Discovery of (*E*)-3-(4-(Diethylamino) phenyl)-1-phenyl-2-phenylsulfonyl)prop-2-en-1-one as Novel Cannabinoid Receptor 2 Ligands

by

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Cannabinoids (CB) are defined as a class of compounds that can act on Cannabinoid receptors 1 or 2 (CB_1 or CB_2) and affect human physiology. Both CB_1 and CB_2 receptors belong to the rhodopsin-like family of G-Protein Coupled Receptors (GPCRs). However, CB_1 receptor is mainly expressed in the central nervous system, while CB_2 receptor is dominantly located in the peripheral nervous system and immune cells. By now, scientists have discovered many CB ligands that have therapeutic potentials, but the limitation of non-selective ligands is the psychiatric side effect mediated by the activation of CB_1 receptor. Although CB_1 receptor is crucial in analgesic and anti-inflammatory effects, strategies of designing CB_2 selective ligands are made by medicinal chemists to avoid undesirable effects in clinic. In this thesis, we discovered novel CB_2 lead compounds with new chemical scaffolds; designed and synthesized four series of analogues for the structure-activity relationship (SAR) studies; tested their binding affinity to both CB_2 and CB_1 receptors; conducted *in-vitro* functional studies; and evaluated their potentials for therapeutic treatment.

In total, four series of (*E*)-3-(4-Ethoxy-3-methoxyphenyl)-2-((4-methoxyphenyl) sulfonyl)-1-phenylprop-2-en-1-one have been identified as novel cannabinoid ligands. Physicochemical properties were predicted and docking studies using our CB_2 model was conducted. 29 derivatives were then synthesized to conduct SAR studies. The binding affinity and selectivity

for cannabinoid receptor CB_1 and CB_2 were then evaluated. Four compounds showed high CB_2 binding affinity (K_i of 10-60 nM) and good selectivity (CB_1/CB_2 of 20- to 1305 fold). Their off-targets effects were also predicted. Overall, these sulfone derivatives can be used to develop novel therapeutic CB_2 ligands.

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1.0 INTRODUCTION

1.1 ENDOCANNABINOID SYSTEM AND CANNABINOID RECEPTORS

1.1.1 ENDOCANNABINOID SYSTEM

Cannabinoids (CB) are defined as a class of compounds that can act on CB receptors and affect human physiology. The active component of *Cannabis sativa* Δ9-Tetrahydrocannabinol (THC) is the very first cannabinoid isolated and structure-elucidated in 1964 [1]. However, the CB receptors and their binding sites are not identified until 1988 [2]. Later on, endogenous CB ligands such as Arachidonoylethanolamine (AEA) and 2-Arachidonoylglycerol (2-AG) were recognized and people start uncovering the veils of endocannabinoids system, which is later proved to play a key role in a large spectrum of diseases such as inflammation, stroke, cancer and osteoporosis [1, 3].

It is now understood that endocannabinoid system is composed of two kinds of cannabinoid receptors, varies endogenous ligands (endocannabinoids) which target those receptors, and many enzymes that synthesize or degrade the endocannabinoids. By now, many endocannabinoids have been discovered including Arachidonoylethanolamine (AEA), 2-Arachidonoylglycerol (2-AG), *N*-arachidonoyl dopamine (NADA) and *N*-palmitoylethanolamine (PEA) as shown in **Figure 1.1**. Among them, AEA and 2-AG have the highest binding affinity towards CB

receptors and are also believed to be the most abundant ones in human body. In 1992, AEA was synthesized and identified as the first endogenous ligand for the cannabinoid receptor which can inhibit the binding of radiolabeled cannabinoid probe. Additionally, it has similar function as THC to inhibit the electrically evoked twitch response to the mouse vas deferens in a concentration-dependent manner. In 1994, 2-AG was isolated from canine intestines and was characterized as second endocannabinoid which is demonstrated to share similar ability with THC as well [4, 5].

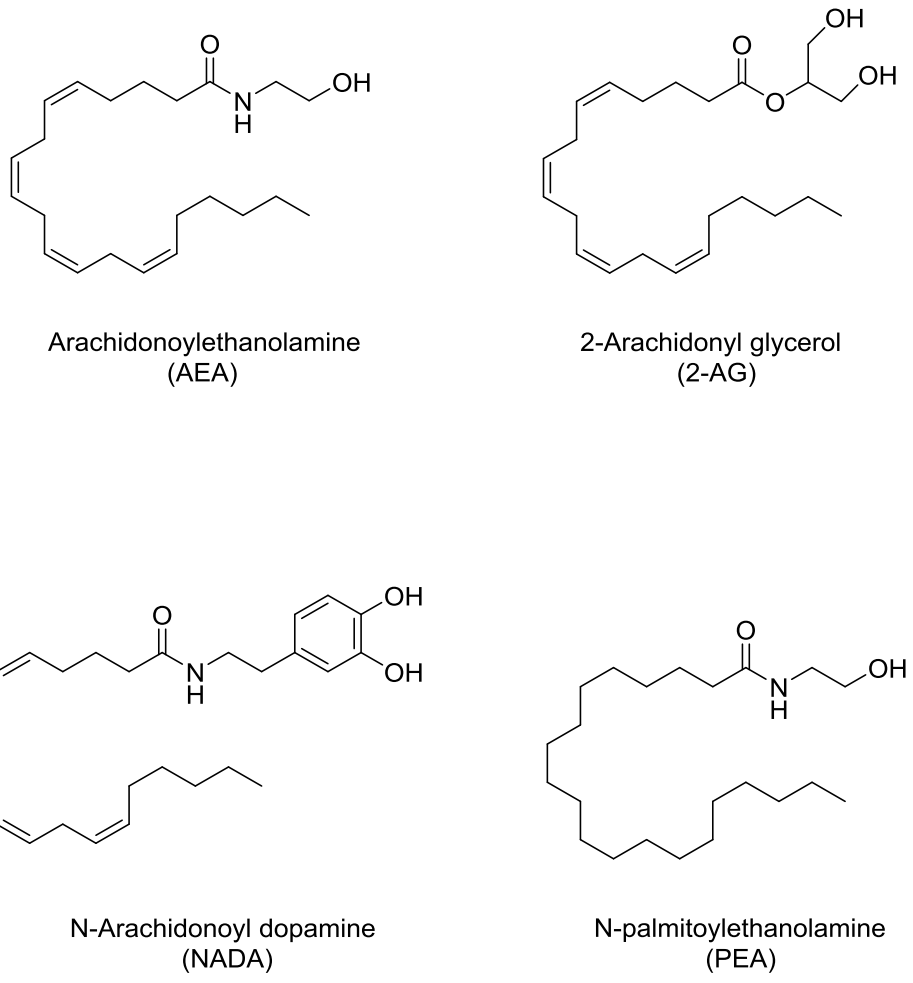


Figure 1.1 Structures of several endocannabinoids

These endogenous ligands are lipid messengers that have been found not only in the brain but also in peripheral tissues. Rather than stored in the vesicles, they are synthesized in the postsynaptic cells when needed, be released from the postsynaptic cells and then act on the CB receptors located in the presynaptic membrane. It is believed that these messengers are crucial in the control of body movements [6] and the function of memories and pain perception [7, 8]. Moreover, they are involved in the regulations of cardiovascular, gastrointestinal, hypothalamic and reproductive functions too [9-11].

1.1.2 GPCRs AND CANNABINOID RECEPTOR

GPCRs are seven transmembrane receptors. Based on their sequence and function similarities, they can be grouped into six classes: Class A - Rhodopsin-like family; Class B - Secretin receptor family; Class C - Metabotropic glutamate family; Class D - Fungal mating pheromone receptors; Class E - Cyclic AMP receptors; Class F – Frizzled family [12, 13]. There are three common regions in GPCRs: extracellular region which contains three extracellular loops (EL1-EL3) and an N' terminal; the transmembrane region which contains seven transmembrane α-helices; intracellular region which contains three intracellular loops (ICL1-ICL3) and a C' terminal (**Figure 1.2**) [14]. They are activated by external ligands or mediators and are involved in many biological processes such as immune responses, blood pressure regulation and even tumor metastasis. Additionally, it is estimated that GPCRs are the targets of about 40% of all modern drugs [15].

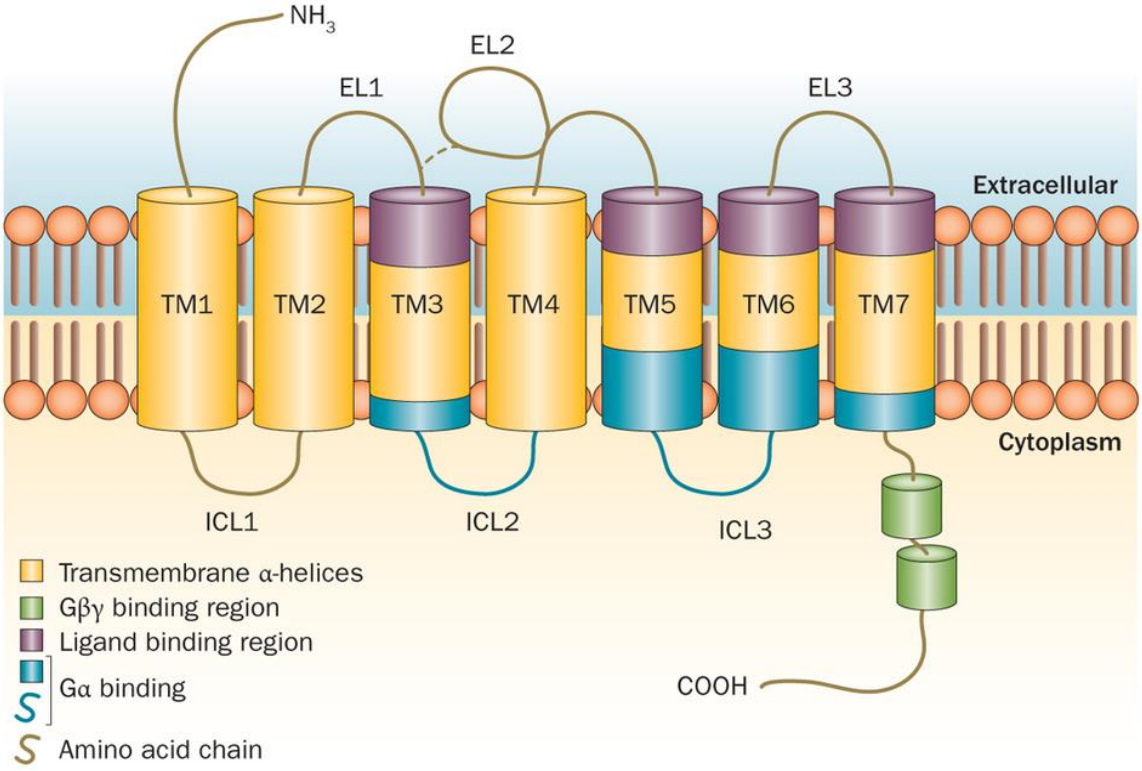


Figure 1.2 Systematic structure of GPCR

Common structure of GPCR: seven transmembrane domains (TM1-TM7), three extracellular loops (EL1-EL3), three intracellular loops (ICL1-ICL3), extracellular N' terminal and intracellular C' terminal (The figure was adapted from [15]).

As GPCRs, cannabinoid receptors belong to Class-A (Rhodopsin-like) G-protein coupled receptors family, which is the most common target of FDA approved drugs as shown in **Figure 1.3** [16].

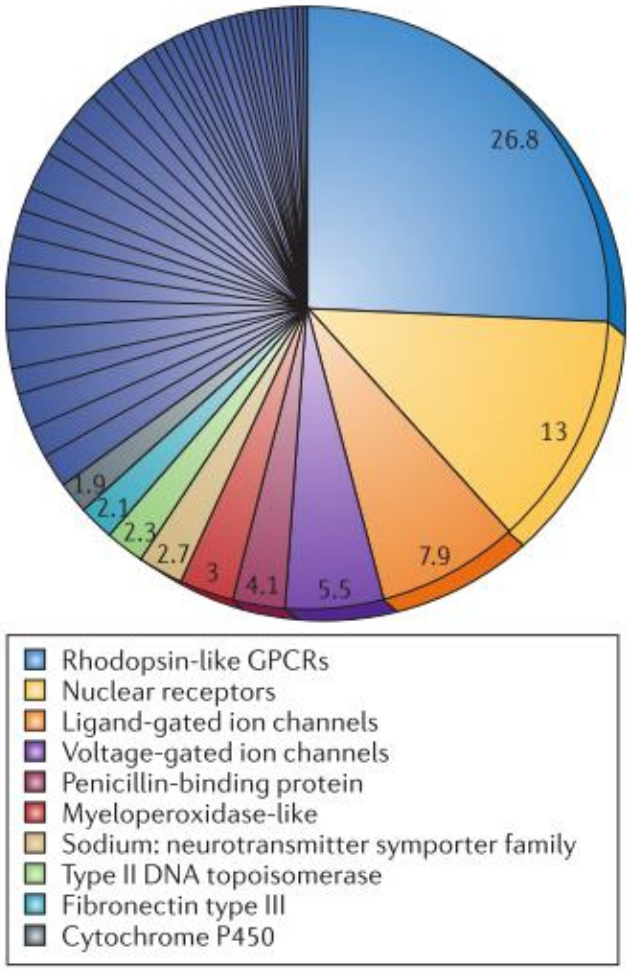


Figure 1.3 Gene-family distribution of drugs

The family share as a percentage of all FDA-approved drugs is displayed for the top ten families. (The figure was adapted from [16])

At present, there are two known subtypes of cannabinoids receptors, termed Cannabinoid receptor 1 (CB_1) and Cannabinoid receptor 2 (CB_2). CB_1 receptor is a 472 amino acid polypeptide while CB_2 receptor is a 360 amino acid polypeptide. They have 44% amino acid sequence homology for the whole protein and 68% in terms of the transmembrane region [17]. In 1990, Matsuda's group first identified CB_1 receptor and isolated it from rat cerebral cortex [18]. One year later, Gerard and colleagues discovered CB_1 receptors in human brain and testis [19]. In 1993, Munro and coworkers cloned CB_2 receptors from human promyelocytic leukemia cells [17].

Although the two receptors have been identified, cloned and studied as potential targets for decades, there are still debates on where they are located and how CB ligands produce pharmacological effects through them [2, 20-22]. Currently, it is believed that CB_1 receptor is most commonly expressed in the central nervous system, especially in the neuron terminals of the basal ganglia, cerebellum, hippocampus, neocortex, hypothalamus and limbic cortex, where they can enhance or prevent "depolarization-induced suppression of excitation/inhibition" mediated by CB ligands [23, 24]. On the other hand, CB_2 receptor is believed to be expressed predominantly in peripheral cells and tissues derived from immune system such as spleens and thymus gland [25, 26], where they can modulate immune suppression, apoptosis or cell migration etc. [26].

1.1.3 CANNABINOID RECEPTOR SIGNALING PATHWAYS

GPCR activation:

G proteins (guanine nucleotide-binding proteins) are a family of proteins that act as switches inside a cell. By attaching to the receptors on the cell membrane, they work as signal transducers which can connect receptors to variety of stimuli outside the cell and therefore, to intracellular signaling pathways.

G proteins are made up of three subunits (α, β and γ subunits), where Gα subunit is bound to guanosine diphosphate (GDP) at basal state. Both CB_1 and CB_2 receptors are coupled with G protein and upon activation by cannabinoid agonists, the extracellular signals will cause conformational change of transmembrane region and thus facilitate the replacement of GDP with guanosine triphosphate (GTP) on Gα subunit. Consequently, Gα subunit will dissociate with Gβγ subunit and both of them will lead to numerous downstream signaling such as regulation of adenylyl cyclase, ceramide, mitogen-activated protein kinase (MAPK), intracellular $Ca^{2+,}$ and ion channels. After the activation, the GTP on Gα subunit will be hydrolyzed by GTPase into GDP, which allow it to recombine to Gβγ subunit and start a new cycle [21, 27].

Regulation of adenylyl cyclase:

There are four families of Gα subunits $G\alpha_s$, $G\alpha_i/G\alpha_o$, $G\alpha_q/G\alpha_{11}$, and $G\alpha_{12}/G\alpha_{13}$ in which both CB_1 and CB_2 receptors are coupled with $G\alpha_i/G\alpha_o$. $G\alpha_i/G\alpha_o$ family is known to inhibit the function of adenylyl cyclase (AC). So when activated by cannabinoid agonists, $G\alpha_i/G\alpha_o$ will be isolated from $G_{\beta\gamma}$ subunit, coupled with GTP and inhibit AC activity [28]. Down regulation of AC will decrease the production of cyclic adenosine monophosphate (cAMP), which is a second messenger that affects cellular activity by regulating the activation of protein kinase, the function

of ion channel or the effects of adrenaline and glucagon [29-32]. In addition, the level of AC or cAMP in a cell is critical in terms of characterizing a ligand's efficacy towards CB receptors. The inhibition of AC by THC in neuroblastoma cells is the very first signal transduction response discovered by Howlett and Fleming in1980s. Also, evidence showed that agonists such as AEA, WIN 55,212-2 or CP 55,940 can inhibit adenylyl cyclase activity and thus decrease the level of cAMP in rat cerebellar membranes [33, 34], where inverse agonists or antagonists such as SR141716 and cannabidiol can inhibit the dissociation of Gα subunit from $G_{\beta\gamma}$ subunit and therefore upregulate the amount of cAMP [35].

Regulation of MAPK:

MAPK pathway is one of the most important pathways that regulate cell proliferation, apoptosis, differentiation and gene expression [36]. Initiated by the activation of tyrosine kinase-linked receptors, G protein Ras is then activated. This will lead to a signal cascades that activate serine/threonine kinase Raf, which will later on activate MAPK and regulate the phosphorylation of many other proteins [21]. In 1995, Bernard and colleagues found that cannabinoid can induce the activation of MAPK in Chinese Hamster Ovary cells (CHO cells) that express human CB_1 receptors [37]. In 1996, the same group showed that cannabinoids can also activate MAPK in CHO cells expressing CB_2 receptors [38].

Additionally, by using WIN55212-2 in N1E-115 neuroblastoma cells, it is suggested that there might be other mechanisms for the regulation of MAPK pathway by cannabinoids. It was demonstrated that the inhibition of cAMP accumulation and the down regulation of Protein Kinase A (PKA) can increase the dephosphorylation of c-Raf. Consequently the Raf kinase will activate mitogen-activated protein kinase kinase (MAPKK) in the p42/p44 MAPK kinase [39, 40].

Modulation of ion channels:

Ion channels exist in the membrane of all cells. By controlling the flow of ions through the membrane, they are involved in many physiological processes such as the conduction across the synapses, the regulation of cell volume and the establishing of membrane potentials [41]. CB_1 agonists AEA, WIN 55,212-2 and CP 55,940 were demonstrated to inhibit N-type voltage operated calcium channels (VOCCs) and therefore decrease the Ca^+ influx in NG 108-15 cells [42]. In addition, AEA was proved to inhibit Q-type Ca^+ flow in AtT-20 pituitary tumor cells that expressed CB_1 receptors [43]. Another experiment in rat hippocampal neurons also showed that AEA, WIN 55,212-2 and CP 55,940 can inhibit N-type or P/Q-type Ca^+ current [44]. Besides Ca^+ channels, there are many ion channels (such as K^+ channels and Na^+ channels) that have also been proved to be inhibited by CB ligands [45, 46].

1.2 THERAPEUTICAL POTENTIAL OF CANNABINOID LIGANDS

As discussed above, endocannabinoid system plays an important role in various physiological processes, and CB receptors are promising targets for a wide span of diseases. Several drugs targeting CB_1 or CB_2 receptors are already in the market and there are also quite a few in clinical trials. More than 30 years ago, two cannabinoid derivatives dronabinol (Marinol®) and nabilone (Cesamet®) were approved by FDA for the treatment of nausea and vomiting [47, 48]. In 2005, a combination of THC and cannabidiol was licensed in Canada as Sativex® to relieve the neuropathic pain in adults with multiple sclerosis [49]. One year later, the only cannabinoid receptor inverse agonist SR141716, known as Rimonabant (Acomplia®) was introduced to the

European clinics for the treatment of obesity [50, 51]. Unfortunately, side effects including headache, nausea, vomiting, diarrhoea, insomnia, influenza, feelings of anxiety and depression were observed in patients [48]. Moreover, it will increase the incidence of depression and suicidality. As a result, it was withdrawn from the market in 2008.

Although there are still challenges in developing potent and selective cannabinoids as novel drugs, an increasing number of ligands have shown great potentials for the treatment of multiple myeloma, tumor or osteoporosis etc. [52, 53].

Cannabinoids and tumor:

Many researchers have found relationships between endocannabinoid system and all kinds of cancer including prostate cancer, breast cancer and multiple myeloma. It has also been reported that both CB receptors and endogenous cannabinoids are upregulated in tumor tissues compared to non-tumor tissues [54]. In addition, there are many signaling pathways that have been validated in terms of the anti-tumor effect of cannabinoid ligands. Several main signaling cascades such as ERK pathways activation, ceramide synthesis and cAMP inhibition are shown in

Figure 1.4 Figure 1.4 [55]. They will eventually ameliorate the symptom of cancer by blocking cell cycle arrest, reducing cell proliferation and preventing tumor progression.

Specifically, it is reported that several breast cancer cell lines (MDA-MB231, MDA-MB231-luc, and MDA-MB468) overexpress CB_1 and CB_2 receptors. Also, *in vivo* tests in various mouse model showed that cannabinoid agonists such as JWH-133 and WIN-55,212-2 can inhibit breast cancer cells' proliferation and migration [56]. In addition, another study showed that human ErbB2-positive breast tumors express CB_2 receptors and likewise, THC and JWH-133 can

induce cancer cell apoptosis, reduce cancer cell proliferation, and impair tumor angiogenesis [57].

Similarly, it is reported by Sami Sarfaraz and colleagues that the expression of both CB_1 and CB_2 receptors are significantly increased in human prostate cancer cells (LNCaP, DUI45, PC3, and CWR22RN1) than in other human prostate epithelial cells. They also mentioned that with an induction of apoptosis, WIN-55,212-2 (CB agonist) treatment with androgen-responsive LNCaP cells can lead to a dose-dependent inhibition of cell growth. Another group demonstrated that THC can induce apoptosis in PC-3 cells, leading to morphological and biochemical alteration and therefore cause cell death. Additionally, people found that AEA analogues methanandamide (MET), as well as JWH-015 (CB agonist) can inhibit PC-3 cell proliferation too [58-62].

Other evidences showed that there are also relationships between endocannabinoid system and blood cancer or bone cancer. So according to these data, cannabinoids could be valuable in terms of the inhibition of tumor growth.

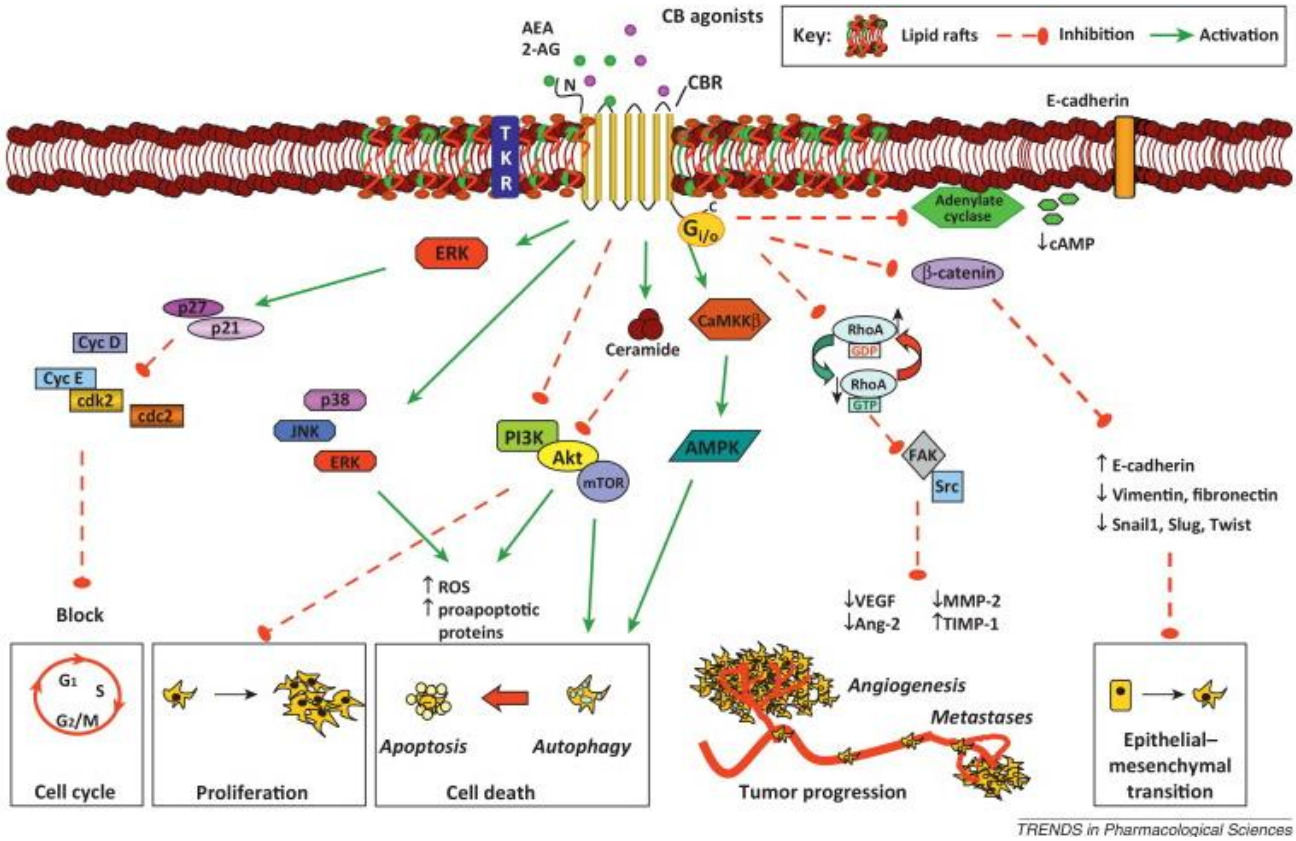


Figure 1.4 Important signaling pathways of anti-tumor effect of cannabinoid ligands

Main downstream signaling cascades of CB receptor are shown in this figure. The activation of CB receptor will eventually affect many crucial features of cancer including 1) cell-cycle arrest; 2) the inhibition of cell proliferation; 3) the induction of cell death; 4) the prevention of tumor progression; 5) the inhibition of the epithelial–mesenchymal transition (The figure was adapted from [55]).

Cannabinoids and osteoporosis:

Osteoporosis is a disease where broken bone may happen due to the decrease of bone density. It is associated with the imbalance between bone resorption and bone formation in the bone remodeling process. In this process, osteoclast cells will first be activated. They can resorb bones (remove old bones) and create bone cavities. Then, osteoblast cells will be activated, which can lead to bone formation. However in osteoporosis, more bone resorption occurs and as a result, it can cause bone loss. It is first reported in 2011 that human osteoclast cells express both CB_1 and CB_2 receptors, which affect osteoclast differentiation *in vitro* [63]. Also, two endocannabinoids AEA and 2-AG were found not only in the trabecular compartment of skeleton, but also in both osteoclast and osteoblast cells. In addition, there are a number of studies including our lab indicated that synthetic cannabinoid ligands such as SR141716 , AM251, AM630, CP55 940, JWH133 and HU308 can influence bone disease by changing bone cells' activity and differentiation [63-68]. The underlying mechanism is quite complex and is concluded in **Figure 1.5** [64]. It is currently known that both CB agonists and CB inverse agonists can benefit the formation of bones due to their effects including increase the number of osteoblast cells, decrease the number of osteoclast cells or reduce the activity of osteoclast cells [63, 65, 69-72].

Considering the limitations of current therapies such as increasing the risk of getting cancer (Calcitonin) or decreasing the osteoblast cells' function in long term uses (bisphosphonates) [73], it is essential to develop a better understanding of the bone remodeling process, as well as explore new chemical entities that have synergistic effects in terms of the regulation of both osteoblast and osteoclast cells.

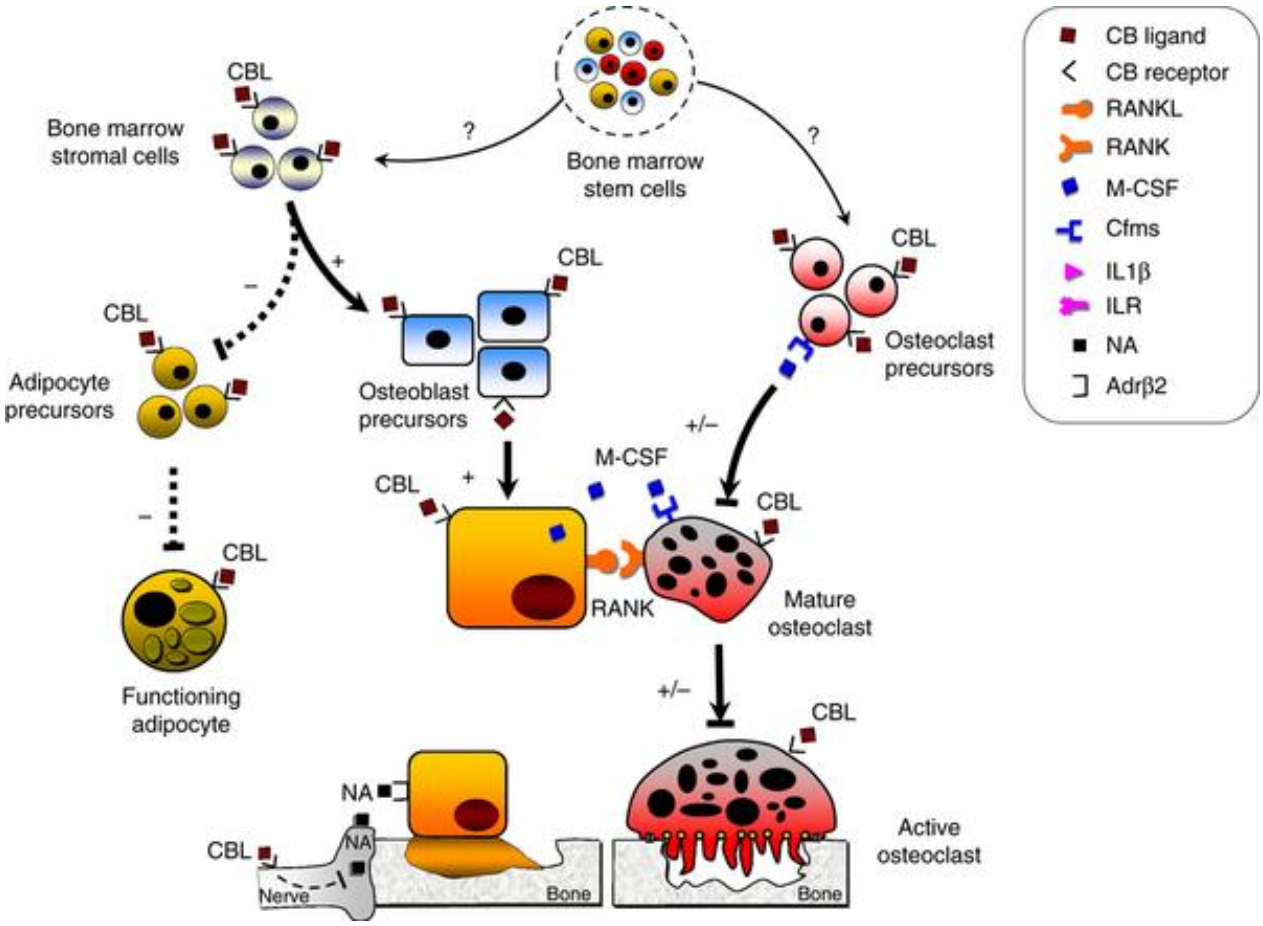


Figure 1.5 Regulation of bone cell activities by the CB ligands

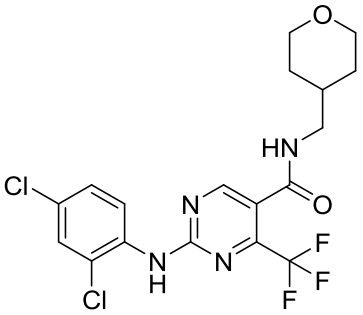
Cannabinoid receptor agonists can simultaneously inhibit and stimulate osteoclast formation and bone resorption by targeting directly on mature osteoclasts and their precursors. They can also stimulate osteoblast proliferation and function by targeting on the CB receptor expressed in the pre-osteoblasts cells. In addition, they regulate the function of osteoblast indirectly by inhibiting the accumulation of the Noradrenaline (NA), an inhibitor of osteoblast. Mature osteoblasts can produce endocannabinoids (AEA and 2-AG) and receptor activator of nuclear factor kappa-B ligand (RANKL), which will stimulates osteoclast formation too (The figure was adapted from [64]).

1.3 CANNABINOID RECEPTOR LIGANDS AND THEIR RECENT DEVELOPMENT

By now, scientists have discovered many CB ligands that have therapeutic potentials, but the limitation of non-selective ligands is the psychiatric side effect mediated by the activation of CB_1 receptor [74]. Although CB_1 receptor is crucial in analgesic and anti-inflammatory effects, strategies of designing CB_2 selective ligands or peripheral restricted CB_1 ligands are made by medicinal chemists to avoid undesirable effects in clinic.

In the past decade, over 400 CB_2 chemical patents, 1500 new chemical ligands have been developed and many of them went into clinical trials or into the market. However recently, legalization of cannabis in many states aroused many discussions and debates. Alaska, Colorado, Oregon, Washington and Washington, D.C. have legalized cannabis use for adults, and 23 states have legalized cannabis for non-FDA-approved medicinal uses under state law (https://www.whitehouse.gov/ondcp/state-laws-related-to-marijuana). Drug abuse become an important issue and the development of novel cannabinoid ligands with potent activity and less addictive effect becomes urgent.

Although the study of cannabinoid lasted for decades, there are still many novel CB ligands or novel indications of classic molecules in the past year. **Table 1.1** shows various cannabinoid ligands with novel chemical scaffolds or CB ligands with novel indications**. Figure 1.6** indicates detailed structures of these ligands.



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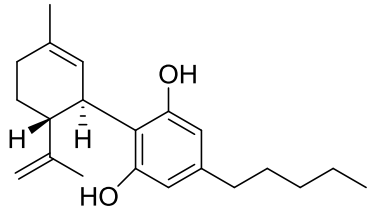
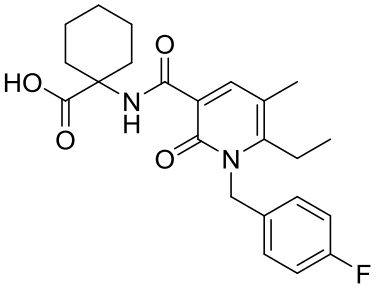
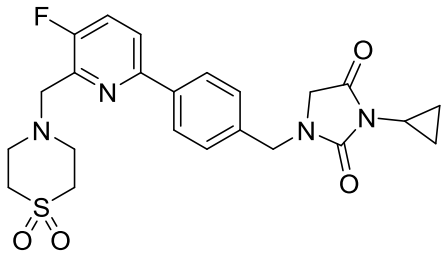


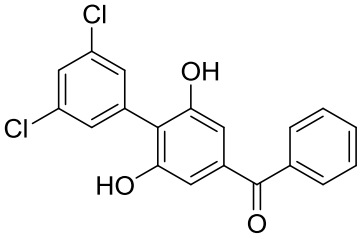
GWP42003-P (Cannabidiol)



S-777469



LEI-101



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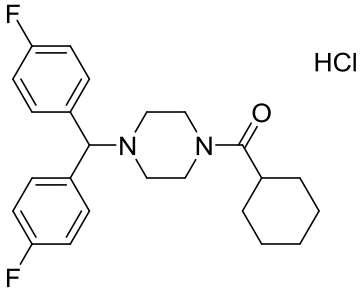

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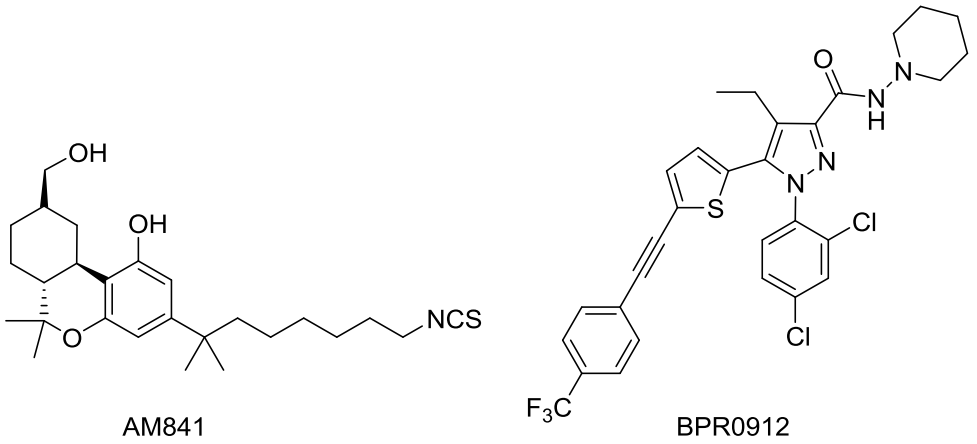


Figure 1.6 Structures of cannabinoid ligands

Table 1.1 Recent development of cannabinoid ligands

Drug Name	Target & Efficacy	Indication	Phase	Reference
GWP42003-P (Cannabidiol)	GPR55 antagonist	Epilepsy Dravet Syndrome	Phase III	[24]
GW842166	CB_2 receptor agonist	Osteoarthritis	Phase II	clinicaltrials.gov NCT00479427
S-777469	CB_2 receptor agonist	Atopic Dermatitis	Phase II	[75]
LEI-101	CB_2 receptor partial agonist	Inflammation and oxidative stress	Pre-clinical	[76]
SMM189	CB_2 receptor inverse agonist	Neurodegenerative diseases and traumatic brain injury.	Pre-clinical	[77]
AM4113	CB_1 receptor neutral antagonist	Tobacco dependence and cannabis dependence.	Pre-clinical	[78]
LDK1229	CB_1 receptor inverse agonist	Addiction and disorders associated with CB_1	Pre-clinical	[79]
AM841	Irreversible CB_1 receptor agonist	Slows gastrointestinal motility	Pre-clinical	[80]
BPR0912	CB_1 receptor neutral antagonist	Metabolic disorders Obesity	Pre-clinical	[81]

2.0 METHODS AND MATERIALS

2.1 COMPUTER MODELING

All molecular docking studies, pharmacophore modeling, and virtual screening procedures were performed using SYBYL X 1.3 (Tripos, Inc.). Molecular docking studies were performed using the Surflex-Dock GemX™ module in the SYBYL software. The docking interactions were analyzed based on the FlexX-Pharm Docking/CScore [82]. Our CB_2 homology model was utilized for the docking and virtual screening procedures. Refined figures and contours were obtained using PyMol 1.7 (Schrödinger®, LCC) platform [83]. Physicochemical properties were predicted using ChemBioDraw Ultra 14.0.Ink.

2.2 CHEMISTRY

All reagents were purchased from commercial suppliers and used without further purification. Analytical thin-layer chromatography (TLC) was performed on SiO_2 plates 200 μm on EMD Millipore Precoated Aluminum-Backed TLC Sheets. Visualization was accomplished by ultra-violet (UV) irradiation at 254 nm. The purification experiments were conducted using Flash chromatography (Biotage, Isolera Inc.). Flash column chromatography was performed using the Biotage Isolera flash purification system with SiO_2 60 89 (particle size 0.040−0.055 mm,

230−400 mesh). Proton and carbon NMR were determined on Bruker 400 MHz or Bruker 600 MHz NMR spectrometer. Chemical shifts are reported as delta (δ) values in parts per million (ppm) as referenced to residual solvent. ^{1}H-NMR spectra are reported as follows: chemical shift, number of protons, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet), and coupling constant. Chemical shifts are reported relative to that of tetramethylsilane at 0.00 ppm.

2.3 BIOLOGICAL EVALUATION

2.3.1 RADIO-LIGAND COMPETITION BINDING ASSAY

The competitive radio-ligand binding assays for CB_1 and CB_2 receptors were performed as described previously using a Perkin Elmer 96-well Top Counter to determine the cannabinoid receptor binding affinity (*K*i) for CB_1 or CB_2 ligands by displacing [^{3}H]-CP 55,940. [84]. Briefly, the compounds to be tested are diluted in binding buffer (50 mM Tris-HCl (pH 7.4), 5 mM MgCl2, 2.5 mM EGTA, and 0.1% (w/v) fatty acid free BSA), supplemented with 10% dimethyl sulfoxide and 0.4% methylcellulose. Each assay plate well contains a total of 200 μL of reaction mixture comprising 5 μg of CB_1 (or CB_2) membrane protein, labeled [^{3}H]-CP 55,940 at a final concentration of 3 nM, and the unlabeled ligand at its varying dilutions. Plates were incubated at 30 ℃ for 1 h with gentle shaking. The reaction was terminated by rapid filtration through Unifilter GF/B filter plates using a UniFilter cell harvester (PerkinElmer). After the plate was allowed to dry overnight, 30 μL MicroScint-0 cocktail (PerkinElmer) was added to each well and the radioactivity was counted by using a PerkinElmer TopCount. All assays were performed in triplicate and data points represented as mean ± S.E.M. The K_i was calculated by using nonlinear

regression analysis (Prism 5, GraphPad) utilizing the Cheng-Prusoff equation: K_i = IC_{50} / 1 + ([L]/K_d), with the K_d values determined from saturation binding experiments.

2.3.2 cAMP FUNCTIONAL ASSAY

Cellular cAMP levels were measured according to reported method with modifications using LANCE cAMP 384 kits (PerkinElmer). The assay is based on competition between a europium-labeled cAMP trace complex and total cAMP for binding sites on cAMP-specific antibodies labeled with a fluorescent dye. CB_2 receptor wild type (WT) transfected CHO cells were seeded in 384- well white ProxiPlates with a density of 2000 cells per well in 5 μL of RPMI-1640 medium containing 1% dialyzed FBS, 25 mM HEPES, 100 μg/mL penicillin, 100 U/mL streptomycin, and 200 μg/mL of G-418. After culture overnight, 2.5 μL of cAMP antibody and RO20-1724 (final concentration, 50 μM) in stimulation buffer (DPBS 1x, containing 0.1% BSA) was added to each well, followed by addition of either 2.5 μL of compound or forskolin (final 5 μM) for an agonist-inhibited adenylate cyclase (AC) activity assay. After incubation at room temperature for 45 min, 10 μL of detection reagent was added into each well. The plate was then incubated for 1 h at room temperature and measured in a Synergy H1 hybrid reader (BioTek) with excitation at 340 nm and emission at 665 nm. Each cAMP determination was made via at least two independent experiments, each in triplicate. EC_{50} values were determined by nonlinear regression, dose−response curves (GraphPad Prism 5) [25, 85].

3.0 RESULTS AND DISCUSSION

3.1 LEAD DISCOVERY

In-silico virtual screening was used to develop novel, potent and selective CB_2 ligands. The strategy led to the discovery of a novel CB_2 scaffold (NCI374672) shown in **Figure 3.1**. In order to confirm that our compound worth further optimization, its physicochemical properties was predicted, docking studies as well as biological evaluations were also conducted.

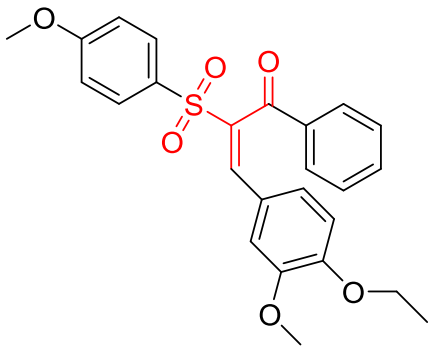


Figure 3.1 Chemical structure of lead compound.

The lead compound NCI374672 exhibited a novel chemical structure containing β-keto sulfones and α, β double bond, which shown in red (**Figure 3.1**).

First of all, we calculated its properties using ChemBioDraw. It shows good physicochemical properties with molecular weight (MW) equals to 452.52, LogP equals to 3.92 and total polar surface area (tPSA) equals to 78.9. In addition, we used Qikprop, a module in Schrodinger software, to predict its drug-like properties and found that it meets all criteria.

Molecular docking using our CB_2 homology model [83] indicated the detailed interaction between lead compound and CB_2 receptor (**Figure 3.2**). Results showed that lead compound formed strong hydrogen bonds with Thr114 (~1.8 Å) and Ser285 (~1.6 Å). Also, it has a strong π-π interaction with Phe281 (~3.4 Å). All these results were consistent with previous studies [83, 86].

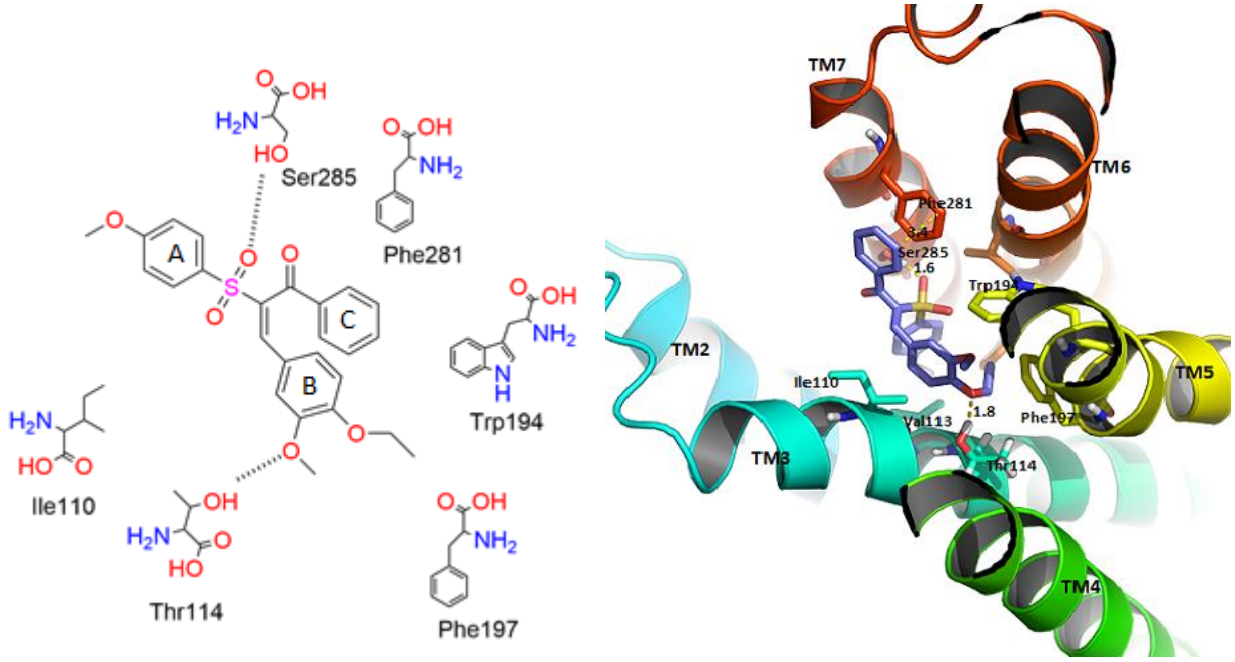


Figure 3.2 The detailed binding pose of lead compound at CB_2 receptor

Additionally, to validate its binding affinity and selectivity towards CB_2 receptor, radio-ligands competition binding assay was conducted. Competition binding assay was conducted on membrane protein harvested from CHO cells expressing CB_1 or CB_2 receptors. Competitive displacement of the [^{3}H]-CP 55,940 was obtained by using an increased amount of cold ligands.

The lead compound showed relatively high binding activity against CB_2 receptor and moderate selectivity over CB_1 receptor (CB_2 K_i = 105 nM, CB1 K_i = 836 nM, Selectivity index > 8) (**Figure 3.3)**.

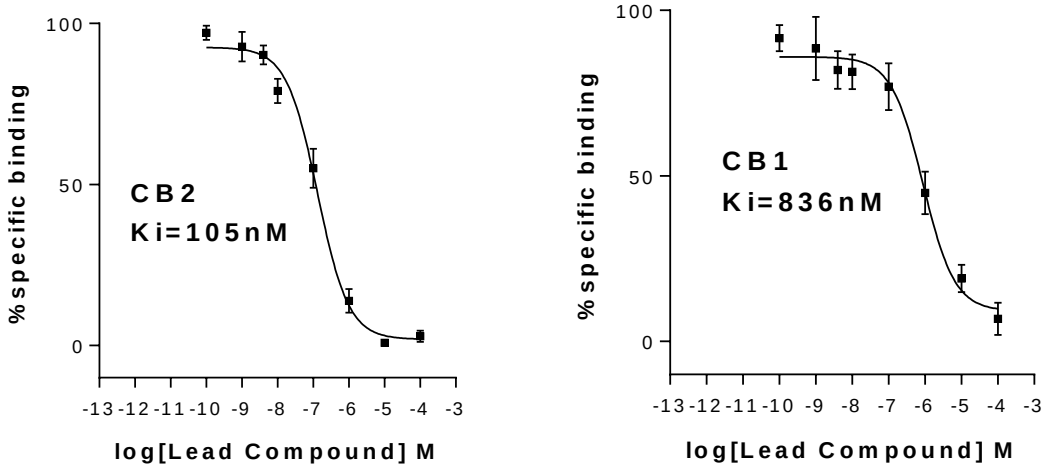


Figure 3.3 Competition binding assay for the lead compound.

Competition binding experiments were performed on membrane proteins harvested from CHO cells stably expressing CB_1 or CB_2 receptors. Competitive displacement of the [^{3}H]-CP 55,940 was obtained by using an increased amount of cold ligands.

CB_2 functional activity of the lead compound was investigated by using a cell-based LANCE cAMP assay, which is a useful method to distinguish between agonists, inverse agonists, and neutral antagonists. A cellular bioassay was carried out to measure the functional activities of the CB_2-selective compounds as described above in the method part. As shown in **Figure 3.4**, increasing concentrations of lead compound cause the reduction of the signal with an ED_{50} of 7.7 μM, which indicates that the lead compound acts as a CB_2 inverse agonist.

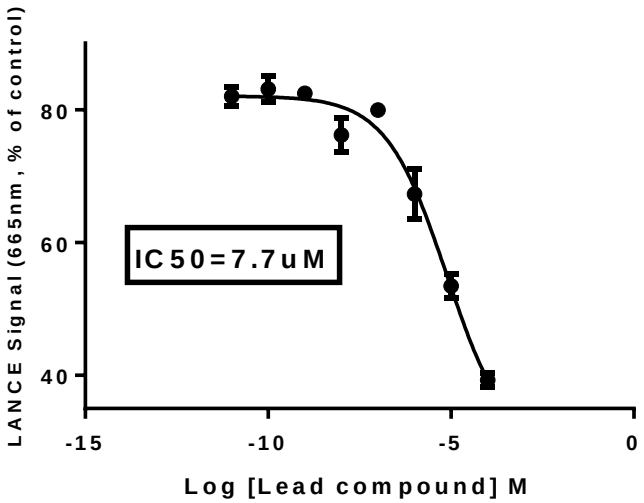


Figure 3.4 LANCE cAMP signal of the lead compound

Stably transfected CHO cells expressing hCB_2 receptors were used in LANCE cAMP assay. Assay was performed in triplicate. Data represented as mean ±SEM

3.2 STRATEGIES FOR NEW COMPOUNDS DESIGN

The ultimate goal of lead optimization is to develop potent and selective CB_2 ligands with better pharmacological and physiological properties. Based on previous docking study of lead compound, different strategies were used to conduct SAR studies (**Figure 3.5**):

1) As shown in **Figure 3.2**, ring C and oxygen in sulfone group show strong interactions with key residues (Phe281 and Ser285) of CB_2 receptor. So we retained the β-keto sulfone and benzene ring C while introduced different functional groups (such as -F, -CH_3 etc) on ring A and ring B;

2) Ring A in lead compound did not show strong interactions with certain residues so we replaced ring A with different aromatic systems as well as linear chains to see if *p*-methoxyl

benzene ring is essential. Moreover, we can have a concept of whether three aromatic ring systems is essential for CB_2 affinity;

3) We also used functional groups such as -N $(CH_3CH_2)_2$ in our modifications, based on our previous study which can not only increase selective CB_2 binding affinity, but also improve compound solubility [25, 87].

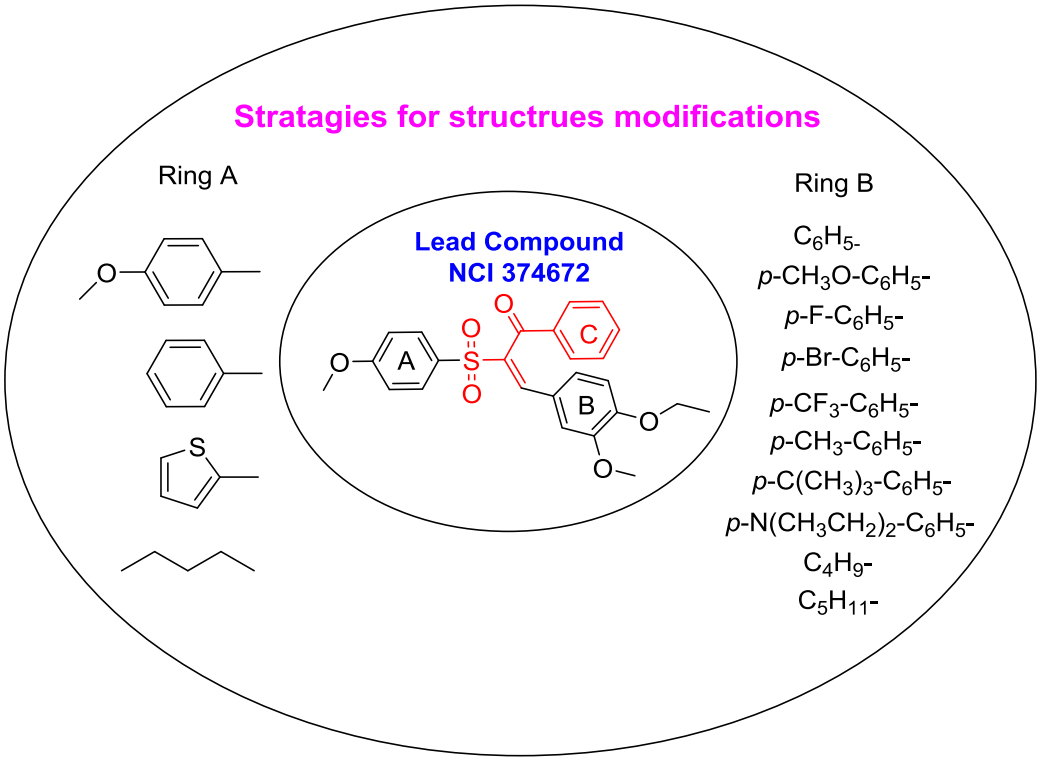


Figure 3.5 Strategies for lead modifications

After designing four series of compounds, we predicted their physicochemical properties and then conducted molecular docking for all the compounds that we designed. **Table 3.1** shows 29 compounds we selected (structure will be shown in SAR study part) which have a docking score higher than lead compound (higher than 6.02). Notably, most of them have a slightly lower or

similar ClogP compared to lead compound, which indicate a better or similar solubility. **Figure 3.6** shows the detailed interactions of one of the best scored compounds (compound **6d**) we designed. Similarly, it forms strong hydrogen bonds with Thr114 (~2.1 Å) and Ser285 (~1.6 Å). The strong π-π interaction with Phe281 (~3.6 Å) also exists. In addition it has relatively strong hydrophobic interactions with key residue Trp194 too. At the same time, it indicates that the replacement of aromatic ring A with linear chain did not harm the binding affinity.

All these results suggest the potential of our lead compound and newly designed compounds. So we synthesized these compounds to further study their SAR.

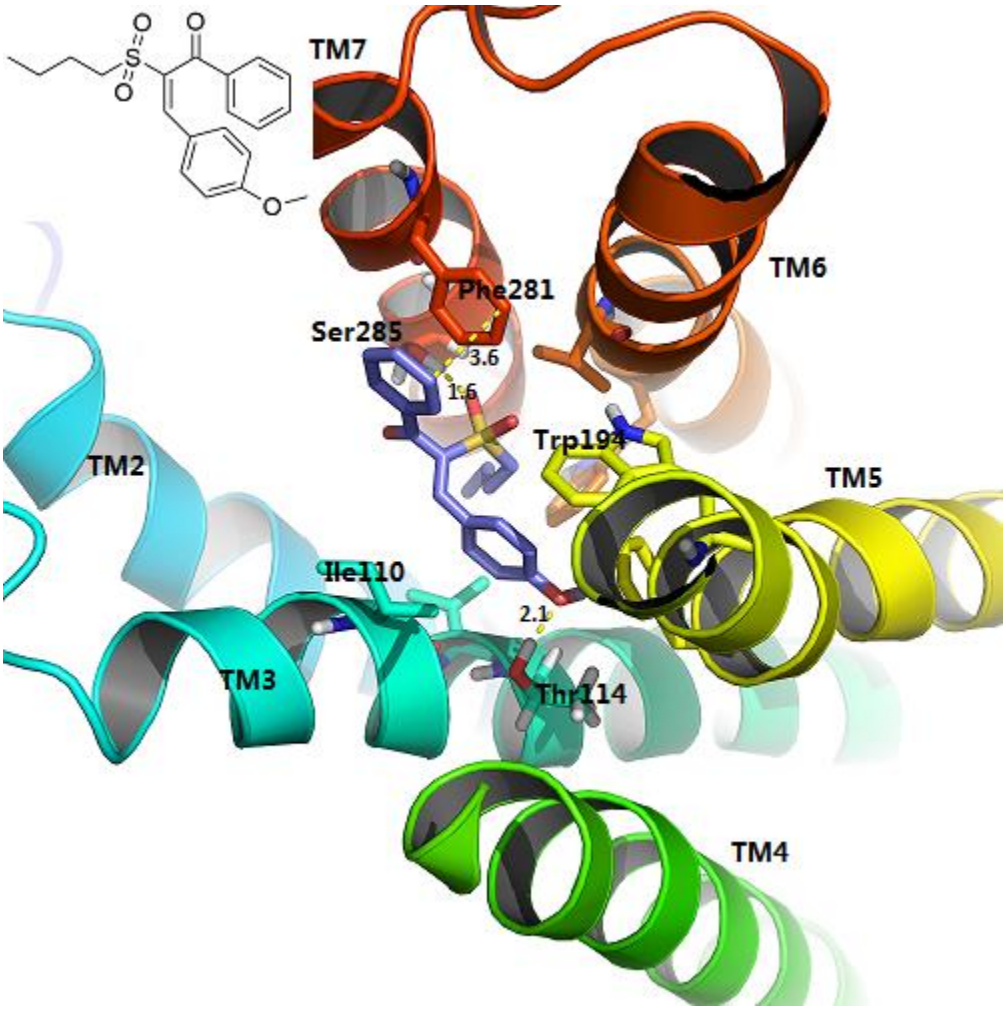


Figure 3.6 The detailed binding pose of compound 6d at CB_2 receptor.

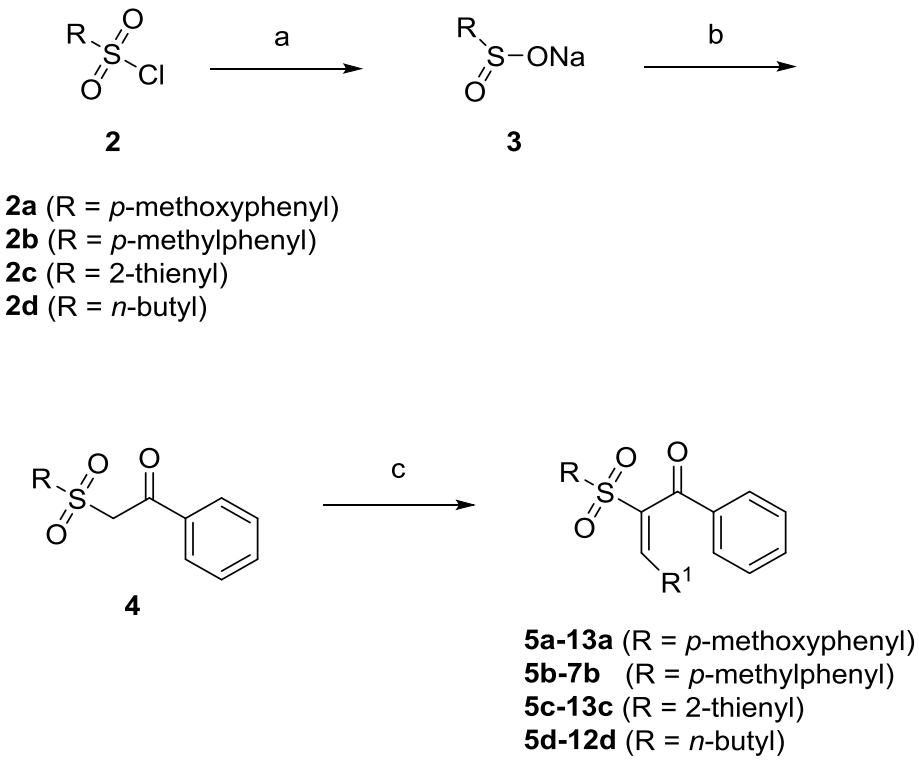
Table 3.1 Calculated properties and docking score of lead and designed compounds

Compound	Calculated Properties				Docking Score
	MW	ClogP	LogS	tPSA	
Lead	452	4.50	-6.39	78.9	6.02
5a	378	3.83	-6.17	60.4	7.73
6a	408	3.70	-6.24	69.7	8.26
7a	396	3.99	-6.41	60.4	7.87
8a	457	4.66	-6.99	60.4	7.49
9a	446	4.75	-7.13	60.4	8.32
10a	434	5.53	-7.61	60.4	8.33
11a	449	4.79	-7.26	63.7	8.67
12a	358	3.89	-5.76	60.4	7.82
13a	372	4.31	-6.18	60.4	8.15
5b	348	3.96	-6.13	51.2	6.70
6b	362	4.44	-6.49	51.2	6.76
7b	419	4.92	-7.21	54.4	7.62
5c	354	4.07	-5.56	51.2	6.03
6c	368	4.56	-5.92	51.2	6.89
7c	372	4.23	-5.77	51.2	6.26
8c	433	4.90	-6.37	51.2	6.59
9c	422	4.99	-6.49	51.2	6.89
10c	410	5.78	-7.00	51.2	6.88

11c	425	5.03	-6.64	54.4	8.22
12c	334	4.13	-5.14	51.2	6.69
13c	348	4.55	-5.56	51.2	8.14
5d	328	3.19	-5.43	51.2	7.59
6d	358	4.09	-5.46	60.4	8.00
7d	342	3.67	-5.79	51.2	7.80
8d	346	3.34	-5.64	51.2	7.61
9d	407	4.02	-6.24	51.2	7.73
10d	384	4.89	-6.86	51.2	7.62
11d	399	4.15	-6.50	54.4	8.14
12d	308	3.25	-5.02	51.2	7.30

3.3 CHEMICAL SYNTHESIS

The general synthesis route of target compounds was shown in **Scheme 1** including three steps. Starting from four different sulfonyl chlorides (**2**), appropriate sodium sulfinates (**3**) were obtained according to the reaction with sodium sulfite and sodium bicarbonate under reflux condition. Corresponding β-keto sulfones (**4**) were then obtained via alkylation reaction with bromo-methyl ketones at 100 ℃. Knoevenagel condensations using a variety of aldehydes with piperdine, acetic acid and toluene were then conducted at 120 ℃ to get four series of different sulfone derivatives (**5a-13a**, **5b-7b**, **5c-13c**, **5d-12d**, [88, 89]). The final compounds were purified by flash chromatography and all the purity is higher than 95%. Their chemical structures were confirmed by NMR as well as LC-MS.



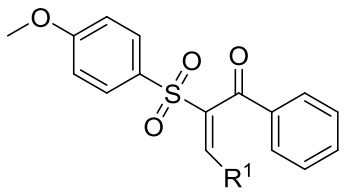
Scheme 1: General synthesis route for sulfone derivatives.

Reagent and conditions: (a) Na_2SO_3 (1.7 equiv), $NaHCO_3$ (1.7 equiv), H_2O, 100 ^oC, 24h; (b) 2-bromo-1-phenylethan-1-one (0.9 equiv), EtOH, H_2O, 100 ^oC, 24h; (c) R^1-CHO (1.1 equiv), piperdine (0.2 equiv), acetic acid (0.5 equiv), toluene, reflux, 120 ^oC, 24h.

3.4 STRUCTURE ACTIVITY RELATIONSHIP ANALYSIS

In the first cycle of SAR study, we held R group constant as p-methoxphenyl (**Table 3.2**). The removal of methoxyl group on ring A resulted in the decrease of CB_2 binding affinity (Compound **5a** vs.**6a**). Additionally, the replacements of methoxyl group on benzene rings to halogens or trifluoromethyl group also reduced the binding activity (Compound **6a** vs. **7a, 8a, 9a**). Interestingly, replacements of tertiary butyl group or diethyl amine group on the benzene ring dramatically increased the CB_2 receptor binding affinity (Compound **10a** and **11a**) when compound **11a** bearing a *p*-$N(CH_3CH_2)_2$ group also showed greatly improved CB_1/CB_2 selectivity (Selectivity Index = 311). Furthermore, we introduced alkyl chain instead of benzene ring (Compound **12a** and **13a**), which decreased the CB_2 binding activities compared to the lead compound. This result indicated that the aromatic ring B may be essential to keep a good activity. These results also confirm that electron donating groups plays an important role to maintain better binding affinity at R^1 (Compound **6a**-**11a**). Also, bulky groups are tolerated and may benefit the binding activity as well.

Table 3.2 Radioligand competition binding affinity and physicochemical properties of 5a-13a.

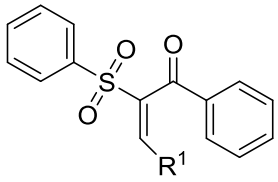


Compound	R^1	MW	CLogP	Ki (CB_2), nM[a]	Ki (CB_1), nM[b]	SI[c]
5a	C_6H_5-	378	3.83	420.4	2650	6.3
6a	p-CH_3O-C_6H_5-	408	3.70	134.6	2959	21.9
7a	p-F-C_6H_5-	396	3.99	478.0	5206	10.9
8a	p-Br-C_6H_5-	457	4.66	507.8	1007	1.9
9a	p-CF_3-C_6H_5-	446	4.75	948.7	1104	1.2
10a	p-$C(CH_3)_3$-C_6H_5-	434	5.53	58.7	1153	19.6
11a	p-$N(CH_3CH_2)_2$-C_6H_5-	449	4.79	43.9	13650	311.9
12a	C_4H_9-	358	3.89	910.1	4462	4.9
13a	C_5H_{11}-	372	4.31	300.3	1537	5.1
Lead	-	452	4.50	105	836	7.9
CP 55,940	-	-	-	0.35	NT	-
SR144528	-	-	-	2.05	NT	-
SR141716	-	-	-	NT	5.6 [90]	-

[a,b] Binding affinities of compounds for CB_1 and CB_2 receptors were evaluated using a [^{3}H]-CP 55,940 radioligand competition binding assay. [c] SI =selectivity index for CB_2, calculated as Ki(CB_1)/Ki(CB_2) ratio. NT: Not tested. CB_2 reference compound CP 55,940, SR144528 and CB_1 reference compound SR141716.

To continue investigate the effect of substituent on the benzene ring A, we retained the benzene ring and continued our SAR study (**Table 3.3**). Compared with compound **5a** bearing a *p*-methoxyl group, compound **5b** exhibit little difference in terms of CB_2 binding affinity, which indicates that methoxyl group may not be essential. Importantly, similar as compound **11a**, the new compound **7b** bearing a diethyl amine group also showed very good CB_2 binding affinity as well as selectivity index, which is consistent with previous results [25, 87]. This result further confirmed the methoxyl group on ring A is not essential to keep a good CB_2 binding affinity and selectivity.

Table 3.3 Radioligand competition binding affinity and physicochemical properties of **5b-7b**.



Compound	R^1	MW	CLogP	Ki (CB_2), nM[a]	Ki (CB_1), nM[b]	SI[c]
5b	C_6H_5-	348	3.96	411.7	781.5	1.8
6b	p-CH_3-C_6H_5-	362	4.44	127.2	543.5	4.3
7b	p-$N(CH_3CH_2)_2$-C_6H_5-	419	4.92	10.9	14220	1304.5
Lead	-	452	4.50	105	836	7.9
CP 55,940	-	-	-	0.35	NT	-
SR144528	-	-	-	2.05	NT	-
SR141716	-	-	-	NT	5.6[90]	-

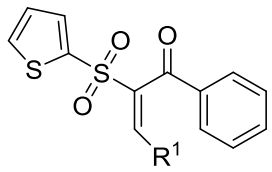
[a,b] Binding affinities of compounds for CB_1 and CB_2 receptors were evaluated using a [^{3}H]-CP 55,940 radioligand competition binding assay. [c] SI =selectivity index for CB_2, calculated as Ki(CB_1)/Ki(CB_2) ratio. NT: Not tested. CB_2 reference compound CP 55,940, SR144528 and CB_1 reference compound SR141716.

To further explore the importance of the benzene ring A, we replaced it with a heterocyclic thiophene ring (**Table** 3.4). Compounds **10c** and **11c** with a butyl group or a diethyl amine group showed the best CB_2 binding affinity as expected. The introduction of a fluorine group decreased the CB_2 binding activity while interestingly a bromine substituent enhanced the binding affinity (Compounds **7c** and **8c**). Additionally, a linear chain significantly reduced the binding affinity in this series than previous ones (Compound **12c** and **13c**). This result confirmed that the aromatic ring B plays important role to keep a good CB_2 binding affinity. Taken together, most compounds in this series exhibit a little decreased affinity compared to previous two series. This indicates that the benzene ring A may be crucial for remaining a better CB_2 binding activity.

Then we replaced this aryl ring A with alkyl chain (butyl) to see whether our previous conclusion is right (**Table 3.5**). In this series, compounds containing a butyl group or a diethyl amine group also exhibited the best CB_2 binding affinity (Compounds **10d** and **11d**). But their selectivity index is decreased compared to previous series. This is an interesting result and lead to a new hypothesis that replacing ring A with a linear chain may be a good strategy to generate new CB_1 potential compounds, which is a good direction for further studies. Similarly, a bromine substitution on the benzene ring (Compound **9d**) showed better activity than a fluorine substitution (Compound **8d**), which indicated that a bigger group or bulky substituent is better to get higher CB_2 binding affinity. Moreover, if both R group and R^1 group of the compound were replaced by a linear chain (Compound **12d**), it completely lost its CB_2 binding activity. This suggests the importance of at least two aromatic rings in our scaffolds. Additionally, there is a dramatic decrease of affinity if we compare compound **5d** with **5a**, **8d** with **7a** and **12d** with **12a**, which indicates that the aryl ring A is better for the CB_2 binding activity. Interestingly, other two

compounds of these two series (Compounds **9d** and **8a**, **6d** and **6a**) showed similar activity, which need further study.

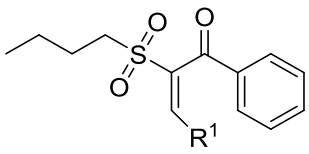
Table 3.4 Radioligand competition binding affinity and physicochemical properties of 5c-13c.



Compound	R^1	MW	CLogP	Ki (CB_2), nM[a]	Ki (CB_1), nM[b]	SI[c]
5c	C_6H_5-	354	4.07	1200	2276	1.9
6c	p-CH_3-C_6H_5-	368	4.56	1000	1883	1.9
7c	p-F-C_6H_5-	372	4.23	2900	1769	0.6
8c	p-Br-C_6H_5-	433	4.90	380.8	1305	3.4
9c	p-CF_3-C_6H_5-	422	4.99	1300	1840	1.4
10c	p-$C(CH_3)_3$-C_6H_5-	410	5.78	32.6	648.4	20
11c	p-$N(CH_3CH_2)_2$-C_6H_5-	425	5.03	30.7	13350	435
12c	C_4H_9-	334	4.13	3700	12620	3.4
13c	C_5H_{11}-	348	4.55	2000	5858	2.9
Lead	-	452	4.50	105	836	7.9
CP 55,940	-	-	-	0.35	NT	-
SR144528	-	-	-	2.05	NT	-
SR141716	-	-	-	NT	5.6[90]	-

[a,b] Binding affinities of compounds for CB_1 and CB_2 receptors were evaluated using a [^{3}H]-CP 55,940 radioligand competition binding assay. [c] SI =selectivity index for CB_2, calculated as Ki(CB_1)/Ki(CB_2) ratio. NT: Not tested. CB_2 reference compound CP 55,940, SR144528 and CB_1 reference compound SR141716.

Table 3.5 Radioligand competition binding affinity and physicochemical properties of 5d-12d.



Compound	R^1	MW	CLogP	Ki (CB_2), nM[a]	Ki (CB_1), nM[b]	SI[c]
5d	C_6H_5-	328	3.19	1380	10100	7.3
6d	p-CH_3O-C_6H_5-	358	4.09	197.5	1748	8.9
7d	p-CH_3-C_6H_5-	342	3.67	704.1	2356	3.3
8d	p-F-C_6H_5-	346	3.34	4300	5803	1.3
9d	p-Br-C_6H_5-	407	4.02	561.2	773.5	1.4
10d	p-$C(CH_3)_3$-C_6H_5-	384	4.89	79.1	281.2	3.6
11d	p-$N(CH_3CH_2)_2$-C_6H_5-	399	4.15	16.2	1592	98.2
12d	C_4H_9-	308	3.25	28700	306.3	0.01
Lead	-	452	4.50	105	836	7.9
CP 55,940	-	-	-	0.35	NT	-
SR144528	-	-	-	2.05	NT	-
SR141716	-	-	-	NT	5.6[90]	-

[a,b] Binding affinities of compounds for CB_1 and CB_2 receptors were evaluated using a [^{3}H]-CP 55,940 radioligand competition binding assay. [c] SI =selectivity index for CB_2, calculated as Ki(CB_1)/Ki(CB_2) ratio. NT: Not tested. CB_2 reference compound CP 55,940, SR144528 and CB_1 reference compound SR141716.

3.5 OFF-TARGET EFFECTS PREDICTION

Then, we use several of our best compounds **11a, 7b, 10c** and **11d** to study the off-target (either bad or good) effects of this series of novel scaffolds. By utilizing high-throughput docking (HT-docking) and online databases our lab created (**http://www.cbligand.org/OP/**) (**http://www.cbligand.org/MM/**), we were able to find many osteoporosis or multiple myeloma related targets that have a high docking score with our compound (**7b**).

Also, we confirm it by looking into the interaction networks of these representative compounds and their potential targets (**Figure 3.7**, **Figure 3.8**). Many overlapping targets which related to osteoporosis or multiple myeloma are shown. (**Table 3.6**, **Table 3.7**)

For example, selective estrogen receptor modulators were reported to have relationship with postmenopausal osteoporosis [91] while vitamin D3 receptor was reported to be crucial in the balance between bone resorption and bone formation [92]. Similarly, heat shock protein 90 inhibitor NVP-AUY922 and Aurora Kinase Inhibitor Alisertib (MLN8237) was reported as monotherapy or in combination with bortezomib for the treatment of relapsed or refractory multiple myeloma [93, 94]. These results not only indicate other potential targets that our compounds might have, but also confirm the therapeutic effects of our compound in osteoporosis and multiple myeloma. Moreover, the underlying mechanism of treating osteoporosis and multiple myeloma using this novel scaffold can be further studied based on these findings.

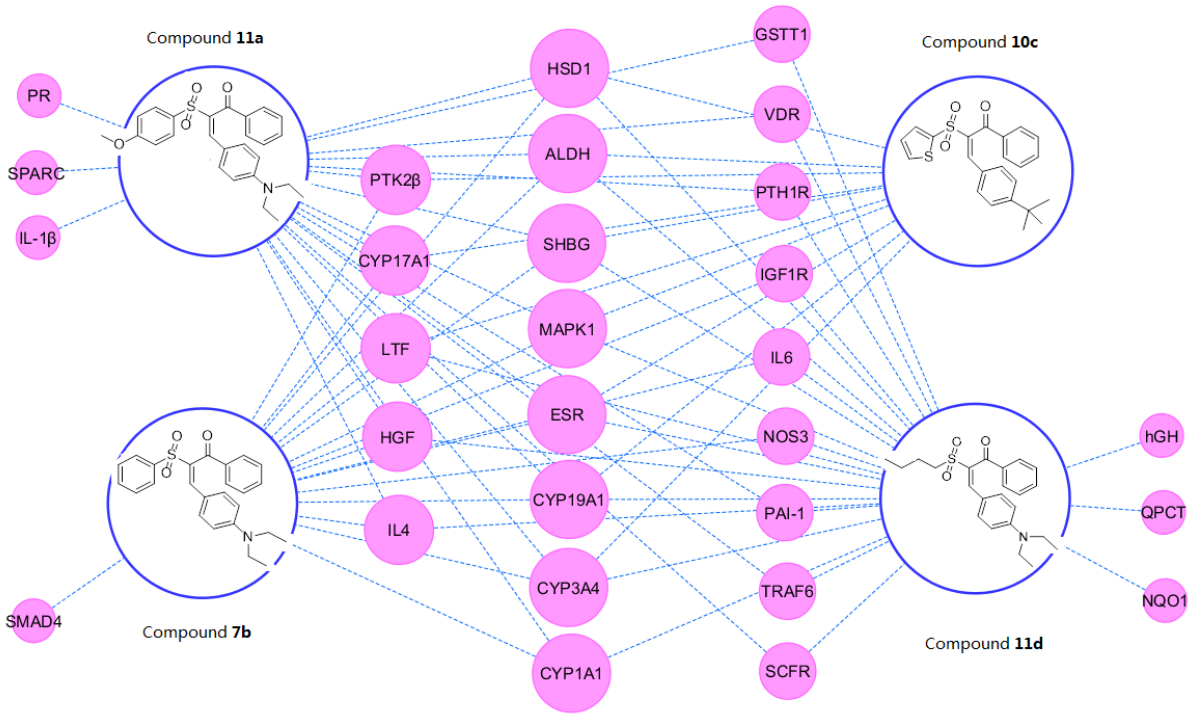


Figure 3.7 Interaction network of representative compounds and their potential targets for OP

Four large circles with chemical structures represent the three representative molecules we selected with best affinity. Each constitute is linked to its predicted targets, represented by nodes.

Abbreviation: PR, Progesterone receptor; SPARC, Serum secreted protein acidic and rich in cysteine; IL-1β, Interleukin-1β; SMAD4, Mothers against decapentaplegic homolog 4; PTK2β, Protein-tyrosine kinase 2β; CYP17A1, Steroid 17-alpha-hydroxylase/17,20 lyase; LTF, Lactotransferrin; HGF, Hepatocyte growth factor; IL4, Interleukin-4; HSD1, Corticosteroid 11-beta-dehydrogenase isozyme 1; ALDH, Aldehyde dehydrogenase, mitochondrial; SHBG, Sex hormone-binding globulin; MAPK1, Mitogen-activated protein kinase 1; ESR, Estrogen receptor; CYP19A1, Cytochrome P450 19A1; CYP3A4, Cytochrome P450 3A4; CYP1A1, Cytochrome P450 1A1; GSTT1, Glutathione S-transferase theta-1; VDR, Vitamin D3 receptor; PTH1R, Parathyroid hormone/parathyroid hormone-related peptide receptor; IGF1R, Insulin-like growth factor 1 receptor; IL6, Interleukin-6; NOS3, Nitric oxide synthase, endothelial; PAI1, Plasminogen activator inhibitor 1; TRAF6, TNF receptor-associated factor 6; SCFR,

Mast/stem cell growth factor receptor Kit; hGH, Somatotropin; QPCT, Glutaminyl-peptide cyclotransferase; NQO1, NAD(P)H dehydrogenase [quinone] 1.

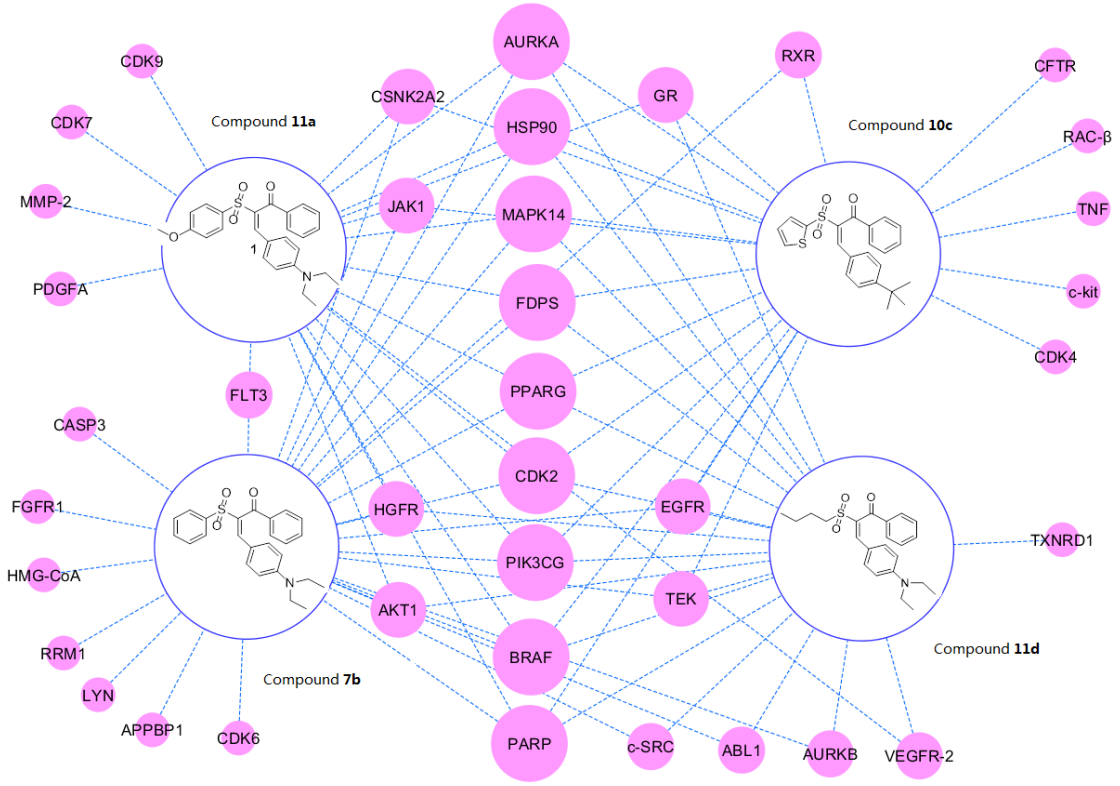


Figure 3.8 Interaction network of representative compounds and their potential targets for MM

Four large circles with chemical structures represent the three representative molecules we selected with best affinity. Each constitute is linked to its predicted targets, represented by nodes.

Abbreviation: CDK9, Cyclin-dependent kinase 9; CDK7, Cyclin-dependent kinase 7; MMP-2, 72 kDa type IV collagenase; PDGFA, Platelet-derived growth factor subunit A; CASP3, Caspase-3; FGFR1, Fibroblast growth factor receptor 1; HMG-CoA, 3-hydroxy-3-methyl-glutaryl-CoA reductase; RRM1, Ribonucleoside-diphosphate reductase large subunit; LYN, Tyrosine-protein kinase Lyn; APPBP1, NEDD8-activating enzyme E1 regulatory subunit; CDK6, Cyclin-dependent kinase 6; FLT3, receptor-type tyrosine-protein kinase; CSNK2A2, Casein kinase II subunit alpha; JAK1, tyrosine kinase; HGFR, hepatocyte growth factor receptor; AKT1, RAC-alpha serine/threonine-protein kinase; AURKA, Aurora

kinase A; HSP90, Heat shock protein 90; MAPK14, Mitogen-activated protein kinase 14; FDPS, Dimethylallyltranstransferase; PPARG, Peroxisome proliferator-activated receptor gamma; CDK2, Cyclin-dependent kinase 2; PIK3CG, Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit gamma isoform; BRAF, serine/threonine-protein kinase B-Raf; PARP, Poly (ADP-ribose) polymerase; GR, glucocorticoid receptor; EGFR, epidermal growth factor receptor; TEK, Angiopoietin-1 receptor; c-SRC, Proto-oncogene tyrosine-protein kinase Src; ABL1, Abelson murine leukemia viral oncogene homolog 1; AURKB, Aurora B kinase; VEGFR-2, Kinase insert domain receptor; RXR, retinoid X receptor; CFTR, Cystic fibrosis transmembrane conductance regulator; RAC-β, RAC-beta serine/threonine-protein kinase; TNF, Tumor necrosis factors; c-kit, Mast/stem cell growth factor receptor; CDK4, Cyclin-dependent kinase 4; TXNRD1, Thioredoxin reductase 1.

Table 3.6 HT-docking of compound 7b using chemogenomics database for osteoporosis

PDB ID	PROTEIN	DOCKING SCORE
1err	Estrogen receptor	8.51
3tkc	Vitamin D3 receptor	8.43
2ojg	Mitogen-activated protein kinase 1	8.13
2c3q	Glutathione S-transferase theta-1	7.91
1xu7	Corticosteroid 11-beta-dehydrogenase isozyme 1	7.68
4i4h	Cytochrome P450 3A4	7.59

Table 3.7 HT-docking of compound 7b using chemogenomics database for multiple myeloma

PDB ID	PROTEIN	DOCKING SCORE
2ye7	Heat shock protein HSP 90-alpha	8.93
3uok	Aurora kinase A	8.62
2r3o	Cyclin-dependent kinase 2	8.62
3skc	Serine/threonine-protein kinase B-raf	8.15

4.0 CONCLUSION

In this study, many ligand discovery tools were utilized in order to identify novel CB_2 selective compounds. High-throughput virtual screening was used to discover the lead compound (NCI374672) with novel structure (β-keto sulfones and α, β double bond). Physicochemical property predication, molecular docking study and biological validations were then conducted. To get more drug-like compounds, various optimization strategies were considered based on the docking score, binding mode and the solubility of novel scaffolds. As a result, we designed and synthesized 29 analogues using a similar three-step synthetic route, and evaluated their binding affinity against two subtypes of cannabinoid receptors CB_1 and CB_2 as well as their CB_1/CB_2 selectivity. Among these new analogues, four compounds showed high CB_2 binding affinity (K_i < 60 nM) and good selectivity (CB_1/CB_2 of 20- to 1305 fold). The best compound **7b** showed a CB_2 binding affinity (K_i) equals to 10.9 nM and selectivity index more than 1000 folds. Moreover, off-target effects were predicted using HT-docking and chemogenomics databases.

In summary, these studies are significant for future drug development and this novel series of CB_2 ligands are promising as potential treatment for osteoporosis and multiple myeloma.

5.0 FUTURE PROSPECTIVES

We have already got several novel potent CB_2 selective compounds with K_i value around 10nM. In order to get more drug-like CB_2 oral drug, further optimizations are needed to improve the solubility or bioavailability of our compounds. We will also test the compounds' efficacy (using [^{35}S]GTPγS binding assay) and toxicity (using MTT assay). *In vivo* assay might also be conducted for the best compounds. Additional SAR of this series of compounds will be studied based on new bioactivity data.

Additionally, scaffold hopping and pharmacophore model will be utilized for the design of CB ligands with new structure. Based on the results from chemogenomics database for multiple myeloma (http://www.cbligand.org/MM/) and chemogenomics database for osteoporosis (http://www.cbligand.org/OP), we can also design new chemical entities that can act on other targets related to multiple myeloma and osteoporosis. To further confirm our speculations, biological validations will also be conducted.

Moreover, to distinguish CB agonists from antagonists/inverse agonists based on their structures are quite difficult. We will build computational methods to predict whether a special structure is more likely to make a compound act as an agonist or an inverse agonist before its efficacy is actually tested. Dockings and pharmacophore model will also be used to study the correlation between functional groups and compounds' efficacies. This will help us design druggable CB ligands which can be used in certain diseases with certain functions and less side effects.

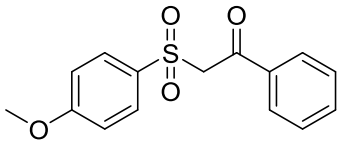
To enhance the selectivity of our compounds, we will also look into the design of novel CB_2 allosteric modulators and study their mechanisms in terms of altering the conformation of CB_2 receptor.

6.0 EXPERIMENTAL

6.1 CHEMISTRY

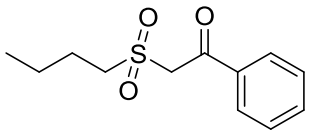
6.1.1 General synthesis procedure of β-keto sulfones

2-((4-Methoxyphenyl) sulfonyl)-1-phenylethan-1-one



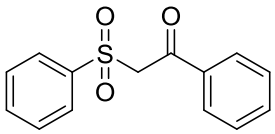
4-Methoxybenzenesulfonyl chloride (5.25 g, 25.5 mmol) was added in water (145 mL). Na_2SO_3 (5.4 g, 43.4 mmol) and $NaHCO_3$ (3.6 g, 43.4 mmol) were added to the solution. The mixture was heated under reflux at 100 ℃ for 24 h. Then an ethanolic solution of 2-bromo-1-phenylethan-1-one (4.57 g, 22.95 mmol) was added slowly to the mixture. After 24 h, reaction was cooled down to room temperature. Diluted HCl was used to neutralize the solution and gave solid product. The product was filtered and washed by ethanol to give the desired compound (2.6 g, Yield: 35.1%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.94-7.97 (m, 2H), 7.80-7.83 (m, 2H), 7.66-7.70 (m, 1H), 7.50- 7.54 (m, 2H), 7.12-7.15 (m, 2H), 5.25 (s, 2H), 3.86 (s, 3H).

2-(Butylsulfonyl)-1-phenylethan-1-one



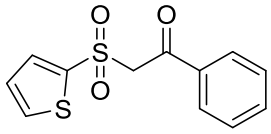
Yield: 73.1%. ^{1}HNMR (400 MHz, DMSO-d_6) δ 8.05-8.07 (m, 2H), 7.70-7.75 (m, 1H), 7.57-7.60 (m, 2H), 5.08 (s, 2H), 3.28-3.30 (m, 2H), 1.69-1.77 (m, 2H), 1.38-1.48 (m, 2H), 0.90-0.94 (m, 3H)

1-Phenyl-2-(phenylsulfonyl)ethan-1-one



Yield: 34.0%. ^{1}HNMR (400 MHz, DMSO-d_6) δ 7.96 (d, J = 8Hz, 2H), 7.92 (d, J = 7.2Hz, 2H), 7.62-7.76 (m, 4H), 7.52 (dd, J = 8Hz, 2H), 5.35 (s, 2H)

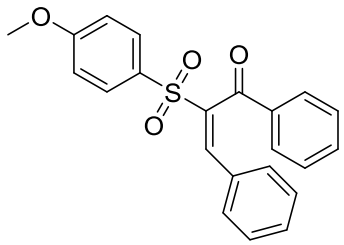
1-Phenyl-2-(thiophen-2-ylsulfonyl)ethan-1-one



Yield: 48.3%. ^{1}HNMR (400 MHz, DMSO-d_6) δ 8.09-8.11 (m, 1H), 7.96-7.99 (m, 2H), 7.79-7.80 (m, 1H), 7.67-7.71 (m, 1H), 7.53 (dd, J = 7.6Hz, 2H), 7.24-7.26 (m, 1H), 5.41 (s, 2H)

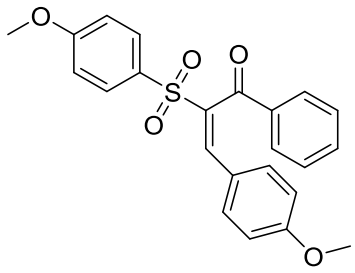
6.1.2 General synthesis procedure of sulfone derivatives

(E)-2-((4-Methoxyphenyl)sulfonyl)-1,3-diphenylprop-2-en-1-one (5a)



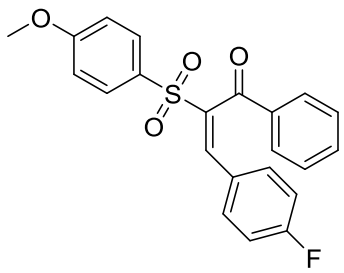
2-((4-methoxyphenyl) sulfonyl)-1-phenylethan-1-one (290mg, 1mmol) was dissolved by toluene (20ml). Benzaldehyde (117mg, 1.1mmol) was then added to the solution. Piperidine (16mg, 0.2mmol) and acetic acid (30mg, 0.5mmol) were added to the mixture. The reaction system was heated at reflux for 24h (115℃-120℃). After the reaction is completed, cool down the system and add toluene to dissolve the mixture. Flash column was run afterwards. Desired compound is given (180mg, Yield: 47.6%). ^{1}HNMR (400 MHz, DMSO-d_6) δ 8.11 (s, 1H), 7.76-7.79 (m, 4H), 7.61-7.65 (m, 1H), 7.43-7.47 (m, 2H), 7.28-7.35 (m, 5H), 7.14-7.16 (m, 2H), 3.87 (s, 3H)

(E)-3-(4-Methoxyphenyl)-2-((4-methoxyphenyl)sulfonyl)-1-phenylprop-2-en-1-one (6a)



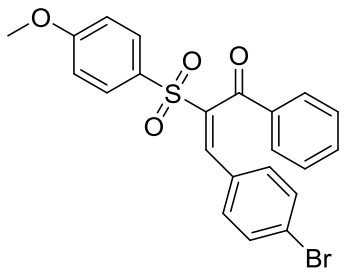
Yield: 25.0%. ^{1}HNMR (400 MHz, DMSO-*d6*) δ 8.02 (s, 1H), 7.74-7.80 (m, 3H), 7.63-7.67 (m, 1H), 7.46-7.47 (m, 2H), 7.13-7.19 (m, 5H), 6.86- 6.88 (d, J = 8Hz, 2H), 3.86 (s, 3H), 3.71 (s, 3H)

(E)-3-(4-Fluorophenyl)-2-((4-methoxyphenyl)sulfonyl)-1-phenylprop-2-en-1-one (7a)



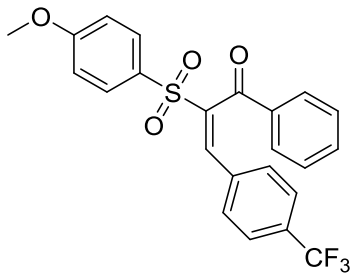
Yield: 67.0%. ^{1}HNMR (400 MHz, DMSO-d_6) δ 8.13 (s, 1H), 7.75-7.78 (m, 4H), 7.62-7.66 (m, 1H), 7.39-7.48 (m, 4H), 7.13-7.19 (m, 4H), 3.87 (s, 3H)

(E)-3-(4-Bromophenyl)-2-((4-methoxyphenyl)sulfonyl)-1-phenylprop-2-en-1-one (8a)



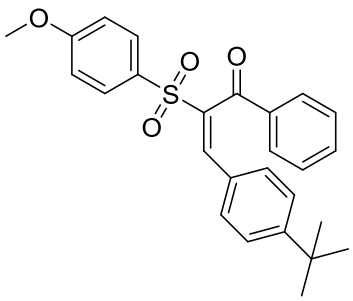
Yield: 5.9%. ^{1}HNMR (400 MHz, DMSO-d_6) δ 8.15-8.16 (m, 2H), 7.76-7.78 (m, 3H), 7.63-7.66 (m, 1H), 7.53-7.55 (m, 2H), 7.46-7.49 (m, 2H), 7.30-7.32 (m, 2H), 7.25-7.27 (m, 1H)

(E)-2-((4-Methoxyphenyl)sulfonyl)-1-phenyl-3-(4-(trifluoromethyl)phenyl)prop-2-en-1-one (9a)



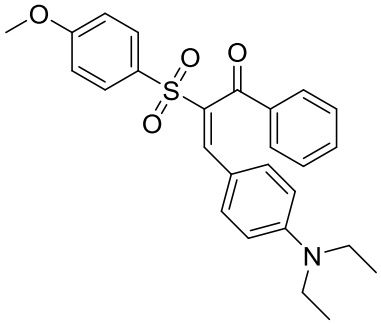
Yield: 43.0%. ^{1}HNMR (400 MHz, DMSO-d_6) δ 8.22 (s, 1H), 7.74-7.8 (m, 4H), 7.61-7.69 (m, 3H), 7.54-7.56 (d, J = 8.4Hz, 2H), 7.43-7.47 (t, J = 8Hz, 2H), 7.14- 7.17 (m, 2H), 3.87 (s, 3H)

(E)-3-(4-(Tert-butyl)phenyl)-2-((4-methoxyphenyl)sulfonyl)-1-phenylprop-2-en-1-one (10a)



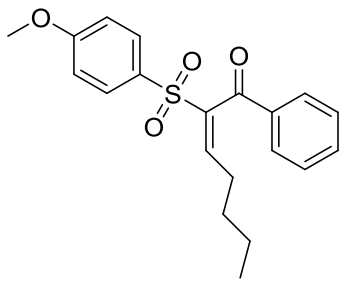
Yield: 50.5%. ^{1}HNMR (400 MHz, DMSO-d_6) δ 8.03 (s, 1H), 7.79-7.82 (m, 2H), 7.73-7.76 (m, 2H), 7.64-7.66 (m, 1H), 7.47-7.50 (m, 2H), 7.27-7.35 (m, 4H), 7.12-7.14 (m, 2H), 3.33 (s, 3H), 1.18 (s, 9H)

(E)-3-(4-(Diethylamino)phenyl)-2-((4-methoxyphenyl)sulfonyl)-1-phenylprop-2-en-1-one (11a)



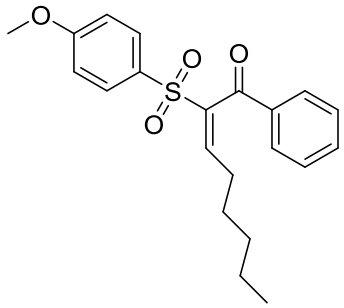
Yield: 51.0%. ^{1}HNMR (400 MHz, DMSO-d_6) δ 7.80-7.84 (m, 3H), 7.71-7.74 (m, 2H), 7.63-7.66 (m, 1H), 7.46-7.50 (m, 2H), 7.10-7.12 (m, 4H), 6.51-6.53 (d, J = 8.8Hz 2H), 3.85 (s, 3H), 3.29-3.33 (m, 4H), 0.99-1.02 (t, J = 7.8Hz, 6H)

(E)-2-((4-Methoxyphenyl)sulfonyl)-1-phenylhept-2-en-1-one (12a)



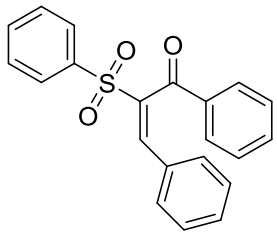
Yield: 9.3%. ^{1}HNMR (400 MHz, DMSO-d_6) δ 8.05-8.08 (m, 2H), 7.69-7.72 (m, 3H), 7.52-7.57 (m, 2H), 7.13-7.15 (m, 2H), 6.20-6.23 (m, 1H), 3.86 (s, 3H), 1.97-2.00 (m, 2H), 1.25-1.28 (m, 4H), 0.78 (t, J = 7.6Hz, 3H)

(E)-2-((4-Methoxyphenyl)sulfonyl)-1-phenyloct-2-en-1-one (13a)



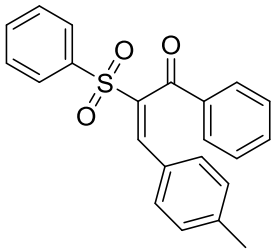
Yield: 11.4%. ^{1}HNMR (400 MHz, DMSO-d_6) δ 8.06-8.08 (m, 2H), 7.70-7.72 (m, 3H), 7.52-7.56 (m, 2H), 7.13-7.15 (m, 2H), 6.20-6.23 (m, 1H), 3.82 (s, 3H), 1.99-2.00 (m, 2H), 1.12-1.24 (m, 6H), 0.82 (t, J = 7.2Hz, 3H)

(E)-1,3-Diphenyl-2-(phenylsulfonyl)prop-2-en-1-one (5b)



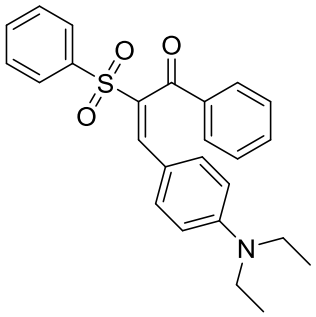
Yield: 3.3%. ^{1}HNMR (400 MHz, DMSO-d_6) δ 8.19 (s, 1H), 7.86-7.88 (m, 2H), 7.76-7.78 (m, 2H), 7.62-7.65 (m, 3H), 7.42-7.46 (m, 2H), 7.28-7.37 (m, 6H)

(E)-1-Phenyl-2-(phenylsulfonyl)-3-(p-tolyl)prop-2-en-1-one (6b)



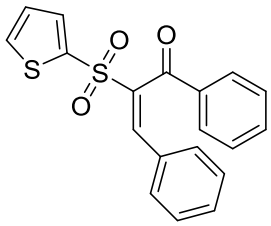
Yield: 5.3%. ^{1}HNMR (400 MHz, DMSO-d_6) δ 8.12 (s, 1H), 7.84-7.87 (m, 2H), 7.71-7.78 (m, 3H), 7.61-7.65 (m, 3H), 7.42-7.47 (m, 2H), 7.24 (d, J = 8Hz, 2H), 7.11 (d, J = 8Hz, 2H), 2.22 (s, 3H)

(E)-3-(4-(Diethylamino)phenyl)-1-phenyl-2-(phenylsulfonyl)prop-2-en-1-one (7b)



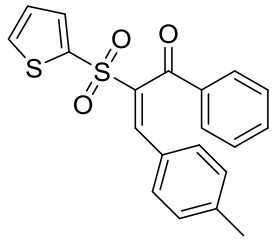
Yield: 13.5%. ^{1}HNMR (400 MHz, DMSO-d_6) δ 7.90 (s, 1H), 7.79-7.83 (m,4H), 7.58-7.70 (m, 4H), 7.48 (dd, J = 7.6Hz, 2H), 7.13 (d, J = 8.8Hz, 2H), 6.52 (d, J = 9.2Hz, 2H), 3.23-3.31 (m, 4H), 1.00 (t, J = 7.2Hz, 6H)

(E)-1,3-Diphenyl-2-(thiophen-2-ylsulfonyl)prop-2-en-1-one (5c)



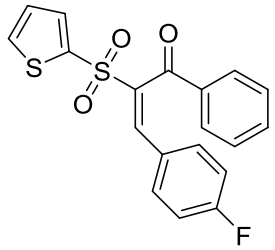
Yield: 6.9%. ^{1}HNMR (400 MHz, DMSO-d_6) δ 8.13-8.14 (m, 2H), 7.75-7.78 (m, 3H), 7.61-7.65 (m, 1H), 7.24-7.47 (m, 8H)

(E)-1-Phenyl-2-(thiophen-2-ylsulfonyl)-3-(p-tolyl)prop-2-en-1-one (6c)



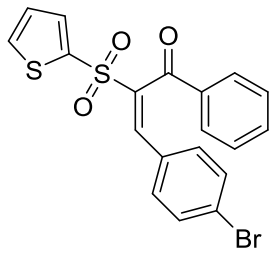
Yield: 11.6%. ^{1}HNMR (400 MHz, DMSO-d_6) δ 8.12-8.14 (m, 1H), 8.09 (s, 1H), 7.73-7.78 (m, 3H), 7.62-7.65 (m, 1H), 7.43-7.48 (m, 2H), 7.23-7.27 (m, 3H), 7.11-7.13 (m, 2H), 2.22 (s, 3H)

(E)-3-(4-Fluorophenyl)-1-phenyl-2-(thiophen-2-ylsulfonyl)prop-2-en-1-one (7c)



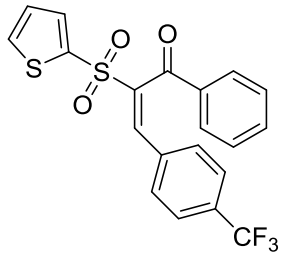
Yield: 3.3%. ^{1}HNMR (400 MHz, DMSO-d_6) δ 8.16 (s, 1H), 8.13-8.15 (m, 1H), 7.74-7.78 (m, 3H), 7.62-7.64 (m, 1H), 7.43-7.48 (m, 4H), 7.15-7.26 (m, 3H)

(E)-3-(4-Bromophenyl)-1-phenyl-2-(thiophen-2-ylsulfonyl)prop-2-en-1-one (8c)



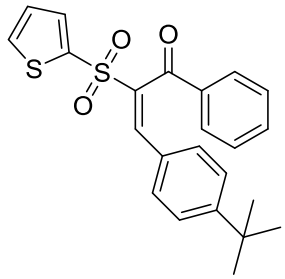
Yield: 5.9%. ^{1}HNMR (400 MHz, DMSO-d_6) δ 8.15-8.16 (m, 2H), 7.76-7.78 (m, 3H), 7.63-7.66 (m, 1H), 7.53-7.55 (m, 2H), 7.46-7.49 (m, 2H), 7.30-7.32 (m, 2H), 7.25-7.27 (m, 1H)

(E)-1-Phenyl-2-(thiophen-2-ylsulfonyl)-3-(4-(trifluoromethyl)phenyl)prop-2-en-1-one (9c)



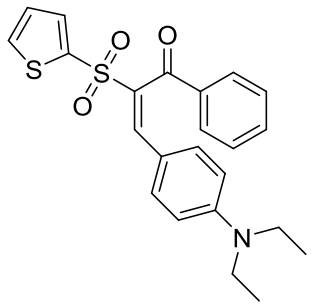
Yield: 5.0%. ^{1}HNMR (400 MHz, DMSO-d_6) δ 8.27 (s, 1H), 8.17-8.18 (m, 1H), 7.76-7.80 (m, 3H), 7.57-7.70 (m, 5H), 7.43-7.46 (m, 2H), 7.26-7.28 (m, 1H)

(E)-3-(4-(Tert-butyl)phenyl)-1-phenyl-2-(thiophen-2-ylsulfonyl)prop-2-en-1-one (10c)



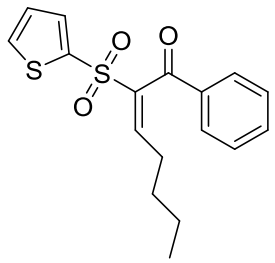
Yield: 13.3%. ^{1}HNMR (400 MHz, DMSO-d_6) δ 8.12-8.14 (m, 1H), 8.07 (s, 1H), 7.81-7.83 (m, 2H), 7.72-7.73 (m, 1H), 7.65-7.68 (m, 1H), 7.48-7.52 (m, 2H), 7.31-7.37 (m, 4H), 7.23-7.25 (m, 1H), 1.18 (s, 9H)

(E)-3-(4-(Diethylamino)phenyl)-1-phenyl-2-(thiophen-2-ylsulfonyl)prop-2-en-1-one (11c)



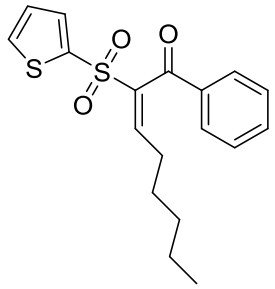
Yield: 1.5%. ^{1}HNMR (400 MHz, DMSO-d_6) δ 8.05-8.06 (m, 1H), 7.81-7.87 (m, 3H), 7.63-7.66 (m, 2H), 7.47-7.53 (m, 2H), 7.14-7.22 (m, 3H), 6.53-6.55 (m, 2H), 3.28-3.30 (m, 4H), 1.01 (t, J = 8Hz, 6H)

(E)-1-Phenyl-2-(thiophen-2-ylsulfonyl)hept-2-en-1-one (12c)



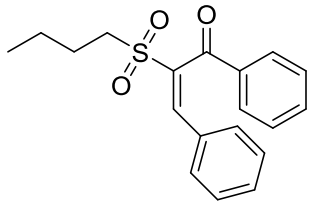
Yield: 20.6%. ^{1}HNMR (400 MHz, DMSO-d_6) δ 8.08-8.16 (m, 3H), 7.70-7.71 (m, 2H), 7.56-7.58 (m, 2H), 7.26-7.28 (m, 1H), 6.35-6.38 (m, 1H), 2.00-2.03 (m, 2H), 1.35-1.46 (m, 4H), 0.81 (t, J = 7.2Hz, 3H)

(E)-1-Phenyl-2-(thiophen-2-ylsulfonyl)oct-2-en-1-one (13c)



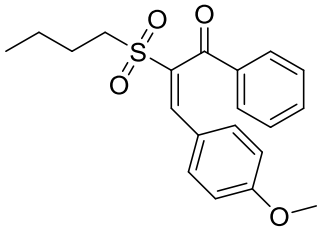
Yield: 9.3%. ^{1}HNMR (400 MHz, DMSO-d_6) δ 8.08-8.16 (m, 3H), 7.69-7.71 (m, 2H), 7.54-7.58 (m, 2H), 7.26-7.28 (m, 1H), 6.35-6.37 (m, 1H), 2.00-2.04 (m, 2H), 1.17-1.25 (m, 6H), 0.82-0.85 (m, 3H)

(E)-2-(Butylsulfonyl)-1,3-diphenylprop-2-en-1-one (5d)



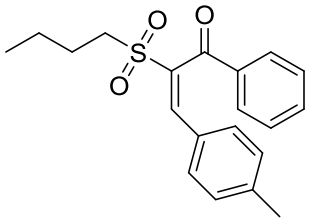
Yield: 11.2%. ^{1}HNMR (400 MHz, DMSO-d_6) δ 7.94 (s, 1H), 7.87 (d, J = 7.2Hz, 2H), 7.65 (dd, J = 7.6Hz, 1H), 7.49 (dd, J = 7.6Hz, 2H), 7.29-7.38 (m, 5H), 3.41 (t, J = 8Hz, 2H), 1.69-1.77 (m, 2H), 1.41-1.50 (m,2H), 0.92 (t, J = 7.2Hz, 3H)

(E)-2-(Butylsulfonyl)-3-(4-methoxyphenyl)-1-phenylprop-2-en-1-one (6d)



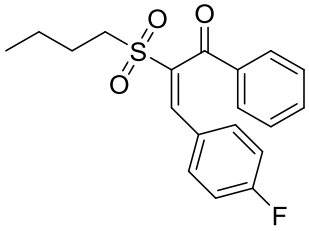
Yield: 36.2%. ^{1}HNMR (400 MHz, DMSO-d_6) δ 7.89 (d, J = 7.6Hz, 2H), 7.84 (s, 1H), 7.67 (dd, J = 7.2Hz, 1H), 7.52 (dd, J = 8Hz, 2H), 7.33 (d, J = 9.2Hz, 2H), 6.88 (d, J = 8.8Hz, 2H), 3.72 (s, 3H), 3.36-3.39 (m, 2H), 1.67-1.75 (m, 2H), 1.38-1.49 (m,2H), 0.91 (t, J = 7.2Hz, 3H)

(E)-2-(Butylsulfonyl)-1-phenyl-3-(p-tolyl)prop-2-en-1-one (7d)



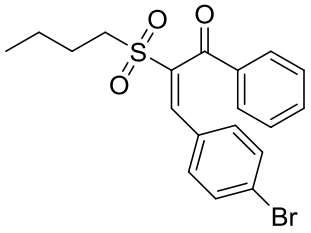
Yield: 15.3%. ^{1}HNMR (400 MHz, DMSO-d_6) δ 7.86-7.89 (m, 3H), 7.64-7.68 (m,1H), 7.50 (dd, J = 7.6Hz, 2H), 7.26 (d, J = 8Hz, 2H), 7.13 (d, J = 8Hz, 2H), 3.36-3.40 (m, 2H), 2.23 (s, 3H), 1.68-1.75 (m, 2H), 1.40-1.50 (m, 2H), 0.91 (t, J = 7.6Hz, 3H)

(E)-2-(Butylsulfonyl)-3-(4-fluorophenyl)-1-phenylprop-2-en-1-one (8d)



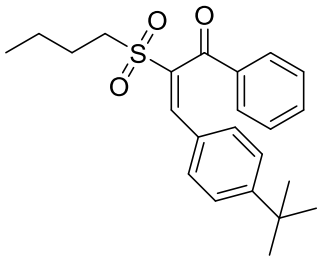
Yield: 17.2%. ^{1}HNMR (400 MHz, DMSO-d_6) δ 7.95 (s, 1H), 7.87 (d, J = 8Hz, 2H), 7.64-7.69 (m, 2H), 7.48-7.52 (m, 2H), 7.43-7.46 (m,2H), 7.16-7.20 (m, 2H), 3.40-3.42 (m,2H), 1.69-1.77 (m,2H), 1.41-1.50 (m,2H), 0.92 (t, J = 7.2Hz, 3H)

(E)-3-(4-Bromophenyl)-2-(butylsulfonyl)-1-phenylprop-2-en-1-one (9d)



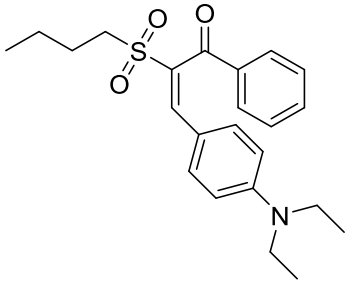
Yield: 28.1%. ^{1}HNMR (400 MHz, DMSO-d_6) δ 7.93 (s, 1H), 7.86 (d, J = 8Hz, 2H), 7.62-7.71 (m,1H), 7.48-7.55 (m, 4H), 7.31 (d, J = 8.4Hz, 2H), 3.42 (t, J = 7.6Hz, 2H), 1.69-1.76 (m, 2H), 1.43-1.48 (m, 2H), 0.92 (t, J = 7.2Hz, 3H)

(E)-3-(4-(Tert-butyl)phenyl)-2-(butylsulfonyl)-1-phenylprop-2-en-1-one (10d)



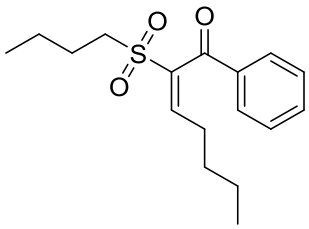
Yield: 25.2%. ^{1}HNMR (400 MHz, DMSO-d_6) δ 7.90-7.92 (m, 2H), 7.84 (s, 1H), 7.66-7.70 (m, 1H), 7.51-7.55 (m, 2H), 7.30-7.37 (m, 4H), 3.36-3.38 (m, 2H), 1.67-1.75 (m, 2H), 1.39-1.49 (m, 2H), 1.16-1.20 (m, 2H), 0.90 (t, J = 7.2Hz, 3H)

(E)-2-(Butylsulfonyl)-3-(4-(diethylamino)phenyl)-1-phenylprop-2-en-1-one (11d)



Yield: 34.3%. ^{1}HNMR (400 MHz, DMSO-d_6) δ 7.90-7.92 (m, 2H), 7.65-7.69 (m, 2H), 7.51-7.54 (m, 2H), 7.13 (d, J=8.8Hz, 2H), 6.53 (d, J = 9.2Hz, 2H), 3.26-3.31 (m, 6H), 1.64-1.72 (m, 2H), 1.38-1.48 (m, 2H), 1.00-1.03 (m, 6H), 0.90 (t, J = 7.2Hz, 3H)

(E)-2-(Butylsulfonyl)-1-phenylhept-2-en-1-one (12d)



Yield: 5.4%. ^{1}HNMR (400 MHz, DMSO-d_6) δ 7.91 (d, J = 7.6Hz, 2H), 7.77 (dd, J = 7.2Hz, 1H), 7.63 (dd, J = 7.6Hz, 2H), 7.04 (t, J = 8Hz, 1H), 3.25-3.31 (m, 2H), 1.97-2.03 (m, 2H), 1.61-1.69 (m, 2H), 1.34-1.48 (m, 4H), 1.06-1.20 (m, 2H), 0.89-0.93 (m, 3H), 0.73 (t, J = 7.2Hz, 3H)

APPENDIX. ABBREVIATIONS

2-AG	2-Arachidonoylglycerol
2-AGE	2-Arachidonyl glyceryl ether
AC	Adenylyl cyclase
ACEA	Arachidonyl-2'-chloroethylamide
ACPA	Arachidonylcyclopropylamide
AEA	Arachidonoylethanolamine
cAMP	Cyclic adenosine monophosphate
cLogP	Calculated logarithm of octanol/water partition coefficient
CB	Cannabinoid
CB_1	Cannabinoid receptor 1
CB_2	Cannabinoid receptor 2
CHO cells	Chinese Hamster Ovary cells
COMFA	Comparative molecular field analysis
DMSO	Dimethyl sulfoxide
ED_{50}	Median effective dose
EL	Extracellular loops
ERK	Extracellular regulated protein kinases
EtOH	Ethanol
FDA	Food and drug administration
G protein	Guanine nucleotide-binding proteins
GDP	Guanosine diphosphate
GPCRs	G-Protein coupled receptors
GTP	Guanosine triphosphate
HT-docking	High-Throughput docking
ICL	Intracellular loops

IL	Interleukin
LC-MS	Liquid chromatography–mass spectrometry
MAPK	Mitogen-activated protein kinase
MAPKK	Mitogen-activated protein kinase kinase
MET	Methanandamide
MHz	Megahertz
MM	Multiple myeloma
MW	Molecular Weight
NA	Noradrenaline
NADA	*N*-arachidonoyl dopamine
nM	Nanomolar
NMR	Nuclear magnetic resonance
OP	Osteoporosis
PEA	*N*-palmitoylethanolamine
PKA	Protein Kinase A
QSAR	Quantitative structure activity relationship
RANKL	Receptor activator of nuclear factor kappa-B ligand
SAR	Structure activity relationship
THC	Δ9-Tetrahydrocannabinol
TLC	Thin-layer chromatography
TM	Transmembrane domains
tPSA	Topological polar surface area
UV	Ultra-violet
VOCCs	Voltage operated calcium channels

BIBLIOGRAPHY

1. Mechoulam, R. and Y. Gaoni, *The absolute configuration of delta-1-tetrahydrocannabinol, the major active constituent of hashish.* Tetrahedron Lett, 1967. **12**: p. 1109-11.
2. Devane, W.A., et al., *Determination and characterization of a cannabinoid receptor in rat brain.* Mol Pharmacol, 1988. **34**(5): p. 605-13.
3. Grotenhermen, F., *Cannabinoids.* Curr Drug Targets CNS Neurol Disord, 2005. **4**(5): p. 507-30.
4. Devane, W.A., et al., *Isolation and Structure of a Brain Constituent That Binds to the Cannabinoid Receptor.* Science, 1992. **258**(5090): p. 1946-1949.
5. Mechoulam, R., et al., *Identification of an Endogenous 2-Monoglyceride, Present in Canine Gut, That Binds to Cannabinoid Receptors.* Biochemical Pharmacology, 1995. **50**(1): p. 83-90.
6. van der Stelt, M. and V. Di Marzo, *The endocannabinoid system in the basal ganglia and in the mesolimbic reward system: implications for neurological and psychiatric disorders.* Eur J Pharmacol, 2003. **480**(1-3): p. 133-50.
7. *Hampson, Robert E., and Sam A. Deadwyler. "Cannabinoids, hippocampal function and memory." Life sciences 65.6 (1999): 715-723.*
8. Iversen, L. and V. Chapman, *Cannabinoids: a real prospect for pain relief?* Curr Opin Pharmacol, 2002. **2**(1): p. 50-5.
9. Randall, M.D., et al., *Cardiovascular effects of cannabinoids.* Pharmacol Ther, 2002. **95**(2): p. 191-202.
10. Maccarrone, M. and T. Wenger, *Effects of cannabinoids on hypothalamic and reproductive function.* Handb Exp Pharmacol, 2005(168): p. 555-71.
11. Di Carlo, G. and A.A. Izzo, *Cannabinoids for gastrointestinal diseases: potential therapeutic applications.* Expert Opin Investig Drugs, 2003. **12**(1): p. 39-49.
12. Attwood, T.K. and J.B. Findlay, *Fingerprinting G-protein-coupled receptors.* Protein Eng, 1994. **7**(2): p. 195-203.
13. Foord, S.M., et al., *International Union of Pharmacology. XLVI. G protein-coupled receptor list.* Pharmacol Rev, 2005. **57**(2): p. 279-88.
14. Neumann, E., K. Khawaja, and U. Muller-Ladner, *G protein-coupled receptors in rheumatology.* Nat Rev Rheumatol, 2014. **10**(7): p. 429-36.
15. *Filmore, David. "It's a GPCR world." Modern drug discovery 7.11 (2004): 24-28.*
16. Overington, J.P., B. Al-Lazikani, and A.L. Hopkins, *Opinion - How many drug targets are there?* Nature Reviews Drug Discovery, 2006. **5**(12): p. 993-996.
17. Munro, S., K.L. Thomas, and M. Abu-Shaar, *Molecular characterization of a peripheral receptor for cannabinoids.* Nature, 1993. **365**(6441): p. 61-5.

18. Matsuda, L.A., et al., *Structure of a cannabinoid receptor and functional expression of the cloned cDNA.* Nature, 1990. **346**(6284): p. 561-4.
19. Gerard, C.M., et al., *Molecular cloning of a human cannabinoid receptor which is also expressed in testis.* Biochem J, 1991. **279 (Pt 1)**: p. 129-34.
20. Onaivi, E.S., et al., *Discovery of the presence and functional expression of cannabinoid CB2 receptors in brain.* Ann N Y Acad Sci, 2006. **1074**: p. 514-36.
21. Demuth, D.G. and A. Molleman, *Cannabinoid signalling.* Life Sci, 2006. **78**(6): p. 549-63.
22. Begg, M., et al., *Evidence for novel cannabinoid receptors.* Pharmacol Ther, 2005. **106**(2): p. 133-45.
23. Sachs, J., E. McGlade, and D. Yurgelun-Todd, *Safety and Toxicology of Cannabinoids.* Neurotherapeutics, 2015.
24. Rosenberg, E.C., et al., *Cannabinoids and Epilepsy.* Neurotherapeutics, 2015.
25. Yang, P., et al., *Lead discovery, chemistry optimization, and biological evaluation studies of novel biamide derivatives as CB2 receptor inverse agonists and osteoclast inhibitors.* J Med Chem, 2012. **55**(22): p. 9973-87.
26. Galiegue, S., et al., *Expression of central and peripheral cannabinoid receptors in human immune tissues and leukocyte subpopulations.* Eur J Biochem, 1995. **232**(1): p. 54-61.
27. Neves, S.R., P.T. Ram, and R. Iyengar, *G protein pathways.* Science, 2002. **296**(5573): p. 1636-1639.
28. Wettschureck, N. and S. Offermanns, *Mammalian G proteins and their cell type specific functions.* Physiological Reviews, 2005. **85**(4): p. 1159-1204.
29. Meinkoth, J.L., et al., *Signal-Transduction through the Camp-Dependent Protein-Kinase.* Molecular and Cellular Biochemistry, 1993. **128**: p. 179-186.
30. Walsh, D.A. and S.M. Vanpatten, *Multiple Pathway Signal-Transduction by the Camp-Dependent Protein-Kinase.* Faseb Journal, 1994. **8**(15): p. 1227-1236.
31. Kaupp, U.B. and R. Seifert, *Cyclic nucleotide-gated ion channels.* Physiological Reviews, 2002. **82**(3): p. 769-824.
32. Childers, S.R. and S.A. Deadwyler, *Role of cyclic AMP in the actions of cannabinoid receptors.* Biochemical Pharmacology, 1996. **52**(6): p. 819-827.
33. Childers, S.R., T. Sexton, and M.B. Roy, *Effects of Anandamide on Cannabinoid Receptors in Rat-Brain Membranes.* Biochemical Pharmacology, 1994. **47**(4): p. 711-715.
34. Childers, S.R., et al., *Opioid and cannabinoid receptor inhibition of adenylyl cyclase in brain.* Ann N Y Acad Sci, 1992. **654**: p. 33-51.
35. Barth, F., *CB1 cannabinoid receptor antagonists.* Annual Reports in Medicinal Chemistry, Vol 40, 2005. **40**: p. 103-118.
36. Pearson, G., et al., *Mitogen-activated protein (MAP) kinase pathways: regulation and physiological functions.* Endocr Rev, 2001. **22**(2): p. 153-83.
37. Bouaboula, M., et al., *Activation of mitogen-activated protein kinases by stimulation of the central cannabinoid receptor CB1.* Biochem J, 1995. **312 (Pt 2)**: p. 637-41.
38. Bouaboula, M., et al., *Signaling pathway associated with stimulation of CB2 peripheral cannabinoid receptor - Involvement of both mitogen-activated protein kinase and induction of Krox-24 expression.* European Journal of Biochemistry, 1996. **237**(3): p. 704-711.

39. Derkinderen, P., et al., *Regulation of extracellular signal-regulated kinase by cannabinoids in hippocampus.* J Neurosci, 2003. **23**(6): p. 2371-82.
40. Davis, M.I., J. Ronesi, and D.M. Lovinger, *A predominant role for inhibition of the adenylate cyclase/protein kinase A pathway in ERK activation by cannabinoid receptor 1 in N1E-115 neuroblastoma cells.* J Biol Chem, 2003. **278**(49): p. 48973-80.
41. Camerino, D.C., D. Tricarico, and J.F. Desaphy, *Ion channel pharmacology.* Neurotherapeutics, 2007. **4**(2): p. 184-98.
42. Caulfield, M.P. and D.A. Brown, *Cannabinoid receptor agonists inhibit Ca current in NG108-15 neuroblastoma cells via a pertussis toxin-sensitive mechanism.* Br J Pharmacol, 1992. **106**(2): p. 231-2.
43. Mackie, K., et al., *Cannabinoids activate an inwardly rectifying potassium conductance and inhibit Q-type calcium currents in AtT20 cells transfected with rat brain cannabinoid receptor.* J Neurosci, 1995. **15**(10): p. 6552-61.
44. Twitchell, W., S. Brown, and K. Mackie, *Cannabinoids inhibit N- and P/Q-type calcium channels in cultured rat hippocampal neurons.* J Neurophysiol, 1997. **78**(1): p. 43-50.
45. Nicholson, R.A., et al., *Sodium channel inhibition by anandamide and synthetic cannabimimetics in brain.* Brain Res, 2003. **978**(1-2): p. 194-204.
46. Maingret, F., et al., *The endocannabinoid anandamide is a direct and selective blocker of the background K+ channel TASK-1.* Embo Journal, 2001. **20**(1-2): p. 47-54.
47. Todaro, B., *Cannabinoids in the treatment of chemotherapy-induced nausea and vomiting.* J Natl Compr Canc Netw, 2012. **10**(4): p. 487-92.
48. Pertwee, R.G., *Emerging strategies for exploiting cannabinoid receptor agonists as medicines.* Br J Pharmacol, 2009. **156**(3): p. 397-411.
49. Vermersch, P., *Sativex((R)) (tetrahydrocannabinol + cannabidiol), an endocannabinoid system modulator: basic features and main clinical data.* Expert Rev Neurother, 2011. **11**(4 Suppl): p. 15-9.
50. Christensen, R., et al., *Efficacy and safety of the weight-loss drug rimonabant: a meta-analysis of randomised trials.* Lancet, 2007. **370**(9600): p. 1706-13.
51. Despres, J.P., I. Lemieux, and N. Almeras, *Contribution of CB1 blockade to the management of high-risk abdominal obesity.* Int J Obes (Lond), 2006. **30 Suppl 1**: p. S44-52.
52. XIE, X., R. Feng, and P. Yang, *Novel cannabinoid receptor 2 (cb2) inverse agonists and therapeutic potential for multiple myeloma and osteoporosis bone diseases*. 2013, Google Patents.
53. Xie, X., J. Chen, and Y. Zhang, *Ligands specific for cannabinoid receptor subtype 2*. 2011, Google Patents.
54. Guindon, J. and A.G. Hohmann, *The endocannabinoid system and cancer: therapeutic implication.* Br J Pharmacol, 2011. **163**(7): p. 1447-63.
55. Pisanti, S., et al., *The endocannabinoid signaling system in cancer.* Trends Pharmacol Sci, 2013. **34**(5): p. 273-82.
56. Qamri, Z., et al., *Synthetic cannabinoid receptor agonists inhibit tumor growth and metastasis of breast cancer.* Mol Cancer Ther, 2009. **8**(11): p. 3117-29.
57. Caffarel, M.M., et al., *Cannabinoids reduce ErbB2-driven breast cancer progression through Akt inhibition.* Molecular Cancer, 2010. **9**.
58. Sarfaraz, S., et al., *Cannabinoid receptor as a novel target for the treatment of prostate cancer*. Cancer Research, 2005. **65**(5): p. 1635-1641.

59. Nithipatikom, K., et al., *2-arachidonoylglycerol: a novel inhibitor of androgen-independent prostate cancer cell invasion.* Cancer Res, 2004. **64**(24): p. 8826-30.
60. Olea-Herrero, N., et al., *Inhibition of human tumour prostate PC-3 cell growth by cannabinoids R(+)-Methanandamide and JWH-015: involvement of CB2.* Br J Cancer, 2009. **101**(6): p. 940-50.
61. Ruiz, L., A. Miguel, and I. Diaz-Laviada, *Delta9-tetrahydrocannabinol induces apoptosis in human prostate PC-3 cells via a receptor-independent mechanism.* FEBS Lett, 1999. **458**(3): p. 400-4.
62. Velasco, L., et al., *delta(9)-Tetrahydrocannabinol increases nerve growth factor production by prostate PC-3 cells. Involvement of CB1 cannabinoid receptor and Raf-1.* Eur J Biochem, 2001. **268**(3): p. 531-5.
63. Whyte, L.S., et al., *Cannabinoids and bone: endocannabinoids modulate human osteoclast function in vitro.* Br J Pharmacol, 2012. **165**(8): p. 2584-97.
64. Idris, A.I., *The promise and dilemma of cannabinoid therapy: lessons from animal studies of bone disease.* Bonekey Rep, 2012. **1**: p. 224.
65. Rossi, F., et al., *The endovanilloid/endocannabinoid system in human osteoclasts: possible involvement in bone formation and resorption.* Bone, 2009. **44**(3): p. 476-84.
66. Tam, J., et al., *The cannabinoid CB1 receptor regulates bone formation by modulating adrenergic signaling.* FASEB J, 2008. **22**(1): p. 285-94.
67. Bab, I., et al., *Endocannabinoids and the regulation of bone metabolism.* J Neuroendocrinol, 2008. **20 Suppl 1**: p. 69-74.
68. Yang, P., et al., *Novel Triaryl Sulfonamide Derivatives as Selective Cannabinoid Receptor 2 Inverse Agonists and Osteoclast Inhibitors: Discovery, Optimization, and Biological Evaluation.* Journal of Medicinal Chemistry, 2013. **56**(5): p. 2045-2058.
69. Ofek, O., et al., *Peripheral cannabinoid receptor, CB2, regulates bone mass.* Proc Natl Acad Sci U S A, 2006. **103**(3): p. 696-701.
70. Idris, A.I., et al., *Regulation of bone mass, bone loss and osteoclast activity by cannabinoid receptors.* Nat Med, 2005. **11**(7): p. 774-9.
71. Idris, A.I., et al., *Regulation of bone mass, osteoclast function, and ovariectomy-induced bone loss by the type 2 cannabinoid receptor.* Endocrinology, 2008. **149**(11): p. 5619-26.
72. Idris, A.I., et al., *Cannabinoid receptor type 1 protects against age-related osteoporosis by regulating osteoblast and adipocyte differentiation in marrow stromal cells.* Cell Metab, 2009. **10**(2): p. 139-47.
73. Sellmeyer, D.E., *Atypical Fractures as a Potential Complication of Long-term Bisphosphonate Therapy.* Jama-Journal of the American Medical Association, 2010. **304**(13): p. 1480-1484.
74. Bertini, S., et al., *Synthesis, biological activity and molecular modeling of new biphenylic carboxamides as potent and selective CB2 receptor ligands.* Eur J Med Chem, 2015. **90**: p. 526-36.
75. Haruna, T., et al., *S-777469, a novel cannabinoid type 2 receptor agonist, suppresses itch-associated scratching behavior in rodents through inhibition of itch signal transmission.* Pharmacology, 2015. **95**(1-2): p. 95-103.
76. Mukhopadhyay, P., et al., *The novel, orally available and peripherally restricted selective cannabinoid CB2 receptor agonist LEI-101 prevents cisplatin-induced nephrotoxicity.* Br J Pharmacol, 2016. **173**(3): p. 446-58.

77. Presley, C., et al., *Preclinical evaluation of SMM-189, a cannabinoid receptor 2-specific inverse agonist.* Pharmacol Res Perspect, 2015. **3**(4): p. e00159.
78. Schindler, C.W., et al., *Blockade of Nicotine and Cannabinoid Reinforcement and Relapse by a Cannabinoid CB1-Receptor Neutral Antagonist AM4113 and Inverse Agonist Rimonabant in Squirrel Monkeys.* Neuropsychopharmacology, 2016.
79. Mahmoud, M.M., et al., *(4-(Bis(4-fluorophenyl)methyl)piperazin-1-yl)(cyclohexyl)methanone hydrochloride (LDK1229): a new cannabinoid CB1 receptor inverse agonist from the class of benzhydryl piperazine analogs.* Mol Pharmacol, 2015. **87**(2): p. 197-206.
80. Keenan, C.M., et al., *AM841, a covalent cannabinoid ligand, powerfully slows gastrointestinal motility in normal and stressed mice in a peripherally restricted manner.* Br J Pharmacol, 2015. **172**(9): p. 2406-18.
81. Hsiao, W.C., et al., *A novel peripheral cannabinoid receptor 1 antagonist, BPR0912, reduces weight independently of food intake and modulates thermogenesis.* Diabetes Obes Metab, 2015. **17**(5): p. 495-504.
82. Chen, J.Z., J. Wang, and X.Q. Xie, *GPCR structure-based virtual screening approach for CB2 antagonist search.* J Chem Inf Model, 2007. **47**(4): p. 1626-37.
83. Feng, Z., et al., *Modeling, molecular dynamics simulation, and mutation validation for structure of cannabinoid receptor 2 based on known crystal structures of GPCRs.* J Chem Inf Model, 2014. **54**(9): p. 2483-99.
84. Leifert, W.R., et al., *Radioligand binding assays: application of [(125)I]angiotensin II receptor binding.* Methods Mol Biol, 2009. **552**: p. 131-41.
85. Zhang, Y., et al., *Mutagenesis and computer modeling studies of a GPCR conserved residue W5.43(194) in ligand recognition and signal transduction for CB2 receptor.* Int Immunopharmacol, 2011. **11**(9): p. 1303-10.
86. Alqarni, M., et al., *Examining the critical roles of human CB2 receptor residues Valine 3.32 (113) and Leucine 5.41 (192) in ligand recognition and downstream signaling activities.* Biochem Biophys Res Commun, 2014. **452**(3): p. 334-9.
87. Yang, P., et al., *Novel triaryl sulfonamide derivatives as selective cannabinoid receptor 2 inverse agonists and osteoclast inhibitors: discovery, optimization, and biological evaluation.* J Med Chem, 2013. **56**(5): p. 2045-58.
88. Swenson, R.E., T.J. Sowin, and H.Q. Zhang, *Synthesis of substituted quinolines using the dianion addition of N-Boc-anilines and alpha-tolylsulfonyl-alpha,beta-unsaturated ketones.* Journal of Organic Chemistry, 2002. **67**(26): p. 9182-9185.
89. Curti, C., et al., *Rapid synthesis of sulfone derivatives as potential anti-infectious agents.* European Journal of Medicinal Chemistry, 2007. **42**(6): p. 880-884.
90. Rinaldi-Carmona, M., et al., *SR141716A, a potent and selective antagonist of the brain cannabinoid receptor.* FEBS Lett, 1994. **350**(2-3): p. 240-4.
91. Komm, B.S. and A.A. Chines, *An update on selective estrogen receptor modulators for the prevention and treatment of osteoporosis.* Maturitas, 2012. **71**(3): p. 221-6.
92. Voskaridou, E. and E. Terpos, *Osteoprotegerin to soluble receptor activator of nuclear factor kappa-B ligand ratio is reduced in patients with thalassaemia-related osteoporosis who receive vitamin D3.* Eur J Haematol, 2005. **74**(4): p. 359-61.
93. Seggewiss-Bernhardt, R., et al., *Phase 1/1B trial of the heat shock protein 90 inhibitor NVP-AUY922 as monotherapy or in combination with bortezomib in patients with relapsed or refractory multiple myeloma.* Cancer, 2015. **121**(13): p. 2185-92.

94. Hay, A.E., et al., *A phase II study of AT9283, an aurora kinase inhibitor, in patients with relapsed or refractory multiple myeloma: NCIC clinical trials group IND.191*. Leuk Lymphoma, 2015: p. 1-4.