buprenorphine is a thebaine derivative partial agonist of the \( \mu \)-opioid receptor with high binding affinity at the \( \mu \)- and \( \kappa \)-opioid receptors producing a powerful analgesic effect. It binds with weak morphine-like activity. Clinically it is used as an analgesic in pain management and as an alternative to methadone in the treatment of opioid dependence (Woods 2010).

Varenicline (Chantix), a modified cytisine, marketed by Pfizer as a partial agonist of the \( \alpha_4\beta_2 \) subtype of the nicotinic acetylcholine receptor. In addition it acts on \( \alpha_3\beta_4 \) and weakly on \( \alpha_3\beta_2 \) and \( \alpha_6 \)-containing receptors. It is used as an alternative for traditional nicotine replacement therapies (NRT) such as nicotine gum or the nicotine patch. It results in the reduction of cravings and reduces the pleasurable effects of smoking. In combination with counseling it has proven to be an effective way to treat nicotine addiction (Faessel 2010).

Buspirone is an anxiolytic prescribed for the treatment of General Anxiety Disorder (GAD) marketed by Bristol Meyer Squibb. It is a partial agonist of serotonin 5-HT\(_{1A} \) receptor. It functions as a dopamine \( D_2 \), as well as \( \alpha_1 \), and \( \alpha_2 \)-adrenergic receptor antagonist to a lesser degree. Buspirone has also shown that there is no potential for addiction or dependence, and cross-tolerance to benzodiazepines, barbiturates, and alcohol, as well as other GABAergic, has not been observed (Dhillon 2008).

Partial agonists are an emerging field in drug discovery. With their dual mode properties it is an attractive target, but remains elusive because of the difficulty in characterization and selectivity of receptors. Traditionally replacement therapy or elimination of the drug of abuse
are the typical treatment for drug addiction. An alternative to replacement therapy are partial agonists and are proving to be a valuable therapeutic.

These medications are an example of partial agonists in the market and its success in the treatment of disease/disorders. This concept could be applied to the cannabinoid receptor for the treatment of marijuana addiction or abuse.

The cannabinoid receptors are bound to a G-inhibitory protein therefore when activated, agonists produce a decrease in cAMP levels, antagonists increase in cAMP and a partial agonist produces a response intermediate to both.

There have been few partial agonists reported for the cannabinoid receptor. ∆⁹-Tetrahydrocannabinol (∆⁹-THC) has been reported as a partial agonist. There have been other cannabinoids of the classical cannabinoid class that have been identified as cannabinoid partial agonist. This is most likely due to the difficulty in characterizing partial agonist activity in the CB1 and CB2 receptors. Figure 1.16 shows representative classical cannabinoid partial agonists; ∆⁸-THC, O-1184, and O-1238.

![Figure 1.15: Classical Cannabinoid partial agonists](image)

CB receptors are part of the family of Gᵢ protein coupled receptors. Activation produces a net decrease in GTPᵣ levels and subsequently cAMP as well. *In vitro* functional assays
measuring the level of cAMP and GTP gives us an understanding of whether compounds act as a full agonist, partial agonist or antagonist.

*In vivo* characterization is more difficult. Agonistic stimulation of the receptor resulting in behavior consistent with that previously reported. Although it is difficult to discern which behaviors are considered partial unless in comparison with a known full agonist. Another useful method is to test the antagonistic properties of a potential partial agonist. It is expected that a partial agonist would antagonize the effects of a full agonist.

Bayer Pharmaceuticals has reported BAY59-3074 as a partial agonist of the CB1 receptor. This compound differs greatly in structure from the classical dibenzopyran core with a biaryl ether core. They have comparable binding affinities. DeVry et al. reported partial agonist by testing in GTPγS assay.

Figure 1.16: BAY59-3074, CP55940, and Δ⁹-THC GTPγS assays (DeVry et.al, 2004)

Their results, shown in Figure 1.17, report the findings of GTPγS results of BAY59-3074, CP55940 and Δ⁹-THC. Their findings show BAY59-3074 as intermediate to full agonist CP55940 and partial agonist Δ⁹-THC. These results are consistent in rat brain, human cortex
membranes and recombinant human CB2 assays. DeVry et al further characterized the antiallydonia and antihyperalgesic effects of BAY59-3074.

1.6 Summary and Objectives

The objective of this proposal is to design and synthesize a series of novel ligands based on the BAY-59-3074 and BAY-30-7271 compounds, which contain a biaryl ether core. This broad objective was accomplished through the exploration of the ligand-binding pocket of the CB1 and CB2 receptors by completing the following specific aims.

1. Structure-activity relationship (SAR) studies of structurally novel partial agonist BAY 59-3074 cannabinoid analogs were pursued to explore the active site within the CB1 receptor and understand the factors leading to high affinity and CB receptor subtype selectivity.

2. SAR studies of biaryl ether template were pursued and these ligands were used to explore the active sites of the endocannabinoid proteins.

3. The binding affinities of the novel ligands for rat CB1, human CB1, mouse CB2 and human CB2 (using [3H]CP55,940 as the radioligand) were determined and selectivity evaluated.

4. IC_{50} values of inhibitors of endocannabinoid deactivation were determined for FAAH.
CHAPTER TWO:
STRUCTURE ACTIVITY RELATIONSHIP (SAR) STUDY OF BIARYL ETHER CANNABINOID PARTIAL AGONIST BAY 59-3074
2.1 Structurally novel Biaryl ether cannabinoids

The cannabinoid (CB) receptor family consists of two receptors: CB1 found in the central nervous system (CNS) and periphery; and, CB2 found almost exclusively in the periphery, predominantly in the immune system. Because of the CB1 receptor’s prevalence in the CNS, it is an attractive therapeutic target.

BAY59-3074 was identified as a structurally novel cannabinoid by Bayer Pharmaceuticals (De Vry, Denzer et al. 2004; De Vry and Jentzsch 2004). This compound acts as a CB1/CB2 partial agonist with antihyperalgesic and antiallodynic effects. Partial agonists bind and activate a receptor, but have only partial efficacy at the receptor relative to a full agonist. They may also be considered ligands which display both agonistic and antagonistic effects. When both a full agonist and partial agonist are present, the partial agonist acts as a competitive antagonist, competing with the full agonist for receptor occupancy and producing a net decrease in the receptor activation observed with the full agonist alone. These unique characteristics of partial agonist and this unexplored class of cannabinergic ligands warrant further investigation of its potential.

![Figure 2.1: Full agonist BAY38-7271 and partial agonist BAY59-3074](image-url)
There have been few reported CB partial agonists, primarily because of the difficulty in characterizing the partial agonist activity. The main psychoactive ingredient in the plant *Cannabis Sativa* is considered a partial agonist and therefore will act as a reference for our work.

In this chapter, research effort was focused on the Structure Activity Relationship (SAR) studies of this class of structurally unique biaryl ether cannabinergic ligands, to identify the necessary pharmacophores for receptor-ligand binding as well as further assist our understanding of the factors that enhance receptor subtype selectivity. In addition, we designed, synthesized and evaluated the binding profiles of a series of side chain analogs.

### 2.2 Design

We chose BAY59-3074 as our lead partial agonist compound. To date there has been no reported SAR of this particular class of biaryl ether cannabinergic ligands. The two structures of BAY38-7271 and BAY 59-3074 are very similar and only differ in the A ring substitutions, with the addition of a C ring the BAY compound becomes a full agonist.

Our approach for the structural modifications made to the lead compound **BAY 59-3074** was to retain the substitutions on the A ring, while making modifications at four possible pharmacophoric positions. These positions are; (Figure 2.2)

1. Side chain lengthening and branching
2. Terminal functional group,
3. Hydrogen-bond acceptor, and
4. Biaryl linker
At these positions, bioisosteric replacements were made and using already well reported SAR on the classical cannabinoid compounds like, Δ⁹THC to determine the necessity of each group to ensure binding affinity to the CB receptors.

![Chemical Structure](image)

**Figure 2.2**: Structural Modification of partial agonist BAY59-3074

### 2.3 Chemistry

#### 2.3.1 Synthesis of BAY-59-3074

BAY-59-3074 was synthesized to test in our own in-house competitive inhibition binding assays and as a comparison for future analogs. We started with the conventional heating of 2-fluoro-6-(trifluoromethyl)benzonitrile (1) and 3-methoxyphenol (2) with sodium hydride in DMSO at 130°C for 12h to yield the key intermediate 3 (Scheme 2.1). This reaction was optimized under microwave conditions at 100°C for 5 minutes, producing comparable yields and reduced reaction time drastically. This method was used for future scale up of the key intermediate 3. Demethylation was accomplished with boron trichloride and tetrabutylammonium iodide in DCM cooled to -78°C warming to RT overnight in 92% yield. Next the BAY side chain was incorporated with 4,4,4-trifluorobutane-1-sulfonyl chloride, TBAB and 45% aqueous NaOH solution in DCM cooled to 0°C warming to RT over 2h.
Scheme 2.1: Synthesis of BAY-59-3074

Reagents and conditions: (a) NaH, DMSO, 130°C, 12h, 80% (b) BCl₃, TBAI, DCM -78°-rt, 3h, 92% (c) 4,4,4-trifluorobutane-1-sulfonyl chloride, TBAB, 45%NaOH, DCM 0°C – RT, 2h, 86%

2.3.2 Side Chain

In Δ⁹-THC SAR, the side chain is considered the most important pharmacophore. The side chain length was modified to explore what length the binding pocket would accommodate at this position while maintaining binding affinity. Side chain variations include homologation and end carbon substitution. Chain length variation ranged from 3 to 5 carbons, while the terminal carbons were substituted with methyl, chloro and cyano groups.

Scheme 2.2: Synthesis of side chain modifications
2.3.3 Terminal group

Initial modifications of BAY59-3074 started with substitution of the terminal trifluoromethyl functional group with methyl, chloro and cyano while maintaining the 3 carbon chain length. Using the appropriate sulfonyl chloride, unsubstituted methyl analogs were achieved in 60-69% yield. Addition of the side chain with chloro terminal group, were synthesized with TBAB and 45% aqueous NaOH in DCM from intermediate 4 yielded AM7504. Lastly the CN was incorporated by displacement with excess NaCN in DMF for 1 day at room temperature.

2.3.4 Side chain lengthening and branching

We elected to explore the optimal chain length and branching for CB1 affinity. Compounds were synthesized using intermediate 4 and commercially available sulfonyl chlorides of varying carbon chain length and methyl branching. Starting from intermediate 4 side
chains were incorporated by mixing with the appropriate sulfonyl chloride, TBAB, and 45% aqueous NaOH in DCM from 0°C-RT over 2h.

Scheme 2.3: Synthesis of branched side chain analogs

Reagents and conditions: (a) sulfonyl chloride TBAB, 45% aq. NaOH, DCM 0°C-RT, 2h, 57%-67%

2.3.5 H-bond acceptor

In the lead Bayer compound the sulfonate group may act as a hydrogen bond acceptor in the binding pocket. Spatially how much room could this position accommodate while retaining binding affinity to CB1? In order to address this we synthesized analogs with bioisosteric replacements at the sulfonate position. These replacements are ether, phenolic carbonate, ester and carbamate moieties at the meta-position with respect to the aryl ether bond with varying side chain lengths. These replacement groups are still capable of making hydrogen bond interactions.

This synthesis utilized the common intermediate 4 to incorporated side chains by different methods. Phenolic carbonates were synthesized from the treatment of intermediate 4 with NEt$_3$ to deprotonate the phenolic OH and addition of the appropriate chloroformate stirred at room temperature for 5 min (Scheme 2.4).

Carbamates were synthesized starting from intermediate 4 mixed with triphosgene to form the acid chloride, followed by addition of the appropriate alkyl amine. Ether compounds
were synthesized from intermediate 4 mixed with K$_2$CO$_3$ base, the appropriate alkyl halide and subjected to microwave irradiation for 15 min. This reaction was incredibly clean, fast and easily purified.

Scheme 2.4: Synthesis of bioisoteric/isoelectronic replacement of sulfonate group

Reagents and conditions: (a) DIPEA, acid chloride, DCM, 2h, 89-96%; (b) chloroformate, NEt$_3$, RT, 5 min, 60-80%; (c) alkyl amine, triphosgene, NEt$_3$, 0°C - RT, 4h, 24-38%; (d) alkyl bromide, K$_2$CO$_3$, µw, 160°C, 15 min, 14-70%.

2.3.6 Biaryl linker

BAY59-3074 contains an ether linker between the two aromatic rings. To determine if this oxygen is necessary, modifications were made to extend the oxygen outwards with a carbonyl group. Also we eliminated the oxygen and replaced it with a methylene linker and eliminated the ether moiety completely.

Initially Scheme 2.5 was devised toward the synthesis of carbonyl analogs. Treatment of 2-Chloro-3-(trifluoromethyl)benzonitrile with Grignard reagent 3-methoxyphenyl magnesium bromide in the presence of catalytic Copper (I) bromide gave dibenzophenone 13 in 58 % yield.
Followed by carbonyl protection of the dibenzophenone as dithiolane by treatment of ethane 1,2-dithiol gave 14. Attempts were made to convert the aryl chloride to cyanide by treatment with nickel chloride and NaCN under microwave conditions, but were unsuccessful. There was trace amounts of product identified by LC/MS, but were unrecoverable. Therefore we chose to deprotect 14 by exposing to BCl₃-TBAI conditions to give the free phenol. Sulfonylation of phenol is achieved under phase transfer conditions using TBABr, 45% aqueous NaOH and 4,4,4-trifluorobutane-1-sulfonyl chloride to give AM7513 (16). Conveniently the dithiolane protecting group fell off during this step and a separate deprotection step was not necessary.

Scheme 2.5: Synthesis of carbonyl linker

Reagents and Conditions: (a) Cu(I)Br, THF, reflux, 1h, 58%; (b) ethane-1,2-dithiolane, BF₃OEt₃, DCM, 0°C – RT, 3hr, 62%; (c) NaCN, NiBr, NMP, μw, 80 min, 200°C, NO RXN; (d) BCl₃, TBAI, DCM -78°- RT, overnight, 83%; (e) 4,4,4-trifluorobutane-1-sulfonyl chloride, TBAB, 45%NaOH, DCM 0°C – RT, 2h, 74%
An alternate route to our desired carbonyl analog is summarized in Scheme 2.6. Starting from 3-methoxybenzoic acid (17) treated with oxalyl chloride to form the acid chloride. This was followed by coupling with 19 under lithiation conditions at low temperature to give intermediate 20. Now we had the desired cyano substituent on the A-ring, but there was considerable difficulty in deprotection of the methyl ether by standard lewis acid conditions (BBr₃ and BCl₃/TBAI).

Scheme 2.6: Revised synthesis of carbonyl linker analog

![Chemical Structure]

**Reagents and Conditions:** (a) oxalyl chloride, DCM, 3h, 0°C – RT, %; (b) 2,2,6,6 TMP n-BuLi, THF, -78°C – RT, overnight, % (c) BCl₃, TBAI, DCM -78°C– RT, or BBr₃, DCM

Utilizing the same route as Scheme 2.6, replacing the methyl ether protecting group with the easily removable silyl ether protecting group, outlined in Scheme 2.7. Starting with 3-hydroxybenzoic acid (17) and protected the phenol with excess TBSCl, washing with 1N HCl to give the monoprotected product, 23. The acid chloride was formed by treatment of oxalyl chloride and coupled with 2-(trifluoromethyl)benzonitrile to yield our dibenzophenone core. The protecting group was easily removed with TBAF. The final step was addition of the side chain under basic conditions to give our desired carbonyl analog AM7534 (26).

Scheme 2.7: Optimized carbonyl linker analog synthesis
Reagents and Conditions  
(a) TBSCI, DCM, 1N HCl, 3.5h, 94%;  
(b) oxalyl chloride, DCM, 3h, 0°C - RT;  
(c) 2,2,6,6 TMP n-BuLi, THF, -78°C–RT, overnight, 27%;  
(d) TBAF, THF, 0°C – RT, 1h, 84%;  
(e) 4,4,4-trifluorobutane-1-sulfonyl chloride, TBAB, 45% aq. NaOH, DCM 0°C – RT, 2h, 44%.

Next, attempts were made to synthesize the methylene analog from the dibenzophenone analog intermediate (14) through a Wolf-Kishner reduction. Unfortunately only starting material remained after treatment of excess hydrazine, base and refluxing overnight. (Scheme 2.8).

Scheme 2.8: Wolf-Kishner reduction

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Reagents and Conditions (a) NH₂NH₂, H₂O/KOH, ethylene glycol, No reaction

An alternate route was proposed to couple commercially available 2-(5,5-dimethyl-1,3,2-dioxaborinan-2-yl)-6-(trifluoromethyl)benzonitrile (19) with 1-(bromomethyl)-3-methoxybenzene (18) through microwave accelerated Suzuki coupling to form the methylene
template (20) (Scheme 2.7). Demethylation was accomplished with boron trichloride and tetrabuylammonium iodide in 91% yield and lastly addition of the side chain by standard procedure.

Scheme 2.9: Synthesis of methylene linker analog

Reagents and Conditions (a) PBr3, DCM, RT, 2h, 89%; (b) Pd(PPh3)4, (BaOH)2•8H2O, DME/H2O, µw; 15 min, 120°C, 70%; (c) BCl3, TBAI, DCM -78ºC - RT, 3h, 91%; (d) 4,4,4-trifluorobutane-1-sulfonyl chloride, TBAB, 45% NaOH, DCM 0ºC – RT, 2h, 93%

Lastly, we eliminated the bond between the two aromatic rings completely while retaining the side chain at the meta-position with respect to the biaryl bond. This was achieved through microwave accelerated Suzuki reaction by the coupling of commercially available 2-(5,5-dimethyl-1,3,2-dioxaborinan-2-yl)-6-(trifluoromethyl)benzonitrile (29) with 3-bromoanisole (33) in good yields to give the biphenyl core template. This was followed by demethylation with boron trichloride and lastly functionalizing with the BAY side chain.

Scheme 2.10: Synthesis of biphenyl analog
We also synthesized analogs that were similar in structure to the classical cannabinoid $\Delta^9$-THC. As shown in Scheme 2.9, 2-fluoro-6-((trifluoromethyl)benzonitrile (1) was coupled with 3,5 dimethoxyphenol (37) under basic conditions to give intermediate 38, which was deprotected under standard conditions and the trifluoromethylpropyl sulfonyl side chain was incorporated under careful reaction conditions to yield the mono substituted product (40) with the free phenolic moiety.

Scheme 2.11: Synthesis of phenolic OH analog

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**Reagents and Conditions**

(a) NaH, DMSO, 130°C, 3h, 87%;
(b) BCl$_3$, TBAI, DCM, -78°C - RT, 3h, 73%;
(c) 4,4,4-trifluorobutane-1-sulfonyle chloride, TBAB, 45% NaOH, DCM 0°C – RT, 2h, 45%.

---

Reagents and Conditions: (a) Pd(PPh$_3$)$_4$, (BaOH)$_2$$\cdot$8H$_2$O, DME/H$_2$O, µw; 15 min, 120°C, 58%;
(b) BCl$_3$, TBAI, DCM, -78°C – RT, overnight, 85%;
(c) 4,4,4-trifluorobutane-1-sulfonyle chloride, TBAB, 45% NaOH, DCM 0°C – RT, 2h, 60%.
In addition to the free phenolic OH analog, we synthesized a side chain similar to Δ⁹-THC, a pentyl side chain. This was accomplished by a one-step synthesis, coupling of 2-fluoro-6-(trifluoromethyl)benzonitrile and olivitol in refluxed 5h in the presence of sodium hydride.

Scheme 2.12: Synthesis of olivitol analog

Reagents and Conditions: (a) NaH, DMSO, 100°C, 5h, 41%

2.4 Results and Discussion

The binding affinities of novel biaryl ether cannabinoids were determined for the CB1 and CB2 receptors using membrane preparations from rat brain or HEK293 cells transfected with mouse or human CB2 via competition-equilibrium binding with [³H]CP55940 as the radioligand, and are summarized in Tables 2.1 and 2.2. The results were analyzed using nonlinear regression to determine the actual IC₅₀ of the ligand (Prizm by GraphPad Software, Inc.) and the Ki values were calculated from the IC₅₀.

Our results indicate that those compounds with terminal side chain modifications produced compounds that had good binding affinity to both CB receptors.

We examined the effect of side chain lengthening and shortening. We observed the order of affinity for the CB1 receptor was 4 carbons (AM7551) > 5 carbons (AM7542) > 3 carbons (AM7549). The 4 carbon chain is the optimal carbon chain length for CB1. CB2 affinity was
optimal at 4 carbons (AM7551) > 3 carbons (AM7549) > 5 carbons (AM7542) in mouse CB2. Human CB2 binding affinities were higher with binding affinity 5 carbon (AM7542) > 4 carbon (AM7551) > 3 carbon (AM7549) lengths. This shows some species selectivity in these series of analogs.

Next, we examined the effect of substitution at the terminal carbon in the side chain. We retained the sulfonate group and the 3 carbon chain length and substituted with methyl, chloro and cyano groups. The substituents have lower affinity than our lead compound BAY59-3074 to the CB1 receptor, but still retain good affinity. The cyano compound has 2x binding affinity to the CB1 receptor than the chloro compound. CB2 affinity has dropped off and binding affinity is reversed.

Compounds with branching at the 3’ position with carbon length extension exhibited CB2 affinity and no CB1 affinity (899nM to >1000nM). The order of mCB2 affinity is 3 carbons AM7544 > 4 carbons AM7543 > 2 carbons AM7547. However, when testing for hCB2 affinity, binding for all compounds are >1000nM. Showing this variation of compounds are also species specific. These results were a little surprising because branching in side chain of classical cannabinoids are well tolerated. This may indicate a different binding domain for biaryl ether compounds.

Table 2.1: Side chain modified biaryl ether cannabinoids

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
<th>Ki (nM)</th>
<th>rCB1/mCB2</th>
<th>rCB1/hCB2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

56
<table>
<thead>
<tr>
<th>Compound</th>
<th>rCB1</th>
<th>mCB2</th>
<th>hCB2</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAY59-3074 (reported)</td>
<td>55.4</td>
<td>--</td>
<td>45.5</td>
</tr>
<tr>
<td>AM7507</td>
<td>16.25</td>
<td>62.91</td>
<td>91.29</td>
</tr>
<tr>
<td>AM7504</td>
<td>89.08</td>
<td>46.23</td>
<td>--</td>
</tr>
<tr>
<td>AM7505</td>
<td>42.71</td>
<td>--</td>
<td>150.8</td>
</tr>
<tr>
<td>AM7506</td>
<td>83.6</td>
<td>48.59</td>
<td>--</td>
</tr>
<tr>
<td>AM7542</td>
<td>171.6</td>
<td>102.2</td>
<td>114.3</td>
</tr>
<tr>
<td>AM7551</td>
<td>27.66</td>
<td>48.39</td>
<td>154.3</td>
</tr>
<tr>
<td>AM7549</td>
<td>183.5</td>
<td>83.12</td>
<td>167.4</td>
</tr>
<tr>
<td>AM7543</td>
<td>&gt;1000</td>
<td>204</td>
<td>1467</td>
</tr>
<tr>
<td>AM7544</td>
<td>899</td>
<td>60.45</td>
<td>1171</td>
</tr>
<tr>
<td>AM7547</td>
<td>&gt;1000</td>
<td>~400</td>
<td>--</td>
</tr>
<tr>
<td>AM7545</td>
<td>2304</td>
<td>296.7</td>
<td>1822</td>
</tr>
</tbody>
</table>

The general trends with sulfonate side chains show that as the carbon side chain is shortened less than 4 carbons the compounds become more CB2 selective. Other terminal substitutions are generally well tolerated and binding affinity remains although to a slightly lesser extent. When branching is introduced the compounds become more species selective for mouse CB2.
The replacement of the trifluoromethyl terminal side chain with a methyl produced comparable binding affinity. Presumably the addition of the trifluoromethyl terminal functionality is to prolong metabolic stability as well as improve physiochemical properties for better absorption. ChemDraw clogp of BAY59-3074 is 4.766, while the methyl analog AM7551 is slightly higher at 5.252. According to this, one would expect good membrane permeability.

We also elected to substitute the sulfonate group with other hydrogen bond acceptor functional groups. These groups include esters, phenolic carbonates, ethers and carbamates (Table 2). These bioisosteric replacements did not show significant binding to either of the CB receptors with the exception of AM7520, it exhibits good binding affinity for both receptors. The clogp of AM7520 is 4.445, lower than BAY59-3074 and may account for its binding affinity. Analogs AM7524 and AM7525 showed no CB2 binding but < 1000 nM binding affinity for CB1. From this data it shows that the sulfonate group is the optimal functional group at this position.

Table 2.2: Modified hydrogen-bond acceptor biaryl ether cannabinoids

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
<th>Ki (nM)</th>
<th>rCB1/mCB2</th>
<th>rCB1/hCB2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>rCB1</td>
<td>mCB2</td>
<td>hCB2</td>
</tr>
<tr>
<td>AM7508</td>
<td>O</td>
<td>26805</td>
<td>1712</td>
<td>--</td>
</tr>
</tbody>
</table>

58
| AM7509 | | 11692 | 17011 | -- | 6.8 | -- |
| AM7518 | | >1000 | >1000 | -- | -- | -- |
| AM7519 | | >1000 | >1000 | -- | -- | -- |
| AM7520 | CN | 96.1 | 51.6 | -- | 1.9 | -- |
| AM7521 | | >1000 | >1000 | -- | -- | -- |
| AM7522 | | >1000 | >1000 | -- | -- | -- |
| AM7523 | CF<sub>3</sub> | >1000 | >1000 | >1000 | -- | -- |
| AM7517 | | >1000 | >1000 | -- | -- | -- |
| AM7524 | | 200-1000 | >1000 | -- | -- | -- |
| AM7525 | | 150-850 | >1000 | -- | -- | -- |

Carbamate analogs were also tested in enzyme inhibition assays and produced promising results. These results are further explored in Chapter 5.

The biaryl substitution between the two aromatic rings we chose functional group replacements capable of hydrogen bonding like a carbonyl group and groups incapable of hydrogen bonding like methylene and elimination of a group completely. It is interesting to see that the carbonyl compound (AM7534) does not bind to either of the CB receptors.

When the ether group is replaced with methylene group (AM7539) there is moderate binding affinity for the CB receptors. Affinity for the mCB2 over the rCB1 is almost 3 times as
much, but in comparison to the hCB2 the rCB1 affinities are similar. Lastly the ether group was eliminated, while keeping the side chain at the \textit{meta}-position. AM7552 showed good affinity to the rCB1, mCB2 and hCB2 receptors comparable to the reported affinities for the BAY 59-3074 compound without any species selectivity. Further work on this biphenyl class is presented in Chapter 4.

Table 2.3: Modified Biaryl linkers binding affinities

<table>
<thead>
<tr>
<th>Compound</th>
<th>X</th>
<th>Ki (nM)</th>
<th>rCB1/mCB2</th>
<th>rCB1/hCB2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>rCB1</td>
<td>mCB2</td>
<td>hCB2</td>
<td></td>
</tr>
<tr>
<td>AM7507</td>
<td>O</td>
<td>16.25</td>
<td>62.91</td>
<td>--</td>
</tr>
<tr>
<td>AM7534</td>
<td>C=O</td>
<td>&gt;1000</td>
<td>&gt;1000</td>
<td>--</td>
</tr>
<tr>
<td>AM7539</td>
<td>CH₂</td>
<td>424</td>
<td>150</td>
<td>394</td>
</tr>
<tr>
<td>AM7552</td>
<td>Nothing</td>
<td>49.69</td>
<td>40.23</td>
<td>31.59</td>
</tr>
</tbody>
</table>

Phenolic OH compounds, AM7546 and AM7556 do not show any significant binding to either of the CB receptors (>1000nM). This is a very interesting discovery because in previously reported SAR of CC, the phenolic OH is an essential pharmacophore for receptor binding affinity. This finding points towards the idea that biphenyl compounds may not bind into the receptor as CC do.
Table 2.4: Phenolic OH Binding Affinities

<table>
<thead>
<tr>
<th>Compound</th>
<th>Ki (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>rCB1</td>
</tr>
<tr>
<td>AM7546</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>AM7556</td>
<td>&gt;1000</td>
</tr>
</tbody>
</table>

Functional Assay cAMP

The CB receptors are part of the superfamily of G-protein coupled receptors and are coupled to a G-inhibitory protein. By measuring the effect of a ligand the functionality can be assessed. Ligands that show a marked decrease in cAMP are agonists and ligands that increase cAMP are antagonists.

AM7507 was evaluated in rCB1 and mCB2 cAMP assays. There is approximately a 40% decrease in cAMP levels in the rCB1 with an EC$_{50}$ = 50.4 nM. This profile is characteristic of an agonist.

![Figure 2.3: rCB1 cAMP](image)
The mCB2 cAMP assay produces an approximately 20% decrease in cAMP with an EC$_{50}$ = 24.9 nM. While this is a lower change observed compared to the rCB1 assay, it points towards an agonistic profile as well.

Biphenyl compound AM7552 was also evaluated in cAMP assay. The results show a 60% decrease in cAMP in rCB1, with an EC$_{50}$ = 39.4 nM. Again, such a large change in cAMP levels is characteristic of an agonist compound. This effect is a greater change observed than in our lead AM7507. hCB2 cAMP assay shows no change in the cAMP levels and presumably considered as neutral agonist at the CB2 receptor. These results are very promising and can be a potential therapeutic as a selective ligand.
The data suggests AM7520 is an antagonist of the CB1 receptor. This is supported by the cAMP, β-arrestin and rat in vivo assays. There is no pronounced change in hypothermia, which is to be expected in an antagonist. The in vitro functional assay shows a slight increase in cAMP levels at high concentrations.
The cAMP pathway and the beta-arrestin pathway are two different pathways. AM7520 shows slight antagonism at high concentrations in cAMP and produces an antagonist effect in beta-arrestin assay. This ligand can activate different signal transduction pathways to elicit responses.

WIN55212-2 is an aminoalkylindole cannabinoid with potent agonist activity at CB receptors. It has been found to be a potent analgesic in neuropathic pain. This compound has been used as a standard in many cannabinoid assays because of its well defined properties. It is used as the competitive agonist in the β-arrestin assays. Pretreatment with 1μM in Antagonist mode assays. Data analysis was readout in the formation or loss of (agonist/antagonist respectively) fluorescent intracellular aggregates containing cannabinoid receptor/beta-arrestin-GFP complexes. Duplicate points were collected for each concentration to enable the determination of a dose response curve. Image wavelet analysis was performed using the Duke Batchmode computer software to assess the response at each point. Dose response data were fit by nonlinear regression analysis using GraphPad Prism to determine potency and efficacy in comparison to the WIN55212-2 of the compounds.

AM7504 is an analog with a substitution at the terminal group for the more reactive chloro group. β-arresting assay results produce an EC50 value of 4.559x10^{-6} in the agonist mode. In antagonist mode the EC50 value of 7.884x10^{-6}.
Figure X: AM7504 β-arrestin CB1 Agonist mode

Figure X: AM7504 β-arrestin CB2 Antagonist mode

Figure 2.7: CB1 β-arrestin assay
**In vivo studies**

The tetrad test is a series of behavioral paradigms in which rodents are treated with potential drugs and tested for cannabinoid receptor-mediated effects in rodents. These four behavioral components are spontaneous activity, catalepsy, hypothermia and analgesia. Agonist in the tetrad test has shown to exhibit hypomotility, catalepsy, hypothermia, and analgesia in rodents.

Here we report a modified tetrad test hypothermic, diuretic, analgesia, and rate effects. A distinct advantage of the tetrad is that its components can be measured sequentially after drug administration, greatly simplify behavioral assay design. Because of the robustness and relative ease of use, the tetrad has assumed a place of central importance in behavioral assays of cannabinoid action.

The tetrad test was performed for AM7507, the lead BAY59-3074 compound. In addition to our lead compound, Δ⁹-THC, another reported partial agonist, and AM4054, a full CB1 agonist, was included as a comparison.

In this study female Sprague Dawley rats were used; n = 6 except for operant studies where n = 4-6. For the hypothermia test, temperature changes were measured by thermal probes inserted to a depth of 7.5 cm. The temperature was recorded twice before injection, then every 30-60 minutes for 6h after injection. Dose-effect curve takes the maximum decrease in temperature recorded within 6h after injection.

Agonists of the CB receptors are expected to exhibit a decrease in temperature. AM7507 shows a decrease of 4 degrees in temperature at doses 1.0 mg/kg to 10 mg/kg, indicative of an agonist.
Figure 2.7: Hypothermia Effect

![Hypothermia Effect Graph]

To measure the diuretic effect, urine was collected over two hours, starting within 1 min after injection. Cannabinoid agonists have been reported to produce an increase in urine. Results show that AM7507 produced a small increase in urine at high dose, approximately 20 g/kg at 10 mg/kg. There is still much controversy over the significance of this effect, but the general trend for all the compounds tested is an increase in urine.

Figure 2.8: Diuretic Effect

![Diuretic Effect Graph]

Analgesia was measured by the tailflick test. Latencies were recorded at ½, 1, 2, 3, 6 h after injection. Baseline latencies range from 1.8-2.2 sec, cut-off is 6 sec. Dose-effect curve
takes the maximum increase in tailflick latency recorded within 6h after injection. Operant responding was measured by schedule is FR30 for 0.1 ml of 20% sweetened condensed milk, followed by 30 sec TO. Sessions are 1h, and start at 1 or 3h after injection. The data from 24h are next day effects control rates range from 89-195 responses/min. The antinociception test and rate effect test show that AM7507 effect is most similar to THC.

Figure 2.9: Antinociception

Figure 2.10: Rate effects
Overall AM7507 is most similar to AM4054, a CB1 full agonist, albeit with a very steep dose-effect function. Δ⁹THC seems a lot like AM7507, except for minimal effects on temperature.

*In vivo* studies were also performed on non-human primate models. The two compounds investigated are AM7507, our lead, and AM7505, a cyano analog. Primates trained on AM4054, a full agonist, were administered AM7507 intravenously. In this discrimination test, primates were asked if they could distinguish between the two compounds by lever presses. Therefore if AM7507 produced an effect that the primate associate with AM4054, they would press the lever associated with that feeling. Results from this test shows that the primates recognized AM7507 as AM4054.

Figure 2.11: AM4054 Discrimination test with AM7507
AM7505 was not recognized as AM4054 even with a dose up to 1 mg/kg intravenously. These are very interesting results and warrant further investigation.

Figure 2.12: AM4054 Discrimination of AM705
Further work investigates the ability of AM7505 to block effects of AM4054, which is what one would expect of a partial agonist. Primates were pretreated with AM7505 and then AM4054 was administered. If AM7505 antagonizes the effects of AM4054, the animals will not press the lever associated with the physiological effect. As seen in Figure 2.13, there is not a decrease in lever presses, so AM7505 does not antagonize AM4054.
There is a marked difference in invivo effect by the substitution of the trifluromethyl group with cyano group. As mentioned previously, the physiochemical properties are improved with the cyano ether side chain of AM7520 and it is the only side chain without sulfonate group that is well tolerated.

Therefore a preliminary in vivo study was done, by testing the hypothermic effect of AM7520, shown in Figure 2.14. There is no change in temperature up to a 10 mg/kg dose of AM7520. From these results we can infer that AM7520 does not have a central CB1 agonist effect.
2.5 Covalent Probes

To date, there has not been a reported solved crystal structure of the CB receptors. Therefore, scientists must rely on homology models as a good estimate of the CB receptors and their ligand binding domains. Recently, the Makriyannis group has reported using photoactivable and electrophilic templates to covalently bind with the active site amino acid residues.

Covalent probes are capable of forming irreversible bonds with amino acid residues in or around the ligand binding site. Subsequent isolation of ligand-protein complex, digestion of the protein, and mass spectrophotometric analysis of the digested fragments has led to the precise identification of the amino acid residues involved. We therefore directed our effort to the design and synthesis of CB1 selective covalent probes, based on the BAY template, that may help determine the amino acid residues and the transmembrane helix(es) involved in binding this class of cannabinoids.
Covalent probes were synthesized to determine if this molecule binds at the same position as Δ⁹-THC or other cannabinergic ligands. We synthesized the original compound, substituting the trifluoromethyl moiety at the terminus for azide (AM7553) and isothiocyanate (AM7554). The azido compound was synthesized from chloro compound, AM7504, by treatment with excess NaN₃ overnight in DMF at room temperature. In addition, isothiocyanates were synthesized by reaction of the corresponding azide with triphenylphosphine and carbon disulfide in THF (Scheme 2.15).

Scheme 2.13: Synthesis of biaryl ether covalent probes

This 3 carbon length covalent probe produced binding affinities similar to that of the lead BAY compound. However to produce reliable data in a covalent assay a Ki value of < 30 nM is desirable. AM7553 and AM7554 binding affinity are > 30nM. It is possible that with the addition of the azido and isothiocyanate groups, the tail of the compound extends too far into the binding pocket and loses binding affinity. Therefore we synthesized a covalent probe with a shortened 2-carbon chain and azido terminal functional group (Scheme 2.14)

We initially attempted to couple 2-chloroethanesulfonyl chloride with the biaryl ether template (4) but immediately produced the elimination product (AM7558). Attempts were made to modify reaction conditions by reducing the reaction temperature to 0⁰C and -78⁰C in order to
slow the elimination step, but did not produce the desired product, elimination occurs instantly. The conjugation of the double bond with the phenyl ring favors the elimination product. In order to reach our desired product, the elimination product (AM7558) was treated with hydrazoic acid, producing anti-Markovnikov addition across the double bond to successfully give the desired covalent probe. This was submitted as AM7578 for binding affinity testing.

Scheme 2.14: Synthesis of shortened chain azide analog

![Scheme 2.14: Synthesis of shortened chain azide analog](image)

Reagents and conditions (a) 2-chloroethanesulfonyl chloride, 45%NaOH, DCM 0°C – RT, 2h, %; (b) Hydrazoic acid 87%

Table 2.5: Biaryl ether Covalent Probes

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
<th>Ki (nM)</th>
<th>rCB1/mCB2</th>
<th>rCB1/hCB2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>rCB1</td>
<td>mCB2</td>
<td>hCB2</td>
</tr>
<tr>
<td>AM7553</td>
<td></td>
<td>77.4</td>
<td>55.44</td>
<td>119</td>
</tr>
<tr>
<td>AM7554</td>
<td></td>
<td>-900 (2pt)</td>
<td>&gt;1000</td>
<td>--</td>
</tr>
</tbody>
</table>
2.6 Molecular Modeling

The compounds synthesized from this biaryl ether SAR study were imputed into Discovery Studio by Accelrys to form a Quantitative Structure Activity Relationship (QSAR) model. This model will assist in mapping the active site of the receptor. Figure 2.15 illustrated the QSAR model. Blue spheres are representative of hydrophobic regions. Green spheres are where there exist hydrogen bond regions. The gray spheres are representative of size exclusion regions. These regions are where if the ligand were to extend to, binding to the receptor does not occur. Most likely these areas are occupied by the receptor.
Figure 2.15: Quantitative Structure Activity Relationship (QSAR) model

We validate this model by plotting the predicted log($IC_{50}$) vs Experimental log ($IC_{50}$) shown in Figure 2.16. With the exception of a couple of outliers, there is good linear correlation.
2.7 Conclusions

In summary, SAR study of biaryl ether compounds as cannabinergic ligands resulted in a better understanding of factors that confer receptor subtype selectivity. Results from this study indicate that the side chain is an important pharmacophore in binding affinity. Modifications that do not incorporate a sulfonate moiety are not well tolerated, with the exception of AM7520.

Furthermore, these ligands have led toward the development of a new model that can be utilized in future drug design. Potential ligands can be investigated *in silico* before synthesis in the wet lab. Leads were also identified from this SAR that is further discussed in this dissertation.

Further functional assays are also required to determine if these compounds are full, partial, or inverse agonist. As well as diverse set of assay to determine which downstream
pathways are activated. In addition, further experiments will be required to identify the amino acid residues that form covalent bonds with this class of compounds.

2.8 Experimental

2-(3-Methoxyphenoxy)-6-(trifluoromethyl)benzonitrile (3)

To a solution of sodium hydride (275 mg, 6.8 mmol, 60% dispersion in mineral oil) in anhydrous DMSO (15 mL) was added 3-methoxyphenol (2) (852 mg, 6.8 mmol) dissolved in anhydrous DMSO (3 mL) through cannula and allowed to stir at room temperature. After 30 minutes, 2-fluoro-6-trifluoromethyl benzonitrile (1) (1.0 g, 5.2 mmol) in anhydrous DMSO (5 mL) was added through cannula. The reaction mixture was refluxed at 130°C overnight. The reaction mixture was cooled to room temperature and water was added, extracted with diethyl ether (3x), organic layers combined and washed with brine, dried (MgSO₄) and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (20% diethyl ether in hexanes) to give compound 3 as a yellow oil (1.20 g; 80%).

Microwave procedure: In a microwave reaction vessel a solution of 3-methoxyphenol (2) (852 mg, 6.8 mmol) in anhydrous DMSO (10 mL) was added NaH (275 mg, 6.8 mmol, 60% dispersion in mineral oil) and degassed with nitrogen gas. After 10 min 2-fluoro-6-trifluoromethyl benzonitrile (1) (1.0 g, 5.2 mmol) was added and subjected to microwave irradiation at 100°C for 2 min. Reaction was quenched with water, extracted with diethyl ether (3x). Combined organic layer was washed with brine, dried (MgSO₄) and concentrated under reduced pressure. The crude was purified by flash column chromatography on silica gel.
(20% diethyl ether in hexanes) to give the title compound as a yellow solid (1.27 g, 85%) yield.

$^1$H NMR (500MHz, CDCl$_3$) $\delta$ 7.56 (t, $J = 8.0$ Hz, 1H), 7.45 (d, $J = 7.5$ Hz, 1H), 7.33 (t, $J = 8.0$ Hz, 1H), 7.09 (d, $J = 9.0$ Hz, 1H), 6.82 (ddd, $J = 8.5$ Hz, $J = 2.5$ Hz, $J = 1.0$ Hz, 1H), 6.70 – 6.66 (m, 2H), 3.82 (s, 3H, OMe) HRMS (ESI) for C$_{15}$H$_{11}$NO$_2$F$_3$ Measured Mass: 294.0742
Calculated Mass: 294.0741

2-(3-Hydroxyphenoxy)-6-(trifluoromethyl)benzonitrile (4)

To a solution of 3 (600 mg, 2.0 mmol) and TBAI (2.27 g, 6.1 mmol) in anhydrous DCM (20 mL) at -78°C, under argon, was added a solution of BCl$_3$ (6.1 mL, 6.1 mmol, 1.0M solution in DCM) dropwise over 5 min. The reaction mixture was stirred at -78°C for 30 min, then warmed to room temperature over 3 hours and quenched with water. The organic layer was separated and the aqueous phase extracted with diethyl ether (2x). The combined organic layer was washed with brine, dried (MgSO$_4$) and concentrated under reduced pressure. The crude was purified by flash column chromatography on silica gel (30% diethyl ether in hexanes) to give 4 as an off-white solid (525 mg, 92%). $^1$H NMR (500MHz CDCl$_3$) $\delta$ 7.58 (t, $J = 8.0$ Hz, 1H), 7.46 (d, $J = 8.0$ Hz, 1H), 7.36 (t, $J = 8.0$ Hz, 1H), 7.09 (d, $J = 9.0$ Hz, 1H), 7.02 (dd, $J = 8.0$ Hz, $J = 2.0$ Hz, 1H), 6.87 (t, $J = 2.0$ Hz, 1H), 6.78 (dd, $J = 8.0$ Hz, $J = 2.0$ Hz, 1H), 5.74 (s, 1H, OH) HRMS (ESI) for C$_{14}$H$_9$NO$_2$F$_3$ Calculated Mass: 280.0585 Measured Mass: 280.0580

3-(2-Cyano-3-(trifluoromethyl)phenoxy)phenyl 3-chloropropane-1-sulfonate (AM7504, 5b)

To a solution of 4 (300 mg, 1.0 mmol) in anhydrous DCM (10 mL) was added TBAB (173.3 mg, 0.5 mmol) and 45% aqueous NaOH solution (1.5 mL) and cooled to 0°C. 3-chloropropane-1-sulfonyl chloride (209 mg, 1.1 mmol) dissolved in anhydrous DCM (5 mL)
was added through cannula. The reaction mixture was allowed to stir for 15 min. at 0°C then warmed to room temperature, and stirred for an additional 1.5 hours and poured into water. The organic layer was separated and the aqueous phase extracted with diethyl ether (2x). The combined organic layer was washed with brine, dried (MgSO₄) and concentrated under reduced pressure. The crude was purified by flash column chromatography on silica gel (40% diethyl ether in hexanes) to give the title compound as a white solid (383 mg; 85%) mp. 65-66⁰C ¹H NMR (500MHz, CDCl₃) δ 7.63 (t, J = 8.5 Hz, 1H), 7.52 (d, J = 8.0 Hz, 1H), 7.49 (t, J = 8.5 Hz, 1H), 7.21 (ddd, J = 8.5 Hz, J = 2.0 Hz, J = 1.0 Hz, 1H), 7.15 (d, J = 8.0 Hz, 1H), 7.12 – 7.07 (m, 2H), 3.74 (t, J = 6.0 Hz, 2H), 3.49 (t, J = 8.0 Hz, 2H), 2.50 – 2.42 (m, 2H) HRMS (ESI) for C₁₇H₁₄ClF₃NO₄S Calculated Mass: 420.0284 Found Mass: 420.0274

3-(2-Cyano-3-(trifluoromethyl)phenoxy)phenyl 3-cyanopropane-1-sulfonate (AM7505, 5c)

To a solution of 5b (100 mg, 0.2 mmol) in anhydrous DCM (3.0 mL) was added NaCN (58 mg, 1.1 mmol) and warmed to 50°C for 4 h then poured into water. The organic layer was separated and the aqueous phase extracted with diethyl ether (2x). The combined organic layer was washed with brine, dried (MgSO₄) and concentrated under reduced pressure. The crude was purified by flash column chromatography on silica gel (40% diethyl ether in hexanes) to give the title compound as an oil (15 mg; 16%) ¹H NMR (500MHz, CDCl₃) δ 7.65 (t, J = 8.0 Hz, 1H), 7.54 (d, J = 7.5 Hz, 1H), 7.50 (t, J = 8.0 Hz, 1H), 7.22 (ddd, J = 9.0 Hz, J = 2.0 Hz, J = 1.0 Hz, 1H), 7.16 (d, J = 8.0 Hz, 1H), 7.12 – 7.07 (m, 2H), 3.46 (t, J = 7.5 Hz, 2H), 2.67 (t, J = 7.0 Hz, 1H), 2.36 (quint., J = 7.0 Hz, 2H) HRMS (ESI) for C₁₈H₁₄F₃N₂O₄S Calculated Mass: 411.0626 Measured Mass: 411.0634
3-(2-Cyano-3-(trifluoromethyl)phenoxy)phenyl 3,3,3-trifluoropropane-1-sulfonate (AM7506, 5d)

The synthesis was carried out as described for 5b, using 4 (100 mg, 0.3 mmol) in anhydrous DCM (10 mL), TBAB (57.7 mg, 0.17 mmol), 45% aqueous NaOH solution (0.5 mL), 3,3,3-trifluoropropane-1-sulfonyl chloride (77 mg, 0.39 mmol) in anhydrous DCM (5 mL). The crude was purified by flash column chromatography on silica gel (40% diethyl ether in hexanes) to give the title compound as a solid (135 mg; 86%) mp. 103-105°C. $^1$H NMR (500MHz, CDCl$_3$) δ 7.64 (t, $J$ = 8.0 Hz, 1H), 7.57 (t, $J$ = 8.0 Hz, 1H), 7.51 (t, $J$ = 8.5 Hz, 1H), 7.2 (dd, $J$ = 8.0 Hz, $J$ = 2.5 Hz, 1H), 7.15 (d, $J$ = 8.5 Hz, 1H), 7.08 (dd, $J$ = 8.5 Hz, $J$ = 2.5 Hz, 1H), 7.06 (t, $J$ = 2.5 Hz, 1H), 4.13 (q, $J$ = Hz, 2H), 1.26 (t, $J$ = 7.0 Hz, 2H).

3-(2-Cyano-3-(trifluoromethyl)phenoxy)phenyl 4,4,4-trifluorobutane-1-sulfonate (AM7507, 5a)

The synthesis was carried out as described for 5b, using 4 (150 mg, 0.5 mmol), TBAB (86.5 mg, 0.2 mmol), 45% aqueous NaOH solution (0.5 mL) in anhydrous DCM (10 mL) and 4,4,4-trifluorobutane-1-sulfonyl chloride (123 mg, 0.59 mmol). The crude was purified by flash column chromatography on silica gel (40% diethyl ether in hexanes) to give the title compound as a white solid (209 mg; 86%). mp. 53-54°C. $^1$H NMR (500MHz, CDCl$_3$) δ 7.64 (t, $J$ = 8.0 Hz, 1H), 7.53 (d, $J$ = 7.5 Hz, 1H), 7.49 (t, $J$ = 8.5 Hz, 1H), 7.19 (ddd, $J$ = 8.0 Hz, $J$ = 2.5 Hz, $J$ = 1.0 Hz, 1H), 7.15 (d, $J$ = 8.5 Hz, 1H), 7.08 (ddd, $J$ = 8.5 Hz, $J$ = 2.5 Hz, $J$ = 1.5 Hz, 1H), 7.06 (t, $J$ = 2.5 Hz, 1H), 3.38 (t, $J$ = 7.0 Hz, 2H), 2.42 – 2.28 (m, 2H), 2.31 – 2.24 (m, 2H). HRMS (ESI) for C$_{18}$H$_{14}$F$_6$NO$_4$S Calculated Mass:454.0548 Measured mass:454.0545

3-(2-cyano-3-(trifluoromethyl)phenoxy)phenyl pentane-1-sulfonate (AM7542, 5e)
The synthesis was carried out as described for 5b using 4 (80mg, 0.28 mmol), TBAB (46.2 mg, 0.13 mmol), 45% aqueous NaOH solution (0.4 mL) and pentyl sulfonyl chloride (53.8 mg, 0.31 mmol) in anhydrous DCM (5 mL). The crude was purified by flash column chromatography on silica gel (10% → 30% ethyl acetate in hexane) to give the title compound as a clear oil (81 mg; 69%). 1H NMR (500MHz, CDCl3) δ 7.62 (t, J = 9.0 Hz, 1H), 7.51 (d, J = 8.0 Hz, 1H), 7.48 (t, J = 8.8 Hz, 1H), 7.20 (ddd, J = 8.0 Hz, J = 3.0 Hz, J = 1.0 Hz, 1H), 7.14 (d, J = 8.8 Hz, 1H), 7.10 - 7.04 (m, 2H), 3.33 - 3.23 (m, 2H), 2.02 - 1.94 (m, 2H), 1.52 - 1.43 (m, 2H), 1.38 (sxt, J = 7.2 Hz, 2H), 0.94 (t, J = 7.1 Hz, 3H, CH3) HRMS (EI) for C19H18F3NO4S Calculated Mass:413.0909 Found Mass:413.0926

3-(2-cyano-3-(trifluoromethyl)phenoxy)phenyl butane-1-sulfonate (AM7551, 5f)

The synthesis was carried out as described for 5b using 4 (70 mg, 0.25 mmol), TBAB (25.3 mg, 0.25 mmol), 45% aqueous NaOH solution (0.4 mL) and butane-1-sulfonyl chloride (43.2 mg, 0.27 mmol in anhydrous DCM (5 mL). The crude was purified by flash column chromatography on silica gel (20% → 30% ethyl acetate in hexanes) to give the title compound as a yellow oil (60 mg; 60%). 1H NMR (500MHz, CDCl3) δ 7.62 (t, J = 8.5 Hz, 1H) 7.51 (d, J = 7.8 Hz, 1H) 7.48 (t, J = 8.5 Hz, 1H) 7.20 (ddd, J = 8.0 Hz, J = 2.5 Hz, J = 1.0 Hz, 1H) 7.14 (d, J = 8.3 Hz, 1H) 7.10 - 7.04 (m, 2H) 3.33 - 3.24 (m, 2H) 2.02 - 1.92 (m, 2H) 1.54 - 1.48 (m, 2H) 0.99 (t, J = 7.3 Hz, 3H, CH3) HRMS (EI) for C19H16F3NO4S Calculated Mass: 399.0752 Found Mass:399.0754

3-(2-cyano-3-(trifluoromethyl)phenoxy)phenyl propane-1-sulfonate (AM7549, 5g)
The synthesis was carried out as described for 5b using 4 (70 mg, 0.25 mmol), TBAB (25.3 mg, 0.25 mmol), 45% aqueous NaOH solution (0.4 mL) and propane-1-sulfonyl chloride (39 mg, 0.27 mmol) in anhydrous DCM (5 mL). The crude was purified by flash column chromatography on silica gel (20% → 30% ethyl acetate in hexanes) to give the title compound as a yellow oil (65 mg; 67%). 1H NMR (500 MHz, CDCl3) δ 7.63 (t, J = 8.0 Hz, 1H) 7.52 (d, J = 7.8 Hz, 1H) 7.48 (t, J = 8.5 Hz, 1H) 7.20 (dd, J = 7.5 Hz, J = 2.5 Hz, 1H) 7.14 (d, J = 8.3 Hz, 1H) 7.10 - 7.04 (m, 2H) 3.34 - 3.22 (m, 2H, CH2), 2.01-1.91 (m, 2H, CH2) 1.14 (t, J = 7.57 Hz, 3H, CH3)

3-(2-cyano-3-(trifluoromethyl)phenoxy)phenyl propane-2-sulfonate (AM7547, 6a)

The synthesis was carried out as described for 5b, using 4 (60 mg, 0.21 mmol) in anhydrous DCM (5.0 mL), TBAB (34.6mg, 0.10mmol), 45% aqueous NaOH solution (0.4mL) and a solution of isopropyl sulfonyl chloride(36.8 mg, 0.25 mmol) in anhydrous DCM (1.5mL). The crude was purified by flash column chromatography on silica gel (10% → 30% ethyl acetate in hexanes) to give the title compound as oil (50 mg; 61%). 1H NMR (500 MHz, CDCl3) δ 7.62 (t, J = 7.5 Hz, 1H), 7.50 (d, J = 8.0 Hz, 1H), 7.47 (t, J = 8.5 Hz, 1H), 7.20 (dd, J = 9.0 Hz J = 1.5 Hz, 1H), 7.14 (d, J = 8.5 Hz, 1H), 7.08 – 7.04 (m, 2H), 3.56 – 3.46 (m, 2H), 1.56 (t, J = 7.0 Hz, 6H, (CH3)2)

3-(2-cyano-3-(trifluoromethyl)phenoxy)phenyl butane-2-sulfonate (AM7544, 6b)

The synthesis was carried out as described for 5b, using 4 (80 mg, 0.28 mmol) in anhydrous DCM (5mL), TBAB (46.2mg, 0.13mmol), 45% aqueous NaOH solution (0.4 mL) and a solution of sec-butyl sulfonyl chloride (49.3 mg, 0.31 mmol) in anhydrous DCM (1.5 mL). The crude
was purified by flash column chromatography on silica gel (10% → 30% ethyl acetate in hexanes) to give the title compound as oil (65 mg; 57%). $^1$H NMR (500MHz, CDCl$_3$) $\delta$ 7.66 - 7.58 (m, 1H), 7.51 (d, $J$ = 7.8 Hz, 1H), 7.47 (t, $J$ = 8.8 Hz, 1H), 7.20 (d, $J$ = 8.8 Hz, 1H), 7.14 (d, $J$ = 8.8 Hz, 1H), 7.09 - 7.02 (m, 2H), 3.33 - 3.22 (m, 1H), 2.27 - 2.15 (m, 1H), 1.83 - 1.71 (m, 1H), 1.56 (s, 3H), 1.11 (t, $J$ = 7.6 Hz, 3H) HRMS for C$_{18}$H$_{16}$F$_3$NO$_4$S Calculated Mass: 399.0752 Measured Mass: 399.0751

3-(2-cyano-3-(trifluoromethyl)phenoxy)phenyl pentane-2-sulfonate (AM743, 6c)

The synthesis was carried out as described for 5b, using 4 (80 mg, 0.28 mmol) in anhydrous DCM (5.0 mL), TBAB (46.2 mg, 0.13 mmol), 45% aqueous NaOH solution (0.4 mL), pentane-2-sulfonyl chloride (57.2 mg, 0.31 mmol) in anhydrous DCM (1.5 mL). The crude was purified by flash column chromatography on silica gel (10% → 30% ethyl acetate in hexanes) to give the title compound as oil (79 mg; 67%). $^1$H NMR (500MHz, CDCl$_3$) $\delta$ 7.66 - 7.58 (m, 1H), 7.51 (d, $J$ = 7.8 Hz, 1H), 7.47 (t, $J$ = 8.5 Hz, 1H), 7.20 (d, $J$ = 8.3 Hz, 1H), 7.14 (d, $J$ = 8.8 Hz, 1H), 7.09 - 7.03 (m, 2H), 3.40 - 3.27 (m, 1H), 2.18 - 2.05 (m, 1H), 1.78 - 1.65 (m, $J$ = 4.9, 9.6, 9.6, 14.1 Hz, 1H), 1.66 - 1.57 (m, 1H), 1.56 (s, 3H), 1.50 - 1.37 (m, 1H), 0.99 (t, $J$ = 7.3 Hz, 3H)

3-(2-cyano-3-(trifluoromethyl)phenoxy)phenyl cyclopropanesulfonate (AM7545)

The synthesis was carried out as described for 5b, using 4 (80 mg, 0.28 mmol) in anhydrous DCM (5.0 mL), TBAB (46.2 mg, 0.13 mmol), 45% aqueous NaOH solution (0.4 mL) and a solution of cyclopropane sulfonyl chloride (44.2 mg, 0.31 mmol) in anhydrous DCM (1.5mL). The crude was purified by flash column chromatography on silica gel (10% → 30% ethyl acetate in hexanes) to give the title compound as oil (85 mg; 78%). $^1$H NMR (500MHz, CDCl$_3$) $\delta$ 7.67 -
To a solution of 4 (100 mg, 0.3 mmol) in anhydrous DCM (10 mL) was added DIPEA (50.9 mg, 0.39 mmol) and cooled to -5°C. A solution of valeryl chloride (64.8 mg, 0.5 mmol) in anhydrous DCM (5 mL) was added through cannula to the reaction mixture and stirred at room temperature for 2 h and then poured into water. The organic layer was separated and the aqueous phase extracted with diethyl ether (2x). The combined organic layer was washed with brine, dried (MgSO₄) and concentrated under reduced pressure. The crude was purified by flash column chromatography (10% diethyl ether in hexanes) to give the title compound as an off white solid (124 mg; 96%) mp. 38-40°C. ¹H NMR (500MHz, CDCl₃) δ 7.59 (t, 8.5 Hz, 1H), 7.47 (d, J = 8.0 Hz, 1H), 7.44 (t, J = 8.0 Hz, 1H), 7.13 (d, J = 8.5 Hz, 1H), 7.02 (ddd, J = 8.0 Hz, J = 2.0 Hz, J = 1.0 Hz, 1H), 6.99 (ddd, J = 8.5 Hz, J = 2.5 Hz, J = 1.0 Hz, 1H), 6.90 (t, J = 2.5 Hz, 1H), 2.56 (t, J = 7.5 Hz, 2H), 1.73 (quint, J = 7.5 Hz, 2H), 1.44 (sxt, J = 8.0 Hz, 2H), 0.97 (t, J = 7.5 Hz, 3H, CH₃) HRMS (EI) for C₁₉H₁₆F₃NO₃  Calculated Mass:363.10823  Found Mass:363.10928

3-(2-Cyano-3-(trifluoromethyl)phenoxy)phenyl butyrate (AM7509, 7b)

The synthesis was carried out as described for 7a using 4 (100 mg,0.3 mmol), DIPEA (50.9 mg, 0.39 mmol) and butyrl chloride (57 mg, 0.5 mmol) in anhydrous DCM (10 mL). The crude was purified by flash column chromatography (20% diethyl ether in hexanes) to give the title
compound as an off white solid (111 mg; 89%) mp. 50-52\(^{\circ}\)C. \(^1\)H NMR (500MHz, CDCl\(_3\)) \(\delta\) 7.59 (t, \(J = 8.5\) Hz, 1H), 7.48 (d, \(J = 8.0\) Hz, 1H), 7.44 (t, \(J = 8.0\) Hz, 1H), 7.14 (d, \(J = 9.0\) Hz, 1H), 7.02 (dd, \(J = 8.0\) Hz, \(J = 1.0\) Hz, 1H), 6.99 (dd, \(J = 8.5\) Hz, \(J = 1.0\) Hz, 1H), 6.91 (t, \(J = 2.0\) Hz, 1H), 2.54 (t, \(J = 7.0\) Hz, 2H), 1.78 (sxt, \(J = 7.4\) Hz, 2H), 1.04 (t, \(J = 7.5\) Hz, 3H, CH\(_3\)) HRMS (EI) for C\(_{18}\)H\(_{14}\)F\(_3\)NO\(_3\) Calculated Mass: 349.09258 Found Mass: 349.09138

**3-(2-cyano-3-(trifluoromethyl)phenoxy)phenyl ethyl carbonate (AM7518, 8a)**

To a solution of 4 (100 mg, 0.1 mmol) in anhydrous toluene (10 mL) was added NEt\(_3\) (0.06 mL, 0.43 mmol) and ethyl chloroformate (46 mg, 0.43 mmol) and stirred at room temperature for 5 min and then poured in water. The organic layer was separated and the aqueous phase extracted with diethyl ether (2x). The combined organic layer was washed with brine, dried (MgSO\(_4\)) and concentrated under reduced pressure. The crude was purified by flash column chromatography on silica gel (10% \(\rightarrow\) 20% diethyl ether in hexanes) to give the title compound as an off white solid (100 mg; 80%). \(^1\)H NMR (500 MHz, CDCl\(_3\)) \(\delta\) 7.59 (t, \(J = 8.0\) Hz, 1H), 7.48 (d, \(J = 8.0\) Hz, 1H), 7.45 (t, \(J = 7.0\) Hz, 1H), 7.13 (ddd, \(J = 8.5\) Hz, \(J = 2.5\) Hz, \(J = 1.0\) Hz, 2H), 7.02 (ddd, \(J = 8.0\) Hz, \(J = 2.5\) Hz, \(J = 1.0\) Hz, 2H), 4.33 (q, \(J = 7.0\) Hz, 2H, CH\(_2\)), 1.39 (t, \(J = 7.0\) Hz, 3H, CH\(_3\)) HRMS (EI) for C\(_{17}\)H\(_{12}\)F\(_3\)NO\(_4\) Calculated Mass: 351.07184 Found Mass: 351.07136

**3-(2-cyano-3-(trifluoromethyl)phenoxy)phenyl isobutyl carbonate (AM7519, 8b)**

The synthesis was carried out as described for 8a using 4 (100 mg, 0.1 mmol), NEt\(_3\) (0.06 mL, 0.43 mmol) and isobutyl chloroformate (58 mg, 0.43 mmol) in anhydrous toluene (10 mL) The crude was purified by flash column chromatography (7% \(\rightarrow\) 15% diethyl ether in hexanes) to give the title compound as a white solid (81 mg; 60%). \(^1\)H NMR (500 MHz, CDCl\(_3\)) \(\delta\) 7.60 (t, \(J
To a solution of 4 (100 mg, 0.3 mmol) in anhydrous DCM (5 mL), cooled to 0°C, was added triphosgene (105 mg, 0.3 mmol) and NEt₃ (0.25 mL, 1.7 mmol) dropwise via syringe and allowed to stir. After 1 h, propylamine (25 mg, 0.4 mmol) was added and the reaction mixture was allowed to stir for 4 hr and poured into water. The organic layer was separated and the aqueous phase extracted with diethyl ether (2x). The combined organic layer was washed with brine, dried (MgSO₄) and concentrated under reduced pressure. The crude was purified by flash column chromatography on silica gel (20% diethyl ether in hexanes) to give the title compound as a white solid (49 mg; 38%). ¹H NMR (500 MHz, CDCl₃) δ HRMS (ESI) for C₁₉H₁₆F₃NO₄ Calculated Mass: 379.10314 Found Mass: 379.10351

3-(2-cyano-3-(trifluoromethyl)phenoxy)phenyl propylcarbamate (AM7517, 9a)

The synthesis was carried out as described for 9a using 4 (25 mg, 0.1 mmol), triphosgene (27 mg, 0.1 mmol), NEt₃ (0.07 mL, 0.5 mmol) and n-butylamine (7.8 mg, 0.1 mmol) in anhydrous DCM (5 mL). The crude was purified by flash column chromatography on silica gel (30% → 50% diethyl ether in hexanes) to give the title compound as a clear oil (9.8 mg; 29%). ¹H NMR (500 MHz, CDCl₃) δ 7.58 (t, J = 7.5 Hz, 1H), 7.46 (d, J = 7.8 Hz, 1H), 7.37 - 7.44 (m, 1H), 7.13 (d, J = 8.7 Hz, 1H), 7.07 (dd, J = 8.3 Hz, J = 2.0 Hz, J = 1.0 Hz, 1H), 6.91 - 6.99 (m,
2H), 5.02 (br. s., 1H), 3.27 (q, J = 6.84 Hz, 2H), 1.45 - 1.34 (m, 2H), 1.30 - 1.16 (m, 2H), 0.95 (t, J = 7.3 Hz, 3H, CH₃) HRMS (EI) for C₁₉H₁₇F₃N₂O₃ Calculated Mass:378.1191 Found Mass: 378.1191

3-(2-cyano-3-(trifluoromethyl)phenoxy)phenyl pentyloxycarbamate (AM7524, 9c)
The synthesis was carried out as described for 9a using 4 (30 mg, 0.1 mmol), triphosgene (31.9 mg, 0.1 mmol), NEt₃ (0.07 mL, 0.5 mmol), and amylamine (0.01 mL, 0.12 mmol) in anhydrous DCM (5 mL) The crude was purified by flash column chromatography on silica gel (20% diethyl ether in hexanes) to give the title compound as a white solid (10 mg; 24%). ¹H NMR (500 MHz,CDCl₃) δ 7.58 (t, J = 7.5 Hz, 1H), 7.46 (d, J = 7.8 Hz, 1H), 7.41 (t, J = 8.5 Hz, 1H), 7.13 (d, J = 8.7 Hz, 1H), 7.07 (dd, J = 8.0, J = 2.0 Hz, J = 1.0 Hz, 1H), 7.00 – 6.90 (m, 2 H), 5.03 (br. s., 1H), 3.26 (q, J = 6.8 Hz, 2H), 1.58 (d, J = 6.8 Hz, 2H), 1.30 - 1.40 (m, 4H), 0.92 (t, J = 6.84 Hz, 3H, CH₃)

2-(3-(4-cyanobutoxy)phenoxy)-6-(trifluoromethyl)benzonitrile (AM7520, 10a)
In an oven dried microwave reaction vessel, a solution of 4 (50 mg, 0.17 mmol) in acetonitrile (1.5 mL) was added potassium carbonate (29 mg, 0.21 mmol), 5-bromopentanenitrile (34 mg, 0.21 mmol) and subjected to microwave irradiation for 15 min. at 160ºC. Reaction mixture was cooled and then poured into water. The organic layer was separated and the aqueous phase extracted with diethyl ether (2x). The combined organic layer was washed with brine, dried (MgSO₄) and concentrated under reduced pressure. The crude was purified by flash column chromatography on silica gel (7% → 15% diethyl ether in hexanes) to give the title compound as an oil (45 mg; 70%). ¹H NMR (500 MHz,CDCl₃) δ 7.58 (t, J = 8.0 Hz, 1 H), 7.46 (d, J = 7.8
Hz, 1H), 7.33 (t, $J = 8.3$ Hz, 1H), 7.10 (d, $J = 8.3$ Hz, 1H), 6.80 (dd, $J = 8.3$ Hz, $J = 1.9$ Hz, 1H), 6.69 (ddd, $J = 7.8$ Hz, $J = 3.0$ Hz, $J = 1.0$ Hz, 1H), 6.65 (t, $J = 2.0$ Hz, 1H), 4.01 (t, $J = 5.6$ Hz, 2H), 2.46 (t, $J = 7.0$ Hz, 2H), 2.00 – 1.92 (m, 2H), 1.92 - 1.83 (m, 2H) HRMS (EI) for C$_{19}$H$_{15}$F$_3$N$_2$O$_2$ Calculated Mass: 360.10857 Found Mass: 360.10851

2-(3-(hexyloxy)phenoxy)-6-(trifluoromethyl)benzonitrile (AM7521, 10b)

The synthesis was carried out as described for 10a using 4 (50 mg, 0.17 mmol), potassium carbonate (29 mg, 0.21 mmol) and 1-bromohexane (35 mg, 0.21 mmol) in anhydrous acetonitrile (1.5 mL). The crude was purified by flash column chromatography on silica gel (7% → 15% diethyl ether in hexanes) to give the title compound as a white solid (45 mg; 69%). $^1$H NMR (500 MHz,CDCl$_3$) δ 7.57 (t, $J = 8.0$ Hz, 1H), 7.44 (d, $J = 7.5$ Hz, 1H), 7.31 (t, $J = 8.5$ Hz, 1H), 7.09 (d, $J = 8.5$ Hz, 1H), 6.80 (dd, $J = 8.0$ Hz, $J = 2.5$ Hz, 1H), 6.68 (ddd, $J = 8.0$ Hz, $J = 3.0$ Hz, $J = 1.0$Hz, 1H), 6.64 (t, $J = 2.0$ Hz, 1H), 4.00 (t, $J = 5.5$ Hz, 2H), 2.45 (d, $J = 2.5$ Hz, 2H), 1.84 - 1.73 (m, 2H), 1.51 - 1.39 (m, 2H), 1.38 - 1.28 (m, 4H), 0.96 - 0.85 (m, 3H, CH$_3$)

2-(3-(heptan-2-yloxy)phenoxy)-6-(trifluoromethyl)benzonitrile (AM7522, 10c)

The synthesis was carried out as described for 10a using 4 (50 mg, 0.17 mmol), potassium carbonate (29 mg, 0.21 mmol) and 2-bromooctane (41.5 mg, 0.21 mmol) in anhydrous acetonitrile (1.5 mL). The crude was purified by flash column chromatography on silica gel (15% → 25% diethyl ether in hexanes) to give the title compound as a clear oil (45 mg; 67%). $^1$H NMR (500 MHz,CDCl$_3$) δ 7.56 (t, $J = 8.0$ Hz, 1H), 7.43 (d, $J = 8.0$ Hz, 1H), 7.30 (t, $J = 8.5$ Hz, 1H), 7.10 (d, $J = 8.0$ Hz, 1H), 6.78 (dd, $J = 8.0$ Hz $J = 1.0$ Hz, 1H), 6.64 (dt, $J = 8.0$ Hz $J =
2.0 Hz, 2H), 4.34 (sxt, J = 6.0 Hz, 1H), 1.40 – 1.25 (m, 11H), 0.88 (t, J = 7.0 Hz, 3H, CH₃)

HRMS (EI) for C₂₁H₂₂F₃NO₂ Calculated Mass: 377.1603 Found Mass: 377.1601

2-(3-(6,6,6-trifluorohexyloxy)phenoxy)-6-(trifluoromethyl)benzonitrile (AM7523, 10d)

The synthesis was carried out as described for 10a using 4 (50 mg, 0.17 mmol), potassium carbonate (29 mg, 0.21 mmol) and 6-bromo-1,1,1-trifluorohexane (46.8 mg, 0.21 mmol) in acetonitrile (1.5 mL). The crude was purified by flash column chromatography on silica gel (15% → 25% diethyl ether in hexanes) to give the title compound as solid (10 mg; 14%). ¹H NMR (500 MHz, CDCl₃) δ 7.56 (t, J = 8.3 Hz, 1H), 7.45 (d, J = 7.3 Hz, 1H), 7.32 (t, J = 8.3 Hz, 1H), 7.09 (d, J = 8.3 Hz, 1H), 6.80 (dd, J = 8.3 J = 1.9 Hz, 1H), 6.68 (ddd, J = 8.0 Hz, J = 2.5 Hz, J = 1.0 Hz, 1H), 6.64 (t, J = 2.5 Hz, 1H), 3.96 (t, J = 6.3 Hz, 2H), 2.18 – 2.03 (m, 2H), 1.88 - 1.76 (m, 2H), 1.70 - 1.60 (m, 2H), 1.57 – 1.50 (m, 2H) HRMS (EI) for C₂₀H₁₇F₆NO₂ Calculated Mass: 417.11633 Found Mass: 417.11623

(2-chloro-3-(trifluoromethyl)phenyl)(3-methoxyphenyl)methanone (13)

To a solution of 11 (100 mg, 0.48 mmol) and (3-methoxyphenyl)magnesium bromide (0.73 mL, 0.73 mmol) in anhydrous THF (5.0 mL) was added Cu(I)Br (6.9 mg, 0.048 mmol) and refluxed for 4h. Reaction mixture was quenched with 15% aqueous H₂SO₄ solution and extracted with ethyl acetate (2x). The combined organic layer was washed with brine, dried (MgSO₄) and concentrated under reduced pressure. The crude was purified by flash column chromatography (0% → 5% ethyl acetate in hexanes) to give the title compound (90 mg; 58%). ¹H NMR (500 MHz, CDCl₃) δ 7.90 (d, J = 8.0 Hz, 1H), 7.80 (d, J = 8.0 Hz, 1H), 7.63 (t, J = 8.0 Hz, 1H), 7.41
(t, J = 8.0 Hz, 1H), 7.35 (t, J = 3.0 Hz, 1H), 7.30 (dt, J = 7.5 Hz J = 1.0 Hz, 1H), 7.17 (ddd, J = 8.5 Hz J = 3.0 Hz J = 1.0 Hz, 1H) 3.88 (s, 3H)

2-(2-chloro-3-(trifluoromethyl)phenyl)-2-(3-methoxyphenyl)-1,3-dithiolane (14)

To a solution of 13 (100 mg, 0.3 mmol) in anhydrous DCM (3.0 mL) was added ethane-1,2-dithiolane (0.04 mL, 0.47 mmol) and cooled to 0°C. Then BF$_3$OEt$_3$ (0.02 mL, 0.15 mmol) was added and warmed to room temperature for 3 h. Reaction quenched with sat. NaHCO$_3$ and extracted with diethyl ether (2x). The combined organic layer was washed with brine, dried (MgSO$_4$) and concentrated under reduced pressure. The crude was purified by flash column chromatography (0% → 7% ethyl acetate in hexanes) to give the title compound (77 mg; 62%).

$^1$H NMR (500 MHz, CDCl$_3$) δ 7.71 (d, J = 7.5 Hz, 1H), 7.48 (d, J = 7.5 Hz, 1H), 7.38 (t, J = 8.0 Hz, 1H), 7.21 (t, J = 8.0 Hz, 1H), 7.18 (t, J = 1.5 Hz, 1H), 7.10 (dt, J = 8.0 Hz J = 1.0 Hz, 1H), 6.80 (ddd, J = 8.5 Hz J = 2.5 Hz J = 1.0 Hz, 1H), 3.78 (s, 3H, OCH$_3$), 3.40 (m, 4H)

3-(2-(2-chloro-3-(trifluoromethyl)phenyl)-1,3-dithiolan-2-yl)phenol (15)

The synthesis was carried out as described for 4 using 14 (100 mg, 0.25 mmol) TBAI (142 mg, 0.38 mmol) and BCl$_3$ (0.38 mL, 0.38 mmol, 1.0 M soln in DCM) in anhydrous DCM (5 mL). The crude was purified by flash column chromatography (10% → 20% ethyl acetate in hexanes) to give the title compound (80 mg, 83%).

$^1$H NMR (500 MHz, CDCl$_3$) δ 7.71 (d, J = 7.5 Hz, 1H), 7.50 (d, J = 7.5 Hz, 1H), 7.38 (t, J = 8.5 Hz, 1H), 7.17 (t, J = 8.5 Hz, 1H), 7.10 (m, 2H), 6.72 (ddd, J = 8.0 Hz J = 2.5 Hz J = 1.0 Hz, 1H), 4.78 (s, 1H), 3.43 (m, 4H)
3-(2-chloro-3-(trifluoromethyl)benzoyl)phenyl 4,4,4-trifluorobutane-1-sulfonate (AM7513, 16)

The synthesis was carried out as described for 5b using 15 (100mg, 0.26 mmol), TBAB (43 mg, 0.13 mmol), 45% aqueous NaOH solution (0.5 mL), and 4,4,4-trifluorobutane-1-sulfonyl chloride (61 mg, 0.29mmol) in anhydrous DCM (3.0 mL). The crude was purified by flash column chromatography on silica gel (15% → 20% ethyl acetate in hexanes) to give the title compound (93 mg, 74%).

1H NMR (500 MHz, CDCl$_3$) δ 7.98 (d, $J = 8.0$ Hz, 1H), 7.88 (d, $J = 8.0$ Hz, 1H), 7.75 (dt, $J = 2.5$ Hz $J = 1.5$ Hz, 1H), 7.69 (t, $J = 2.0$ Hz, 1H), 7.66 (t, $J = 8.0$ Hz, 1H), 7.59 (t, $J = 8.0$ Hz, 1H), 7.54 (ddd, $J = 8.5$ Hz $J = 2.5$ Hz $J = 1.0$ Hz, 1H), 3.40 (t, $J = 7.5$ Hz, 2H), 2.42 – 2.35 (m, 2H), 2.35 – 2.25 (m, 2H)

HRMS (EI) for C$_{18}$H$_{13}$O$_4$F$_6$SCl Calculated Mass: 474.01271 Measured Mass: 474.01122

3-methoxybenzoyl chloride (18)

To a solution of 3-methoxybenzoic acid (mg, mmol) in anhydrous DCM (mL) cooled to 0°C was added oxalyl chloride (mL, mmol) and stirred for 3h. The solvent was evaporated under reduced pressure to give the title compound as a liquid.

1H NMR (500 MHz, CDCl$_3$) δ

2-(3-methoxybenzoyl)-6-(trifluoromethyl)benzonitrile (20)

In a flame dried 50mL round bottom flask, under static N$_2$ atmosphere, was added 2,2,6,6-tetramethyl piperidine (0.23 mL, 1.4 mmol) in anhydrous THF (20 mL) and cooled to -10°C. Then n-BuLi (0.87 mL, 1.4 mmol) was added and stirred at -10°C for 15min, then cooled to -78°C. Then 2-(trifluoromethyl)benzonitrile (19) (200 mg, 1.1 mmol) in anhydrous THF (5.0 mL) was added to the reaction mixture and stirred for 30 min. Then 18 (410 mg, 1.5 mmol) in
anhydrous THF (5.0 mL) was added. Reaction was slowly warmed to room temperature overnight. Reaction mixture was poured into water and extracted with ethyl acetate (3x). The combined organic layer was washed with brine, dried (MgSO₄) and concentrated under reduced pressure. The crude product was purified by flash column chromatography on silica gel (0% → 10% ethyl acetate in hexanes) to give the title compound (mg; %). ¹H NMR (500 MHz, CDCl₃) δ

3-(tert-butyl-dimethylsilyloxy)benzoic acid (23)

To a solution of 3-hydroxybenzoic acid (22) (1.0 g, 7.2 mmol) in anhydrous DMF (40 mL) was added NaH (869 mg, 18.1 mmol, 60% dispersion in mineral oil) and stirred at room temperature for 30 min. A solution of TBSCl (2.53 mg, 18.1 mmol) in anhydrous DMF (10 mL) was then added to the reaction mixture and stirred for an additional 3 hours. The reaction mixture was quenched with 1N HCl and extracted with ethyl acetate (2x). The combined organic layer was washed with half-saturated brine, dried (MgSO₄) and concentrated under reduced pressure. The crude was purified by flash column chromatography on silica gel (7% → 30% ethyl acetate in hexanes) to give the title compound as a white solid (1.71 g; 94%). ¹H NMR (500 MHz, CDCl₃) δ 7.72 (dt, J = 7.5 Hz, J = 1.5 Hz, 1H), 7.57 (t, J = 2.5 Hz, 1H), 7.33 (t, J = 8.0 Hz, 1H), 7.09 (ddd, J = 8.0 Hz, J = 2.0 Hz, J = 1.0 Hz, 1H), 1.00 (s, 9H, Si(CH₃)₃), 0.23 (s, 6H, Si(CH₃)₂)

3-(tert-butyl-dimethylsilyloxy)benzoyl chloride (24)

To a solution of 23 (800 mg, 3.1 mmol) in anhydrous DCM (30 mL), cooled to 0°C, was added oxalyl chloride (0.41 mL, 4.7 mmol). After 10 min., the reaction was removed from ice bath and allowed to stir at room temperature for 3h. The solvent was evaporated under reduced pressure
to give the title compound as a liquid. $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 7.73 (dd, $J = 8.0$ Hz, $J = 1.0$ Hz, 1H), 7.55 (t, $J = 2.5$ Hz, 1H), 7.36 (t, $J = 8.0$ Hz, 1H), 7.15 (ddd, $J = 7.5$ Hz, $J = 2.5$ Hz, $J = 1.0$ Hz, 2H), 0.98 (s, 9H, Si(CH$_3$)$_3$), 0.22 (s, 6H, Si(CH$_3$)$_2$).

2-(3-(tert-butyldimethylsilyloxy)benzoyl)-6-(trifluoromethyl)benzonitrile (25)

In a flame dried 50mL round bottom flask, under static N$_2$ atmosphere, was added 2,2,6,6-tetramethyl piperidine (0.23 mL, 1.4 mmol) in anhydrous THF (20 mL) and cooled to -10°C. Then n-BuLi (0.87 mL, 1.4 mmol) was added and stirred at -10°C for 15 min, then cooled to -78°C. Then 2-(trifluoromethyl)benzonitrile (19) (200 mg, 1.1 mmol) in anhydrous THF (5.0 mL) was added to the reaction mixture and stirred for 30 min. Then 3,4-bis(tert-butyldimethylsilyloxy)benzoyl chloride (24) (410 mg, 1.5 mmol) in anhydrous THF (5.0 mL) was added. Reaction was slowly warmed to room temperature overnight. Reaction mixture was poured into water and extracted with ethyl acetate (3x). The combined organic layer was washed with brine, dried (MgSO$_4$) and concentrated under reduced pressure. The crude product was purified by flash column chromatography on silica gel (0% $\rightarrow$ 10% ethyl acetate in hexanes) to give the title compound as a pale yellow solid (127 mg; 27%). $^1$H NMR (500 Hz, CDCl$_3$) $\delta$ 7.97 (d, $J = 7.8$ Hz, 1H), 7.83 (t, $J = 8.0$ Hz, 1H), 7.89 (d, $J = 7.5$ Hz, 1H), 7.28 - 7.32 (m, 2H), 7.15 (ddd, $J = 8.3$ Hz, $J = 2.0$ Hz, $J = 0.5$ Hz, 1H), 0.98 (s, 9H, Si(CH$_3$)$_3$), 0.22 (s, 6H, Si(CH$_3$)$_2$). HRMS (ESI) Calculated mass for C$_{21}$H$_{23}$NO$_2$F$_3$Si: 406.1450 Measured mass: 406.1451

2-(3-hydroxybenzoyl)-6-(trifluoromethyl)benzonitrile (21)

To a solution of 25 (500 mg, 1.2 mmol) in anhydrous THF (25 mL), cooled to 0°C was added TBAF (0.5 mL, 0.5 mmol) and stirred for 1 h. Reaction mixture was quenched with saturated
aqueous NaHCO₃ solution and extracted with ethyl acetate (2x). The combined organic layer was washed with brine, dried (MgSO₄) and concentrated under reduced pressure. The crude was purified by flash column chromatography on silica gel (15% → 60% ethyl acetate in hexanes) to give the title compound as a yellow solid (301 mg; 84%). ¹H NMR (500 MHz, CDCl₃) δ 7.97 (d, J = 7.8 Hz, 1H), 7.84 (d, J = 8.0 Hz, 1H), 7.80 (t, J = 8.0 Hz, 1H), 7.31 - 7.41 (m, 2H), 7.25 (d, J = 7.8 Hz, 1H), 7.16 (dd, J = 8.3 Hz, J = 2.4 Hz, 1H).

3-(2-cyano-3-(trifluoromethyl)benzoyl)phenyl 4,4,4-trifluorobutane-1-sulfonate (AM7534, 26)

The synthesis was carried out as described for 5b using 21 (100 mg, 0.34 mmol), TBAB (55.3 mg, 0.17 mmol), 45% NaOH solution (0.5 mL), and 4,4,4-trifluorobutan-1-sulfonyl chloride (86 mg, 0.41 mmol) in anhydrous DCM (10 mL). The crude was purified by flash column chromatography on silica gel (20% → 40% ethyl acetate in hexanes) to give the title compound as a yellow oil (70 mg; 44%). ¹H NMR (500 MHz, CDCl₃) δ 7.74 (d, J = 7.8 Hz, 1H), 7.64 (t, J = 7.8 Hz, 1H), 7.60 (s, 1H), 7.55 (d, J = 7.3 Hz, 1H), 7.44 - 7.36 (m, 2H), 7.22 (s, 1H), 3.37 (t, J = 7.3 Hz, 2H, CH₂), 2.42 - 2.29 (m, 2H, CH₂), 2.20 - 2.30 (m, 2H, CH₂) HRMS (EI) for C₁₉H₁₃F₆NO₄S Calculated Mass:465.04693 Found Mass:465.04744

1-(bromomethyl)-3-methoxybenzene (28)

To a solution of 3-methoxybenzyl alcohol (27) (300 mg, 2.1 mmol) in anhydrous DCM (20 mL) was added phosphorus tribromide (0.42 mL, 2.1 mmol) and stirred at room temperature for 2 hours and then poured in water and extracted with ethyl acetate (2x). The combined organic layer was washed with sat. aqueous NaHCO₃ solution, dried (MgSO₄) and concentrated under reduced pressure. The crude was purified by flash column chromatography on silica gel (0% →
20% ethyl acetate in hexanes) to give the title compound as oil (388 mg; 89%). 1H NMR (500 MHz, CDCl3) δ 7.25 (t, J = 7.5 Hz, 1H), 6.97 (d, J = 7.5 Hz, 1H), 6.92 (t, J = 2.5 Hz, 1H), 6.84 (dd, J = 8.5 J = 2.5 Hz, 1H), 4.47 (s, 2H, CH2), 3.82 (s, 3H, OMe) HRMS (EI) for C8H9OBr
Calculated Mass: 199.98368 Measured Mass: 199.98485

2-(3-methoxybenzyl)-6-(trifluoromethyl)benzonitrile (30)
In an oven dried microwave reaction vessel, a solution of 28 (213 mg, 1.05 mmol) in DME (3mL)/H2O(0.75mL) was added 2-cyano-3(trifluoromethyl)phenyl boronic acid neopentyl glycol ester (29) (200 mg, 0.7 mmol) and degassed with N2 for 10min. Then Pd(PPh3)4 (81.6 mg, 0.07 mmol) and Ba(OH)2•8H2O (445 mg, 1.41 mmol) was added and degassed with N2 for 5min. and subjected to microwave irradiation for 15min. at 120ºC. The reaction was poured in water and extracted with ethyl acetate (2x). The combined organic layer was washed with brine, dried (MgSO4) and concentrated under reduced pressure. The crude was purified by flash column chromatography on silica gel (0% → 20% ethyl acetate in hexanes) to give the title compound as a white solid (144 mg; 70%). 1H NMR (500 MHz, CDCl3) δ 7.65 (d, J = 8.0 Hz, 1H), 7.60 (t, J = 8.0 Hz, 1H), 7.47 (d, J = 8.0 Hz, 1H), 7.25 (t, J = 7.5 Hz, 1H), 6.82 (d, J = 8.5 Hz, 1H), 6.78 (d, J = 11.0 Hz, 1H), 4.27 (s, 2H, CH2) 3.79 (s, 3H, OCH3) HRMS (ESI) Calculated mass for C16H13NOF3: 292.0949 Measured mass: 292.0949

2-(3-hydroxybenzyl)-6-(trifluoromethyl)benzonitrile (31)
The synthesis was carried out as described as for 4, using 30 (130 mg, 0.44 mmol), TBAI (329 mg, 0.89 mmol) and BCl3 (0.89 mL, 0.89 mmol, 1.0M soln in DCM) in anhydrous DCM (5 mL). The crude was purified by flash column chromatography (20% → 50% ethyl acetate in hexanes) to give the title compound as a brown solid (113 mg; 91%). 1H NMR (500 MHz, CDCl3) δ 7.65
(d, J = 7.5 Hz, 1H), 7.61 (t, J = 7.5 Hz, 1H), 7.49 (d, J = 7.5 Hz, 1H), 7.20 (t, J = 8.0 Hz, 1H),
6.81 (d, J = 7.0 Hz, 1H), 6.73 (dd, J = 8.5 Hz J = 2.5 Hz, 1H) 6.71 (t, J = 2.0 Hz, 1H), 4.87 (br.
s., 1H, OH), 4.25 (s, 2H, CH₂) HRMS (ESI) Calculated mass for C₁₅H₁₁NOF₃: 278.0793
Measured mass: 278.0795

3-(2-cyano-3-(trifluoromethyl)benzyl)phenyl 4,4,4-trifluorobutane-1-sulfonate (AM7539, 32)
The synthesis was carried out as described for 5b using 31 (50 mg, 0.18 mmol), TBAB (29 mg,
0.09 mmol), 45% NaOH (0.5 mL) and 4,4,4-trifluorobutane-sulfonyl chloride (38 mg, 0.21
mmol) in anhydrous DCM (5 mL). The crude was purified by flash column chromatography
(20% to 50% ethyl acetate in hexanes) to give the title compound as a yellow oil (76 mg; 93%).
¹H NMR (500 MHz, CDCl₃) δ 7.62 - 7.73 (m, 2H), 7.52 (d, J = 7.8 Hz, 1H), 7.35 - 7.43 (m, 1H),
7.19 (dd, J = 7.0 Hz, J = 4.1 Hz, 2H), 7.16 (s, 1H), 3.34 (t, J = 7.3 Hz, 2H, CH₂), 2.30 - 2.41 (m,
2H, CH₂), 2.20 - 2.30 (m, 2H, CH₂)

5'-methoxy-3-(trifluoromethyl)biphenyl-2-carbonitrile (34)
The synthesis was carried out as described as for 30, using 3-bromoanisole (33) (145 mg, 0.77
mmol), 2-cyano-3(trifluoromethyl)phenyl boronic acid neopentyl glycol ester (200 mg, 0.7
mmol), Pd(PPh₃)₄ (81.6 mg, 0.07 mmol) and Ba(OH)₂•8 H₂O (222 mg, 0.7 mmol) in DME
(3mL)/H₂O (0.75 mL), The crude was purified by flash column chromatography (0% → 20%
ethyl acetate in hexanes) to give the title compound as a white solid (113 mg; 58%). ¹H NMR
(500 MHz, CDCl₃) δ 7.80 (d, J = 7.0 Hz, 1H), 7.75 (t, J = 7.8 Hz, 1H), 7.71 (d, J = 7.0 Hz, 1H),
7.43 (t, J = 7.8 Hz, 1H), 7.11 (d, J = 7.8 Hz, 1H), 7.06 (t, J = 1.5 Hz, 1H), 7.05 - 7.01 (m, 1H),
3.88 (s, 3H, OMe) HRMS (ESI) C_{15}H_{11}NOF_{3} Calculated Mass: 278.0793 Measured Mass: 278.0792

5'-hydroxy-3-(trifluoromethyl)biphenyl-2-carbonitrile (35)
The synthesis was carried out as described as for 4, using 34 (100 mg, 0.3 mmol), TBAI (199 mg, 0.5 mmol) and BCl_{3} (0.5 mL, 0.5 mmol) in anhydrous DCM (5 mL). The crude was purified by flash column chromatography on silica gel (10% ethyl acetate in hexanes) to give the title compound as a clear oil (80 mg; 85%). ^{1}H NMR (500 MHz, CDCl_{3}) δ 7.80 (d, J = 6.5 Hz, 1H), 7.75 (t, J = 7.8 Hz, 1H), 7.72 - 7.67 (m, 1H), 7.39 (t, J = 7.8 Hz, 1H), 7.10 (d, J = 7.8 Hz, 1H), 7.00 (s, 1H), 6.97 (d, J = 8.3 Hz, 1H), 4.98 (s, 1H, OH) HRMS (ESI) Calculated mass for C_{14}H_{9}NOF_{3}: 264.0636 Measured mass: 264.0634

6'-cyano-5'--(trifluoromethyl)biphenyl-3-yl 4,4,4-trifluorobutane-1-sulfonate (AM7552, 36)
The synthesis was carried out as described for 5b using 35 (100 mg, 0.38 mmol), TBAB (61 mg, 0.19 mmol), 45% NaOH (0.5 mL) and 4,4,4-trifluorobutane-sulfonyl chloride (96 mg, 0.45 mmol) in anhydrous DCM (5 mL). The crude was purified by flash column chromatography (20% → 50% ethyl acetate in hexanes) to give the title compound as a yellow oil (99 mg; 60%). ^{1}H NMR (500 MHz, CDCl_{3}) δ 7.85 (d, J = 7.0 Hz, 1H), 7.81 (t, J = 8.0 Hz, 1H), 7.73 (d, J = 7.8 Hz, 1H), 7.60 (t, J = 8.0 Hz, 1H), 7.51 (d, J = 7.8 Hz, 1H), 7.48 (t, J = 2.0 Hz, 1H), 7.45 (ddd, J = 8.3 Hz, J = 2.5 Hz, J = 1.0 Hz, 1H), 3.42 (t, J = 7.3 Hz, 2H), 2.44 - 2.32 (m, 2H), 2.32 - 2.24 (m, 2H) HRMS (ESI) for C_{18}H_{14}F_{6}NO_{3}S Calculated Mass 438.0599 Found Mass:438.0606

2-(3,5-dimethoxyphenoxy)-6-(trifluoromethyl)benzonitrile (38)
To a solution of 3,5-dimethoxyphenol (37) (120 mg, 0.64 mmol) in anhydrous DMSO (6.0 mL) was added NaH (37 mg, 0.77 mmol, 60% dispersion in mineral oil) and nitrogen gas was bubbled through. After 5 min., 2-fluoro-6-(trifluoromethyl)benzonitrile (1) (100 mg, 0.52 mmol) in anhydrous DMSO (1 mL) was added. Reaction mixture stirred at room temperature for 3 h. Reaction was quenched with 1N HCl and extracted with ethyl acetate (2x). The combined organic layer was washed with brine, dried (MgSO₄) and concentrated under reduced pressure. The crude was purified by flash column chromatography on silica gel (0% → 10% ethyl acetate in hexanes) to give the title compound as oil (148 mg; 87%). ¹H NMR (500 MHz, CDCl₃) δ 7.54 (t, J = 7.5 Hz, 1H), 7.44 (d, J = 7.5 Hz, 1H), 7.14 (d, J = 8.5 Hz, 1H), 6.36 (t, J = 2.5 Hz, 1H), 6.25 (d, J = 2.0 Hz, 2H), 3.80 (s, 6H, OCH₃)

2-(3,5-dihydroxyphenoxy)-6-(trifluoromethyl)benzonitrile (39)

The synthesis was carried out as described as for 4, using 38 (180 mg, 0.55 mol), TBAI (513 mg, 1.4 mmol) and BCl₃ (1.4 mL, 1.4 mmol) in anhydrous DCM (5 mL). The crude was purified by flash column chromatography on silica gel (30% to 60% ethyl acetate in hexanes) to give the title compound as oil (120 mg; 73%). ¹H NMR (500 MHz,CDCl₃) δ 7.66 (t, J = 8.5 Hz, 1H), 7.47 (d, J = 9.0 Hz, 1H), 6.24 (t, J = 2.0 Hz, 1H), 6.18 (d, J = 2.0 Hz, 2H), 4.90 (s, 2H, OH)

3-(2-cyano-3-(trifluoromethyl)phenoxy)-5-hydroxyphenyl 4,4,4-trifluorobutane-1-sulfonate (AM7546, 40)

The synthesis was carried out as described for 5a, using 39 (100 mg, 0.33 mmol), TBAB (54 mg, 0.16 mmol), 45% NaOH soln (0.5 mL) and 4,4,4-trifluorobutane-1-sulfonyl chloride (64
mg, 0.30 mmol) in anhydrous DCM (10 mL). The crude product obtained after workup was purified by flash column chromatography on silica gel (30% ethyl acetate in hexanes) to give the title compound as oil (71 mg; 45%). $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 7.66 (t, $J = 8.5$ Hz, 1H), 7.55 (d, $J = 7.8$ Hz, 1H), 7.22 (d, $J = 8.3$ Hz, 1H), 6.69 (s, 1H), 6.60 (s, 1H), 6.57 (s, 1H), 5.37 (br. s., 1H, OH), 3.38 (t, $J = 7.6$ Hz, 2H, CH$_2$), 2.45 - 2.30 (m, 2H, CH$_2$), 2.30 - 2.21 (m, 2H, CH$_2$) HRMS (EI) for C$_{18}$H$_{13}$F$_6$NO$_5$S Calculated Mass: 469.0418 Found Mass: 469.0417

2-(3-hydroxy-5-pentylphenoxy)-6-(trifluoromethyl)benzonitrile (AM7556, 42)

To a solution of olivitol (50 mg, 0.27 mmol) in anhydrous DMSO (2.0 mL) was added NaH (28 mg, 0.58 mmol). After 10 min, 2-fluoro-6-(trifluoromethyl)benzonitrile (52 mg, 0.27 mmol) was added and stirred at room temperature for 5h. The reaction was poured into water and extracted with ethyl acetate (2x). The combined organic layers was washed with brine and dried (MgSO$_4$). The crude was purified by flash column chromatography using silica gel (0% $\rightarrow$ 40% ethyl acetate in hexanes) to give the title compound as and oil (40 mg; 41%). $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ HRMS (EI) for C$_{19}$H$_{18}$O$_2$NF$_3$ Calculated Mass: 319.12896 Measured Mass: 349.12880

3-(2-cyano-3-(trifluoromethyl)phenoxy)phenyl 3-azidopropane-1-sulfonate (AM7553)

To a solution of 3-(2-cyano-3-(trifluoromethyl)phenoxy)phenyl 3-chloropropane-1-sulfonate (70 mg, 0.16 mmol) in DMF (5.0 mL) was added NaN$_3$ (16 mg, 0.25 mmol) and stirred at 45°C for 2 days. Reaction poured into water and extracted with ethyl acetate (2x). The combined organic layers was washed with brine and dried (MgSO$_4$). The crude was purified by flash column chromatography using silica gel (20% $\rightarrow$ 50% ethyl acetate in hexanes) to give the title
compound as an oil (50 mg; 71%)

\[ ^1H \text{NMR (500MHz, CDCl}_3 \] \( \delta \) 7.67 - 7.61 (m, 1H), 7.53 (d, \( J = 7.8 \) Hz, 1H), 7.50 (t, \( J = 8.1 \) Hz, 1H), 7.21 (d, \( J = 7.3 \) Hz, 1H), 7.15 (d, \( J = 8.3 \) Hz, 1H), 7.11 - 7.06 (m, 2H), 3.57 (t, \( J = 6.1 \) Hz, 2H), 3.44 - 3.36 (m, 2H), 2.28 - 2.19 (m, 2H) HRMS (EI) for C\(_{17}\)H\(_{13}\)O\(_4\)N\(_4\)F\(_3\)S Calculated Mass: 426.06096 Measured Mass: 426.05928

3-(2-cyano-3-(trifluoromethyl)phenoxy)phenyl 3-isothiocyanatopropane-1-sulfonate (AM7554)

To a solution of - 3-(2-cyano-3-(trifluoromethyl)phenoxy)phenyl 3-azidopropane-1-sulfonate (40 mg, 0.09 mmol) in THF (5.0 mL) was added carbon disulfide (0.16 mL, 2.6 mmol) and triphenyphosphine (37 mg, 0.14 mmol) and stirred at room temperature for 2 days. Reaction poured into water and extracted with ethyl acetate (2x). The combined organic layers was washed with brine and dried (MgSO\(_4\)). The crude was purified by flash column chromatography using silica gel (5% \( \rightarrow \) 30% ethyl acetate in hexanes) to give the title compound as an oil (21 mg; 50%). \( ^1H \text{NMR (500MHz, CDCl}_3 \) \( \delta \) 7.65 (t, \( J = 8.1 \) Hz, 1H), 7.56 - 7.47 (m, 2 H), 7.24 - 7.19 (m, 1 H), 7.17 (d, \( J = 8.3 \) Hz, 1 H), 7.12 - 7.07 (m, 2 H), 3.82 (t, \( J = 6.8 \) Hz, 2 H) HRMS (EI) for C\(_{18}\)H\(_{14}\)F\(_3\)N\(_2\)O\(_4\)S\(_2\) Calculated Mass: 443.0347 Measured Mass: 443.0353

3-(2-cyano-3-(trifluoromethyl)phenoxy)phenyl ethenesulfonate (AM7558)

The synthesis was carried out as described for AM7550, using 4 (60 mg, 0.21 mmol) in anhydrous DCM (5.0 mL), NaH (10mg, 0.21mmol) and a solution of prop-2-ene-1-sulfonyl chloride 2-chloroethanesulfonyl chloride (35 mg, 0.21 mmol) in anhydrous DCM (1.0 mL) The crude was purified by flash column chromatography on silica gel (20% \( \rightarrow \) 30% ethyl acetate in
hexanes) to give the title compound as oil (51 mg; 65%). $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 7.59 - 7.68 (m, 1H), 7.53 (d, $J = 7.8$ Hz, 1H), 7.47 (t, $J = 8.3$ Hz, 1H), 7.17 (dd, $J = 8.3$ $J = 1.9$ Hz, 1H), 7.13 (d, $J = 8.3$ Hz, 1H), 7.07 (d, $J$=8.30 Hz, 1 H), 6.99 - 7.04 (m, 1 H), 6.68 (dd, $J = 16.6$ Hz $J = 9.7$ Hz, 1H), 6.43 (d, $J = 16.6$ Hz, 1H), 6.23 (d, $J = 10.2$ Hz, 1H) HRMS (EI) for C$_{16}$H$_{10}$O$_4$NF$_3$S Calculated Mass: 369.02826 Measured Mass: 369.02855
CHAPTER THREE:
TRICYCLIC BIARYL ETHER CANNABINIODS
3.1 Tricyclic Cannabinoid Receptor 1 (CB1) Agonist

The endocannabinoid system plays a vital role in many pathophysiological processes and includes two cannabinoid receptors (CB) receptors, CB1 and CB2, their endogenous ligands, arachidonylethanolamine (AEA, anandamide) and 2-arachidonoylglycerol (2-AG) as well as several enzymes involved in their biosynthesis and bioinactivation. CB1 and CB2 belong to class-A (rhodopsin-like) of the superfamily of G-protein coupled receptors (GPCRs). While CB1 is the most abundant GPCR in the brain and also present in several peripheral tissues (Kunos, G. 2009; Pacher, P. 2005) CB2 is mainly found in the periphery (Guindon, J., 2008) and its expression is upregulated during early inflammation (Cabral, G. A, 2009). (-) $\Delta^9$-Tetrahydrocannabinol ($\Delta^9$-THC; Figure 3.1), the main psychoactive constituent of marijuana (Gaoni, Y. M., 1964) (Cannabis sativa L.), produces its physiological effects through interaction with both CB1 and CB2, while its psychoactive effects are attributable to interaction with CB1 receptors in the CNS. Existing data suggest the potential usefulness of CB agonists for treating pain, gastrointestinal (GI) disorders, glaucoma, nausea and vomiting induced by chemotherapeutic agents, atherosclerosis, addiction, MS and tumorigenesis (Pertwee, R. G., 2009).

Using high throughput screening (HTS), Bayer Pharmaceuticals identified a structurally novel chemotype that exhibits agonism at both CB receptors. BAY 38-7271( (-)-(R)-3-(2-hydroxymethylindanyl-4-oxy)phenyl-4,4,4-trifluoro-1-sulfonate), a structurally novel cannabinergic ligand, is a high affinity ($K_i = 0.46\text{-}1.85\text{nM}$), full agonist at both CB receptors with pronounced neuroprotective properties (DeVry, J.; 2002). Structural modification of the cyclopentyl ring fused to the aromatic ring led to another structurally novel, orally active, CB1/CB2 agonist, BAY 59-3074 that exhibits moderate affinity ($K_i = 55.4, 48.3, \text{and } 45.5 \text{nM}$).
for rat CB1, human CB1 and human CB2 receptors, respectively) and partial agonist properties at these receptors in \[^{35}\text{S}]\text{GTP} \gamma \text{S} binding assays (DeVry, J., 2004). BAY 59-3074 was shown to be selective for CB receptors with no interaction of comparable potency with other targets in a 214-target receptor, and enzyme activity screen. In rat models of chronic neuropathic and inflammatory pain, BAY59-3074 exhibits pronounced antihyperalgesic and antiallodynic properties (DeVry, J. 2004).

Figure 3.1: Structures of \(\Delta^9\)-THC and BAY compounds

Bayer Pharmaceuticals reported a potent CB1/CB2 agonist of a structurally novel class of cannabinoids. It has been characterized \textit{in vitro} and \textit{in vivo} to have a pronounced neuroprotective efficacy in a rat traumatic brain injury model.

There is limited SAR information on the two BAY templates in the literature. As both structures share common pharmacophoric features including a biaryl ether template and the \textit{meta}-sulfonyl alkyl side chain, we hypothesized that both molecules may interact with the CB1 receptor in a similar fashion. With the objective of improving the affinity and potency of BAY 59-3074 and increasing our understanding of the conformational requirements for CB1 receptor
binding and activation, we synthesized a novel series of analogs using the strategy of conformational restriction as a tool for molecular modification and design.

The focus of this chapter is the design and synthesis of a series of such analogs in which rotation around the C1- O bond is restricted and orientation of the side chain is varied (Figure 3.2). This was achieved by connecting the two rings of BAY59-3074 through a single C2-C6' (4a) or C4-C6' (45) bonds to form a bis-benzofuran ring, or alternatively through a C2-C6' methylene bridge (71) (Figure 3.2). To obtain information on the relative orientation of the side chain with regard to the tricyclic template we also synthesized their respective regioisomers 44 and 46.

3.2 Constrained Analogs

Our approach for the design of conformationally constrained analogs of biaryl ether compound BAY59-3074 involves restricting the free rotation about the ether bond between the two aromatic rings. This is accomplished by incorporation of a C-C bond between aromatic rings A and B to form a dibenzofuran class of compounds. By incorporating this constraint we also change the conformation of the compound to planar. We are electing to maintain the key features on both aromatic rings that we believe to be of pharmacological importance. These features include the nitrile and trifluoromethyl substituents on the A ring as well as the 4,4,4-trifluorobutane-1-sulfonate side chain on the B ring. To obtain information on the relative orientation of the side chain with regard to the tricyclic template we also synthesized their respective regioisomers 44 and 46.
3.2.1 Retrosynthetic analysis of dibenzofuran compounds

In the retrosynthetic analysis of constrained analogs of BAY59-3074 (Figure 3.3) there are two positions we can make disconnections from. We can choose to disconnect to form the ether bond first (Route 1) or disconnect to form the carbon-carbon bond first (Route 2).

3.3 Chemistry

3.3.1 Synthesis of dibenzofuran compounds
From the retrosynthetic analysis we elected to form the ether bond first (Scheme 3.1). The commercially available 3-methoxyphenol was first MOM protected. Next, n-BuLi was used to selectively brominate ortho to the methoxy and MOM groups. This was followed by deprotection of the MOM group to give the desired starting material (50). Then coupled with commercially available 2-fluoro-6-(trifluoromethyl)benzonitrile (51) with sodium hydride in DMSO under microwave accelerated conditions for 2 min at 100°C to yield 52 in 61% yield.

Multiple attempts were made to close the ring, initially using Ames conditions. To a solution of 52 in anhydrous DMA was added Pd (II) acetate and Na$_2$CO$_3$ and subjected to CEM microwave irradiation for 10 min at 180°C. Pd(II)acetate (4.2 mg, 0.018 mmol) and Na$_2$CO$_3$ After a further literature research of carbon-carbon coupling a procedure reported by Campeau, L.-C. et al using palladium catalyzed reaction conditions was tried. This method involved the use of 1 equivalent K$_2$CO$_3$, catalytic amounts of PCy$_3$-HBF$_4$, and Pd(OAc)$_2$ in DMA under microwave irradiation. This reaction was also performed at varying lengths of microwave irradiation, up to 40 min and no reaction occurred. These reactions were also performed under conventional heating without success. These methods did not prove fruitful so an alternate route was devised.

Scheme 3.1: Route 1 synthesis of dibenzofuran analog
Reagents and Conditions: (a) MOM-Cl, K₂CO₃, 18-crown-6, ACN, RT, 3h, 84%; (b) n-BuLi, Br₂C₂Cl₄, Et₂O, RT, 18h, 55%; (c) 3N HCl, THF, RT, 18h, 75%; (d) NaH, DMSO, microwave; 2 min 100°C, 61%; (e) Pd(OAc)₂, Na₂CO₃, No Rxn.

After failed attempts to cyclize the ring, we decided to switch gears to the second proposed route. We chose to form the carbon-carbon bond first before attempting to close the ring, summarized in Scheme 3.2.

Scheme 3.2: Route 2 Synthesis of Dibenzofuran

Reagents and Conditions: (a) Pd(PPh₃)₂, Ba(OH)₂•8H₂O, DME/H₂O, microwave, 120°C, 15 min, 42-64%; (b) BCl₃, TBABr, DCM, -78°C-RT, overnight, 65-87% (c) NaH, DMSO, RT, 1h, 65-81% (d) ClO₂SO(CH₂)₃CF₃, 45% NaOH, TBABr, DCM, 74-90%.

The common starting material 3-chloro-2-fluoro-6-(trifluoromethyl)benzonitrile (54) was prepared by dehydration of the corresponding benzamide using phosphorous oxychloride to give a white crystalline solid. The biaryl intermediates, 56a-d, were obtained by microwave accelerated Suzuki coupling of commercially available methoxyphenyl boronic acids 55a-d with 54 in 42-64% yields. Deprotection of both methoxy groups in 56 by treatment with BCl₃ and
TBAI in dichloromethane at -78°C led to biaryl phenols 57a-d (obtained in 65-87% yield). These were then subjected to intramolecular cyclization by treatment with sodium hydride in anhydrous DMSO at room temperature to give dibenzofurans 58 in 65-81%. It is important to note, that this step does not tolerate high reaction temperatures. Refluxing results in a mixture of decomposed products and loss of cyano group. Treatment of 10 with commercially available 4,4,4-trifluorobutane-1-sulfonyl chloride in the presence of tetrabutyl ammonium bromide and 45% aq. NaOH in dichloromethane gave the desired sulfonates 43-46 in 74-90% yield.

3.3.2 Synthesis of dibenzopyran compound

The conformation of the dibenzopyran compounds adopt a near planar geometry, therefore we decided to further expand the oxygen containing ring by including a methylene bridge between C2 and C6' to explore a corresponding motif that allows for a limited conformational flexible bridge. Our initial attempts are outline in Scheme 3.3.

Synthesis started with the commercially available compound 2-fluoro-6-(trifluoromethyl)benzonitrile (1) treatment with LDA in THF at -78°C and quenched with DMF, stirred overnight warming to room temperature to form aldehyde (59). In situ formation of LDA by addition of BuLi to diisopropylamine in THF at low temperatures (-78°C - 0°C). This was followed by oxidation of the newly formed aldehyde using oxone to form the carboxylic acid (60). Caution must be used to not introduce moisture to the reaction system. All glassware was scrupulously dried in the oven and flame dried twice to insure anhydrous conditions. Otherwise yields are significantly reduced.

Alternatively, the direct bubbling of CO₂ gas into the reaction mixture of lithiated 2-fluoro-6-(trifluoromethyl)benzonitrile to give 60 in 1 step. This was followed by direct synthesis
of Weinreb amide (61) using triphosgene in 89% yield. Next, 61 was coupled with 62 to form the dibenzophenone intermediate, 63. Universal deprotection was tried with treatment of excess boron trichloride and tetrabutyl ammonium iodide, but only gave the monoprotected product 63a. The dibenzophenone was subjected to intramolecular catalyzed cyclization by addition of sodium hydride to give our desired xanthenone core (64). Attempts were made to reduce the carbonyl by sodium cyanoborohydride reduction but proved unsuccessful. While it is not our intended path, the introduction of this carbonyl is another site to synthetically build off of.

Consistently low yields leading up to the reduction step only provided small amount of material to work with so decided to use alternate route to the end compound.

Scheme 3.3: Synthesis of xanthenone

Reagents and Conditions: (a) LDA THF, DMF, -78°C-RT, overnight, 48%; (b) oxone, MeOH, overnight, 88%; (c) LDA, THF, CO₂, -78°C-RT, 35%; (d) triphosgene, NEt₃, N,O-dimethylhydroxylamine HCl, DCM 0°C, 89% (e) TMP, LDA, -78°C-RT, 4h, 30%; (f) NaH, DMSO, RT, 2h, 71%; (g) NaCNBH₄, MeOH, RT, NO RXN
Alternatively we sought to reduce methyl ether to couple with a side chain. After multiple attempts of reducing the methyl ether by traditional lewis acid catalyzed means with boron tribromide or boron trichloride and tetrabutyl ammonium iodide yielded unsuccessful results this route was abandoned and the synthesis in Scheme 3.4 was devised. Also due to the low yields of the initial steps there was a lack of material to carry on with the synthesis.

The synthesis of xanthane analog (71) is outlined in Scheme 3.4. Boronic acid 66 was obtained by ortho-metallation of commercially available 2-fluoro-6-(trifluoromethyl) benzonitrile (1) by treatment with LDA followed by quenching with trimethyl borate. 1-(bromomethyl)-2,3-dimethoxybenzene (67) prepared from corresponding alcohol was then coupled with boronic acid 66 under microwave accelerated Suzuki conditions to give 68 in 34% yield. Treatment of 68 with BCl₃/TBAI gave catechol 69 in 65% yield. Intramolecular cyclization of 69 led to 70 in 54% yield under basic conditions. Sulfonate 71, was obtained in 75% yields by treatment of tricyclic compound 69 with 4,4,4-trifluorobutane-1-sulfonyl chloride in the presence of tetrabutylammonium bromide under basic conditions.

Scheme 3.4: Synthesis of dibenzopyran analog

Reagents and Conditions: (a) LDA, THF, -78°C, 2h, trimethyl borate, -78°C-RT, 16h, 32%; (b) Pd(PPh₃)₄, Ba(OH)₂•8H₂O, DME: H₂O, μwave, 34%; (c) BBr₃, CH₂Cl₂, -78°C-RT, 65%; (d) NaOH, DMSO, RT, 54%; (e) 4,4,4-trifluorobutane-1-sulfonyl chloride, TBAB, 45% NaOH, CH₂Cl₂, 0°C–RT, 2h, 75%.
3.3.3 Synthesis of Carbazole

Substitution of the oxygen in the dibenzofuran analogs with nitrogen gave us a carbazole analog (Scheme 13). We started with microwave accelerated Suzuki coupling of 1-bromo-2-nitro-4-(trifluoromethyl)benzene (72) with para-methoxyphenylboronic acid to give 4'-methoxy-2-nitro-4-(trifluoromethyl)biphenyl. Treatment with triphenylphosphine at reflux conditions cyclized the compound to give our carbazole template (76). Unmasking of the phenolic group was accomplished by treatment with BCl$_3$/TBAI. Lastly the sulfonate side chain was incorporated through standard basic conditions to yield 77 in 47%.

Scheme 3.5: Synthesis of carbazole

Reagents and Conditions: (a) Pd(PPh$_3$)$_4$, Ba(OH)$_2$•8H$_2$O, DME/H$_2$O, µwave; 15 min 120ºC, 91%; (b) PPh$_3$, DMA, reflux overnight, 69%; (c) BCl$_3$, TBAI, DCM, -78ºC – RT, overnight, 67%; (d) 4,4,4-trifluorobutane-1-sulfonyl chloride, TBAB, 45%NaOH, DCM 0ºC – RT, 2hr., 47%

3.4 Results and Discussion

We explored pharmacophoric implications of constraining the rotation about the ether bond as well as the positioning of the side chain. The affinities of all new compounds for both CB1 and CB2 cannabinoid receptors are listed in Table 3.1. rCB1, mCB2 and hCB2 binding
affinities were determined by radioligand competition binding experiments using \([^3 \text{H}]\text{CP55,940}\), as the radioligand.

All conformationally constrained analogs had significantly reduced affinities for both receptors. The binding affinity in comparison to BAY59-3074 at the cannabinoid receptors is weaker, but the receptor selectivity is more evident as the side chain position is varied. BAY59-3074 has no reported selectivity for either of the two cannabinoid receptor. Although, all constrained analogs exhibited reduced binding affinity at both cannabinoid receptor subtypes, 45, 46 and 71 exhibited 3- to 12-fold selectivity for hCB2 over rCB1 receptors. These two chemotypes provide new leads for the development of CB2-selective cannabinergics.

We also explore the replacement of the oxygen in dibenzofuran compounds for nitrogen, in a carbazole analog AM7541. Again, there is a reduced affinity to CB1, but interestingly species selectivity is exhibited for mCB2.

This series of compounds reinforces the necessity of the free rotatable bond to maintain binding affinity in this class of compounds. This was also illustrated previously with AM7534 with the incorporation of the carbonyl linker the binding affinity completely drops off at both CB receptors.

Table 3.1: Affinities for CB1 and CB2 receptors

<table>
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<tr>
<th>Compound</th>
<th>Ki (nM)</th>
<th>rCB1/mCB2</th>
<th>rCB1/hCB2</th>
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<tr>
<td></td>
<td>rCB1</td>
<td>mCB2</td>
<td>hCB2</td>
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<tr>
<td>43 AM7526</td>
<td>1900</td>
<td>676</td>
<td>1805</td>
</tr>
<tr>
<td></td>
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<td></td>
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<tr>
<td>77</td>
<td>&gt;1000</td>
<td>66.6</td>
<td>1646</td>
</tr>
</tbody>
</table>

To explore the significant loss of affinity of the novel conformationally constrained analogs when compared to the parent ligand, BAY59-3074, we compared their respective preferred conformations (Figure 3.4) using Discovery Studio by Acceleryx. It is clear that for analog 45 the tricyclic ring system is planar. Expansion of the five membered furan ring of 45 to a six membered ring in xanthane analog 71 avails some, albeit modest, conformational flexibility to the ring system. Here too, the overall tricyclic system is also fixed into a quasi-planar conformation (2.16° from planar). Conversely, the parent compound assumes a conformation in which the planes of the two aryl rings are at 4.52° from planar and are capable of further thermodynamically allowable conformations capable of reacting favorably with the CB1 or CB2 targets. These results argue for a pharmacophoric conformation in which aryl rings A and B are not coplanar and may explain the loss of affinity for the novel conformationally constrained analogs described here.
3.4 Preferred Conformations of BAY 59-3074, AM7528 and AM7585

Preferred conformations of BAY 59-3074 (purple), AM7528 (magenta) and AM7585 (blue) using their minimized conformations based on steric and electrostatic properties to help to visualize the geometric conformations of each compound. The red frames are used as references slab consistent in all three figures.

3.5 Conclusions
In summary, to probe the bioactive conformation of BAY59-3074 and to improve its binding profile at CB receptors, a series of conformationally constrained analogs were synthesized successfully. These constrained analogs represent a new CB chemotype of dibenzofuran class, with AM7536 (46) exhibiting selectivity for both mouse and human CB2 receptors. These constrained analogs are a starting point for further development of CB2 selective ligands.

This work has also shown that rotational freedom about the biaryl ether bond is required for binding to the CB1 receptor. We can also postulate that the side chain positioning plays a vital role in affinity for the CB receptor.

3.6 Experimental

1-methoxy-3-(methoxymethoxy)benzene (48)

To a solution of 3-methoxyphenol (4.0 g, 32 mmol) in anhydrous ACN (50 mL) was added potassium carbonate (8.9 g, 64 mmol) and was cooled to -5°C. Then 18-crown-6 (2.1 g, 8.0 mmol) and MOM-Cl (2.7 mL, 35 mmol) was added and stirred at room temperature for 18 h. Reaction mixture was filtered and concentrated under reduced pressure. The crude was purified by flash column chromatography on silica gel (5% diethyl ether in hexanes) to give the title compound as a yellow oil (4.5 g; 84%) ¹H NMR (500 MHz, CDCl₃) δ 7.18 (t, J = 7.5 Hz, 1H), 6.64 (dd, J = 7.0 Hz J = 2.5 Hz, 1H), 6.61 (t, J = 2.5 Hz, 1H), 6.57 (ddd, J = 8.5 Hz J = 2.5 Hz J = 1.0 Hz, 1H), 5.17 (s, 2H), 3.79 (s, 3H), 3.48 (s, 3H)

2-bromo-1-methoxy-3-(methoxymethoxy)benzene (49)
To a solution of 1-methoxy-3-(methoxymethoxy)benzene (1.0 g, 5.9 mmol) dissolved in anhydrous ether (60 mL) was added n-BuLi (2.8 mL, 7.1 mmol) and refluxed for 2h. Cooled to room temperature and Br$_2$C$_2$Cl$_4$ (2.9 g, 8.9 mmol) was added and stirred at room temperature for 30 min under inert atmosphere. Reaction was poured into water and the organic layer was separated and the aqueous phase extracted with diethyl ether (3x). The combined organic layer was washed with brine, dried (MgSO$_4$) and concentrated under reduced pressure. The crude was purified by flash column chromatography on silica gel (10% diethyl ether in hexanes) to give the title compound as a yellow oil (1.07 g; 73%) $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 7.21 (t, $J$ = 8.0 Hz, 1H), 6.79 (dd, $J$ = 8.5 Hz $J$ = 1.0 Hz, 1H), 6.61 (dd, $J$ = 8.5 Hz $J$ = 1.0 Hz, 1H), 5.25 (s, 2H), 3.90 (s, 3H), 3.52 (s, 3H)

2-bromo-3-(methoxy)phenol (50)

To a solution of 2-bromo-1-methoxy-3-(methoxymethoxy)benzene (150 mg, 0.6 mmol) in anhydrous THF (8.0 mL) was added 3N HCl, under Nitrogen and stirred at room temperature for 24 h. Reaction was quenched with sat. NaHCO$_3$ and extracted with diethyl ether (3x). The combined organic layer was washed with brine, dried (MgSO$_4$) and concentrated under reduced pressure. The crude was purified by flash column chromatography on silica gel (7% $\rightarrow$ 15% diethyl ether in hexanes) to give the title compound as oil (89 mg; 72%) $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 7.17 (t, $J$ = 8.0 Hz, H), 6.68 (dd, $J$ = 8.0 Hz $J$ = 1.5 Hz, 1H), 6.48 (dd, $J$ = 8.0 Hz $J$ = 1.5 Hz, 1H), 5.63 (s, 1H), 3.89 (s, 3H) HRMS (EI) for C$_7$H$_7$O$_2$Br Calculated Mass: 201.96294 Measured Mass: 201.96459

2-(2-bromo-3-methoxyphenoxy)-6-(trifluoromethyl)benzonitrile (52)
To a solution of 2-bromo-3-(methoxymethoxy)phenol (68 mg, 0.5 mmol) in anhydrous DMSO (3.0 mL), in a microwave reaction vessel, under Nitrogen, was added NaH (33 mg, 0.8 mmol), followed by 2-fluoro-6-(trifluoromethyl)benzonitrile (104 mg, 0.5 mmol) was added and subjected to microwave irradiation for 2 min at 100°C. The reaction was quenched with dilute HCl and extracted with diethyl ether (3x). The combined organic layer was washed with brine, dried (MgSO$_4$) and concentrated under reduced pressure. The crude was purified by flash column chromatography on silica gel (20% → 40% diethyl ether in hexanes) to give the title compound (81 mg; 61%). $^1$H NMR (500 MHz, CDCl$_3$) δ 7.53 (t, $J = 7.5$ Hz, 1 H), 7.44 (d, $J = 7.5$ Hz, 1H), 7.36 (t, $J = 8.0$ Hz, 1H), 6.86 (t, $J = 8.5$ Hz, 2H), 6.81 (dd, $J = 8.0$ Hz $J = 1.5$ Hz, 1H), 3.96 (s, 3H, OCH$_3$) HRMS (ESI) Calculated for C$_{15}$H$_{10}$NO$_2$BrF$_3$: 371.9847 Measured mass: 371.9849

3-chloro-2-fluoro-6-(trifluoromethyl)benzonitrile (54)

To a stirred solution of 3-chloro-2-fluoro-6-(trifluoromethyl)benzamide (1.0 g, 4.1 mmol) in anhydrous dichloroethane (10 mL) was added phosphorus oxychloride (0.75 mL, 8.2 mmol), condenser attached and allowed to reflux at 100°C overnight. Reaction poured into ice water and extracted with ethyl acetate (2x). The combined organic layer was washed 1N HCl, brine, dried (MgSO$_4$) and concentrated under reduced pressure to give white crystalline solid. (870 mg; 94%)  $^1$H NMR (500 MHz, CDCl$_3$) δ 7.80 (t, $J = 8.5$ Hz, 1H), 7.57 (d, $J = 8.5$ Hz, 1H)

2-fluoro-2',6'-dimethoxy-4-(trifluoromethyl)biphenyl-3-carbonitrile (56a)

In a microwave reaction vessel, a stirred solution of 3-chloro-2-fluoro-6-(trifluoromethyl)benzonitrile (400 mg, 1.8 mmol) in anhydrous dimethoxyethane (6.0 mL) and
water (1.5 mL) was added 2,6-dimethoxyphenylboronic acid (489 mg, 2.6 mmol) and stirred at room temperature with Nitrogen gas bubbled through for 10 min. Then Ph(PPh₃)₄ (206 mg, 0.18 mmol) and Ba(OH)₂·H₂O (1.13 g, 3.58 mmol) was added and subjected to microwave irradiation for 15 min at 120⁰C. Reaction was poured into water and extracted with ethyl acetate (2x). The combined organic layer was washed with brine, dried (MgSO₄) and concentrated under pressure. The crude was purified by flash column chromatography on silica gel (5% → 30% ethyl acetate in hexanes) to give the title compound (372 mg; 64%). ¹H NMR (500MHz,CDCl₃) δ 7.66 (t, J = 8.5 Hz, 1H), 7.58 (d, J = 8.0 Hz, 1H), 7.39 (t, J = 8.0 Hz, 1H), 6.67(s, 1H), 6.65 (s, 1H), 3.76 (s, 6H) HRMS (ESI) for C₁₆H₁₂NO₂F₄ Calculated Mass: 326.0804 Measured Mass: 326.0804

2-fluoro-2',5'-dimethoxy-4-(trifluoromethyl)biphenyl-3-carbonitrile (56b)

The synthesis was carried out as described for 56a, using 3-chloro-2-fluoro-6-(trifluoromethyl)benzonitrile (400 mg, 1.8 mmol) in anhydrous dimethoxyethane (6.0 mL) and water (1.5 mL), 2,5-dimethoxyphenylboronic acid (489 mg, 2.6 mmol), Ph(PPh₃)₄ (206 mg, 0.18 mmol) and Ba(OH)₂·H₂O (1.13 g, 3.58 mmol). The crude was purified by flash column chromatography on silica gel (5% → 30% ethyl acetate in hexanes) to give the title compound (349 mg; 60%). ¹H NMR (500 MHz, CDCl₃) δ 7.73 (t, J = 8.0 Hz, 1 H), 7.63 (d, J = 8.0 Hz, 1 H), 7.12 - 7.21 (m, 1 H), 7.05 (d, J = 8.0 Hz, 1 H), 6.85 (d, J = 8.0 Hz, 1 H), 3.93 (s, 3 H), 3.75 (s, 3 H)

2-fluoro-2',4'-dimethoxy-4-(trifluoromethyl)biphenyl-3-carbonitrile (56c)
The synthesis was carried out as described for 56a, using 1 (400 mg, 1.8 mmol) in anhydrous dimethoxyethane (6.0 mL) and water (1.5 mL), 2,4-dimethoxyphenylboronic acid (489 mg, 2.6 mmol), Ph(PPh₃)₄ (206 mg, 0.18 mmol) and Ba(OH)₂•H₂O (1.13 g, 3.58 mmol). The crude was purified by flash column chromatography on silica gel (5% → 30% ethyl acetate in hexanes) to give the title compound (349 mg; 60%). ¹H NMR (500 MHz, CDCl₃) δ 7.70 (t, J = 7.0 Hz, 1H), 7.59 (d, J = 8.5 Hz, 1H), 7.16 (d, J = 8.5 Hz, 1H), 6.61(dd, J = 8.5 Hz J = 2.5Hz, 1H), 6.57 (d, J = 2.5 Hz, 1H), 3.87 (s, 3H, CH₃), 3.80 (s, 3H, CH₃)

2-fluoro-2',3'-dimethoxy-4-(trifluoromethyl)biphenyl-3-carbonitrile (56d)

The synthesis was carried out as described for 56a, using 1 (400 mg, 1.8 mmol) in anhydrous dimethoxyethane (6.0 mL) and water (1.5 mL), 2,3-dimethoxyphenylboronic acid (489 mg, 2.6 mmol), Ph(PPh₃)₄ (206 mg, 0.18 mmol) and Ba(OH)₂•H₂O (1.13 g, 3.58 mmol). The crude was purified by flash column chromatography on silica gel (5% → 30% ethyl acetate in hexanes) to give the title compound (244 mg; 42%). ¹H NMR (500 MHz, CDCl₃) δ 7.73 (t, J = 7.5 Hz, 1H), 7.63 (d, J = 8.0 Hz, 1H), 7.16 (t, J = 8.0 Hz, 1H), 7.05 (dd, J = 8.5 Hz J = 1.5 Hz, 1H), 6.84 (d, J = 8.0 Hz, 1H), 3.93 (s, 3H, OCH₃), 3.75 (s, 3H, OCH₃)

2-fluoro-2',6'-dihydroxy-4-(trifluoromethyl)biphenyl-3-carbonitrile (57a)

To a stirred solution of 56a (350 mg, 1.0 mmol) in anhydrous dichloromethane (10 mL) was added tetrabutylammonium iodide (993 mg, 2.6 mmol) and cooled to -78°C. Then boron trichloride (2.6 mL, 2.6 mmol, 1.0 M solution in DCM) was added. Reaction stirred overnight warming to room temperature. Reaction poured into water and extracted with ethyl acetate (3x). The combined organic layer was washed with brine, dried (MgSO₄) and concentrated under
reduced pressure The crude was purified by flash column chromatography on silica gel (10% → 50% ethyl acetate in hexanes) to give the title compound (252 mg; 79%). $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 7.84 (t, $J$ = 7.5 Hz, 1H), 7.66 (d, $J$ = 8.0 Hz, 1H), 7.20 (t, $J$ = 8.0 Hz, 1H), 6.53 (d, $J$ = 8.5 Hz, 2H), 4.90 (s, 2H, OH) HRMS (ESI) for C$_{14}$H$_8$NO$_2$F$_4$ Calculated Mass: 298.0491 Measured Mass: 298.0493

2-fluoro-2',5'-dihydroxy-4-(trifluoromethyl)biphenyl-3-carbonitrile (57b)
The synthesis was carried out as described for 57a using 56b (350 mg, 1.0 mmol), in anhydrous dichloromethane (10 mL), tetrabutylammonium iodide (993 mg, 2.6 mmol) and boron trichloride (2.6 mL, 2.6 mmol, 1.0 M solution in DCM). The crude was purified by flash column chromatography on silica gel (10% → 50% ethyl acetate in hexanes) to give the title compound (249 mg; 78%) $^1$H NMR (500MHz ,CDCl$_3$) $\delta$ 7.79 (t, $J$ = 7.5 Hz, 1H), 7.65 (d, $J$ = 8.0 Hz, 1H), 6.89 - 6.79 (m, 2H), 6.75 (d, $J$ = 2.5 Hz, 1H), 4.87 (br. s., 1H, OH), 4.82 (br. s., 1H, OH)

2-fluoro-2',4'-dihydroxy-4-(trifluoromethyl)biphenyl-3-carbonitrile (57c)
The synthesis was carried out as described for 57a, using 56c (350 mg, 1.0 mmol) in anhydrous dichloromethane, tetrabutylammonium iodide (993 mg, 2.6 mmol) and boron trichloride (2.6 mL, 2.6mmol, 1.0 M solution in DCM). The crude was purified by flash column chromatography on silica gel (10% → 50% ethyl acetate in hexanes) to give the title compound (277 mg; 87%). $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 7.76 (t, $J$ = 8.0 Hz, 1H), 7.62 (d, $J$ = 8.0 Hz, 1H), 7.12 (dd, $J$ = 8.5 Hz $J$ = 1.0Hz, 1H), 6.54 (dd, $J$ = 8.5 Hz $J$ = 2.5Hz, 1H), 6.45 (d, $J$ = 2.5 Hz 1H), 4.95 (s, 1H, OH), 4.94 (s, 1H, OH)
2-fluoro-2',3'-dihydroxy-4-(trifluoromethyl)biphenyl-3-carbonitrile (57d)
The synthesis was carried out as described for 57a, using 56d (350 mg, 1.0 mmol) in anhydrous dichloromethane, tetrabutylammonium iodide (993 mg, 2.6 mmol) and boron trichloride (2.6 mL, 2.6 mmol, 1.0 M solution in DCM). The crude was purified by flash column chromatography on silica gel (10% → 50% ethyl acetate in hexanes) to give the title compound (207 mg; 65%). \( ^1H \) NMR (500 MHz, CDCl\(_3\)) \( \delta \): 7.82 (t, \( J = 7.5 \) Hz, 1H), 7.64 (d, \( J = 8.0 \) Hz, 1H), 6.96 (d, \( J = 8.0 \) Hz, 1H), 6.89 (t, \( J = 8.0 \) Hz, 1H), 6.84 (d, \( J = 8.0 \) Hz, 1H), 4.95 (s, 2H, OH)

9-hydroxy-3-(trifluoromethyl)dibenzo[b,d]furan-4-carbonitrile (58a)
To a solution of 57a (250 mg, 0.8 mmol) in anhydrous DMSO (10 mL) was added NaH (101 mg, 2.1 mmol) and stirred at room temperature for 2h. Reaction was quenched with 1N HCl and extracted with ethyl acetate (2x). The combined organic layer was washed with brine, dried (MgSO\(_4\)) and concentrated under reduced pressure. The crude was purified by flash column chromatography on silica gel (5% → 30% ethyl acetate in hexanes) to give the title compound as a white solid (151 mg; 65%). \( ^1H \) NMR (500 MHz, CDCl\(_3\)) \( \delta \): 8.39 (d, \( J = 8.0 \) Hz, 1H), 7.75 (d, \( J = 8.0 \) Hz, 1H), 7.47 (t, \( J = 8.5 \) Hz, 1H), 7.31 (d, \( J = 8.5 \) Hz, 1H), 6.81 (d, \( J = 8.0 \) Hz, 1H), 5.66 (s, 1H, OH) HRMS (ESI) for C\(_{14}\)H\(_{7}\)NO\(_2\)F\(_3\) Calculated Mass: 278.0429 Measured Mass: 278.0429

8-hydroxy-3-(trifluoromethyl)dibenzo[b,d]furan-4-carbonitrile (58b)
The synthesis was carried out as described for 58a, using 57b (250 mg, 0.8 mmol) in anhydrous DMSO (10 mL) and NaH (101 mg, 2.1 mmol). The crude was purified by flash column
chromatography on silica gel (5% → 30% ethyl acetate in hexanes) to give the title compound as white solid (189 mg; 81%). $^1$H NMR (500MHz, CDCl$_3$) δ 8.17 (d, $J = 8.5$ Hz, 1H), 7.74 (d, $J = 8.5$ Hz, 1H), 7.59 (d, $J = 9.0$ Hz, 1H), 7.44 (d, $J = 2.5$ Hz, 1H), 7.14 (dd, $J = 9.0$ Hz $J = 3.0$ Hz, 1H), 5.13 (s, 1H, OH).

7-hydroxy-3-(trifluoromethyl)dibenzo[b,d]furan-4-carbonitrile (58c)

The synthesis was carried out as described for 58a, using 57c (250 mg, 0.8 mmol) in anhydrous DMSO (10 mL) and NaH (101 mg, 2.1 mmol). The crude was purified by flash column chromatography on silica gel (5% → 30% ethyl acetate in hexanes) to give the title compound as white solid (158 mg; 68%). $^1$H NMR (500 MHz, CDCl$_3$) δ 7.93 (d, $J = 8.0$ Hz, 1H), 7.83 (d, $J = 8.0$ Hz, 1H), 7.78 (d, $J = 1.0$ Hz, 1H), 7.58 (dd, $J = 7.0$ Hz $J = 1.0$ Hz, 1H) 7.08 (d, $J = 2.5$ Hz, 1H), 6.89 (dd, $J = 9.0$ Hz $J = 2.5$ Hz, 1H) $^1$H NMR (500MHz, DMSO) δ 10.54 (s, 1H, OH), 8.51 (d, $J = 8.5$ Hz, 1H), 8.13 (d, $J = 8.0$ Hz, 1H), 7.95 (d, $J = 8.5$ Hz, 1H), 7.22 (d, $J = 2.0$ Hz, 1H), 7.00 (dd, $J = 8.5$ Hz $J = 2.0$ Hz, 1H) HRMS (ESI) for C$_{14}$H$_7$NO$_2$F$_3$ Calculated Mass: 278.0429 Measured Mass: 278.0431

6-hydroxy-3-(trifluoromethyl)dibenzo[b,d]furan-4-carbonitrile (58d)

The synthesis was carried out as described for 58a, using 57d (250 mg, 0.8 mmol) in anhydrous DMSO (10 mL) and NaH (101 mg, 2.1 mmol). The crude was purified by flash column chromatography on silica gel (5% → 30% ethyl acetate in hexanes) to give the title compound as white solid (184 mg; 79%). $^1$H NMR (500 MHz, CDCl$_3$) δ 8.24 (d, $J = 8.0$ Hz, 1H), 7.78 (d, $J = 8.5$ Hz, 1H), 7.58 (dd, $J = 7.5$ Hz $J = 1.0$ Hz, 1H), 7.37 (t, $J = 8.0$ Hz, 1H), 7.21 (dd, $J = 6.8$ Hz $J = 1.0$ Hz, 1H)
6-cyano-7-(trifluoromethyl)dibenzo[b,d]furan-1-yl 4,4,4-trifluorobutane-1-sulfonate (AM7526, 43)

To a stirred solution of constrained 58a (30 mg, 0.1 mmol) in anhydrous DCM (5.0 mL) was added TBAB (17.4 mg, 0.05 mmol) and 45% aqueous NaOH (0.1 mL) and cooled to 0°C. Then 4,4,4-trifluorobutane-sulfonyl chloride (27.2 mg, 0.12 mmol) in anhydrous DCM (1.0 mL) was added and stirred at room temperature for 2h. The reaction was poured into water and extracted with ethyl acetate (2x). The combined organic layer was washed with brine, dried (MgSO₄) and concentrated under reduced pressure. The crude was purified by flash column chromatography on silica gel (20% → 60% ethyl acetate in hexanes) to give the title compound as a white solid (39 mg; 79%). ¹H NMR (500 MHz, CDCl₃) δ 8.49 (d, J = 8.0 Hz, 1H), 7.83 (d, J = 8.0 Hz, 1H), 7.74 (d, J = 8.0 Hz, 1H), 7.69 (t, J = 8.0 Hz, 1H), 7.37 (d, J = 7.5 Hz, 1H), 3.56 (t, J = 7.5 Hz, 2H), 2.49 - 2.33 (m, 4H) HRMS (EI) for C₁₈H₁₁O₄NF₆S Calculated Mass: 451.03128 Measured Mass: 451.03181

6-cyano-7-(trifluoromethyl)dibenzo[b,d]furan-2-yl 4,4,4-trifluorobutane-1-sulfonate (AM7535, 44)

The synthesis was carried out as described for 43 using 58b (75 mg, 0.27 mmol) in anhydrous DCM (10 mL), TBAB (43 mg, 0.13 mmol), 45% aqueous NaOH (0.5 mL) and 4,4,4-trifluorobutane-sulfonyl chloride (68 mg, 0.43 mmol) in anhydrous DCM (2.0 mL). The crude was purified by flash column chromatography on silica gel (20% → 50% ethyl acetate in hexanes) to give the title compound as white solid (110 mg; 90%). ¹H NMR (500 MHz, CDCl₃) δ 8.26 (d, J = 8.0 Hz, 1H), 7.98 (s, 1H), 7.82 (d, J = 8.5 Hz, 1H), 7.78 (d, J = 8.5 Hz, 1H), 7.52
The synthesis was carried out as described for 43, using 58c (30 mg, 0.1 mmol) in DCM, TBAB (17.4 mg, 0.05 mmol) and 45% aqueous NaOH (0.1 mL) and, 4,4,4-trifluorobutane-1-sulfonyl chloride (27.2 mg, 0.12 mmol) in DCM (1.0 mL). The crude was purified by flash column chromatography on silica gel (20% → 60% ethyl acetate in hexanes) to give the title compound as a white solid (36 mg; 74%). ¹H NMR (500 MHz, CDCl₃) δ 8.26 (d, J = 8.0 Hz, 1H), 8.08 (d, J = 8.5 Hz, 1H), 7.82 (d, J = 8.5 Hz, 1H), 7.69 (d, J = 2.0 Hz, 1H), 7.43 (dd, J = 9.0 Hz J = 2.5 Hz, 1H), 3.47 (t, J = 8.0 Hz, 2H), 2.46 - 2.38 (m, 2H), 2.38 - 2.30 (m, 2H) HRMS (EI) for C₁₈H₁₁F₆NO₄S Calculated Mass: 451.03128 Measured Mass: 451.03123

6-cyano-7-(trifluoromethyl)dibenzo[b,d]furan-1-yl 4,4,4-trifluorobutane-1-sulfonate

(AM7528, 45)

The synthesis was carried out as described for 43, using 58d (30 mg, 0.1 mmol) in DCM, TBAB (17.4 mg, 0.05 mmol) and 45% aqueous NaOH (0.1 mL) and, 4,4,4-trifluorobutane-1-sulfonyl chloride (27.2 mg, 0.12 mmol) in DCM (1.0 mL) was added. The crude was purified by flash column chromatography on silica gel (20% → 60% ethyl acetate in hexanes) to give the title compound as a white solid (44 mg; 83%). ¹H NMR (500 MHz, CDCl₃) δ 8.30 (d, J = 7.5 Hz, 1H), 8.01 (d, J = 7.5 Hz, 1H), 7.85 (d, J = 8.5 Hz, 1H), 7.64 (d, J = 8.5 Hz, 1H), 7.54 (t, J = 8.0 Hz, 1H) HRMS (EI) for C₁₈H₁₁F₆NO₄S Calculated Mass: 451.03128 Measured Mass: 451.03085

6-cyano-7-(trifluoromethyl)dibenzo[b,d]furan-4-yl 4,4,4-trifluorobutane-1-sulfonate

(AM7536, 46)

The synthesis was carried out as described for 43, using 58d (30 mg, 0.1 mmol) in DCM, TBAB (17.4 mg, 0.05 mmol) and 45% aqueous NaOH (0.1 mL) and, 4,4,4-trifluorobutane-1-sulfonyl chloride (27.2 mg, 0.12 mmol) in DCM (1.0 mL) was added. The crude was purified by flash column chromatography on silica gel (20% → 60% ethyl acetate in hexanes) to give the title compound as a white solid (44 mg; 83%). ¹H NMR (500 MHz, CDCl₃) δ 8.30 (d, J = 7.5 Hz, 1H), 8.01 (d, J = 7.5 Hz, 1H), 7.85 (d, J = 8.5 Hz, 1H), 7.64 (d, J = 8.5 Hz, 1H), 7.54 (t, J = 8.0 Hz, 1H) HRMS (EI) for C₁₈H₁₁F₆NO₄S Calculated Mass: 451.03128 Measured Mass: 451.03085
Hz, 1H), 3.70 (t, J = 6.5 Hz, 2H), 2.64 - 2.43 (m, 4H) HRMS (EI) for C_{18}H_{11}F_{6}NO_{4}S Calculated Mass: 451.03128  Measured Mass: 451.03127

2-fluoro-3-formyl-6-(trifluoromethyl)benzonitrile (59)
To a flame dried, 3-neck round bottom flask, under argon, was added 2-fluoro-6-(trifluoromethyl)benzonitrile (1.0 g, 5.3 mmol) in anhydrous THF (53 mL), cooled to -78°C was added LDA (2.1 mL, 5.3 mmol, 2.5M solution in THF). After 2.5h, DMF (4.0 mL, 53 mmol) was added to the reaction and stirred at room temperature overnight. The reaction mixture was quenched with 1N HCl and extracted with ethyl acetate (3x). The combined organic layer was washed with brine, dried (MgSO_{4}) and concentrated under reduced pressure. The crude was purified by flash column chromatography on silica gel (40% → 100% ethyl acetate in hexanes) to give the title compound (560 mg; 48%). ¹H NMR (500 MHz, CDCl₃) δ 10.42 (s, 1H), 8.25 (t, J = 7.5 Hz, 1H), 7.77 (d, J = 8.5 Hz, 1H) HRMS (EI) for C₉H₂ONF₄ Calculated Mass: 216.00725 Measured Mass: 216.00848

3-cyano-2-fluoro-4-(trifluoromethyl)benzoic acid (60)
To a solution of 59 (200 mg, 0.92 mmol) in methanol (9.0 mL) was added oxone (210 mg, 1.30 mmol) and stirred at room temperature overnight. Solvent was evaporated under reduced pressure, dissolved in ethyl acetate, washed with brine, dried (MgSO₄) and concentrated under reduced pressure. The crude was purified by flash column chromatography on silica gel (40% → 100% ethyl acetate in hexanes) to give the title compound (190 mg, 88 %). Carbon dioxide method -
To a flame dried, 3-neck round bottom flask, under argon, was added 2-fluoro-6-(trifluoromethyl)benzonitrile (1.0 g, 5.3 mmol) in anhydrous THF (53 mL), cooled to -78°C was added LDA (2.1 mL, 5.3 mmol, 2.5M solution in THF). After 2.5h, carbon dioxide gas was bubbled through solution for 2h. The reaction mixture was quenched with careful addition of 1N HCl and extracted with ethyl acetate (3x). The combined organic layer was washed with brine, dried (MgSO₄) and concentrated under reduced pressure. The crude was purified by flash column chromatography on silica gel (40% \(\rightarrow\) 100% ethyl acetate in hexanes) to give the title compound (430 mg; 35%). \(^1\)H NMR (500 MHz, CDCl₃) δ 8.40 (t, \(J = 7.5\) Hz, 1H), 7.73 (d, \(J = 8.5\) Hz, 1H)

HRMS (EI) for C₉H₃O₂NF₄ Calculated Mass: 233.00999 Measured Mass: 233.00821

3-cyano-2-fluoro-4-(trifluoromethyl)benzoyl chloride

To a solution of carboxylic acid 60 (100 mg, 0.43 mmol) in anhydrous DCM (4.0 mL), cooled to 0°C, was added oxalyl chloride (65 mg, 0.51 mmol) and stirred for 2h. The reaction was concentrated under reduced pressure and used without further purification. \(^1\)H NMR (500 MHz, CDCl₃) δ 8.42 (t, \(J = 8.0\) Hz, 1H), 7.78 (d, \(J = 8.0\) Hz, 1H)

3-cyano-2-fluoro-N-methoxy-N-methyl-4-(trifluoromethyl)benzamide (61)

To a solution of carboxylic acid, 60, (100 mg, 0.4 mmol) in CH₂Cl₂ (4.0 mL) at 0°C was added triphosgene (64 mg, 0.2mmol) and triethylamine (216 mg, 2.1 mmol). Then N,O-dimethylhydroxylamine hydrochloride (26 mg, 0.4 mmol) was added to the solution and the ice bath was removed. The reaction mixture was stirred at room temperature for 3h. The triethylamine hydrochloride was removed by suction filtration and filtrate concentrated under
reduced pressure. The crude was purified by flash column chromatography on silica gel (0% → 20% ethyl acetate in hexanes) to give the title compound as a brown solid (105 mg; 89%). $^1$H NMR (500 MHz, CDCl$_3$) δ 7.80 (dd as t, $J$ = 7.0 Hz, 1H), 7.67 (d, $J$ = 8.0 Hz, 1H), 3.56 (s, 3H), 3.41 (s, 3H).

### 3-(2,4-dimethoxybenzoyl)-2-fluoro-6-(trifluoromethyl)benzonitrile

To a solution of 1-bromo-2,4-dimethoxybenzene (100 mg, 0.4 mmol) in THF (4.0 mL) was cooled to -78°C and n-BuLi (0.4 mL, 0.4 mmol) was added. After 2.5 h, weinreb amide 61 (152 mg, 0.5 mmol) was added and stirred overnight warming to room temperature. The reaction mixture was quenched with 1N HCl and extracted with ethyl acetate (3x). The combined organic layer was washed with brine, dried (MgSO$_4$) and concentrated under reduced pressure. The crude was purified by flash column chromatography on silica gel (0% → 20% ethyl acetate in hexanes) to give the title compound (48 mg; 30%). $^1$H NMR (500 MHz, CDCl$_3$) δ 7.88 (dd as t, $J$ = 7.0 Hz, 1H), 7.82 (d, $J$ = 9.0 Hz, 1H), 7.66 (d, $J$ = 8.0 Hz, 1H), 6.62 (dd, $J$ = 9.0 Hz $J$ = 2.0 Hz, 1H), 6.43 (d, $J$ = 1.5 Hz, 1H), 3.90 (s, 3H), 3.62 (s, 3H) HRMS (ESI) Calculated mass for C$_{17}$H$_{12}$NO$_3$F$_4$: 354.0753 Measured mass: 354.0754

### 2-fluoro-3-(2-hydroxy-4-methoxybenzoyl)-6-(trifluoromethyl)benzonitrile (64)

To a solution of 3-(2,4-dimethoxybenzoyl)-2-fluoro-6-(trifluoromethyl)benzonitrile (30 mg, 0.08 mmol) in anhydrous DCM was added TBAI (156 mg, 0.4 mmol) and cooled to -78°C. Then BCl$_3$ (0.4 mL, 0.4 mmol, 1.0M solution in DCM) was added and stirred overnight warming to room temperature. Water was poured into the reaction mixture and extracted with ethyl acetate.
(3x). The combined organic layer was washed with brine, dried (MgSO₄) and concentrated under reduced pressure. The crude was purified by flash column chromatography on silica gel (using 20% → 40% ethyl acetate in hexanes) to give the title compound (20 mg; 71%). 

\[ ^{1}H \text{NMR (500 MHz, CDCl}_3 \text{)} \delta 12.98 (s, 1H, OH), 7.79 (d, J = 8.5 Hz, 1H), 7.40 (d, J = 8.5, 1H), 7.24 (d, J = 8.0 Hz, 1H), 6.65 (dd, J = 8.0 Hz J = 2.0 Hz, 1H), 6.56 (d, J = 1.5 Hz, 1H) \]

HRMS (EI) for C₁₆H₈O₃NF₃ Calculated Mass: 319.04563 Measured Mass: 319.04680

**6-methoxy-9-oxo-3-(trifluoromethyl)-9H-xanthene-4-carbonitrile**

To a solution of 64 (10 mg, 0.03 mmol) in anhydrous DMSO (1.0 mL) was added sodium hydride (28 mg, 0.06 mmol, 60% dispersion in mineral oil) and stirred at room temperature for 2h. Water was added to the reaction mixture and extracted with ethyl acetate (3x). The combined organic layers were washed with brine, dried (MgSO₄) and concentrated under reduced pressure. The crude was purified by flash column chromatography on silica gel (using 20% → 40% ethyl acetate in hexanes) to give the title compound as a white solid (8.0 mg; 85%)

\[ ^{1}H \text{NMR (500 MHz, CDCl}_3 \text{)} 8.65 (d, J = 8.0 Hz, 1H), 8.25 (d, J = 9.0 Hz, 1H), 7.77 (d, J = 8.5 Hz, 1H), 7.07 (d, J = 2.5 Hz, 1H), 7.06 (dd, J = 9.0 Hz J = 2.0 Hz, 1H), 3.99 (s, 3H) \]

**2,3-dimethoxybenzyl bromide (67)**

To a solution of 2,3-dimethoxybenzyl alcohol (500 mg, 2.9 mmol) in anhydrous DCM (29 mL) was added PBr₃ (0.14 mL, 1.4 mmol) and stirred at room temperature for 2.5h. The reaction mixture was then quenched by addition of water and diluted with ethyl acetate. The organic phase was separated and the aqueous phase extracted with ethyl acetate (2x). The combined organic layer was washed with brine, dried (MgSO₄) and concentrated under reduced pressure.
The crude was purified by flash column chromatography on silica gel (0% → 30% ethyl acetate in hexanes) to give the title compound (632 mg; 92%) 1H NMR (500 MHz, CDCl3) δ 7.01 (t, J = 10 Hz, 1H), 6.96 (dd, J = 10 Hz J = 2.0 Hz, 1H), 6.89 (dd, J = 10 Hz J = 2.5 Hz, 1H), 4.57 (s, 2H), 3.96 (s, 3H), 3.87 (s, 3H)

3-cyano-2-fluoro-4-(trifluoromethyl)phenylboronic acid (66)

To a flame dried, 3-neck round bottom flask, under argon, was added 2-fluoro-6-(trifluoromethyl)benzonitrile (5.0 g, 264 mmol) in anhydrous THF (52 mL) and cooled to -78°C and then added LDA (mL, mmol). After 2.5h, trimethyl borate (29.4 mL, 264.5 mmol) was added to the reaction and stirred at room temperature for 2 days. The reaction mixture was quenched with 1N HCl and extracted with ethyl acetate (3x). The combined organic layer was washed with brine, dried (MgSO4) and concentrated under reduced pressure. The crude was purified by flash column chromatography on silica gel (20% → 80% ethyl acetate in hexanes) to give the title compound as yellow solid (1.96 g; 32%) 1H NMR (500 MHz, CDCl3) δ 7.49 (d, J = 8.5 Hz, 1H), 7.33 (d, J = 8.5 Hz, 1H) HRMS (EI) for C8H4O2NF4B Calculated Mass: 233.02712 Measured Mass: 233.02533

3-(2,3-dimethoxybenzyl)-2-fluoro-6-(trifluoromethyl)benzonitrile (68)

In a microwave reaction vessel, a solution of 3-cyano-2-fluoro-4-(trifluoromethyl)phenylboronic acid (200 mg, 0.85 mmol) in DME (3.5 mL) and H2O (1.0 mL) was added 2,3-dimethoxybenzyl bromide (237 mg, 1.0 mmol) and degassed with N2. Then Ba(OH)2•8H2O (405 mg, 1.2 mmol) and Pd(PPh3)4, (99 mg, .085 mmol) was added, capped and subjected to microwave irradiation
for 15 min at 120°C. Reaction mixture was quenched by addition of water and reaction mixture diluted with ethyl acetate. Organic phase was separated and the aqueous phase extracted with ethyl acetate (2x). The combined organic layer was washed with brine, dried (MgSO₄) and concentrated under reduced pressure. The crude was purified by flash column chromatography on silica gel (0% → 30% ethyl acetate in hexanes) to give the title compound (99 mg; 34%) \(^1\)H NMR (500 MHz, CDCl₃) \(\delta\) 7.48 (d, \(J = 9.5\) Hz, 1H), 7.353 (t, \(J = 8.5\) Hz, 1H), 7.10 (t, \(J = 8.5\) Hz, 1H), 7.01 (dd, \(J = 8.5\) Hz \(J = 1.5\) Hz, 1H), 6.95 (dd, \(J = 8.5\) Hz \(J = 1.5\) Hz, 1H), 5.28 (s, 2H), 3.90 (s, 3H), 3.90 (s, 3H) HRMS (ESI) for C\(_{17}\)H\(_{14}\)NO\(_2\)F\(_4\) Calculated Mass: 340.0961 Measured Mass: 340.0964

\textbf{3-(2,3-dihydroxybenzyl)-2-fluoro-6-(trifluoromethyl)benzonitrile (69)}

To a solution of 3-(2,3-dimethoxybenzyl)-2-fluoro-6-(trifluoromethyl)benzonitrile (100 mg, 0.29 mmol) in anhydrous DCM (3.0 mL), cooled to -78°C, was added BBr\(_3\) (0.73 mL, 0.73 mmol, 1.0 M solution in DCM) and stirred overnight. Reaction mixture was then poured into water and extracted with ethyl acetate (2x). The combined organic layer was washed with brine, dried (MgSO₄) and concentrated under reduced pressure. The crude was purified by flash column chromatography on silica gel (0% → 30% ethyl acetate in hexanes) to give the title compound (60 mg; 65%). \(^1\)HNMR (500MHz, CDCl₃) \(\delta\) 7.57 (t, \(J = 7.5\) Hz, 1H), 7.45 (d, \(J = 8.0\) Hz, 1H), 6.75 (m, 3H), 5.52 (s, 1H), 5.20 (s, 1H), 4.07 (s, 2H) HRMS (ESI) C\(_{15}\)H\(_{10}\)NO\(_2\)F\(_4\) Calculated Mass: 312.0648 Measured Mass: 312.0645

\textbf{5-hydroxy-3-(trifluoromethyl)-9H-xanthene-4-carbonitrile (70)}
To a solution of 3-(2,3-dihydroxybenzyl)-2-fluoro-6-(trifluoromethyl)benzonitrile (8.0 mg, 0.025 mmol) in anhydrous DMSO (1.0 mL) was added NaH (3.7 mg, 0.075 mmol) and allowed to stir at room temperature for 20 min. Reaction was quenched with 1N HCl and extracted with ethyl acetate (3x). The combined organic layer was washed with brine, dried (MgSO₄) and concentrated under reduced pressure. The crude was purified by flash column chromatography on silica gel (0% → 30% ethyl acetate in hexanes) to give the title compound as a white solid (4.0 mg; 54%). ¹H NMR (500 MHz, CDCl₃) δ 7.55 (d, J = 7.5 Hz, 1H), 7.47 (d, J = 7.5 Hz, 1H), 7.04 (t, J = 8.0 Hz, 1H), 6.94 (dd, J = 8.0 Hz J = 1.5 Hz, 1H), 6.74 (dd, J = 7.5 Hz, 1H), 5.68 (br. s., 1H), 4.14 (s, 2H) HRMS (ESI) Calculated mass for C₁₅H₉NO₂F₃: 292.0585 Measured mass: 292.0586

5-cyano-6-(trifluoromethyl)-9H-xanthen-4-yl 4,4,4-trifluorobutane-1-sulfonate HT-6-10 (AM7585, 71)

The synthesis was carried out as described as 43 using 70 (10 mg, 0.34 mmol), TBAB (13 mg, 0.41 mmol), 45% aq. NaOH (0.1mL) and 4,4,4-trifluorobutane-1-sulfonyl chloride (9.0 mg, 0.41 mmol). Reaction mixture was then quenched by addition of water and reaction mixture diluted with ethyl acetate. Organic phase was separated and the aqueous phase extracted with ethyl acetate (2x). The combined organic layer was washed with brine, dried (MgSO₄) and concentrated under reduced pressure. The crude was purified by flash column chromatography on silica gel (20% → 30% ethyl acetate in hexanes) to give the title compound as a white solid (12 mg; 75%). ¹H NMR (500 MHz, CDCl₃) δ 7.55 (d, J = 7.5 Hz, 1H), 7.48 (d, J = 8.0 Hz, 1H), 7.34 (t, J = 5.0 Hz, 1H), 7.17 (d, J = 5.5 Hz, 1H), 4.20 (s, 2H), 3.78 (t, J = 7.0 Hz, 2H) 2.40 (m, 4H) HRMS (EI) for C₁₉H₁₉O₄NF₆S Calculated Mass: 465.04693 Measured Mass: 465.04538
4’-methoxy-2-nitro-4-(trifluoromethyl)biphenyl (74)

The synthesis was carried out as 56 using 1-bromo-2-nitro-4(trifluoromethyl)benzene (500 mg, 1.85 mmol) in DME (6.0 mL) and H₂O (1.5 mL), 4-methoxyphenyl boronic acid (337 mg, 2.22 mmol) Pd(PPh₃)₄ (213 mg, 0.18 mmol) and Ba(OH)₂•8H₂O (1.16g, 3.7mmol) The crude was purified by flash column chromatography on silica gel (0% → 20% ethyl acetate in hexanes) to give the title compound (500 mg; 91%). ¹H NMR (500 MHz, CDCl₃) δ 8.07 (s, 1H), 7.84 (dd, J = 8.0 Hz J = 1.5 Hz, 1H), 7.59 (d, J = 8.5 Hz, 1H), 7.27 (dt, J = 9.0 Hz J = 3.0 Hz, 2H), 6.98 (dt, J = 8.5 Hz J = 3.0 Hz, 2H), 3.86 (s, 3H, OCH₃) HRMS (EI) for C₁₄H₁₀O₃NF₃ Calculated Mass: 297.06128 Measured Mass: 297.06149

2-methoxy-7-(trifluoromethyl)-9H-carbazole (75)

To a solution of 4’-methoxy-2-nitro-4-(trifluoromethyl)biphenyl (500 mg, 1.6 mmol) in anhydrous DMA (15 mL) was added PPh₃ (1.1 g, 4.2 mmol). Reaction cooled and poured in water and extracted with ethyl acetate (2x). The combined organic layer was washed with brine, dried (MgSO₄) and concentrated under reduced pressure. The crude was purified by flash column chromatography on silica gel (5% → 30% ethyl acetate in hexanes) to give the title compound as off-white solid (330 mg; 69%). ¹H NMR (500 MHz, CDCl₃) δ 8.14 (br. s., 1H), 8.04 (d, J = 8.5 Hz, 1H), 7.97 (d, J = 8.5 Hz, 1H), 7.65 (s, 1H), 7.46 (d, J = 7.0 Hz, 1H), 6.95 (d, J = 2.0 Hz, 1H), 6.90 (dd, J = 8.5 J = 2.0 Hz, 1H), 3.92 (s, 3H, OCH₃)

7-(trifluoromethyl)-9H-carbazol-2-ol (76)
To a stirred solution of 2-methoxy-7-(trifluoromethyl)-9H-carbazole (330 mg, 1.17 mmol) in anhydrous dichloromethane was added tetrabutylammonium iodide (866 mg, 2.3 mmol) and cooled to -78°C. Boron trichloride (2.3 mL, 2.3 mmo, 1.0 M solution in DMC) was then added. Reaction stirred overnight warming to room temperature. Reaction poured into water and extracted with ethyl acetate (2x). The combined organic layer was washed with brine, dried (MgSO₄) and concentrated under reduced pressure. The crude was purified by flash column chromatography on silica gel (10% → 50% ethyl acetate in hexanes) to give the title compound (270 mg; 91%) ¹H NMR (500 MHz, CDCl₃) δ 8.12 (br. s., 1H, NH), 8.02 (d, J = 8.5 Hz, 1H), 7.94 (d, J = 8.5 Hz, 1H), 7.64 (s, 1H), 7.45 (d, J = 8.5 Hz, 1H), 6.91 (d, J = 2.5 Hz, 1H), 6.80 (dd, J = 8.5 J = 2.0 Hz, 1H), 4.91 (s, 1H, OH)

7-(trifluoromethyl)-9H-carbazol-2-yl 4,4,4-trifluorobutane-1-sulfonate (AM7541, 77)

The synthesis was carried out as described for 43 using 7-(trifluoromethyl)-9H-carbazol-2-ol (100 mg, 0.1 mmol) in DCM (10 mL), TBAB (64 mg, 0.19 mmol), 45% aqueous NaOH (0.5 mL) and 4,4,4-trifluorobutane-1-sulfonyl chloride (84 mg, 0.47 mmol) in DCM (1.0 mL). The crude was purified by flash column chromatography on silica gel (20% → 60% ethyl acetate in hexanes) to give the title compound (80 mg; 47%). ¹H NMR (500 MHz, CDCl₃) δ 8.35 (br. s., 1H, NH), 8.14 (d, J = 8.5 Hz, 1H), 8.12 (d, J = 8.5 Hz, 1H), 7.74 (s, 1H), 7.53 (d, J = 8.5 Hz, 1H), 7.46 (d, J = 2.0 Hz, 1H), 7.17 (dd, J = 9.0 J = 2.5 Hz, 1H), 3.41 (t, J = 8.0 Hz, 2H), 2.42 – 2.36 (m, 2H), 2.36 – 2.28 (m, 2H)
CHAPTER FOUR:
BIPHENYL CANNABINOIDS
4.1 CB2 Selective Cannabinoids

Selective CB2 cannabinoid activation induces analgesia similar in potency to non-selective cannabinergic ligands (Iwamura, H., et al. 2001, Ibrahim, M. M, 2005) and has immunomodulatory effects. Unfortunately much of cannabinoid based therapeutics is hindered by the well-known psychoactive side-effects. Therefore the design and synthesis of selective CB2 agonist has become a very important and attractive target.

CB2 receptor activation inhibits the production of cAMP and stimulation of mitogen activated protein kinase (MAPK) (Pertwee, R.G., 1997). Because of this, it has also been suggested for the pain treatment of multiple sclerosis by inhibition of T cell activation (Sanchez, A.J. et a. 2006). Other therapeutic potentials include treatment of osteoporosis (Bab, I., et al. 2004), inhibition of malignant gliomas growths (Sanchez, C., et al. 2001), arterosclerosis (Steffens, S., et al. 2005) and Alzheimer’s disease (Ramirez, B. G. et al. 2005)

4.2 Biphenyl Cannabinoids

The first report of compounds with the biphenyl template as CB2 selective ligands was disclosed by researchers at Merk Frosst Laboratory (Gareau, Y., et al. 1996). In 2007, Dolle et al. reported a structure activity relationship study of biaryl cannabinoid mimetics. In this work they explored the potential of this underexplored but synthetically more accessible non-classical cannabinoid structure. They explored this template by utilizing structure activity relationship (SAR) from classical cannabinoids. Their synthesis is summarized in Figure 4.1.
Figure 4.1: SAR of biphenyl compounds by Merk Frosst

The biphenyl structure can be divided in three major fragments; 1) A ring 2) B-ring and side chain (Figure 4.1).

Previous work in our lab has identified biphenyl cannabinoids by Lai et al., explored the biphenyl class of compounds. These biphenyls are a modification of the reported Merck-Frosst compounds with different side chain, A-ring and B-ring substitutions. Lai’s cannabinoids also exhibited a significant selectivity for hCB2 receptor.

AM7552 was identified as a lead during a preliminary study of biaryl ether compounds in Chapter 2. Biphenyl AM7552 exhibiting binding affinity similar to that of the BAY59-3074, thus we pursued the biphenyl template further.
This chapter is focused on the structural variations on the two aromatic rings with modification in the A ring (mono- and di-substituted) substitutions and B ring side chain. This is of particular interest to us because of the variety of modifications that the receptor can tolerate.

In previous reports, the incorporation of a free phenolic hydroxyl groups in the A ring was deemed necessary to retain affinity to the CB receptors. In comparison to the identified biphenyl from SAR reported (Figure 4.1) the two biphenyl compounds differ in their A-ring substitution, absence of phenolic hydroxyls and position of side chain. Also substitutions in the A-ring at varying positions and screen in competition binding assay in CB1 and CB2 receptors.

4.3 Chemistry:

The retrosynthetic analysis in Figure 4.2 outlines the synthetic strategy for our desired biphenyl compounds.

\[
\begin{align*}
\text{R}_1 &= \text{CF}_3, \quad \text{R}_2 = \text{CN}, \quad \text{R}_3 = \text{OMe}, \quad \text{X} = \text{halide or boronic acid} \\
\end{align*}
\]

**Figure 4.3**: Retrosynthetic analysis of biphenyl analogs

There are multiple methods to form aryl-aryl bonds, we chose to utilize microwave accelerated Suzuki-Miyaura reactions to establish our core biphenyl because it tolerates a large range of substituents and short reaction times. There are also a large number of commercially available aryl bromide and boronic acids.

4.3.1 Synthesis of A- and B-ring modified biphenyls

139
We started our synthesis with the side chain of our lead compound BAY59-3074 varying side chains could be incorporated. The initial synthesis can be found in Chapter 2. We also elected to synthesize the \textit{para}-substituted side chain.

Scheme 4.1. Synthesis of biphenyl cannabinoid

Reagents and Conditions (a) Pd(PPh$_3$)$_4$, (BaOH)$_2$·8H$_2$O, DME/H$_2$O, µw; 15 min, 120ºC, 48%; (b) BCl$_3$, TBAI, DCM, -78ºC – RT, overnight, 82%; (c) 4,4,4-trifluorobutane-1-sulfonyl chloride, TBAB, 45%NaOH, DCM 0ºC – RT, 2h, 63%.

The commercially available boronic acids (82a-f) were coupled with 1-bromo-3-methoxybenzene (83) to give our core biphenyl under standard Suzuki-Miyaura coupling conditions in good yields (70%-80%). This gave access to the core biphenyl compound. The biphenyl intermediate was treated with 1.5 equivalents of boron trichloride and tetrabutyl ammonium iodide to remove the methyl protecting group and furnish the desired biphenyl with a free phenolic hydroxyl group. Lastly, the standard sulfonate side chain was incorporated with the appropriate sulfonyl chloride, TBAB and 45% aqueous NaOH solution in 53%-70% yield.

Scheme 4.2: Synthesis of A-ring modified biphenyls
Reagents and Conditions: (a) Pd(PPh$_3$)$_4$, Ba(OH)$_2$•8H$_2$O, DME: H$_2$O, microwave 70% - 80%
(b) BCl$_3$, TBAI, DCM, -78°C-RT, overnight, 75% - 89%
(c) ClO$_2$(CH$_2$)$_3$CF$_3$, 45% NaOH, TBABr, DCM, 53% – 70%

Similar to the full agonist, with the fused cyclopentyl moiety, we chose to synthesize the indole biphenyl compound. Following the synthesis outlined in Scheme 4.3, the bromo indole was coupled with 3-methoxyphenyl boronic acid (88) under microwave accelerated conditions.

Scheme 4.3: Synthesis of indole biphenyl
Reagents and Conditions: (a) Pd(PPh\textsubscript{3})\textsubscript{4}, Ba(OH)\textsubscript{2}•8H\textsubscript{2}O, DME/H\textsubscript{2}O, μw, 120°C, 15 min, 60%; (b) BCl\textsubscript{3}, TBAI, DCM, -78°C-RT, overnight, 71%; (c) Cl\textsubscript{2}OSO\textsubscript{2}(CH\textsubscript{2})\textsubscript{3}CF\textsubscript{3}, 45% NaOH, TBABr, DCM, 0°C – RT, 44%.

Alternatively by synthesizing the B-ring first and then coupling to the A-ring biphenyl, we were able to reduce the synthesis by 1 step. From 3-bromophenol (92), the side chain was incorporated under standardized methods and then coupled with the commercially available boronic acids. This scheme reduces the synthesis by 1 step, and can be applied for parallel synthesis.

Scheme 4.4: Shortened synthesis of biphenyls
Reagents and conditions: (a) TBAB, 45%NaOH, DCM 0ºC – RT, 2h; (b) Pd(PPh₃)₄, (BaOH)₂•8H₂O, DME/H₂O, µw; 15 min, 120ºC, 41% - 53%.

4.3.2 Synthesis of covalent biphenyl probes

Overall the biphenyl template template has good affinity to both CB receptors. We chose to synthesize covalent probes, to determine where the ligand may bind in the CB receptors. Covalent probes were also synthesized for the biphenyl class of compounds at the meta and para positions through the same procedure. The procedure is outlined in Scheme 4.4.

Scheme 4.5: Synthesis of biphenyl covalent probes
4.4 Results and Discussion

With AM7552 biphenyl compound as a lead and previous SAR performed by Lai et. al., functional groups were tested on the A-ring for their effects on the CB receptors’ binding affinities and selectivity.

AM7552 with the trifluoromethyl and cyano substitutions on the A-ring gave good binding affinity, with no particular selectivity. In addition to the meta-position side chain a compound with the para-position side chain substitution was also synthesized. AM7555 shows a marked decrease in binding affinity to the rCB1, but better affinity to the mCB2 in comparison to AM7552. Although in human CB2 cells Ki values increases and has a moderate affinity to the CB2 receptor.

Although this is an interesting finding, the majority of the ligands synthesized here are meta- because of the better binding affinities. This trend continues to be observed when comparing AM7574 and AM7575. The para-position is more CB2 selective than the meta-position, although the binding affinity is significantly better in AM7574. Due to the increase in binding affinity observed we chose to synthesize analogs with the chloro terminal functional group.
Table 4.1: Biphenyl cannabinoids

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<th>Compound</th>
<th>R1</th>
<th>R2</th>
<th>R3</th>
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<th>Ki (nM)</th>
<th>rCB1/mCB2</th>
<th>rCB1/hCB2</th>
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<td>hCB2 (HEK)</td>
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### 4.4.1 Functional testing

Functional testing of AM7574 does not show a significant change in cAMP levels. This suggests that AM7574 has a neutral effect at rCB1 receptor.

AM7574 is a biphenyl analog with almost 30x selectivity for the CB2 in competition binding assay. To further investigate the activity of this compound AM7574 was evaluated in *in vitro* and *in vivo* models. In β-arrestin assay the ligand exhibited selective CB2 agonist activity with an EC50 value of $1.502 \times 10^{-6}$. *In vivo* testing shows that there is no hypothermic effect at
the CB1 receptor. According to efficacy results in comparison are to WIN55212-2, AM7574 and the full agonist are near equipotent. This is a

Introduction of the compound AM7576, an azido biphenyl, produced effects consistent with AM7574 results. Displaying effects in both the CB1 antagonist and CB2 agonist modes. These efficacies are less than reported for AM7574, but very similar.

These minor changes make a large difference to the activity of the ligand in the receptors. This suggests that the side chain is a very important pharmacophore in determining the functional activity. Modifications at these points alter the agonist/antagonism.
4.4.2 *In vivo* testing

AM7574 was tested in 6 female rats, doses at low (1.0 mg/kg) and at high concentration (10.0 mg/kg) following subcutaneous injection; temperature was monitored over 6 h. There seems no significant change in temperature over the 6 h experiment. Although AM7574 possesses good affinity for the CB1 receptor it may not have any positive effect or does not cross the blood brain barrier in order to reach the significant CB1 receptor population in the CNS. Though this not to be expected because the Chemdraw generated clop = 4.45815 and tPSA = 67.16, which indicate good permeability and bioavailability.

![Figure 4.7: AM7574 Hypothermia test](image)

4.4.3 Functional Assay
As shown in Figure 4.6, the measurement of cAMP in rCB1 whole cells was tested. CB receptors are coupled to G-inhibitory proteins and therefore inhibit the production of cAMP. Therefore as the concentration of AM7576 increases the measured amount of cAMP produced is decreased. This inverse relationship is to be expected for an agonist. From this assay we observe a 40% change in cAMP levels and an EC$_{50}$ of 57.8 nM, indicative of an agonist.

Figure 4.8: AM7576 rCB1 cAMP assay

Figure 4.9: AM7576 β-arrestin CB2 agonist mode

4.4.4 Covalent labeling
Due to the lack of a solved crystal structure of either of the known CB receptors, we rely mainly on ligand based drug design. Another tool in our arsenal is the method of ligand assisted protein structure (LAPS), a great tool for the direct determination of the ligand receptor binding motif. This method aids in the determination of the structural features involved in the interaction of a ligand with the target protein.

Table 4.2: Biphenyl covalent probes

<table>
<thead>
<tr>
<th>Compound</th>
<th>X</th>
<th>Ki (nM)</th>
<th>rCB1/mCB2</th>
<th>rCB1/hCB2</th>
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<td></td>
<td></td>
<td>rCB1</td>
<td>mCB2</td>
<td>hCB2</td>
</tr>
<tr>
<td>98 7576</td>
<td>N₃</td>
<td>122</td>
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<td>99 7578</td>
<td>NCS</td>
<td>&gt;1000</td>
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</tr>
<tr>
<td>100 7577</td>
<td>N₃</td>
<td>606</td>
<td>70.6</td>
<td>110</td>
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<td>101 7579</td>
<td>NCS</td>
<td>&gt;1000</td>
<td>&gt;1000</td>
<td>--</td>
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</tbody>
</table>
Covalent probes were synthesized of our biphenyl class of compounds incorporating a photoactivatable azide and isothiocyanate at the terminal side chain. It was surprising to see that only the azido (98, 100) compounds exhibited binding affinity to the CB receptors. Therefore AM7576 was tested in the mCB2 receptor for covalent binding affinity. Results of this assay show 24% specific binding at 10nM concentration. This is an important finding and warrants further development to identify key amino acids in the binding domain.

![Figure 4.7: AM7576 mCB2 covalent assay](image)

### 4.5 Conclusions

In summary, the early stage SAR investigations show that biphenyl ligands have good affinity to both CB receptors. Unlike previously reported biphenyl structures the positioning of the substituents is different, while the core remains the same. While the two phenolic hydroxyl groups were reported as necessary for CB binding, it is not in this series.

The positioning of the side chain with respect to the biphenyl bond plays a large role in affinity and selectivity of the compound. Sulfonate side chain in the para position with respect
to the biaryl bond, impart a large selectivity for the CB2 receptor over CB1 receptor. A high affinity ligand, AM7574, for the hCB2 was identified and a covalent ligand AM7576 was identified to be used in future studies and improve upon.

4.5 Experimental

4'-methoxy-3-(trifluoromethyl)biphenyl-2-carbonitrile (79)

In a microwave reaction vessel: To a solution of 4-bromoanisole (145mg, 0.77mmol) in DME(3.0 mL)/H₂O (0.75 mL) was added 2-Cyano-3(trifluoromethyl)phenyl boronic acid neopentyl glycol ester (200 mg, 0.7 mmol) and degassed with N₂ for 10 min. Then Pd(PPh₃)₄ (81.6 mg, 0.07 mmol) and Ba(OH)₂•8 H₂O (222 mg, 0.7 mmol) was added and degassed with N₂ for 5 min. and subjected to microwave irradiation for 15min. at 120°C. The reaction was poured in water and extracted with ethyl acetate (2x). The combined organic layer was washed with brine, dried (MgSO₄) and concentrated under reduced pressure. The crude was purified by flash column chromatography on silica gel (0% → 20% ethyl acetate in hexanes) to give the title compound as white solid (94 mg; 48%) ¹H NMR (500MHz ,CDCl₃) δ 7.78 - 7.74 (m, 1H), 7.74 - 7.70 (m, 1H), 7.70 - 7.65 (m, 1H), 7.49 (d, J = 8.8 Hz, 2H), 7.04 (d, J = 8.8 Hz, 2H), 3.88 (s, 3H) HRMS (ESI) for C₁₅H₁₁NOF₃ Calculated Mass: 278.0793 Measured Mass: 278.0789

4'-hydroxy-3-(trifluoromethyl)biphenyl-2-carbonitrile (80)

To a solution of 4'-methoxy-3-(trifluoromethyl)biphenyl-2-carbonitrile (100 mg, 0.3 mmol) in anhydrous DCM (6.0 mL) was added TBAI (199 mg, 0.5 mmol) and cooled to -78°C, under argon gas. Boron trichloride (0.5mL, 0.5mmol, 1.0 M solution in DCM) was then added. The
reaction mixture was stirred at -78°C for 30min, then warmed to room temperature overnight and quenched with water. The organic layer was separated and the aqueous phase extracted with ethyl acetate (2x). The combined organic layer was washed with brine, dried (MgSO₄) and concentrated under reduced pressure. The crude was purified by flash column chromatography on silica gel (20% → 40% ethyl acetate in hexanes) to give the title compound as white solid (78 mg; 82%) ¹H NMR (500MHz, CDCl₃) δ 7.75 (t, 1H), 7.72 (d, 1H), 7.68 (d, 1H), 7.45 (d, J = 8.8 Hz, 2H), 6.97 (d, J = 8.3 Hz, 2H), 5.01 (s, 1H)

2'-cyano-3'-(trifluoromethyl)biphenyl-4-yl 4,4,4-trifluorobutane-1-sulfonate (AM7555, 81)

To a stirred solution of 4'-hydroxy-3-(trifluoromethyl)biphenyl-2-carbonitrile (20 mg, 0.76 mmol) in anhydrous DCM (7.0 mL) was added TBAB (12 mg, 0.38 mmol) and 45% NaOH (0.5 mL) and cooled to 0°C. Then 4,4,4-trifluorobutane-sulfonyl chloride (19 mg, 0.91 mmol) in anhydrous DCM (1.0 mL) was added and stirred at room temperature for 2h. Reaction was poured into water and extracted with ethyl acetate (2x). Organic layers washed with brine, dried (MgSO₄) and concentrated under reduced pressure. The crude was purified by flash column chromatography on silica gel (20% → 50% ethyl acetate in hexanes) to give the title compound as yellow oil (21 mg; 63%) ¹H NMR (500MHz, CDCl₃) δ 7.87 - 7.82 (m, 1 H), 7.79 (t, J = 7.8 Hz, 1 H), 7.69 (d, J = 7.8 Hz, 1 H), 7.61 (d, J = 8.3 Hz, 2 H), 7.43 (d, J = 8.8 Hz, 2 H), 3.42 (t, J = 7.3 Hz, 2 H), 2.46 - 2.36 (m, 2 H), 2.36 - 2.27 (m, 2 H) HRMS (EI) for C₁₈H₁₃O₃NF₆S Calculated Mass: 437.05202 Measured Mass: 437.05341

2'-cyano-3'-(trifluoromethyl)biphenyl-3-yl 3-chloropropane-1-sulfonate (AM7574, 86a)
The synthesis carried out as described for 81 using 35 (150 mg, 0.57 mmol), TBAB (202 mg, 0.62 mmol), 45% aqueous NaOH solution (0.5 mL) and 3-chloropropane-1-sulfonyl chloride (111 mg, 0.62 mmol) in anhydrous DCM (5.0 mL). The crude was purified by flash column chromatography on silica gel (40% diethyl ether in hexanes) to give the title compound as yellow oil (170 mg; 74%). 1H NMR (500 MHz, CDCl₃) δ 7.85 (d, J = 8.0 Hz, 1H), 7.80 (t, J = 8.0 Hz, 1H), 7.73 (d, J = 7.5 Hz, 1H), 7.60 (t, J = 8.5 Hz, 1H), 7.49 (t, J = 2.0 Hz, 1H), 7.45 (dd, J = 8.0 Hz  J = 1.0 Hz, 1H), 3.74 (t, J = 6.0 Hz, 2H), 3.53 (t, J = 7.5 Hz, 2H), 2.50 – 2.44 (m, 2H) HRMS (ESI) for C₁₇H₁₄NO₃F₃SCl Calculated Mass: 404.0335 Measured Mass: 404.0333

2'-cyano-3'- (trifluoromethyl)biphenyl-4-yl 3-chloropropane-1-sulfonate (AM7575)

The synthesis carried out as described for 81 using 80 (150 mg, 0.57 mmol), TBAB (202 mg, 0.62 mmol), 45% aqueous NaOH solution (0.5 mL), and 3-chloropropane-1-sulfonyl chloride (111 mg, 0.62 mmol) in anhydrous DCM (6.0 mL). The crude was purified by flash column chromatography on silica gel (40% diethyl ether in hexanes) to give the title compound as yellow oil (149 mg; 65%) 1H NMR (500 MHz, CDCl₃) δ 7.86 (d, J = 8.0 Hz, 1H), 7.80 (t, J = 8.0 Hz, 1H), 7.72 (d, J = 7.0 Hz, 1H), 7.63 (dt, J = 8.0 Hz J = 1.0 Hz, 2H). 7.47 (dt, J = 8.0 Hz J = 2.5 Hz, 2H), 3.78 (t, J = 5.5 Hz, 2H), 3.53 (t, J = 7.5 Hz, 2H), 2.50 – 2.44 (m, 2H) HRMS (EI) for C₁₇H₁₃O₃NF₃SCl Calculated Mass: 403.02568 Measured Mass: 403.02466

3-methoxy-3'- (trifluoromethyl)-1,1'-biphenyl (84b)

The synthesis was carried out as described in 79 using 3-bromoanisole (100 mg, 0.53 mmol) and (3-(trifluoromethyl)phenyl)boronic acid (121 mg, 0.64 mmol), Ba(OH)₂•H₂O (252 g, 0.8 mmol)
and Pd(PPh₃)₄ (62 mg, 0.05 mmol) in DME (3.0 mL) and water (1.0 mL). The crude was purified by flash column chromatography on silica gel (0 → 10% ethyl acetate in hexanes) to give the title compound as clear oil (101 mg; 75%). ¹H NMR (500MHz, CDCl₃) δ 7.82 (s, 1H), 7.75 (d, J = 7.0 Hz, 1H), 7.60 (d, J = 8.0 Hz, 1H), 7.55 (t, J = 7.5 Hz, 1H), 7.39 (t, J = 8.0 Hz, 1H), 7.18 (ddd, J = 8.0 Hz J = 2.0 Hz J = 1.0 Hz, 1H), 7.12 (t, J = 2.5 Hz, 1H), 6.94 (ddd, J = 8.0 Hz J = 2.5 Hz J = 1.0 Hz, 1H), 3.88 (s, 3H, OCH₃) HRMS (EI) for C₁₄H₁₁OF₃ Calculated Mass: 252.0721 Measured Mass: 252.07705

3'-methoxy-[1,1'-biphenyl]-3-carbonitrile (84c)
The synthesis was carried out as described in 79 using 3-bromoanisole (100 mg, 0.53 mmol) and (3-cyanophenyl)boronic acid (94 mg, 0.64 mmol), Ba(OH)₂•H₂O (252 mg, 0.8 mmol) and Pd(PPh₃)₄ (62 mg, 0.05 mmol) in DME (3.0 mL) and water (1.0 mL). The crude was purified by flash column chromatography on silica gel (0 → 10% ethyl acetate in hexanes) to give the title compound as clear oil (80 mg; 72%). ¹H NMR (500MHz, CDCl₃) δ 7.86 (t, J = 1.5 Hz, 1H), 7.80 (dt, J = 7.5 Hz J =1.5 Hz, 1H), 7.63 (dt, J = 7.0 Hz J = 1.5 Hz, 1H), 7.54 (t, J = 7.5 Hz, 1H), 7.39 (t, J = 8.5 Hz, 1H), 7.14 (dd, J = 8.0 Hz J = 1.5 Hz, 1H), 7.07 (t, J = 2.5 Hz, 1H), 6.95 (ddd, J = 8.5 Hz J = 2.0 Hz J = 1.0 Hz, 1H), 3.88 (s, 3H, OCH₃) HRMS (ESI) for C₁₄H₁₂NO Calculated Mass: 210.0919 Measured Mass: 210.0916

3'-methoxy-2-(trifluoromethyl)-1,1'-biphenyl (84d)
The synthesis was carried out as described in 79 using 3-bromoanisole (100 mg, 0.53 mmol) and (2-(trifluoromethyl)phenyl)boronic acid (121 mg, 0.64 mmol), Ba(OH)₂•H₂O (252 mg, 0.8
mmol) and Pd(PPh₃)₄ (62 mg, 0.05 mmol) in DME (3.0 mL) and water (1.0 mL). The crude was purified by flash column chromatography on silica gel (0 → 10% ethyl acetate in hexanes) to give the title compound as clear oil (94 mg; 70%). ¹HNMR (500MHz, CDCl₃) δ 7.34 (d, J = 8.0 Hz, 1H), 7.55 (t, J = 7.0 Hz, 1H), 7.47 (t, J = 8.0 Hz, 1H), 7.34 (d, J = 7.0 Hz, 1H), 7.31 (t, J = 8.0 Hz, 1H), 6.92 (t, J = 9.0 Hz, 1H), 6.89 (s, 1H), 3.83 (s, 3H, OCH₃) HRMS (EI) for C₁₄H₁₁OF₃ Calculated Mass: 252.07621 Measured Mass: 252.07570

3'-methoxy-[1,1'-biphenyl]-2-carbonitrile (84e)
The synthesis was carried out as described in 79 using 3-bromoanisole (100 mg, 0.53 mmol) and (2-cyanophenyl)boronic acid (94 mg, 0.64 mmol), Ba(OH)₂•H₂O (252 mg, 0.8 mmol) and Pd(PPh₃)₄ (62 mg, 0.05 mmol) in DME (3.0 mL) and water (1.0 mL). The crude was purified by flash column chromatography on silica gel (0 → 10% ethyl acetate in hexanes) to give the title compound as clear oil (79 mg; 71%). ¹HNMR (500MHz, CDCl₃) δ 7.76 (d, J = 8.0 Hz, 1H), 7.64 (td, J = 8.0 Hz J = 1.5 Hz, 1H), 7.52 (d, J = 8.5 Hz, 1H), 7.45 (td, J = 8.0 Hz J = 1.0 Hz, 1H), 7.40 (t, J = 8.0 Hz, 1H), 7.13 (dd, J = 7.5 Hz J = 1.0 Hz, 1H), 7.09 (t, J = 2.5 Hz, 1H), 6.99 (dd, J = 8.0 Hz J = 2.0 Hz, 1H), 3.87 (s, 3H, OCH₃)

3'-methoxy-3,5-bis(trifluoromethyl)-1,1'-biphenyl (84f)
The synthesis was carried out as described in 79 using 3-bromoanisole (100 mg, 0.53 mmol) and (3,5-bis(trifluoromethyl)phenyl)boronic acid (165 mg, 0.64 mmol), Ba(OH)₂•H₂O (252 mg, 0.8 mmol) and Pd(PPh₃)₄ (62 mg, 0.05 mmol) in DME (3.0 mL) and water (1.0 mL). The crude was purified by flash column chromatography on silica gel (0 → 10% ethyl acetate in hexanes) to give the title compound as clear oil (136 mg; 80 %). ¹HNMR (500MHz, CDCl₃) δ 8.00 (s, 1H), 7.86 (s, 1H), 7.42 (t, J = 8.0 Hz, 1H), 7.18 (dt, J = 7.0 Hz J = 1.0 Hz, 1H), 7.11 (t, J = 2.0 1H),
6.99 (dd, $J = 8.0$ Hz $J = 2.0$ Hz, 1H), 3.89 (s, 3H, OCH$_3$) HRMS (EI) for C$_{15}$H$_{10}$OF$_6$ Calculated Mass: 320.06357 Measured Mass: 320.06285

3'-((trifluoromethyl)-[1,1'-biphenyl]-3-ol (85b)

The synthesis was carried out as described in 80 using 3-methoxy-3'-((trifluoromethyl)-1,1'-biphenyl (70 mg, 0.28 mmol), TBAI (154 mg, 0.41 mmol) and BCl$_3$ (0.41 mL, 0.41 mmol, 1.0 M solution in DCM). The crude was purified by flash column chromatography on silica gel (0 $\rightarrow$ 10% ethyl acetate in hexanes) to give the title compound as oil (50 mg; 75%). $^1$HNMR (500MHz, CDCl$_3$) δ 7.74 (d, $J = 8.0$ Hz, 1H), 7.55 (t, $J = 7.0$ Hz, 1H), 7.47 (t, $J = 7.5$ Hz, 1H), 7.33 (d, $J = 7.0$ Hz, 1H), 7.27 (t, $J = 8.0$ Hz, 1H), 6.90 (d, $J = 7.5$ Hz, 1H), 6.86 (ddd, $J = 8.5$ Hz $J = 2.5$ Hz $J = 1.0$ Hz, 1H), 6.81 (s, 1H), 4.79 (br. s., 1H, OH)

HRMS (ESI) Calculated mass for C$_{13}$H$_{10}$NO: 196.0762 Measured mass: 196.0761

3'-hydroxy-[1,1'-biphenyl]-3-carbonitrile (85c)

The synthesis was carried out as described in 80 using 3'-methoxy-[1,1'-biphenyl]-3-carbonitrile (70 mg, 0.33 mmol), TBAI (185 mg, 0.5 mmol) and BCl$_3$ (0.5 mL, 0.5 mmol, 1.0 M solution in DCM). The crude was purified by flash column chromatography on silica gel (0 $\rightarrow$ 10% ethyl acetate in hexanes) to give the title compound as oil (54 mg; 82%). $^1$HNMR (500MHz, CDCl$_3$) δ 7.85 (t, $J = 2.0$ Hz, 1H), 7.80 (dt, $J = 7.5$ Hz $J = 2.0$ Hz, 1H), 7.64 (dt, $J = 7.0$ Hz $J = 1.5$ Hz, 1H), 7.54 (t, $J = 7.5$ Hz, 1H), 7.35 (t, $J = 8.0$ Hz, 1H), 7.13 (dt, $J = 2.0$ Hz, 1H), 6.88 (ddd, $J = 8.5$ Hz $J = 2.0$ Hz $J = 1.0$ Hz, 1H), 5.02 (br. s., 1H, OH) HRMS (ESI) Calculated mass for C$_{13}$H$_{10}$NO: 196.0762 Measured mass: 196.0761
2'-(trifluoromethyl)-[1,1'-biphenyl]-3-ol (85d)

The synthesis was carried out as described in 80 using 3'-methoxy-2-(trifluoromethyl)-1,1'-biphenyl (70 mg, 0.28 mmol), TBAI (154 mg, 0.41 mmol) and BCl3 (0.41 mL, 0.41 mmol, 1.0 M solution in DCM). The crude was purified by flash column chromatography on silica gel (0 → 10% ethyl acetate in hexanes) to give the title compound as oil (57 mg; 86%). 1HNMR (500MHz, CDCl3) δ 7.74 (d, J = 8.0 Hz, 1H), 7.55 (t, J = 7.5 Hz, 1H), 7.46 (t, J = 7.5 Hz, 1H), 7.34 (d, J = 8.0 Hz, 1H), 7.31 (t, J = 8.0 Hz, 1H), 6.94 (ddd, J = 8.0 Hz J = 2.0 Hz J = 1.0 Hz), 6.91 (d, J = 9.5 Hz, 1H), 6.87 (s, 1H) HRMS (EI) for C13H9OF3 Calculated Mass: 238.06056 Measured Mass: 238.05991

3'-hydroxy-[1,1'-biphenyl]-2-carbonitrile (85e)

The synthesis was carried out as described in 80 using 3'-methoxy-[1,1'-biphenyl]-2-carbonitrile (70 mg, 0.33 mmol), TBAI (185 mg, 0.5 mmol) and BCl3 (0.5 mL, 0.5 mmol, 1.0 M solution in DCM). The crude was purified by flash column chromatography (0% → 30% ethyl acetate in hexanes) to give the title compound as oil (58 mg; 89 %). 1HNMR (500MHz, CDCl3) δ 7.76 (dd, J = 7.5 Hz J = 1.5 Hz, 1H), 7.64 (td, J = 8.0 Hz J = 1.5 Hz, 1H), 7.51 (dd, J = 8.0 Hz J = 1.0 Hz, 1H), 7.44 (td, J = 7.0 Hz J = 1.0 Hz, 1H), 7.30 (t, J = 7.5 Hz, 1H), 7.12 (d, J = 8.0 Hz, 1H), 7.03 (t, J = 2.0 Hz, 1H), 6.62 (ddd, J = 8.5 Hz J = 3.5 Hz J = 1.0 Hz, 1H), 5.15 (br. s., 1H, OH) HRMS (ESI) Calculated mass for C13H10NO: 196.0762 Measured mass: 196.0762

3',5'-bis(trifluoromethyl)-[1,1'-biphenyl]-3-ol (85f)

The synthesis was carried out as described in 80 using 3'-methoxy-3,5-bis(trifluoromethyl)-1,1'-biphenyl (70 mg, 0.22 mmol), TBAI (121 mg, 0.32 mmol) and BCl3 (0.32 mL, 0.32 mmol, 1.0 M solution in DCM). The crude was purified by flash column chromatography on silica gel (0 → 158
10% ethyl acetate in hexanes) to give the title compound as oil (62 mg; 92 %). \(^1\)HNMR (500MHz, CDCl\(_3\)) \(\delta\) 8.0 (s, 1H), 7.86 (s, 1H), 7.38 (t, \(J = 8.0\) Hz, 1H), 7.18 (d, \(J = 7.0\) Hz, 1H), 7.09 (t, \(J = 2.0\) Hz, 1H), 6.92 (dd, \(J = 8.0\) Hz \(J = 2.0\) Hz, 1H), 4.94 (s, 1H, OH) HRMS (EI) for \(\text{C}_{14}\text{H}_{8}\text{OF}_6\) Calculated Mass: 306.04792 Measured Mass: 306.04864

3'-(trifluoromethyl)-[1,1'-biphenyl]-3-yl 3-chloropropane-1-sulfonate (86b)

The synthesis was carried out as described in 81 using 3'-(trifluoromethyl)-[1,1'-biphenyl]-3-ol (10 mg, 0.04 mmol), TBAB (16 mg, 0.05mmol), 45% aqueous NaOH solution (0.1 mL) and 3-chloropropane-1-sulfonyl chloride (11 mg, 0.06 mmol). The crude was purified by flash column chromatography on silica gel (0 \(\rightarrow\) 20% ethyl acetate in hexanes) to give the title compound as oil (11 mg; 70 %). \(^1\)HNMR (500MHz, CDCl\(_3\)) \(\delta\) 7.76 (d, \(J = 7.5\) Hz, 1H), 7.58 (t, \(J = 7.5\) Hz, 1H), 7.51 (t, \(J = 7.5\) Hz, 1H), 7.46 (t, \(J = 8.0\) Hz, 1H), 7.35 (d, \(J = 7.5\) Hz, 1H), 7.30 (d, \(J = 8.0\) Hz, 1H), 3.72 (t, \(J = 6.5\) Hz, 2H), 3.46 (t, \(J = 7.5\) Hz, 2H), 2.41 – 2.48 (m, 2H) HRMS (ESI) for \(\text{C}_{16}\text{H}_{15}\text{O}_3\text{F}_3\text{SCl}\) Calculated Mass: 379.0383 Measured Mass: 379.0382

3'-cyano-[1,1'-biphenyl]-3-yl 3-chloropropane-1-sulfonate (86c)

The synthesis was carried out as described in 81 using 3'-hydroxy-[1,1'-biphenyl]-3-carbonitrile (10 mg, 0.05 mmol), TBAB (8.0 mg, 0.02mmol), 45% aqueous NaOH solution (0.1 mL) and 3-chloropropane-1-sulfonyl chloride (11 mg, 0.06 mmol). The crude was purified by flash column chromatography on silica gel (0 \(\rightarrow\) 20% ethyl acetate in hexanes) to give the title compound as oil (10 mg; 59 %). \(^1\)HNMR (500MHz, CDCl\(_3\)) \(\delta\) 7.85 (t, \(J = 1.5\) Hz, 1H), 7.80 (d, \(J = 7.5\) Hz, 1H), 7.67 (d, \(J = 7.5\) Hz, 1H), 7.58 (t, \(J = 7.5\) Hz, 1H), 7.53 (t, \(J = 6.5\) Hz, 1H), 7.52 (m, 1H), 7.48 (t, \(J = 2.0\) Hz, 1H), 7.38 (m, 1H), 3.76 (t, \(J = 6.5\) Hz, 2H), 3.52 (t, \(J = 7.0\) Hz, 2H), 2.52 – 2.46 (m, 2H)
2'-(trifluoromethyl)-[1,1'-biphenyl]-3-yl 3-chloropropane-1-sulfonate (86d)

The synthesis was carried out as described in 81 using 2'-(trifluoromethyl)-[1,1'-biphenyl]-3-ol (10 mg, 0.04 mmol), TBAB (16 mg, 0.05 mmol), 45% aqueous NaOH solution (0.1 mL) and 3-chloropropane-1-sulfonyl chloride (11 mg, 0.06 mmol). The crude was purified by flash column chromatography on silica gel (0 → 20% ethyl acetate in hexanes) to give the title compound as oil (9.0 mg; 57%). 1H NMR (500MHz, CDCl3) δ 7.76 (d, J = 7.0 Hz, 1H), 7.60 (t, J = 7.5 Hz, 1H), 7.51 (d, J = 8.0 Hz, 1H), 7.47 (t, J = 8.0 Hz, 1H), 7.34 (d, J = 7.5 Hz, 1H), 7.30 (d, J = 7.5 Hz, 1H), 3.72 (t, J = 6.5 Hz, 1H), 3.46 (t, J = 7.5 Hz, 2H), 2.49 – 2.41 (m, 2H).

2'-cyano-[1,1'-biphenyl]-3-yl 3-chloropropane-1-sulfonate (86e)

The synthesis was carried out as described in 81 using 3'-hydroxy-[1,1'-biphenyl]-2-carbonitrile (10 mg, 0.05 mmol), TBAB (8.0 mg, 0.02 mmol), 45% aqueous NaOH solution (0.1 mL) and 3-chloropropane-1-sulfonyl chloride (11 mg, 0.06 mmol). The crude was purified by flash column chromatography on silica gel (0 → 20% ethyl acetate in hexanes) to give the title compound as oil (9.0 mg; 53%). 1H NMR (500MHz, CDCl3) δ 7.81 (dd, J = 8.0 Hz J = 1.0 Hz, 1H), 7.71 (td, J = 7.5 Hz J = 1.5 Hz, 1H), 7.59 (t, J = 8.0 Hz, 1H), 7.56 (d, J = 7.5 Hz, 1H), 7.55 (m, 1H), 7.53 (dd, J = 8.0 Hz, 1H), 7.51 (t, J = 2.0 Hz, 1H), 7.43 (ddd, J = 2.0 Hz, 1H), 3.76 (t, J = 6.5 Hz, 2H), 3.54 (t, J = 7.5 Hz, 2H), 2.52 – 2.47 (m, 2H) HRMS (ESI) Calculated mass for C16H15NO3SCl: 336.0461 Measure mass:336.0461

3',5'-bis(trifluoromethyl)-[1,1'-biphenyl]-3-yl 3-chloropropane-1-sulfonate (86f)
The synthesis was carried out as described in [81] using 3',5'-bis(trifluoromethyl)-[1,1'-biphenyl]-3-ol (10 mg, 0.03 mmol), TBAB (5.2 mg, 0.01 mmol), 45% aqueous NaOH solution (0.1 mL) and 3-chloropropane-1-sulfonyl chloride (6.3 mg, 0.032 mmol). The crude was purified by flash column chromatography on silica gel (0 → 20% ethyl acetate in hexanes) to give the title compound as oil (9.4 mg; 65%). $^1$HNMR (500MHz, CDCl$_3$) $\delta$ 7.99 (s, 2H), 7.90 (s, 1H), 7.58 (d, $J = 1.0$ Hz, 1H), 7.57 (t, $J = 1.0$ Hz, 1H), 7.51 (t, $J = 1.5$ Hz, 1H), 7.39 (m, 1H), 3.76 (t, $J = 6.0$ Hz, 2H), 3.53 (t, $J = 7.5$ Hz, 2H), 2.52 – 2.49 (m, 2H)

3-bromophenyl butane-1-sulfonate (94)

The synthesis was carried out as described for [81] using 3-bromophenol (500 mg, 2.9 mmol), TBAB (465 mg, 1.4mmol), 45% aqueous NaOH solution (1.0 mL) and butane-1-sulfonyl chloride (375mg, 2.4 mmol). The crude was purified by flash column chromatography on silica gel (0 → 5 % ethyl acetate in hexanes) to give the title compound as oil (804 mg; 95 %). $^1$HNMR (500MHz, CDCl$_3$) $\delta$ 7.48 – 7.43 (m, 2H), 7.29 (t, $J = 7.5$ Hz, 1H), 7.23 (ddd, $J = 8.0$ Hz $J = 1.5$ Hz $J = 0.5$ Hz, 1H), 3.25 – 3.22 (m, 2H), 2.00 – 1.92 (m, 2H), 1.53 (sxt, $J = 8.0$ Hz, 2H), 0.99 (t, $J = 7.0$ Hz, 3H, CH$_3$) HRMS (ESI) for C$_{10}$H$_{14}$O$_3$SBr Calculated Mass: 292.9847 Measured Mass: 292.9850

3-bromophenyl 3-chloropropane-1-sulfonate (HT-6-62)

The synthesis was carried out as described in [81] using 3-bromophenol (300 mg, 1.7 mmol), TBAB (670 mg, 2.1 mmol), 45% aqueous NaOH solution (1.0 mL) and 3-chloropropane-1-sulfonyl chloride (368 mg, 2.1mmol). The crude was purified by flash column chromatography
on silica gel (0 → 10% ethyl acetate in hexanes) to give the title compound as clear oil (460 mg; 85%). $^1$HNMR (500MHz, CDCl$_3$) δ 7.50-7.45 (m, 2H), 7.30 (t, $J = 8.5$ Hz, 1H), 7.24 (ddd, $J = 9.0$ Hz $J = 2.0$ Hz $J = 1.0$ Hz, 1H), 3.73 (t, $J = 5.5$ Hz, 2H), 3.47 (t, $J = 7.0$ Hz, 2H), 2.48-2.42 (m, 2H)

**ethyl 3'-(butylsulfonyloxy)-[1,1'-biphenyl]-3-carboxylate (96a)**

This synthesis was carried out as described for 79 using 94 (20 mg, 0.06 mmol) and (3-ethoxycarbonyl)phenyl)boronic acid (16 mg, 0.08mmol), Ba(OH)$_2$•H$_2$O (32 mg, 0.12 mmol) and Pd(PPh$_3$)$_4$ (7.0 mg, 0.006 mmol) in DME (3.0 mL) and water (1.0 mL). The crude was purified by flash column chromatography on silica gel (10% → 30% ethyl acetate in hexanes) to give the title compound as oil (11 mg; 41%). $^1$HNMR (500MHz, CDCl$_3$) δ 8.25 (t, $J = 2.0$ Hz, 1H), 8.07 (dt, $J = 8.0$ Hz $J = 2.0$ Hz, 1H), 7.76 (dt, $J = 8.0$ Hz $J = 1.0$ Hz, 1H), 7.58 (dt, $J = 8.0$ Hz, 1H), 7.52 (quint, $J = 7.5$ Hz, 1H), 7.51 (t, $J = 2.0$ Hz, 1H), 7.30 (ddd, $J = 9.5$ Hz $J = 2.5$ Hz $J = 1.0$ Hz, 1H), 4.42 (q, $J = 7.0$ Hz, 2H), 3.34-3.26 (m, 2H), 2.04-1.97 (m, 2H), 1.54 (sxt, $J = 7.5$ Hz, 2H), 1.42 (t, $J = 7.0$ Hz, 3H, CH$_3$), 0.99 (t, $J = 7.5$ Hz, 3H, CH$_3$) HRMS (ESI) for C$_{19}$H$_{23}$O$_5$S Calculated Mass: 363.1266 Measured Mass: 363.1269

**3'-methoxy-[1,1'-biphenyl]-3-yl butane-1-sulfonate (96b)**

Direct coupling. The synthesis was carried out as described for 79 using 94 (20 mg, 0.06 mmol), 3-methoxyphenyl boronic acid (12 mg, 0.08 mmol), and Ba(OH)$_2$•H$_2$O (32 mg, 0.12 mmol) and Pd(PPh$_3$)$_4$ (7.0 mg, 0.006 mmol) in DME (3.0 mL) and water (1.0 mL). The crude was purified by flash column chromatography on silica gel (0% → 20% ethyl acetate in hexanes) to give the title compound as oil (11 mg; 53%). $^1$HNMR (500MHz, CDCl$_3$) δ 7.53 (dt, $J = 8.0$ Hz $J = 1.5$ Hz, 1H), 7.30 (ddd, $J = 9.5$ Hz $J = 2.5$ Hz $J = 1.0$ Hz, 1H), 4.42 (q, $J = 7.0$ Hz, 2H), 3.34-3.26 (m, 2H), 2.04-1.97 (m, 2H), 1.54 (sxt, $J = 7.5$ Hz, 2H), 1.42 (t, $J = 7.0$ Hz, 3H, CH$_3$), 0.99 (t, $J = 7.5$ Hz, 3H, CH$_3$) HRMS (ESI) for C$_{19}$H$_{23}$O$_5$S Calculated Mass: 363.1266 Measured Mass: 363.1269
Hz, 1H), 7.48 (t, \( J = 8.0 \text{ Hz}, 1 \text{H} \)), 7.47 (t, \( J = 2.5 \text{ Hz}, 1 \text{H} \)), 7.37 (t, \( J = 7.5 \text{ Hz}, 1 \text{H} \)), 7.25 (m, 1H), 7.15 (d, \( J = 7.5 \text{ Hz}, 1 \text{H} \)), 7.10 (t, \( J = 2.5 \text{ Hz}, 1 \text{H} \)), 6.93 (dd, \( J = 8.5 \text{ Hz} J = 2.5 \text{ Hz}, 1 \text{H} \)), 3.87 (s, 3H, OCH\(_3\)), 3.28 (t, \( J = 8.0 \text{ Hz}, 2 \text{H} \)), 2.02 – 1.95 (m, 2H), 1.52 (sxt, \( J = 7.0 \text{ Hz}, 2 \text{H} \)), 0.99 (t, \( J = 8.0 \text{ Hz}, 3 \text{H}, \text{CH}_3\))

HRMS (ESI) for H\(_{17}\)H\(_{21}\)O\(_4\)S Calculated Mass: 321.1161 Measured Mass: 321.1139

**5-(3-methoxyphenyl)-1H-indole (89)**

The synthesis was carried out as described for 79 using 5-bromo-1H-indole (50 mg, 0.25mmol), 3-methoxyphenyl boronic acid (46 mg, 0.30 mmol), Ba(OH)\(_2\)\(\cdot\)H\(_2\)O (120 mg, 0.38 mmol) and Pd(PPh\(_3\))\(_4\) (29 mg, 0.025 mmol) in DME (3.0 mL) and water (1.0 mL). The crude product was purified by flash column chromatography on silica gel (0% to 30% ethyl acetate in hexanes) to give the title compound as oil (34 mg; 60%). \(^1\)HNMR (400MHz, CDCl\(_3\)) \(\delta\) 7.59 (d, \( J = 7.6 \text{ Hz}, 1 \text{H} \)), 7.43 (t, \( J = 8.0 \text{ Hz}, 1 \text{H} \)), 7.35 (t, \( J = 7.2 \text{ Hz}, 1 \text{H} \)), 7.34 (t, \( J = 8.0 \text{ Hz}, 1 \text{H} \)), 7.18 (d, \( J = 6.0 \text{ Hz}, 1 \text{H} \)), 7.13 (t, \( J = 2.0 \text{ Hz}, 1 \text{H} \)), 6.90 (dd, \( J = 8.0 \text{ Hz} J = 2.4 \text{ Hz}, 1 \text{H} \)), 3.86 (s, 3H, OCH\(_3\))

**3-(1H-indol-5-yl)phenol (90)**

The synthesis was carried out as described for 80 using 89 (30 mg, 0.13 mmol), TBAI (74 mg, 0.20 mmol), BCl\(_3\) (0.2 mL, 0.2 mmol, 1.0 M solution in DCM) in anhydrous DCM (5.0 mL). The crude product was purified by flash column chromatography on silica gel (0% to 30% ethyl acetate in hexanes) to give the title compound as oil (20 mg; 71%). \(^1\)HNMR (400MHz, CDCl\(_3\)) \(\delta\) 7.62 (d, \( J = 7.6 \text{ Hz}, 2 \text{H} \)), 7.45 (t, \( J = 8.0 \text{ Hz}, 2 \text{H} \)), 7.32 (t, \( J = 7.2 \text{ Hz}, 1 \text{H} \)), 7.30 (t, \( J = 8.0 \text{ Hz}, 1 \text{H} \)), 7.15 (d, \( J = 6.0 \text{ Hz}, 1 \text{H} \)), 7.10 (t, \( J = 2.0 \text{ Hz}, 1 \text{H} \)), 6.90 (dd, \( J = 8.0 \text{ Hz} J = 2.4 \text{ Hz}, 1 \text{H} \))
3-(1H-indol-5-yl)phenyl 4,4,4-trifluorobutane-1-sulfonate (AM7594, 91)

The synthesis was carried out as described for 81 using 90 (10 mg, 0.04 mmol), TBAB (8 mg, 0.023 mmol), 0.1mL 45% aqueous NaOH solution and 4,4,4-trifluorobutane-1-sulfonyl chloride (10 mg, 0.05 mmol) in anhydrous DCM (10 mL). The crude was purified by flash column chromatography on silica gel (20% \(\rightarrow\) 40% ethyl acetate in hexanes) to give the title compound as oil (8 mg; 44%). \(^{1}\)HNMR (400MHz, CDCl\(_{3}\)) \(\delta\) 7.58 (d, \(J = 7.0\) Hz, 1H), 7.57 (d, \(J = 7.0\) Hz, 1H), 7.56 (dt, \(J = 8.0\) Hz \(J = 1.5\) Hz, 1H), 7.49 (t, \(J = 8.0\) Hz, 2H), 7.46 (t, \(J = 8.0\) Hz, 2H), 7.39 (tt, \(J = 7.5\) Hz \(J = 1.5\) Hz, 1H), 7.25 (ddd, \(J = 8.0\) Hz \(J = 2.5\) Hz \(J = 1.0\) Hz, 1H), 3.38 (t, \(J = 7.5\) Hz, 2H), 2.44 – 2.34 (m, 2H), 2.34 – 2.26 (m, 2H)

2'-cyano-3'-((trifluoromethyl)biphenyl-3-yl 3-azidopropane-1-sulfonate (AM7576, 98)

To a solution of 2'-cyano-3'-((trifluoromethyl)biphenyl-3-yl 3-chloropropane-1-sulfonate (100 mg, 0.24 mmol) in DMF (5.0 mL) was added NaN\(_{3}\) (78 mg, 1.2 mmol) and stirred at room temperature overnight. Reaction poured into water and extracted with ethyl acetate (2x). The combined organic layer was washed with brine and dried (MgSO\(_{4}\)). The crude was purified by flash column chromatography using silica gel (20% \(\rightarrow\) 50% ethyl acetate in hexanes) to give the title compound as and oil (60 mg; 59%). \(^{1}\)H NMR (500 MHz, CDCl\(_{3}\)) \(\delta\) 7.88 (d, \(J = 8.0\) Hz, 1H), 7.83 (t, \(J = 8.0\) Hz, 1H), 7.76 (d, \(J = 7.5\) Hz, 1H), 7.54 (d, \(J = 9.0\) Hz, 1H), 7.52 (dd, \(J = 7.5\) Hz \(J = 2.5\) Hz, 1H), 7.48 (ddd, \(J = 7.5\) Hz \(J = 2.5\) Hz \(J = 1.0\) Hz, 1H), 3.59 (t, \(J = 6.5\) Hz, 2H), 3.46 (t, \(J = 7.5\) Hz, 2H), 2.32 – 2.24 (m, 2H) HRMS (EI) for C\(_{17}\)H\(_{13}\)N\(_{4}\)O\(_{3}\)F\(_{3}\)NaS Calculated Mass: 433.0558 Measured Mass: 433.0560
2'-cyano-3'-(trifluoromethyl)biphenyl-4-yl 3-azidopropane-1-sulfonate (AM7577, 101)

To a solution of 2'-cyano-3'-(trifluoromethyl)biphenyl-4-yl 3-chloropropane-1-sulfonate (100 mg, 0.24 mmol) in DMF (5.0 mL) was added NaN₃ (78 mg, 1.2 mmol) and stirred at room temperature overnight. Reaction poured into water and extracted with ethyl acetate (2x). The combined organic layers was washed with brine and dried (MgSO₄). The crude was purified by flash column chromatography using silica gel (20% → 50% ethyl acetate in hexanes) to give the title compound as an oil (55 mg; 54%). ¹H NMR (500 MHz, CDCl₃) δ 7.84 (d, J = 8.0 Hz, 1H), 7.90 (t, J = 7.5 Hz, 1H), 7.69 (d, J = 6.5 Hz, 1H). 7.60 (dt, J = 9.0 Hz J = 2.0 Hz, 2H), 7.44 (dt, J = 8.5 Hz J = 3.0 Hz, 2H), 3.57 (t, J = 6.5 Hz, 2H), 3.43 (t, J = 7.0 Hz, 2H), 2.30 – 2.20 (m, 2H)

2'-cyano-3'-(trifluoromethyl)biphenyl-3-yl 3-isothiocyanatopropane-1-sulfonate (AM7578, 99)

To a solution of 2'-cyano-3'-(trifluoromethyl)biphenyl-3-yl 3-azidopropane-1-sulfonate (40 mg, 0.09 mmol) in THF (5.0 mL) was added carbon disulfide (0.1 mL, 0.19 mmol) and triphenyphosphine (38 mg, 0.14 mmol) and stirred at room temperature for 1 day. Reaction poured into water and extracted with ethyl acetate (2x). The combined organic layers was washed with brine and dried (MgSO₄). The crude was purified by flash column chromatography using silica gel (10% → 30% ethyl acetate in hexanes) to give the title compound as an oil (25 mg; 60%) ¹H NMR (500MHz ,CDCl₃) δ 7.88 (d, J = 8.0 Hz, 1H), 7.83 (t, J = 8.0 Hz, 1H), 7.76 (d, J = 7.5 Hz, 1H), 7.54 (d, J = 9.0 Hz, 1H), 7.52 (dd, J = 7.5 Hz J = 2.5 Hz, 1H), 7.48 (ddd, J = 7.5 Hz J = 2.5 Hz J = 1.0 Hz, 1H), 3.59 (t, J = 6.5 Hz, 2H), 3.46 (t, J = 7.5 Hz, 2H), 2.32 – 2.24 (m, 2H)
2'-cyano-3'-{(trifluoromethyl)biphenyl-4-y1} 3-isothiocyanatopropane-1-sulfonate (AM7579, 101)

To a solution of 2'-cyano-3'-{(trifluoromethyl)biphenyl-4-y1} 3-azidopropane-1-sulfonate (40 mg, 0.09 mmol) in THF (5.0 mL) was added carbon disulfide (0.1 mL, 0.19 mmol) and triphenyphosphine (38 mg, 0.14 mmol) and stirred at room temperature for 1 day. Reaction poured into water and extracted with ethyl acetate (2x). The combined organic layers was washed with brine and dried (MgSO₄). The crude was purified by flash column chromatography using silica gel (10% → 30% ethyl acetate in hexanes) to give the title compound as an oil (23 mg; 55%). ¹H NMR (500 MHz, CDCl₃) δ 7.84 (d, J = 8.0 Hz, 1H), 7.90 (t, J = 7.5 Hz, 1H), 7.69 (d, J = 6.5 Hz, 1H), 7.60 (dt, J = 9.0 Hz J = 2.0 Hz, 2H), 7.44 (dt, J = 8.5 Hz J = 3.0 Hz, 2H), 3.57 (t, J = 6.5 Hz, 2H), 3.43 (t, J = 7.0 Hz, 2H), 2.30 – 2.20 (m, 2H)
CHAPTER FIVE:
BIARYL ETHER COMPOUNDS AS INHIBITORS OF FATTY ACID AMIDE HYDROLASE (FAAH)
5.1 The Endocannabinoid System

There are two endocannabinoids of primary significance, anandamide (AE) and 2-arachidonylglycerol (2-AG). They are deactivated by the endocannabinoid hydrolyzing enzymes, fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MGL). By inhibiting the action of these proteins, the degradation of endocannabinoids are slowed and in turn the CB effect prolonged. Through the manipulation of the endogenous cannabinoid signaling by the inhibition of FAAH and related enzymes we can elicit the desired effects of cannabinoids, while avoiding undesirable negative effects of CB activation.

These proteins are part of the serine hydrolase super family. This family of enzymes is one of the largest families that catalytic mechanism involves a catalytic triad consisting of a serine nucleophile which is activated by a proton relay mechanism. Subclasses also include proteases, amidases, esterases, lipases, and transacylases. FAAH is responsible for the degradation of fatty acid primary amides and ethanolamides, while MGL is categorized under the lipase sub-class. Because of the many subclasses, selectivity against other sub-classes of serine hydrolase sub-classes is very desirable.

5.2 FAAH Inhibitors

FAAH inhibitors consist of two pharmacophoric subunits responsible for enzyme recognition and inactivation. The hydrophilic (“inhibition”) subunit, bearing an activated sulfonyl, phosphonyl or carbonyl group interacts with the nucleophilic hydroxyl group of the enzyme, and the lipophilic (“binding”) subunit interacts with the hydrophobic region of the catalytic channel through π-bond interaction, aromatic stacking interaction, or hydrophobic bonding
The active site of FAAH is buried deep within the enzyme and is only accessible by two narrow channels. The residues of the catalytic triad (Ser 241, 217, and Lys142), interact through a network of hydrogen bonds that facilitates proton exchange. The hydrolysis reaction involves a protonation of Lys142 via deprotonation of Ser217, which then deprotonates the nucleophilic residue Ser241. These protons are still available to the leaving group of the substrate. It is believed that the ability of FAAH to protonate the leaving group is responsible for FAAH’s ability to hydrolyze amides at a rate comparable to that of esters.

Figure 5.1 illustrates the mechanism for substrate hydrolysis of FAAH. The initial proton exchange occurs when Lys142 deprotonates Ser217, resulting in a zwitterionic intermediate B. The alkoxide of Ser217 removes the proton from Ser241, which then adds to the carbonyl of the substrate to form a tetrahedral intermediate. Transfer of the proton from Ser217 to the substrate’s leaving group to give another zwitterion D, leaves Ser 241 covalently modified as an ester. Transfer of a proton from Lys142 to Ser217 gives E. The exit of ethanolamine and hydrolysis of the acylated Ser241 results in the regeneration of catalytic FAAH.
5.3 Biaryl ether drugs

In the market today there are drugs available that contain the biaryl ether core. One such example is of Fenoprofen. Fenoprofen is a non-steroid anti-inflammatory drug used for symptomatic relief of rheumatoid arthritis, osteoarthritis, and mild to moderate pain. It decreases inflammation, pain, and fever, probably through inhibition of cyclooxygenase activity and prostaglandin synthesis (Borne 2008). Another example is the drug Nimesulide, a selective COX-2 inhibitor for the treatment of acute pain and osteoarthritis, but due to the risk of hepatotoxicity it was withdrawn from the market. (Bessone 2010)

![Figure 5.2 Biaryl ether medications](image)


In this chapter, our efforts are focused on exploring the catalytic site of FAAH by synthesis of biaryl ether cannabinoids. In previous SAR studies, AM7517 was tested as an enzyme inhibitor of Fatty Acid Amide Hydrolase (FAAH) and inhibited with an IC₅₀ of 433 nM. This is our starting point for this research.
5.4 Chemistry

5.4.1 Synthesis of substituted biaryl ether inhibitor

Scheme 5.1: Synthesis of substituted biaryl ether analogs

Reagents and conditions: (a) NaH, DMSO, 130°C, 12h, 80%; (b) BCl$_3$, TBAI, DCM -78º-RT, 3h, 92%; (c) propyl amine, triphosgene, NEt$_3$, 0ºC - RT, 4h, 38%

The synthesis of substituted biaryl ether inhibitor began with the coupling of 1 and 3-methoxyphenol in 80% yield. Reduction of the methyl ether by treatment of boron trichloride and tetrabutyl ammonium iodide in DCM was accomplished in 92% yield. The incorporation of the carbamate side chain by reaction of the phenolic 4 with triethylamine and propylamine.

5.4.2 Synthesis of non-substituted biaryl ether inhibitors

Starting from commercially available 3-phenoxyphenol, coupled with $p$-nitrophenyl chloroformate in toluene to give major intermediate AM7559. From AM7559, a series of compounds with side chains that contain a carbamate functional group were synthesized by coupling AM7559 with the appropriate amine. Synthesis of this key intermediate made it
possible to perform parallel synthesis of this series of compounds. The coupling was accomplished by addition of the appropriate amine in THF stirring at room temperature for 2h, yielding 64 - 97%.

Scheme 5.2: Synthesis of carbamate analogs

Reagents and Conditions: (a) 4-nitrophenyl carbonochloridate NEt₃, RT, 1h, 92%;
(b) amine, THF, RT, 2h, 64 - 97%

5.4 Results and Discussion
All compounds were evaluated initially in 3-point assay at 1μM, 10μM and 100μM concentrations and followed by 8-point inhibition assays for FAAH.

Fluorescent assay has recently gained wide acceptance as a simple and sensitive protocol amenable to HTS to quickly assay large number of compounds synthesized as enzyme inhibitors. The principle of this protocol is based on monitoring the fluorescence produced by hydrolysis of a non-fluorogenic substrate by the enzyme to give a fluorogenic compound. 7-amino-4-methylcoumarin (AMC) is the commonly used fluorescent moiety and various acyl groups, including dodecyl (Kage, K. L., et al. 2007) and arachidonoyl group (Ramarao, M. K., et al. 2005), can be coupled with this moiety to produce non-fluorescent substrates. In a report disclosed by Ramarao et al (Ramarao, M. K., et al. 2005), the ability of human FAAH expressed on CHO cells to hydrolyze arachidonoyl-7-amino-4-methyl-coumarinamide (AAMC) was assessed by monitoring the relative intensity of AMC on a fluorimeter. Stability under assay conditions, substrate specificity, as well as low signal to noise ratio, are key parameters that are necessary for such a substrate. By using this protocol, a series of well-known FAAH inhibitors from different chemical classes have been evaluated and the IC\textsubscript{50} values obtained agree well with those assayed using radioactively labeled endogenous substrates, like anandamide.

Recently developed in the Makriyannis Laboratory, a modification of the Ramarao methodology involves the use of rat ΔTM-FAAH expressed in E. coli as the enzyme source and AAMC as substrate. IC\textsubscript{50} values are then determined by non-linear regression in Prism by Graphpad.

To explore the necessity of substituents on ring A we eliminated the trifluoromethyl and cyano groups to give AM7548. By removing the substituents on the A-ring, while maintaining the propyl carbamate side chain, the IC\textsubscript{50} values dropped from 433 nM to 62.8 nM. This is
nearly a 7-fold increase in inhibition. Therefore we concluded that these unsubstituted biaryl ether compounds have a greater potential to be better inhibitors of FAAH then substituted biaryl ether compounds on the A ring.

Table 5.1: Straight chain carbamate analogs

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
<th>% Inhibition (µM) FAAH</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt;*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 µM</td>
<td>10 µM</td>
</tr>
<tr>
<td>104 AM7560</td>
<td></td>
<td>75.7%</td>
<td>106.3%</td>
</tr>
<tr>
<td>105 AM7548</td>
<td></td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>106 AM7572</td>
<td>1nM</td>
<td>4.0%</td>
<td>10nM 20.8%</td>
</tr>
<tr>
<td>107 AM7561</td>
<td></td>
<td>104.7%</td>
<td>106.4%</td>
</tr>
<tr>
<td>108 AM7562</td>
<td></td>
<td>10.9%</td>
<td>45.0%</td>
</tr>
<tr>
<td>109 AM763</td>
<td></td>
<td>96.4%</td>
<td>105.9%</td>
</tr>
<tr>
<td>110 AM7564</td>
<td>1nM</td>
<td>7.3%</td>
<td>10nM 5.5%</td>
</tr>
<tr>
<td>111 AM7573</td>
<td></td>
<td>5.5%</td>
<td>31.5%</td>
</tr>
</tbody>
</table>

*15 min incubation

With the addition of a hydroxyl group of the carbamate, it was surprising to see that the IC<sub>50</sub> value was so high. The straight alkyl chains were the best.
A variety of carbamate side chains were synthesized including straight alkyl chains and cyclic groups. The IC\textsubscript{50} values decrease as the alky side chain is increased from ethyl to pentyl (2<3<4<5). The pentyl side chain produced the best values with an IC\textsubscript{50} of 18.4 nM. Terminal substitutions were not well tolerated and inhibition values increased.

Table 5.2: Cyclic carbamate side chain

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
<th>% Inhibition (µM) FAAH</th>
<th>IC\textsubscript{50}*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 µM</td>
<td>10 µM</td>
</tr>
<tr>
<td>112 AM7565</td>
<td></td>
<td>(1nM) 9.0%</td>
<td>(10nM) 28.8%</td>
</tr>
<tr>
<td>113 AM7566</td>
<td></td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>114 AM7567</td>
<td></td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>115 AM7568</td>
<td></td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>116 AM7569</td>
<td></td>
<td>100.4%</td>
<td>100.6%</td>
</tr>
<tr>
<td>117 AM7559</td>
<td></td>
<td>-8.3%</td>
<td>12.8%</td>
</tr>
<tr>
<td>118 AM7570</td>
<td></td>
<td>60.4%</td>
<td>96.7%</td>
</tr>
<tr>
<td>119 AM7571</td>
<td></td>
<td>(1nM) 6.6%</td>
<td>(10nM) 6.2%</td>
</tr>
</tbody>
</table>

*15 min incubation

Cyclic structures inhibit FAAH well. Of particular note is the cyclohexane group (IC\textsubscript{50} = 28 nM). The cyclobutyl, -pentyl and –hexyl groups produce IC\textsubscript{50} values between 25-35nM.
The large cyclic adamantyl group is surprisingly still a good inhibitor, but as more polar groups like the morpholine group is added the IC$_{50}$ values begin to trail off.

The carbamate group is essential for inhibition of FAAH activity in this series of compounds. It acts as a leaving group after the nucleophilic attack of the Ser241 residue of the carbonyl of the inhibitors, resulting in enzyme carbamolyation. When replaced by sulfonate side chain the inhibition of FAAH is considerably reduced.

**Molecular Modeling**

The *in vitro* results are further reinforced with the docking of AM7517 into FAAH. FAAH consists of two long channels. There is ample room for the side chain to be accommodated. The ligand is also in close proximity to the catalytic triad.

![Figure 5.3 Molecular Modeling of AM7517 docked in FAAH](image)

### 5.6 Conclusions
Our SAR on the binding subunit has assisted our understanding of the factors that increases inhibitory potency, thus, for our hit compound, we have succeeded in increasing the potency for inhibiting FAAH, from IC$_{50}$ value of 430 nM to an excellent 18.76 nM in 107 (AM7561), a nearly 23-fold increase. In addition, 114 (AM7567) and 116 (AM7569) exhibited low affinity for the CB receptors. It will be interesting to see the in vivo effect of these compounds.

5.7 Experimental

3-phenoxypbenyl propylcarbamate (AM7548, 105)

To an ice cooled solution of 3-phenoxypbenol (100mg, 0.53mmol) and triphosgene (158mg, 0.53mmol) in anhydrous DCM, under argon, was added triethylamine (0.37 mL, 2.6 mmol). After 1h, propylamine (0.05 mL, 0.64 mmol) was added and stirred in ice for 2h. The reaction was diluted in DCM, washed with water (2x), brine (2x), dried over MgSO$_4$ and concentrated under reduced pressure. The crude was purified by flash column chromatography on silica gel (0% $\rightarrow$ 20% ethyl acetate in hexanes) to give the title compound as a white solid (134 mg; 92%) $^1$H NMR (500MHz,CDCl$_3$) δ 7.34 (t, $J = 7.5$ Hz, 1H), 7.28 (t, $J = 8.0$ Hz, 1H), 7.12 (t, $J = 8.0$ Hz, 1H), 7.04 (d, $J = 8.0$ Hz, 2H), 6.88 (dd, $J = 7.5$ Hz J = 1.5 Hz, 1H), 6.83 (dd, $J = 8.5$ Hz $J = 2.0$ Hz, 1H), 6.78 (t, $J = 2.5$ Hz, 1H), 4.97 (br. s., 1H, NH), 3.22 (q, $J = 6.5$ Hz, 2H), 1.59 (sxt, $J = 7.5$ Hz, 2H), 0.96 (t, $J = 7.0$ Hz, 3H, CH$_3$) HRMS (EI) for C$_{18}$H$_{15}$F$_3$N$_2$O$_3$ Calculated Mass: 363.0956 Found Mass: 363.0958

4-nitrophenyl 3-phenoxypbenyl carbonate (AM7559, 117)
To a solution of 3- phenoxyphenol (2.0 g, 10.7 mmol) in anhydrous toluene (40 mL) was added triethylamine (1.79 mL, 12.9 mmol) and p-nitrophenyl chloroformate (2.6 g, 12.9 mmol) and stirred at room temperature for 1 h. The reaction mixture was poured into water and extracted with ethyl acetate (2x). The combined organic layer was washed with brine, dried (MgSO₄) and concentrated under reduced pressure. The crude was purified by flash column chromatography on silica gel (0% → 10% ethyl acetate in hexanes) to give the title compound as an off-white solid (4.08 g; 92%) ³¹H NMR (500 MHz, CDCl₃) δ 8.32 (dt, J = 6.5 Hz J = 1.5 Hz, 2H), 7.49 (dt, J = 7.0 Hz J = 2.0 Hz, 2H), 7.36 (t, J = 5.5 Hz, 2H), 7.24 (t, J = 9.0 Hz, 1H), 7.14 (t, J = 9.0 Hz, 1H), 7.08 – 7.00 (m, 4H) HRMS (EI) for C₁₉H₁₃NO₆ Calculated Mass: 351.0743 Found Mass: 351.0744

3- phenoxyphenyl ethylcarbamate (AM7560, 104)

To a solution of 4-nitrophenyl 3-phenoxyphenyl carbonate (50 mg, 0.14 mmol) in anhydrous THF (3.0 mL) was added ethylamine (7.0 mg, 0.15 mmol) dissolved in THF (1.0 mL) and stirred at room temperature for 2 h, then poured into water, extracted with DCM (2x), dried (MgSO₄) and concentrated under reduced pressure. The crude was purified by flash column chromatography on silica gel (0% → 10% diethyl ether in hexanes) to give the title compound as white solid (24 mg; 64%). Mp: 48-49°C ¹H NMR (500 MHz, CDCl₃) δ 7.34 (t, J = 8.0 Hz, 2H), 7.28 (t, J = 8.0 Hz, 1H), 7.12 (t, J = 8.0 Hz, 1H), 7.04 (d, J = 7.5 Hz, 2H), 6.88 (dd, J = 8.5 Hz J = 2.0 Hz, 1H), 6.83 (dd, J = 8.5 Hz J = 2.0 Hz, 1H), 6.78 (t, J = 2.5 Hz, 1H), 4.95 (br. s., 1H, NH), 3.30 (quin, J = 6.0 Hz, 2H), 1.20 (t, J = 7.5 Hz, 3H, CH₃) HRMS (EI) for C₁₅H₁₅NO₃ Calculated Mass: 257.10520 Found Mass: 257.10561

3- phenoxyphenyl pentylcarbamate (AM7561, 107)
The synthesis was carried out as described for 104, using 4-nitrophenyl 3-phenoxyphenyl carbonate (50 mg, 0.14 mmol), and ethanolamine (9.5 mg, 0.15 mmol) in anhydrous THF (1.0 mL). The crude was purified by flash column chromatography on silica gel (0% \rightarrow 10% ethyl acetate in hexanes) to give the title compound as a white solid (38 mg; 97%) mp: 66-67\degree C. 

\[ \begin{align*}
\text{HRMS (EI) for C}_{15}\text{H}_{15}\text{NO}_4 \quad \text{Calculated Mass: 273.1001} \\
\text{Found Mass: 273.1001}
\end{align*} \]

3-phenoxyphenyl 2-hydroxyethylcarbamate (AM7562, 108)

The synthesis was carried out as described for 104, using 4-nitrophenyl 3-phenoxyphenyl carbonate (50 mg, 0.14 mmol), and ethanolamine (9.5 mg, 0.15 mmol) in anhydrous THF (1.0 mL). The crude was purified by flash column chromatography on silica gel (50% \rightarrow 100% ethyl acetate in hexanes) to give the title compound as a white solid (38 mg; 97%) mp: 43-44\degree C. 

\[ \begin{align*}
\text{HRMS (EI) for C}_{18}\text{H}_{21}\text{NO}_3 \quad \text{Calculated Mass: 299.15215} \\
\text{Found Mass: 299.15357}
\end{align*} \]

3-phenoxyphenyl allylcarbamate (AM7563, 109)

\[ \begin{align*}
\text{HRMS (EI) for C}_{15}\text{H}_{15}\text{NO}_4 \quad \text{Calculated Mass: 273.1001} \\
\text{Found Mass: 273.1001}
\end{align*} \]
The synthesis was carried out as described for 104, using 4-nitrophenyl 3-phenoxyphenyl carbonate (50 mg, 0.14 mmol), and allylamine (8.9 mg, 0.15 mmol) in anhydrous THF (1.0 mL). The crude was purified by flash column chromatography on silica gel (0% → 20% ethyl acetate in hexanes) to give the title compound as a white solid (35 mg; 92%) mp: 38-39°C. 

\[
\text{HNMR (500MHz, CDCl}_3\] \delta 7.35 (t, J = 9.0 Hz, 2H), 7.29 (t, J = 8.0 Hz, 1H), 7.12 (t, J = 8.0 Hz, 1H), 7.04 (dd, J = 8.0 Hz, J = 1.0 Hz, 2H), 6.8 (d, J = 8.5 Hz, 1H), 6.84 (d, J = 8.5 Hz, 1H), 6.79 (d, J = 2.5 Hz, 1H), 5.95 - 5.84 (m, 1H), 5.26 (d, J = 17.5 Hz, 1H), 5.18 (d, J = 10 Hz, 1H), 5.06 (br. s., 1H, NH), 3.89 (br. s., 2H) HRMS (EI) for C_{16}H_{15}NO_3: Calculated Mass: 269.10520 Found Mass: 269.10512

3-phenoxyphenyl prop-2-ynylcarbamate (AM7564, 110)

The synthesis was carried out as described for 104, using 4-nitrophenyl 3-phenoxyphenyl carbonate (50 mg, 0.14 mmol), and propargylamine (8.6 mg, 0.15 mmol) in anhydrous THF (1.0 mL). The crude was purified by flash column chromatography on silica gel (0% → 30% ethyl acetate in hexanes) to give the title compound as a white solid (37 mg; 97%) mp: 46-47°C. 

\[
\text{HNMR (500MHz, CDCl}_3\] \delta 7.35 (t, J = 7.5 Hz, 2H), 7.29 (t, J = 8.5 Hz, 1H), 7.13 (t, J = 8.0 Hz, 1H), 7.04 (d, J = 9.0 Hz, 2H), 6.88 (dd, J = 8.5 Hz, J = 2.0 Hz, 1H), 6.85 (dd, J = 7.5 Hz, 1H), 6.79 (t, J = 2.0 Hz, 1H), 5.18 (br. s., 1H, NH), 4.06 (dd, J = 5.5 J = 2.0 Hz, 2H), 2.29 (s, 1H) HRMS (EI) for C_{16}H_{13}NO_3: Calculated Mass: 267.08955 Found Mass: 267.08954

3-phenoxyphenyl cyclohexylcarbamate (AM7565, 112)

The synthesis was carried out as described for 104, using 4-nitrophenyl 3-phenoxyphenyl carbonate (50 mg, 0.14 mmol), and cyclohexylamine (15.5 mg, 0.15 mmol) in anhydrous THF (1.0 mL). The crude was purified by flash column chromatography on silica gel (0% → 30%
ethyl acetate in hexanes) to give the title compound as white solid (40 mg; 91%) mp: 93-94°C

$^1$HNMR: HT-4-83 $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 7.35 (t, $J = 7.5$ Hz, 2H), 7.28 (t, $J = 7.5$ Hz, 1H), 7.12 (t, $J = 7.5$ Hz, 1H), 7.04 (d, $J = 8.5$ Hz, 2H), 6.88 (ddd, $J = 8.5$ Hz $J = 2.0$ Hz $J = 1.0$ Hz, 1H), 6.84 (d, $J = 8.5$ Hz, 1H), 6.78 (t, $J = 2.0$ Hz, 1H), 4.86 (br. S., 1H), 3.52 - 3.42 (m, 1H), 2.02 - 1.95 (m, 2H), 1.80 - 1.70 (m, 2H), 1.68 - 1.60 (m, 1H), 1.43 - 1.30 (m, 2H), 1.30 - 1.10 (m, 3H) HRMS (ESI) for C$_{19}$H$_{22}$NO$_3$ Calculated Mass: 312.1600 Found Mass: 312.1593

3-phenoxypyphenyl adamantlycarbamate (AM7566, 113)

The synthesis was carried out as described for 104, using 4-nitrophenyl 3-phenoxypyphenyl carbonate (50 mg, 0.14 mmol), and l-adamantylamine (23.6 mg, 0.15 mmol) in anhydrous THF. The crude was purified by flash column chromatography on silica gel (0% $\rightarrow$ 30% ethyl acetate in hexanes) to give the title compound as a white solid (41 mg; 80%) mp: 119-120°C $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 7.34 (t, $J = 7.5$ Hz, 2H), 7.27 (t, $J = 8.0$ Hz, 1H), 7.11 (t, $J = 7.5$ Hz, 1H), 7.04 (d, $J = 7.5$ Hz, 2H), 6.88 (ddd, $J = 9.0$ Hz $J = 2.0$ Hz $J = 1.0$ Hz, 1H), 6.81 (dd, $J = 8.0$ Hz, 1H), 6.78 (s, 1 H), 4.85 (br. s., 1H, NH), 2.10 (s, 3 H), 1.98 (d, $J = 2.5$ Hz, 6H), 1.68 (s, 6H) HRMS (ESI) for C$_{23}$H$_{26}$NO$_3$ Calculated Mass: 364.1913 Found Mass: 364.1909

3-phenoxypyphenyl cyclobutylcarbamate (AM7567, 114)

The synthesis was carried out as described for 104, using 4-nitrophenyl 3-phenoxypyphenyl carbonate (50 mg, 0.14 mmol), and cyclobutylamine (11.1 mg, 0.15 mmol) in anhydrous THF. The crude was purified by flash column chromatography on silica gel (0% $\rightarrow$ 30% ethyl acetate in hexanes) to give the title compound as a white solid (35 mg; 87%). Mp: 72-73°C $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 7.34 (t, $J = 8.0$ Hz, 2H), 7.28 (t, $J = 8.0$ Hz, 1H), 7.12 (t, $J = 8.5$ Hz, 1H),
7.04 (d, J = 8.0 Hz, 2H), 6.87 (dd, J = 8.0 Hz J = 2.0 Hz, 1H), 6.83 (dd, J = 8.0 Hz J = 2.0 Hz, 1H), 6.77 (t, J = 2.0 Hz, 1H), 5.10 (br. s., 1H, NH), 4.20 (q, J = 7.5 Hz, 1H), 2.40 – 2.31 (m, 2H), 1.92 (quint, J = 12.5 Hz, 2H), 1.75 - 1.65 (m, 2H) HRMS (EI) for C₁₇H₁₇NO₃ Calculated Mass:: 283.12085 Found Mass: 283.12108

3-phenoxyphenyl cyclopropylmethylcarbamate (AM7568, 115)

The synthesis was carried out as described for 104, using 4-nitrophenyl 3-phenoxyphenyl carbonate (50 mg, 0.14 mmol), and aminomethylcyclopropane (11.1 mg, 0.15 mmol) in anhydrous THF (1.0 mL). The crude product was purified by flash column chromatography on silica gel (0% → 30% ethyl acetate in hexanes) to give the title compound as a white solid (35 mg; 87%). Mp: 55-56ºC ¹H NMR (500 MHz, CDCl₃) δ 7.35 (t, J = 8.0 Hz, 2H), 7.29 (t, J = 8.5 Hz, 1H), 7.12 (t, J = 7.5 Hz, 1H), 7.04 (d, J = 8.0 Hz, 2H), 6.88 (ddd, J = 8.5 Hz J = 3.0 Hz J = 1.0 Hz, 1H), 6.82 (dd, J = 8.5 Hz J = 2.0 Hz, 1H), 6.79 (t, J = 2.5 Hz, 1H), 5.09 (br. s., 1H, NH), 3.12 (t, J = 6.0 Hz, 2H), 1.09 – 0.96 (m, 1H), 0.54 (q, J = 5.0 Hz, 2H), 0.23 (q, J = 5.5 Hz, 2H) HRMS (EI) for C₁₇H₁₇NO₃ Calculated Mass: 283.12085 Found Mass: 283.1121

3-phenoxyphenyl cyclopentylcarbamate (AM7569, 116)

The synthesis was carried out as described for 104, using 4-nitrophenyl 3-phenoxyphenyl carbonate (50 mg, 0.14 mmol), cyclopentylamine (13.3 mg, 0.15 mmol) in anhydrous THF(1.0 mL). The crude was purified by flash column chromatography on silica gel (0% → 30% ethyl acetate in hexanes) to give the title compound as an oil (38 mg; 90%). ¹H NMR (500 MHz, CDCl₃) δ 7.34 (t, J = 7.0 Hz, 2H), 7.28 (t, J = 8.0 Hz, 1H), 7.12 (t, J = 7.5 Hz, 1H), 7.04 (d, J = 8.0 Hz, 2H), 6.88 (dd, J = 8.5 Hz J = 1.5 Hz, 1H), 6.83 (dd, J = 8.5 Hz J = 2.0 Hz, 1H),
6.78 (t, J = 2.0 Hz, 1H), 4.92 (br. s., 1H, NH), 4.11 – 3.97 (m, 1H), 2.08 – 1.93 (m, 2H), 1.78 – 1.65 (m, 2H), 1.65 – 1.60 (m, 2H), 1.52 – 1.40 (m, 2H) HRMS (EI) for C₁₈H₁₉NO₃ Calculated Mass: 297.1365 Found Mass: 297.1365

3-phenoxyphenyl 2-morpholinoethylcarbamate (AM7570, 118)

The synthesis was carried out as described for AM7560, using 4-nitrophenyl 3-phenoxyphenyl carbonate (50 mg, 0.14 mmol), and 4-(2-Aminoethyl)morphline (20.3 mg, 0.15 mmol) in anhydrous THF (1.0 mL). The crude was purified by flash column chromatography on silica gel (50% → 100% ethyl acetate in hexanes) to give the title compound as an oil (40 mg; 83%).

1H NMR (500 MHz, CDCl₃) δ 7.35 (t, J = 8.0 Hz, 2H), 7.29 (t, J = 8.5 Hz, 1H), 7.13 (t, J = 8.0 Hz, 1H), 7.04 (d, J = 8.0 Hz, 2H), 6.88 (ddd, J = 8.5 Hz J = 2.5 Hz J = 1.0 Hz, 1H), 6.84 (dd, J = 8.0 Hz J = 2.0 Hz, 1H), 6.78 (t, J = 2.5 Hz, 1H), 5.53 (br. s., 1H, NH), 3.72 (t, J = 4.5 Hz, 4H), 3.36 (q, J = 6.0 Hz, 2H), 2.53 (t, J = 6.0 Hz, 2H), 2.48 (br. s., 4H) HRMS (ESI) for C₁₉H₂₃N₂O₄ Calculated Mass: 343.1658 Found Mass: 343.1653

3-phenoxyphenyl 4-hydroxyphenethylcarbamate (AM7571, 119)

The synthesis was carried out as described for 104, using 4-nitrophenyl 3-phenoxyphenyl carbonate (50 mg, 0.14 mmol), and tyramine (20.3 mg, 0.15 mmol) in anhydrous THF (1.0 mL). The crude was purified by flash column chromatography on silica gel (50% → 100% ethyl acetate in hexanes) to give the title compound as a white solid (39 mg; 79%). Mp: 78-79°C

1H NMR (500 MHz, CDCl₃) δ 7.35 (t, J = 7.5 Hz, 2H), 7.28 (t, J = 8.0 Hz, 1H), 7.12 (t, J = 7.5 Hz, 1H), 7.08 (d, J = 9.0 Hz, 2H), 7.02 (d, J = 8.0 Hz, 2H), 6.84 (t, J = 7.5 Hz, 2H), 6.77 (d, J = 8.5 Hz, 2H), 6.76 (t, J = 2.0 Hz, 1H), 4.99 (br. s., 1H, NH), 4.74 (s, 1H, OH), 3.47 (q, J = 6.5 Hz,
2H), 2.80 (t, J = 8.5 Hz, 2H) HRMS (ESI) for C_{21}H_{20}NO_{4} Calculated Mass: 350.1392  Found Mass: 350.1385

3-phenoxyphenyl butylcarbamate (AM7572, 106)

The synthesis was carried out as described for 104, using 4-nitrophenyl 3-phenoxyphenyl carbonate (50 mg, 0.14 mmol) and butylamine (11.4 mg, 0.15 mmol) in anhydrous THF (1.0 mL). The crude was purified by flash column chromatography on silica gel (0% → 30% ethyl acetate in hexanes) to give the title compound as a white solid (39 mg; 97%). Mp: 47-48°C

1H NMR (500 MHz, CDCl3) δ 7.35 (t, J = 8.0 Hz, 2H), 7.28 (t, J = 8.5 Hz, 1H), 7.12 (t, J = 7.5 Hz, 1 H), 7.04 (d, J = 8.0 Hz, 2H), 6.88 (dd, J = 8.0 Hz J = 2.0 Hz, 1H), 6.83 (dd, J = 8.5 Hz J = 2.0 Hz, 1 H), 6.78 (t, J = 2.0 Hz, 1H), 4.96 (br. s., 1H, NH), 3.25 (q, J = 6.0 Hz, 2H), 1.60-1.50 (m, 2H), 1.40-1.35 (m, 2H) 0.95 (t, J = 7.5 Hz, 3H, CH₃) HRMS (EI) for C_{17}H_{19}NO_{3} Calculated Mass: 285.1365 Found Mass: 285.1368

3-phenoxyphenyl 4,4,4-trifluorobutane-1-sulfonate (AM7573, 111)

To a solution of 3-phenoxyphenol (100 mg, 0.53 mmol) in anhydrous DCM (10 mL) was added TBAB (207mg, 0.6 mmol) and 45% aqueous NaOH solution (1.0 mL) and cooled to 0°C. 4,4,4-trifluorobutane-1-sulfonyl chloride (135 mg, 0.6 mmol) dissolved in anhydrous DCM (5.0 mL) was added through cannula. The reaction mixture was allowed to stir for 15 min. at 0°C then warmed to room temperature, and stirred for an additional 1.5 hours and poured into water. The organic layer was separated and the aqueous phase extracted with diethyl ether (2x). The combined organic layer was washed with brine, dried (MgSO₄) and concentrated under reduced pressure. The crude was purified by flash column chromatography on silica gel (40% diethyl
ether in hexanes) to give the title compound as a yellow oil (174 mg; 90%). $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 7.38 (t, $J = 7.5$ Hz, 2H), 7.34 (d, $J = 8.0$ Hz, 1H), 7.17 (t, $J = 7.5$ Hz, 1H), 7.05 (d, $J = 7.5$ Hz, 1H), 6.99 (ddd, $J = 8.5$ Hz $J = 3.0$ Hz $J = 1.0$ Hz, 1H), 6.89 (ddd, $J = 8.0$ Hz $J = 3.0$ Hz $J = 1.0$ Hz, 1H), 6.89 (ddd, $J = 8.0$ Hz $J = 3.0$ Hz $J = 1.0$ Hz, 1H), 3.33 (t, $J = 7.5$ Hz, 2H), 2.40-2.29 (m, 2H), 2.29-2.20 (m, 2H) HRMS (ESI) for C$_{16}$H$_{16}$F$_3$O$_4$S Calculated Mass: 361.0721 Found Mass: 361.0711
CHAPTER SIX:
FUTURE DIRECTIONS
6.1 Ligand based drug design

Biaryl ether compounds have proven to be good ligands for the cannabinoid receptors, as well as to deactivate the hydrolytic enzyme FAAH. Through the Structure Activity Relationship study, reported here, we have shown the importance of the side chain for receptor recognition. Also new chemotypes have been identified for the CB2 receptor.

Over 95 compounds were generated from this study and key compounds should be further evaluated on multiple functional assays; cyclic AMP, \([^{35}S]GTP\gamma S\), and \(\beta\)-arrestin to determine which pathways are activated. It is important to remember that binding affinity does not always correlate with potency or functionality.

While our initial design was to leave the substitution on the A-ring unaltered, due to the assumption that it imparted partial agonist activity, it can also be a potential region to determine the functionality of the ligand. Future work should include bioisosteric replacement of the A-ring with electron withdrawing and donating substituents as well as heterocycles such as pyridine or indole.

![Figure 6.1: A-ring substitution](image)

6.2 Cannabinoids without CNS effects
The CNS effects of cannabinoids have been a large deterrent for the development of CB1 therapeutics. By targeting the CB2, which does not have a presence in the brain, we can circumvent this undesirable effect and develop cannabimimetics without the CNS side effects. The tricylic and biphenyl cannabinoids are good starting points for the development of non-psychoactive cannabinoid based therapeutic agent due to its CB2 selectivity.

The biphenyl moiety is particularly useful as a lipophilic core. Lipophilic binding energies have been recognized as a major component of total binding to target proteins. The biphenyl analogs are an effective template for small molecule lead discovery which can be functionalized with a wide variety of functional groups and avail itself to a variety of spatial configurations. It is also shown here that there are a large variety of substitutions tolerated than previously thought.

![Figure 6.2: biphenyl side chain modifications](image)

6.3 Mapping the CB2 receptor

The biphenyl compounds exhibited high binding affinity to the CB2 receptor and would make good covalent probes. In preliminary study of AM7576, azido containing ligand, showed good covalent labeling of the mCB2 receptor, but unfortunately not hCB2. None the less, this discovery can be the jumping off point to design more covalent probes. While most covalent
probes were incorporated in the flexible side chain region, placement of a covalent probe, N$_3$ or NCS, at the northern portion of the biphenyl compound is a possibility (Figure 6.3).

![Figure 6.3: Covalent probes](image)

Once covalent probes are identified, site directed mutation studies are necessary to determine the binding site of the biphenyl compounds. The resulting information will give us further insight into the specific interactions between functional groups on the ligands and the amino acid residues responsible for activation of CB2. Techniques like this are the only tool available until the crystal structure of the cannabinoid receptor is solved. Membrane proteins are hard to isolate in proper amount and once taken out of the membrane do not behave the same way.

**6.4 Enzyme inhibitors**

We have also shown that biaryl ether ligands as excellent inhibitors of the endocannabinoid system protein FAAH. Our results show that opportunities exist to develop more inhibitors, possibly switching the focus to MGL inhibitors. Further SAR studies may lead to selective inhibitors for the endocannabinoid hydrolytic enzymes. The replacement of carbamates with ureas in this series of compounds would provide another enzyme inhibitor with a longer duration of action. Figure 6.4 is a representative urea analog utilizing the pentyl chain similar to AM7561.
Figure 6.4: Representative Urea Analog
APPENDIX

rCB1, mCB2 and hCB2 binding assay:

Competitive binding assays were performed using rat brain containing CB1 (rCB1) or HEK293 cells transfected with mouse or human CB2 (mCB2 or hCB2 respectively); membrane preparation has been previously described\(^1\). Compounds to be tested (10 mM in DMSO) were diluted in TME buffer (50 mM Tris-HCL, 3 mM MgCl\(_2\), 100 mM NaCl, 0.2 mM EDTA, pH 7.4) with 0.1% BSA and transferred to 96 well plates containing \(^{3}\text{H}\)-CP55940 at a final concentration of 0.76 nM. Non-specific binding was assessed in the presence of 100 nM CP55940. Binding was initiated with the addition of the respective membrane suspension (25 µg membrane protein/well) followed by incubation at 30°C in a shaking water bath for 1 hour. The interaction was terminated by rapid filtration of the membrane suspension over Unifilter GF/B-96 Well Filter Plates (Perkin Elmer Life Sciences) using a Packard Filtermate-196 Cell Harvester. Filter plates were washed five times with ice-cold wash buffer (50 mM Tris-base, 5 mM MgCl\(_2\) with 0.5% BSA) and bound radioactivity was determined using a Packard TopCount Scintillation Counter. The results were analyzed from one triplicate experiment using nonlinear regression to determine the IC\(_{50}\) of the ligand (Prizm by GraphPad Software, Inc.) and the K\(_i\) values were calculated by Prism from the IC\(_{50}\).\(^2\)

Enzyme assay (FAAH)

**FAAH and MGL Preparation**

Rat ΔTM FAAH is expressed in *E. coli* cells and purified using the procedure disclosed by Patricelli et al.\(^3\) Recombinant hexa-histidine-tagged human MGL (hMGL) is expressed in *E. coli* cells and purified following our recently reported procedures.\(^4,5\)
**MGL and FAAH Inhibitor Assay (Fluorescent)**

Human-recombinant FAAH (hFAAH) and MAG lipase (hMAG lipase) are expressed in E. coli and purified as described. A high-throughput fluorometric screening assay for FAAH inhibition using the fluorescent substrate, arachidonoyl 7-amino-4-methylcoumarin amide (AAMCA) is performed as previously reported, while the MGL assays use the fluorescent substrate arachidonoyl, 7-hydroxy-6-methoxy-4-methylcoumarin ester (AHMMCE). IC values are calculated using Prism software (GraphPad).

**GPCR Functional Assay**

**cAMP Assay (In-house)**

HEK-293 cells transfected with rCB1, hCB2 or mCB2 receptors were used for the studies. The cAMP assays were carried out using PerkinElmer’s Lance ultra cAMP kit following the protocol of the manufacturer. Briefly, the assays were carried out in 384-well format using 1000 cells/well. The cells were harvested with a non-enzymatic cell dissociation reagent Versene. They were washed once with HBSS and resuspended in the stimulation buffer. The test compounds (5 μl) in forskolin (2 μM) containing stimulation buffer were added to the plate followed by the cell suspension (5 μl). The cells were stimulated for 30 min at room temperature. Then Eu-cAMP tracer working solution (5 μl) and Ulight-anti-cAMP working solution (5 μl) were added to the plate and incubated at room temperature for 1h. The plates were read on PerkinElmer Envision and the data were analyzed using GraphPad Prism software.

**cAMP (Cerep)**
**Human cannabinoid CB1 receptor – agonist effect –**

The cells are suspended in HBSS buffer (Invitrogen) complemented with 20mM HEPES (pH 7.4), then distributed in microplates at a density of $10^4$ cells/well in the presence of either of the following: HBSS (basal control), the reference agonist at 100 nM (stimulated control) or various concentrations (EC$_{50}$ determination), or the test compounds. Thereafter, the adenylyl cyclase activator NKH 477 is added at a final concentration of 3 μM. Following 10 min incubation at 37$^\circ$C, the cells are lysed and the fluorescence acceptor (D2-labeled cAMP) and fluorescence donor (anti cAMP antibody labeled with europium cryptate) are added. After 60 min at room temperature, the fluorescence transfer is measured at $\lambda_{ex} = 337$ nm and $\lambda_{em} = 620$ and 665 nm using a microplate reader (Rubystar, BMG). The cAMP concentration is determined by dividing the signal measured at 665 nm by that measured at 620 nm (ratio). The results are expressed as a percent of the control response to 100 nM CP55940. The standard reference agonist is CP55940, which is tested in each experiment at several concentrations to generate a concentration response curve from which its EC$_{50}$ value is calculated.

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

The cells are suspended in HBSS buffer (Invitrogen) complemented with 20mM HEPES (pH 7.4), then distributed in microplates at a density of $10^4$ cells/well and preincubated for 5 min at room temperature in the presence of either of the following: HBSS (stimulated control), the reference antagonist AM 281 at 3 μM (basal control) or various concentrations (IC$_{50}$ determination), or the test compounds. Thereafter, the reference agonist CP55940 and the adenylyl cyclase activator NKH 477 are added at respective final concentrations of 10nM and 3 μM AM 281. For basal control measurements, CP55940 is omitted from the wells containing 3
μM AM 281. Following 10 min incubation at 37°C, the cells are lysed and the fluorescence acceptor (D2-labeled cAMP) and fluorescence donor (anti-cAMP antibody labeled with europium cryptate) are added. After 60 min at room temperature, the fluorescence transfer is measured at λex = 337 nm and λem = 620 and 665 nm using a microplate reader (Rubystar, BMG). The cAMP concentration is determined by dividing the signal measured at 665 nm by that measured at 620 nm (ratio). The results are expressed as a percent inhibition of the control response to 10 nM CP55940. The standard reference antagonist is AM 281, which is tested in each experiment at several concentrations to generate a concentration-response curve from which its IC_{50} value is calculated.

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

The cells are suspended in HBSS buffer (Invitrogen) complemented with 20 mM HEPES (pH 7.4), then distributed in microplates at a density of 7.5x10^3 cells/well in the presence of either of the following: HBSS (basal control), the reference agonist at 100 nM (stimulated control) or various concentrations (EC50 determination), or the test compounds. Thereafter, the adenylyl cyclase activator NKH 477 is added at a final concentration of 3 μM. Following 10 min incubation at 37°C, the cells are lysed and the fluorescence acceptor (D2-labeled cAMP) and fluorescence donor (anti-cAMP antibody labeled with europium cryptate) are added. After 60 min at room temperature, the fluorescence transfer is measured at λex = 337 nm and λem = 620 and 665 nm using a microplate reader (Rubystar, BMG). The cAMP concentration is determined by dividing the signal measured at 665 nm by that measured at 620 nm (ratio). The results are expressed as a percent of the control response to 100 nM WIN 55212-2. The standard reference
agonist is WIN 55212-2, which is tested in each experiment at several concentrations to generate a concentration-response curve from which its EC$_{50}$ value is calculated.

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

The cells are suspended in HBSS buffer (Invitrogen) complemented with 20 mM HEPES (pH 7.4), then distributed in microplates at a density of 7.5x10$^3$ cells/well and preincubated for 5 min at room temperature in the presence of either of the following: HBSS (basal control), the reference antagonist AM 630 at 100 μM (basal control) or various concentrations (IC$_{50}$ determination), or the test compounds. Thereafter, the reference agonist WIN55212-2 and the adenyl cyclase activator NKH 477 are added at respective final concentrations of 10 nM and 3 μM. For basal control measurements, WIN 55212-2 is omitted from the wells containing 100 μM AM 630. Following 10 min incubation at 37°C, the cells are lysed and the fluorescence acceptor (D2-labeled cAMP) and fluorescence donor (anti-cAMP antibody labeled with europium cryptate) are added. After 60 min at room temperature, the fluorescence transfer is measured at λ$_{\text{ex}}$ = 337 nm and λ$_{\text{em}}$ = 620 and 665 nm using a microplate reader (Rubystar, BMG). The cAMP concentration is determined by dividing the signal measured at 665 nm by that measured at 620 nm (ratio). The results are expressed as a percent of the control response to 100 nM WIN 55212-2. The standard reference agonist is AM 630, which is tested in each experiment at several concentrations to generate a concentration response curve from which its IC$_{50}$ value is calculated.

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

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

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