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Selective modulation of the cannabinoid type 1 (CB₁) receptor as an emerging platform for the treatment of neuropathic pain

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Introduction

Neuropathic pain is a complex, chronic pain state caused directly by a lesion or disease affecting the somatosensory nervous system.^{1, 2} Neuropathic pain may be spontaneous, such as a painful response to a non-painful stimulus (allodynia), or evoked as an exaggerated response to a painful stimulus (hyperalgesia).³ Diagnosis of neuropathic pain requires a history of injury to the nervous system (the peripheral nerve, dorsal root or dorsal root ganglion, or central nervous system) and is a common consequence of stroke, surgical or chemotherapeutic nerve trauma, and diabetic neuropathy.⁴

Neuropathic pain is estimated to affect one in every ten adults over the age of 30 in the United States,⁵ significantly impacts quality of life,⁶ is associated with a three-fold increase in direct healthcare costs,⁷ and contributes significantly to the \$100 billion annual indirect costs attributed to chronic pain conditions due to absenteeism and decreased productivity.^{8, 9}

The current first-line treatments for neuropathic pain are tricyclic antidepressants (nortriptyline, desipramine)¹⁰ and anticonvulsants (gabapentin, pregabalin);¹¹⁻¹³ however, many patients report incomplete relief as well as dose-limiting adverse effects of these drugs.¹⁴⁻¹⁶ Opioid drugs are a second-line treatment for neuropathic pain since they are ineffective in many patients and chronic opioid use is associated with adverse reactions, tolerance, and addiction.^{17, 18} The current mini-review aims to provide an overview of the chemotypes currently under investigation for the development of novel neuropathic pain treatments targeting CB₁ receptors.

The endocannabinoid system and neuropathic pain models

The endocannabinoid (eCB) system is ubiquitously expressed throughout the body and is responsible for the homeostatic control of many basic physiological processes. Modulation of the endocannabinoid system has been proposed as a promising platform for the treatment of nociceptive pain for over a decade.¹⁹⁻²² The eCB system is comprised of two well-characterized G protein-coupled receptors (GPCRs), the cannabinoid type 1 and type 2 receptors (CB₁ and CB₂

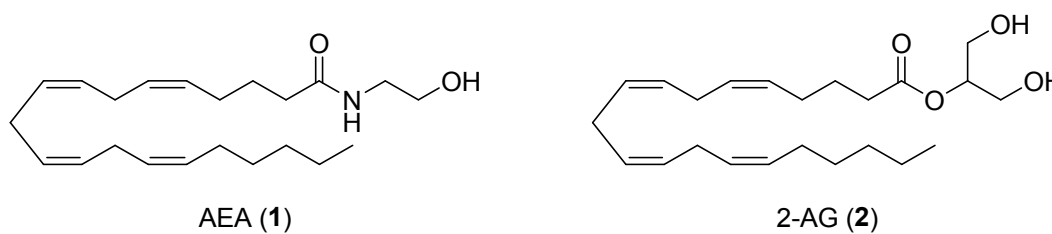


Figure 1. Selected endogenous cannabinoids.

respectively), endogenous signaling lipids such as anandamide (arachidonyl ethanolamide, AEA, **1**, Fig. 1)²³ and 2-arachidonoylglycerol (2-AG, **2**),²⁴ and associated metabolic enzymes like fatty acid amide hydrolase (FAAH)²⁵ and monoacylglycerol lipase (MAGL)²⁶. Both FAAH and MAGL are key enzymes in the hydrolysis of the endocannabinoid 2-arachidonoylglycerol (2-AG). Due to their ability to regulate nociception, they are currently viewed as attractive drug targets for the treatment of pain. Activation of CB₁ and CB₂ receptors is known to reduce nociceptive signaling, and nociceptive processing is typically controlled by endocannabinoids, like AEA and 2-AG.²⁷

Anandamide is believed to serve as natural pain modulator²⁸ as several pertinent anandamide targets within the eCB system have been explored for pain treatment.²⁹ Recent evidence from rodent models indicates that novel approaches targeting the eCB system might be beneficial in refractory neuropathic pain. However, consensus on whether single or multitarget modulators of the cannabinoid system represent the most effective therapeutic platforms for the treatment of neuropathic pain has not been reached.^{30,31}

Translational efforts to date have focused on agonists of CB₁ and CB₂, as well as inhibitors of endocannabinoid metabolism.³² Ligands that activate central CB₁ receptors via its orthosteric site produce psychoactive effects, limiting the broad utility of such drugs. Selective CB₂ agonists have demonstrated efficacy in preclinical models of neuropathic pain, but despite extensive efforts by the pharmaceutical industry, no CB₂ agonist has advanced to market.³³ Selective inhibitors of FAAH and MAGL, as well as dual inhibitors, have also shown promise in preclinical models, but have largely failed to meet end-points in clinical trials.³⁴

Many rodent models of neuropathic pain were developed to evaluate the activity of new drug candidates wherein the efficacy of the eCB system's modulators were noted.³⁵ All rodent models of neuropathic pain involve surgical manipulation of the peripheral nervous system to induce injury and inflammatory response. Although countless other models are available³⁶, the most commonly employed model for persistent neuropathic pain in rodents is partial sciatic nerve injury,³⁷⁻³⁹ which is caused by partial ligation of the sciatic nerve,⁴⁰ chronic constriction injury (CCI),⁴¹ or L5 and L6 spinal nerve ligation (SNL).⁴² Rodent models of neuropathic pain aim to reproduce characteristics of human neuropathic pain, including mechanical allodynia and thermal hyperalgesia, and it is typically these features of pain that are assessed.⁴³

The CB₁ receptor and pain

The cannabinoid 1 (CB₁) receptor is one of the most abundant G protein-coupled receptors (GPCRs) in the central nervous system, with key roles during neurotransmitter release and synaptic plasticity.^{44,46} The CB₁ has been cloned and encodes 473 amino acid protein.⁴⁷ Like other Class A G protein-coupled receptors (GPCRs), the CB₁R possesses six helical transmembrane domains, an extracellular N-terminus and an intracellular C-terminus. Upon ligand activation, CB₁Rs may signal in three different spatiotemporal waves. The first wave, which is transient (<10 minutes) and initiated by heterotrimeric G proteins, is followed by a second wave (>5 minutes) that is mediated by β -arrestins.⁴⁶⁻⁴⁸ The third and final wave occurs at intracellular compartments and could be elicited by G proteins or β -arrestins. This complexity presents multiple challenges, including the correct classification of receptor ligands, the identification of the signaling pathways regulated by each wave, and the underlying molecular mechanisms and physiologic impacts of these waves. It has been shown that upon small molecule agonist-induced activation, β -arrestins are recruited to the plasma membrane to initiate clathrin-mediated endocytosis (CME) and signaling. Compounds that activate CB₁ were suggested as possible treatments for a wide range of medical disorders including pain, inflammation, glaucoma, nausea and emesis, neurodegenerative disorders, anxiety, and hypertension.

Like many GPCRs, the CB₁ receptor is capable of ligand- and heterodimer-directed functional selectivity.⁴⁹ The CB₂ receptor is also known to possess diverse, ligand-directed signaling bias.⁵⁰ There is also the physical and functional interplay between CB₁ receptors and other CNS targets of relevance to pain, including; D₂ dopamine receptors, mu-, kappa-, and delta-opioid receptors (MORs, KORs, and DORs, respectively), the orexin-1 receptor, and the A_{2A} adenosine receptor. It has been seen that following peripheral nerve lesion in rats, there is upregulation of a CB₁-DOR heteromeric complex in the cortex of neuropathic animals, with concomitant changes in the activity of each protein.⁵¹

The development of CB₁ knock-out (KO) mice has provided insights regarding the role of CB₁ receptors in nociception. The CB₁ KO mice are hypoalgesic⁵² and demonstrate a reduced analgesic response to cannabinoids,⁵³ however, CB₁ KO mice still develop neuropathic pain,^{54,55} consistent with the complex pathophysiology of neuropathic pain. Spinal and supraspinal CB₁ receptors are upregulated following nerve injury, and this may account for the efficacy of CB₁ agonists in neuropathic pain.⁵⁶ Indeed, CB₁ receptors are upregulated in many rat models of neuropathic pain.⁵⁷⁻⁵⁹

Phytocannabinoids targeting CB₁

The therapeutic activity of exogenous phytocannabinoids found in the cannabis plant supports the potential of the CB₁ receptor as a relevant platform for the development of novel analgesics.⁶⁰ The earliest indication that modulation of the CB₁ receptor might be effective in the treatment of pain comes from the historical use of the cannabis plant. The recorded medicinal use of the cannabis plant (*Cannabis sativa* L.) for pain and other conditions, dates back to approximately 2,700 BCE.⁶¹ The total number of natural compounds identified in cannabis exceeds 480, of which the most important pharmacologically active members are the phenolic terpenoids collectively known as phytocannabinoids.^{62, 63} The most abundant phytocannabinoids are Δ^9 -tetrahydrocannabinol (Δ^9 -THC, **3**, Figure 2)⁶⁴ and cannabidiol (CBD, **4**), both found in cannabis as their acid derivatives, each of which have found application in the treatment of neuropathic pain.

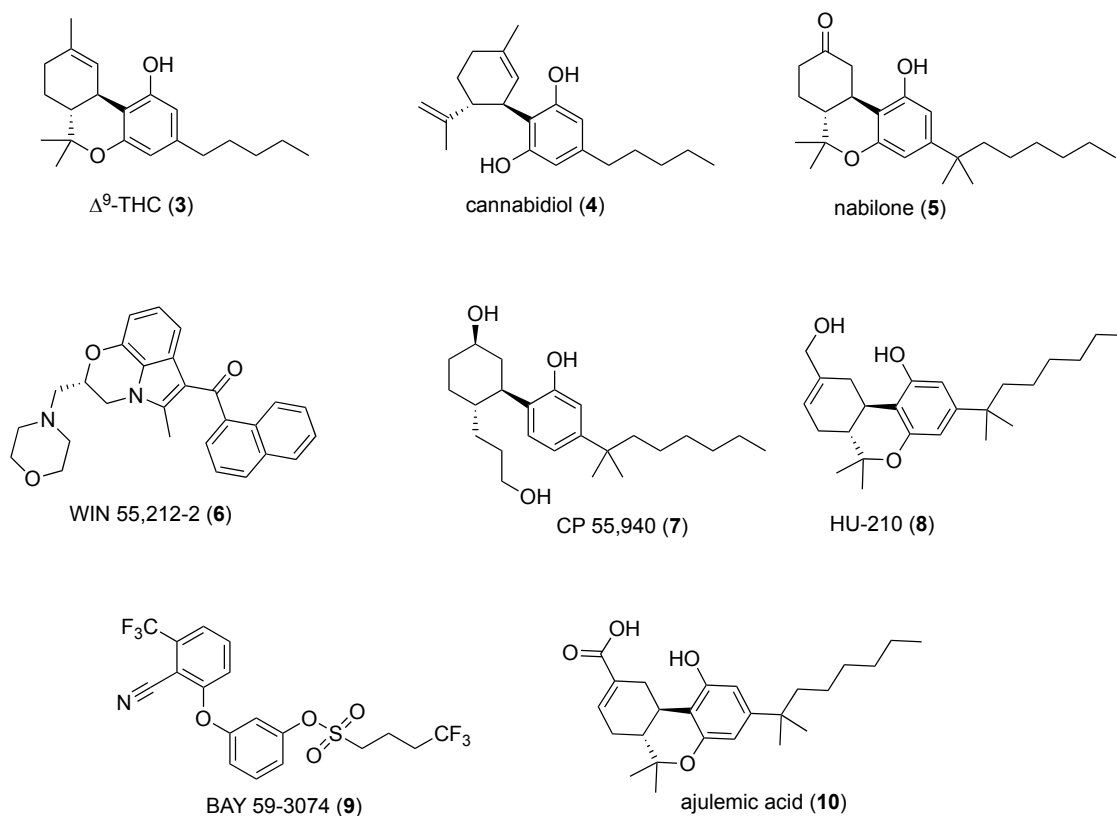


Figure 2. Selected phytocannabinoids and analogs.

Phytocannabinoids interact non-selectively with many targets of the human endocannabinoid system beyond CB₁ receptors. Many also interact with non-cannabinoid targets, some of which are relevant to neuropathic pain.⁶⁵

The efficacy of medical cannabis itself in numerous pain conditions has been established and reviewed elsewhere.^{66,67} The utility of phytocannabinoids as lead structures for the development of novel analgesics targeting CB₁ is confounded by their complex pharmacology.⁶⁸ For example, Δ⁹-THC acts as a potent, low efficacy agonist of CB₁ and CB₂ receptors, while CBD is a negative allosteric modulator (NAM) of CB₁,⁶⁹ yet both compounds produce analgesic effects in rodent models. It is believed that non-cannabinoid targets like spinal α₃ glycine receptors mediate the analgesic properties of CBD.^{70,71}

Despite the therapeutic potential of phytocannabinoid-derived drugs, surprisingly few phytocannabinoids or phytocannabinoid analogues have reached the clinic. For example, oral Δ⁹-THC, known generically as dronabinol (Marinol®, Unimed), was approved by the FDA for

nausea associated with chemotherapy in 1985. Nabiximols (Sativex®, GW Pharmaceuticals), first passed in the United Kingdom in 2010, is an oral spray formulation of Δ^9 -THC and CBD and is now available in numerous countries. Nabiximols is currently undergoing Phase III trials in the US for the treatment of cancer pain. A synthetic analogue of Δ^9 -THC, nabilone (**5**), was approved by the FDA in 1985 and is sold as Cesamet® (Valeant Pharmaceuticals) for the treatment of refractory chemotherapy-induced nausea and vomiting (CINV).

Synthetic cannabinoids targeting CB₁

Beyond phytocannabinoids, many synthetic cannabinoids were explored as potential analgesics in the 1980s, and several pharmaceutical companies had active cannabinoid analgesic research programs. These cannabinoid drug development programs produced some of the earliest CB₁/CB₂ and CB₁-selective agonists still in use as pharmacological tool molecules, including WIN 55,212-2 (**6**, Fig. 2) from Sterling Winthrop and CP 55,940 (**7**) from Pfizer. Mechoulam and colleagues at Hebrew University also produced phytocannabinoid-inspired ligands intended to probe cannabinoid function, such as HU-210 (**8**).⁷²

Like Δ^9 -THC, these cannabinoids are typically orthosteric CB₁ agonists, albeit more potent and efficacious than Δ^9 -THC. For example, in the chronic constriction injury (CCI) model of neuropathic pain in rats, mechanical allodynia and thermal hyperalgesia were strongly attenuated by Δ^9 -THC at doses of 3 mg/kg and 6 mg/kg (Per os p.o.) respectively, whereas CP 55,940 produced similar effects at 0.05 mg/kg and 0.025 mg/kg (Intraperitoneal i.p. injection) respectively.⁷³ Both cannabinoids were more potent than traditional neuropathic pain treatments such as morphine (8 mg/kg and 16 mg/kg p.o. respectively) and gabapentin (50 mg/kg i.p. in both tests). CP 55,940 also attenuated tactile allodynia in the rat spinal nerve ligation (SNL) model of neuropathic pain, an effect that was reversed by a selective CB₁ antagonist.⁷⁴ The antinociceptive and motor effects of CP 55,940 observed in wild-type mice are absent in CB₁ but not CB₂ KO mice, indicating that CP 55,940 exerts its analgesic properties *via* orthosteric activation of CB₁ receptors.⁷⁵ CP 55,940, WIN 55,212-2 and HU-210 all produced complete reversal of mechanical hyperalgesia in the partial sciatic nerve ligation model of neuropathic pain.^{56, 76-81}

The psychoactivity and motor impairment of Δ^9 -THC and synthetic CB₁ orthosteric agonists limits their broad application as an analgesic agent; therefore, CB₁ agonists without central activity have been suggested as therapeutics.⁸² Several strategies for selective modulation of CB₁ have been proposed, with a focus on reducing adverse reactions and psychotropic activity.^{83,84} In this regards, the peripherally restricted CB₁ agonists retain antinociceptive effects without apparent psychoactivity and are currently under development.⁸⁵ Another alternative is the development of CB₁ positive allosteric modulators (PAMs) or functionally selective CB₁ ligands that induce biased signaling.⁸⁶

Peripherally-restricted CB₁ agonists

The use of centrally active CB₁ agonists for neuropathic pain treatment is limited by centrally mediated side effects. The same is true for low efficacy agonists such as Δ^9 -THC and synthetic partial agonists with a similar pharmacological profile, like BAY 59-3074 (**9**).⁸⁷ However, there is pharmacological evidence that both central and peripheral CB₁ receptors mediate CB₁ antihyperalgesia.^{76, 88}

Ajulemic acid (CT-3, **10**), a synthetic analogue of a Δ^9 -THC, is efficacious in neuropathic pain in rodents and humans, and showed activity in a Phase II trial.^{89, 90} Curiously, ajulemic acid is an agonist at both CB₁ and CB₂ receptors,⁹¹ yet it does not produce psychotropic effects.^{89,90} The action mechanisms of ajulemic acid in neuropathic pain are not entirely understood,⁹² but its negligible adverse effect profile is believed to be due to reduced CNS penetration.^{91, 93,94} Other potential peripherally restricted CB₁ antagonists are under development for metabolic disorders and provide proof-of-principle for the development of CB₁ agonists with reduced CNS liabilities.^{95,96} Although ajulemic acid has shown promise in clinical trials, phytocannabinoids are not ideal drug leads for peripherally-restricted CB₁ agonists owing, to their high lipophilicity and limited scope of structural modification.

The aminoalkylindole (AAI) class of CB₁ agonists, typified by drugs like WIN 55,212-2, has served as a useful scaffold for the development of cannabinoid agonists with an array of physicochemical and functional properties. WIN 53,365 (**11**, Fig. 3) was selected as the lead for a generation of peripheral CB₁ agonists by Novartis.⁹⁷ The indole core was substituted with

naphthalene based on previous bioequivalency data,⁹⁸ and a 1,4-disubstitution pattern was selected to maintain optimal spatial orientation of key functional groups (**12**). Investigation of numerous pendant ether substituents was based on prior structure-activity relationships reported for other AAI's,^{99, 100} with a pentyl ether found conferring greater hCB₁ binding (CRA13, **13**, IC₅₀ = 15 nM) than a butyl (**14**, IC₅₀ = 48 nM) or hexyl (**15**, IC₅₀ = 160 nM) ether.

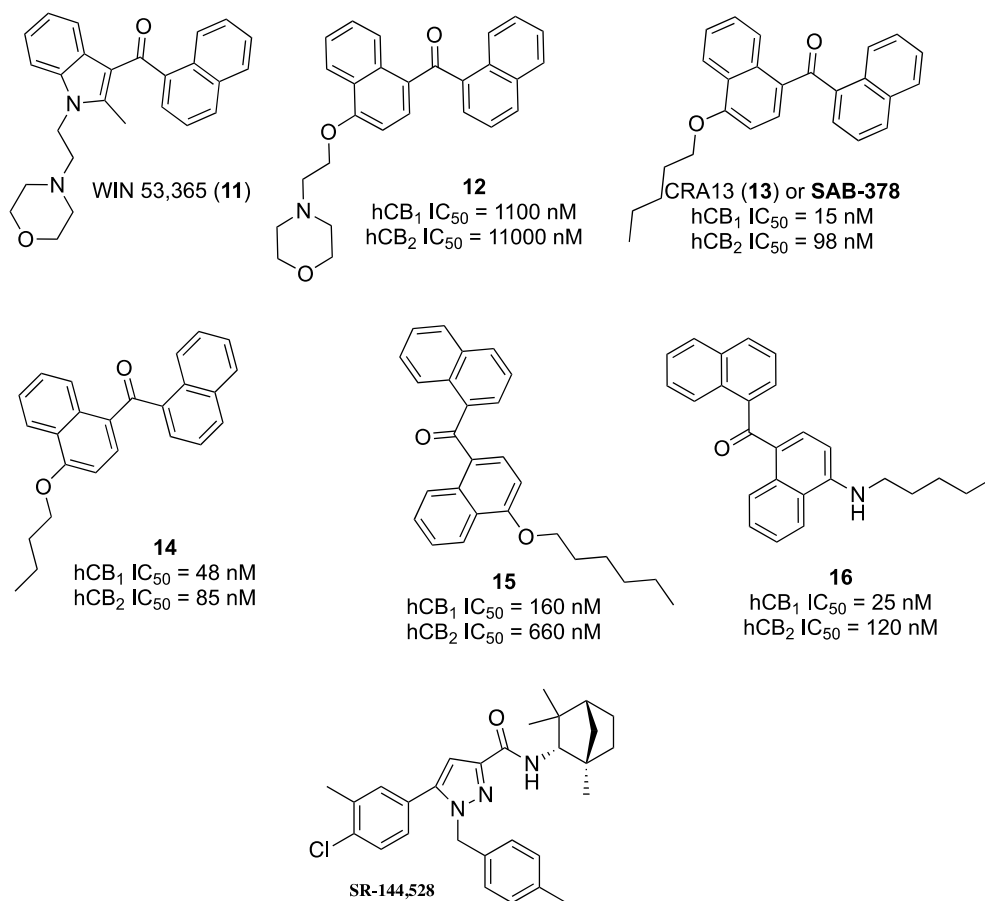


Figure 3. Structures and activities of selected ligands reported during the discovery of CRA13.

CRA13 (also known as SAB-378), i.e., naphthalen-1-yl-(4-pentyloxynaphthalen-1-yl) methanone, was advanced as a development candidate and found to possess potent, orally bioavailable CB₁/CB₂ agonist activity with restricted CNS penetration.¹⁰¹ CRA13 produced up to 90% hyperalgesia reversal when administered orally at 3 mg/kg in a rat neuropathic model of mechanical hyperalgesia with rapid onset, long duration of action, and without apparent CNS-mediated side effects. The antihyperalgesic effects of CRA13 were inhibited by a CB₁-selective inverse agonist/antagonist rimonabant, but not by CB₂-selective antagonist SR 144528 (need

structure). In rats, CRA13 only produced central side effects at doses 170-fold higher than those required for antihyperalgesic activity. The human pharmacokinetic data for CRA13 was reported, and adverse effects were observed at high doses similar to those reported for Δ^9 -THC, which are likely due to CNS penetration at high plasma concentrations.¹⁰²

AstraZeneca has explored many peripherally-restricted CB₁ agonists for their efficacy in rodent models of neuropathic pain, resulting in clinical candidates AZ11713908 (**17**, Fig. 4)¹⁰³ and AZD1940 (**18**),¹⁰⁴ arising from the extensive exploration of 5-sulfonamide benzimidazole scaffold.

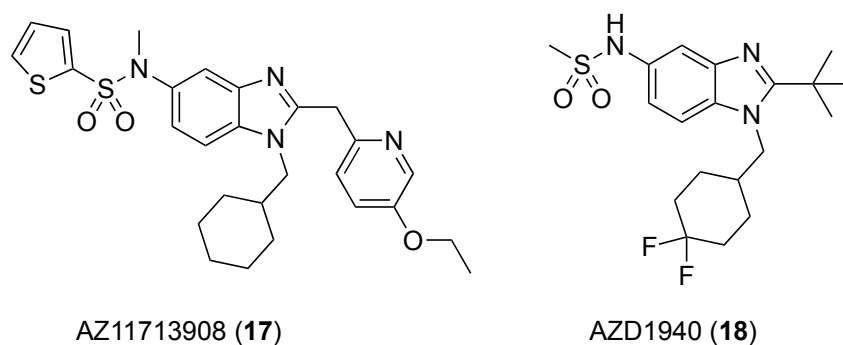


Figure 4. Peripherally-restricted CB₁ agonists under development by AstraZeneca.

AZ11713908 possessed high affinity for CB₁ and CB₂ receptors derived from mouse, rat, and human sources (hCB₁ pIC₅₀ 8.4, hCB₂ pIC₅₀ 9.0), but little selectivity between subtypes.¹⁰³ This drug showed potent, efficacious agonist activity at hCB₁ in the GTP γ S assay (pEC₅₀ 8.0, E_{max} = 115%) compared to WIN 55,212. It was noted that compared to WIN 55,212, which showed 2.2- and 4.2-fold increase in the brain compared to plasma in rat and mouse respectively, AZ11713908 showed just 7% and 5% brain uptake compared to plasma in the same species. In the rat spinal nerve ligation (SNL) model of neuropathic pain, systemic administration of 2.5 μ mol/kg of AZ11713908 reduced mechanical allodynia with 100% efficacy, whereas 8 μ mol/kg of WIN 55,212 was required to achieve the same effect.

AZD1940 is a high affinity hCB₁/hCB₂ agonist with pK_i values of 7.93 and 9.06 at each cannabinoid receptor respectively.¹⁰⁴ AZD1940 has a brain-plasma partition coefficient of 0.04 in rats, comparable to that of AZ11713908. Low brain uptake was confirmed using positron emission tomography (PET) imaging of the carbon-11-labeled isotopologue ([¹¹C] AZD1940).¹⁰⁵ Despite its promising preclinical profile, AZD1940 showed limited analgesic efficacy against

capsaicin-induced pain and hyperalgesia in healthy humans and mild-to-moderate CNS and gastrointestinal adverse effects.¹⁰⁶ AZD1940 also failed to reduce post-operative pain after surgical molar removal and produced central cannabinoid side-effects.¹⁰⁷ Although AZD1940 reached Phase II trials, it discontinued in 2009.¹⁰⁷

By scaffold hopping from a benzimidazole class of cannabinoid ligands,¹⁰⁸ researchers at AstraZeneca identified hit **19** (Fig, 5) as the first of a new γ -carboline class of cannabinoids.¹⁰⁹ **19** possessed moderate affinity for both CB receptors (hCB₁ K_i = 143 nM, hCB₂ K_i = 14 nM), and functioned suitably as an agonist (rCB₁ EC₅₀ = 1440 nM, E_{max} = 47%). Additionally, **18** had reasonable aqueous solubility (>3 mg/mL at pH 7.4), unlike many classes of cannabinoids.

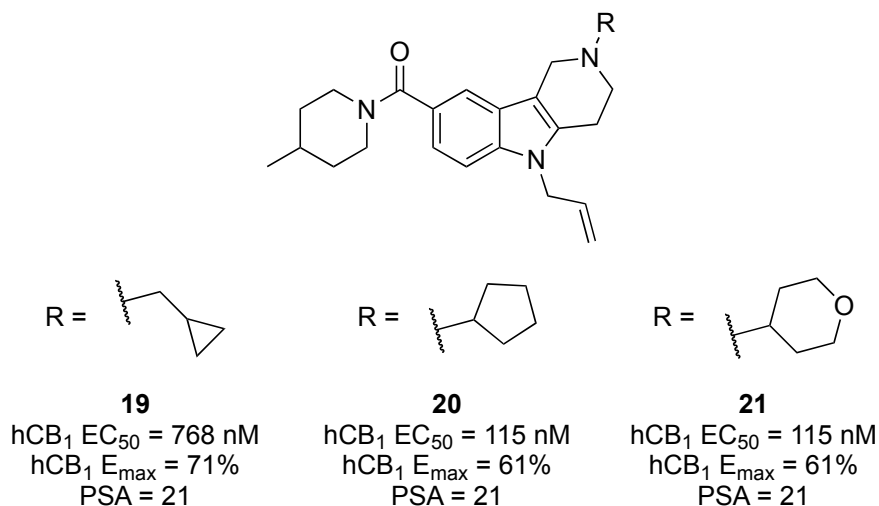


Figure 5. Effect of terminal carboline amine substituent on CB₁ agonist activity and PSA.

Starting from hit **19**, with a moderate brain-to-plasma ratio of 0.74, AstraZeneca aimed to increase polar surface area (PSA) based on the finding that marketed non-CNS drugs have higher PSA values than CNS drugs (mean PSA of 56 and 40, respectively).¹¹⁰ A range of alicyclic, heterocyclic, aromatic, and heteroaromatic groups was explored at the terminal carboline nitrogen, with all leading to increased hCB₁ potency and comparable efficacy, except for various pyridines. The cyclopentyl (**20**, EC₅₀ = 33 nM, E_{max} = 65%) and 4-tetrahydropyranyl (**21**, EC₅₀ = 60 nM, E_{max} = 57%) analogues showed the greatest improvement in potency without

compromising metabolic stability (HLM clearance of 93 and 42 $\mu\text{l}/\text{min}/\text{mg}$ respectively), or decreasing PSA (21 and 30, respectively).

While retaining the cyclopentyl and 4-tetrahydropyranyl groups of **20** and **21**, an array of polar substituents was introduced at the carboline central nitrogen (Fig. 7). All alkyl substituents (methyl, ethyl, and propyl) were tolerated with minimal effect of CB_1 agonist potency, efficacy, or PSA. Compared to propyl analogue **22** ($\text{EC}_{50} = 103 \text{ nM}$, $E_{\text{max}} = 72\%$, PSA 30), introduction of a carbonyl spacer as in **23** increased PSA to 48 with little effect on potency ($\text{EC}_{50} = 117 \text{ nM}$), but efficacy was dramatically reduced ($E_{\text{max}} = 17\%$). PSA was further increased by replacing the carbonyl spacer with a sulfone (**24**, PSA 67), with little impact on potency and reinstatement of efficacy ($E_{\text{max}} = 100\%$). Truncation of the propyl group of **24** to an ethyl group (**25**; $\text{EC}_{50} = 49 \text{ nM}$, $E_{\text{max}} = 120\%$, PSA 67) furnished a potent CB_1 agonist with suitably high PSA, whereas further truncation to a methylsulfone reduced potency by 10-fold (**26**; $\text{EC}_{50} = 473 \text{ nM}$). Attempts to further increase PSA by an introduction of a sulfonamide (e.g., **27**; PSA 83) completely abolished the activity.

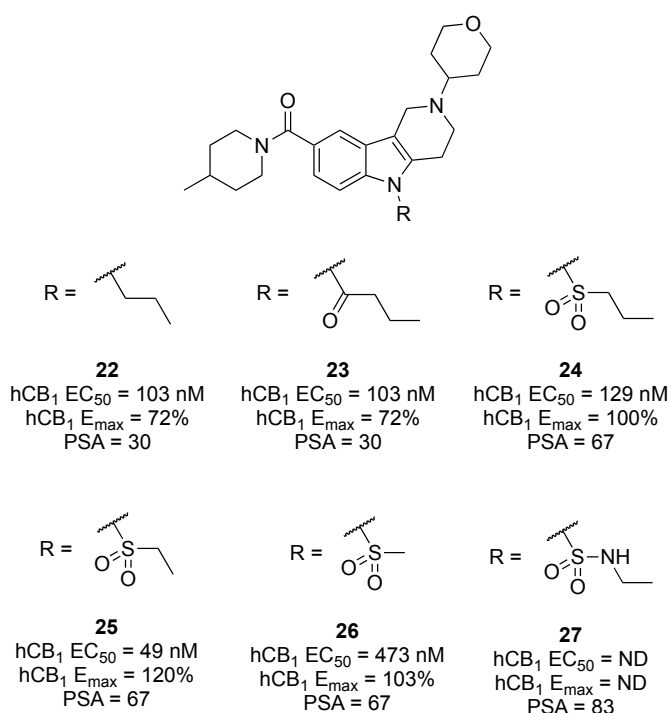


Figure 6. Effect of central carboline amine substituent on CB_1 agonist activity and PSA.

Compound **25** was selected as a potent, efficacious agonist at hCB₁ (EC₅₀ = 49 nM, E_{max} = 120%) and rCB1 (EC₅₀ = 85 nM, E_{max} = 156%), with no appreciable activity at more than 50 other CNS targets, including pain targets. The brain-plasma partition coefficient for **25** was the same as AZ11713908 (0.07), and it showed a promising pharmacokinetic profile as well as good aqueous solubility. In rats, carboline **25** showed reasonable oral bioavailability (16%), low CYP inhibition, but moderate hERG activity. In the rat Carrageenan inflammatory pain model, **25** showed potent, dose-dependent reversal of thermal hyperalgesia with mild hypoactivity observed only at the highest dose (1.4 mg/kg Subcutaneous, s.c.). No further reports of the translation of **25** have appeared.

Researchers at Merck have also employed the strategy of reducing CNS penetration by increasing PSA.¹¹¹ Using MONIKA, a web tool developed internally with Organon, Merck optimized parameters related to both oral bioavailability and CNS permeability (used as exclusion criteria), to further optimized a previously developed indole cannabinoid with desirable properties (**28**, Fig. 7). Compound **28** hydrochloride was a moderately potent CB₁ agonist (pEC₅₀ = 7.4) with good oral bioavailability in the rat. Previous SAR studies had indicated that both oxadiazole and thiadiazoles were tolerated, but **29** showed inferior potency and PSA compared to lead **30**. Introducing a sulfone in place of the tetrahydropyran ether bridge, as in **29**, increased PSA but reduced potency. Introduction of polar groups as pendant substituents of the oxadiazole ring was found to increase potency and PSA (**31** and **32**).

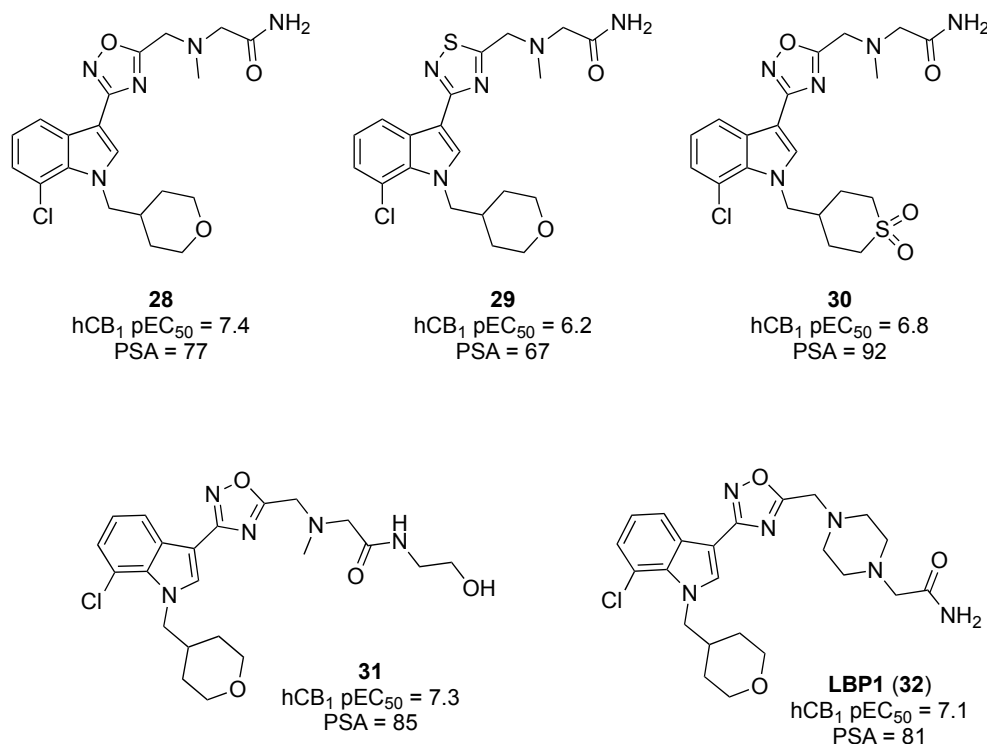


Figure 7. Peripherally-restricted indole-3-heterocycle CB₁ agonists.

Compound **32** is a moderately potent agonist of CB₁ (pK_i 7.7, pEC₅₀ 7.1) with negligible selectivity over CB₂ (pK_i 7.7). LBP1 had suitably low brain-to-plasma ratio in mouse (0.16), and good aqueous solubility (89 mg/L) and oral bioavailability (45%). **32** showed antihyperalgesic effects in the rat Chung Hageaves model and antiallodynic effects in the rat Chung Von Frey model, while CB₁ antagonist rimonabant blocked these effects. As with other centrally penetrant CB₁ agonists, WIN 55,212-2 produced robust catalepsy in rats, whereas **32** induced no effects in the rat catalepsy model up to a maximum oral dose of 160 μ mol/kg. Compound **32** has progressed to Phase I clinical trials, but no further details have been reported.

Inspired by WIN 55,225 (or JWH-200) and to utilize the benefits of CBs to ameliorate neuropathic pain study with a series of 3-alkylated indenes was recently reported, where peripherally restricted cannabinoids were designed and tested for selectivity by an introduction of charge to CB₁. Screening of compounds at various doses and drug administration routes identified 4-{2-[-(1*E*)-1[(4-propylnaphthalen-1-yl)methylidene]-1*H*-inden-3-yl]ethyl}morpholine (PrNMI, **33**) as the most promising compound. (Figure 8).¹¹²

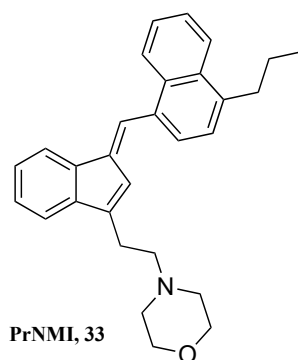


Figure 8. Peripherally restricted CB₁ agonists with efficacy in rodent models of neuropathic pain.

This CB₁ agonist showed repeated suppression of neuropathy symptoms with a lack of side effects mediated by activation of central CB₁ receptors. The potency, peripheral selectivity, in vivo efficacy, and absence of CNS side effects of the peripherally restricted cannabinoids suggests promising further development of such compounds for a viable treatment for neuropathic pain states.

Positive allosteric modulators (PAMs) of CB₁

The therapeutic utility of allosteric interactions with GPCRs is an emerging area of rational drug design enabled by advances in structural and computational biology.¹¹³⁻¹¹⁵ As with many class A GPCRs, CB₁ contains an allosteric site that can be targeted by small molecules.¹¹⁶⁻¹¹⁹ Several endogenous allosteric modulators of CB₁ were recently identified, including pregnenolone (**34**, Fig. 9) lipoxin A4 (**35**)¹²⁰ and the dodecapeptide pepcan-12. The off-target activity of these ligands for CB₁ allosteric modulation limits their utility as lead structures for a rational design of CB₁ PAMs.

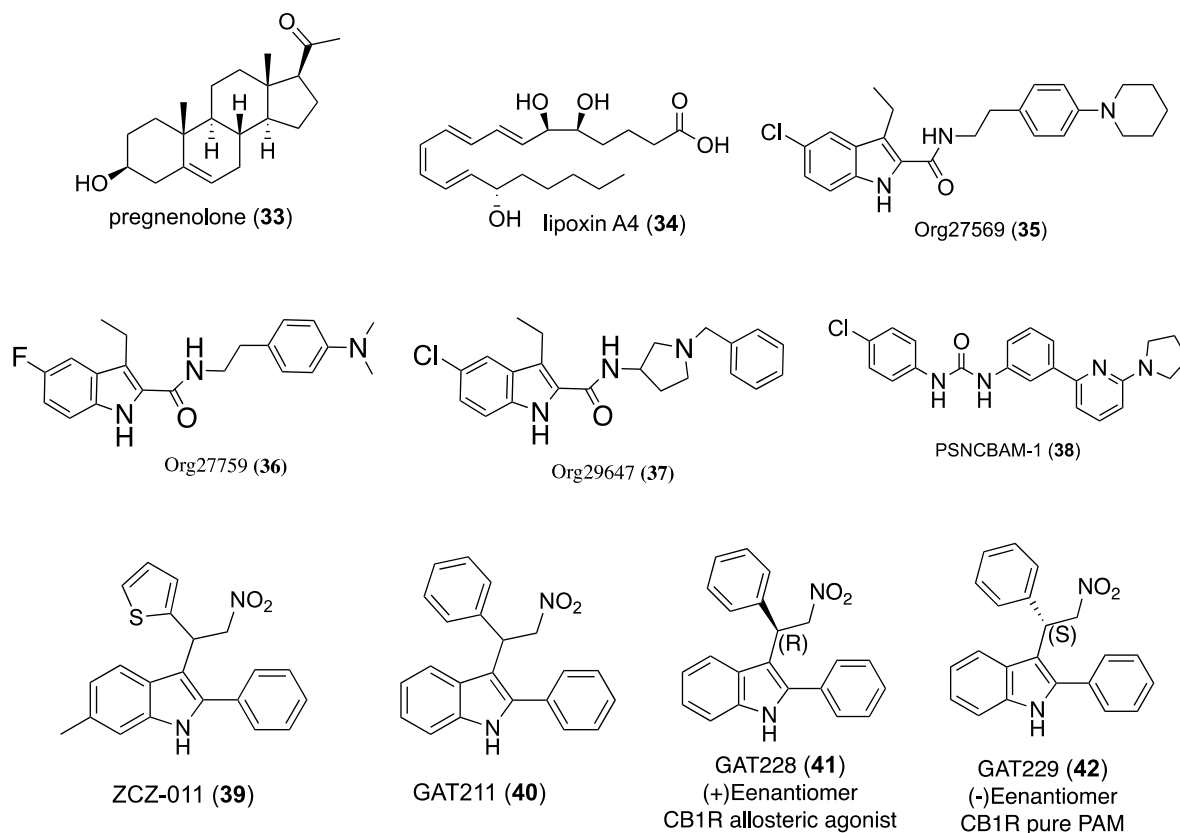


Figure 9. Selected endogenous and exogenous allosteric modulators of CB₁.

The first identified CB₁ AM was the indole-2-carboxamide Org27569 (**35**). Org27569 enhances the CB₁ binding of selective cannabinoid ligands, yet acts as an insurmountable antagonist in several biochemical assays.¹²¹ The activity of Org27569 was recently explored *in vivo*, and it is unclear if Org27569 functions as a positive or negative allosteric modulator of CB₁ in rodents.^{122, 123} Nguyen *et al* found that the modification of 1*H*-indole-2-carboxamides compounds related to Org27569 (**35**), Org27759 (**36**), and Org29647 (**37**), showed that modulation potency of this series at the CB1 receptor was enhanced by the presence of a diethylamino group at the 4-position of the phenyl ring, a chloro or fluoro group at the C₅ position and short alkyl groups at the C₃ position on the indole ring.¹²⁴ The most active compound (**37**) had an IC₅₀ value 79 nM which is ~2.5 and 10 fold more potent than the parent compounds **35** and **36**, respectively. These compounds appeared to be negative allosteric modulators at the CB₁ receptor.¹²⁵ Org27569 has served as a useful scaffold for the development of CB₁ NAMs intended to probe CB₁ structure and function,¹²⁶⁻¹³⁰ and several structurally distinct CB₁ NAMs have also been identified, including PSNCBAM-1 (**38**).^{131, 132}

Although CB₁ NAMs may have utility in obesity and other conditions, fewer CB₁ PAMs are known. By enhancing the signaling of endogenous CB₁ agonists, CB₁ PAMs may represent a relatively safe and efficacious treatment option for neuropathic pain. ZCZ-011 (**39**) was recently shown to enhance CB₁ signaling, and reverse nociceptive behavior in neuropathic and inflammatory pain models, without apparent psychoactive effects in mice.^{133,134} GAT-211 (**40**) is a CB₁ PAM but has not been explored *in vivo*. GAT-211 was also resolved into its (+)- and (-)-enantiomers (GAT-228, **41** and GAT-229, **42**), revealing a stereochemical basis for differences in signaling bias.¹³⁵ Because the endocannabinoid system can affect dopamine neurotransmission and cause hypolocomotion,¹³⁶ anomalous pharmacology of the dopamine transporter (DAT) inhibitor JHW007 (**43**) led to the discovery of RTI-371 (**44**) as the first tropane with PAM activity at CB₁ receptors (Figure10).¹³⁷

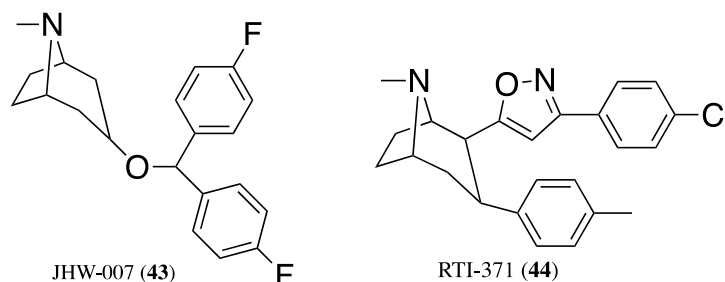


Figure 10. Selected PAM modulator of CB₁.

The potential of biased CB₁ ligands for pain

The cannabinoid CB₁ receptor has been implicated in the treatment of drug addiction, pain, appetite disorders, and other CNS related diseases.¹³⁸⁻¹⁴¹ For example, Huntington disease (HD) is a neurodegenerative disorder in which there is a decrease in the levels of type 1 cannabinoid receptor (CB₁) mRNA and protein in the medium spiny projection neurons of the caudate and putamen.¹⁴²⁻¹⁴⁵ A comparative analysis of six cannabinoids tested for signaling bias in *in vitro* models of medium spiny projection neurons expressing wild-type (*STHdh*Q7/Q7) or mutant huntingtin protein (*STHdh*Q111/Q111), showed that *Gai*/o- and *Gβγ*-selective CB₁ ligands are probably the most therapeutically useful cannabinoids in the treatment of HD. However, highly

potent synthetic cannabinoids, such as WIN, could produce unwanted psychoactive effects and their chronic use would probably result in receptor desensitization or downregulation.^{146,147} When administered directly, endocannabinoids, which enhance *Gai/o*- and *Gβγ*-dependent signaling in the *STHdh* cell culture system, are rapidly metabolized *in vivo* and consequently have limited efficacy.^{148, 149} The inhibitor of endocannabinoid catabolism URB597 has demonstrated limited efficacy at improving motor control deficits in R6/2 HD mice,^{150, 151} but additional studies are needed to understand how elevating endocannabinoid levels affects the signs and symptoms of HD *in vivo*.

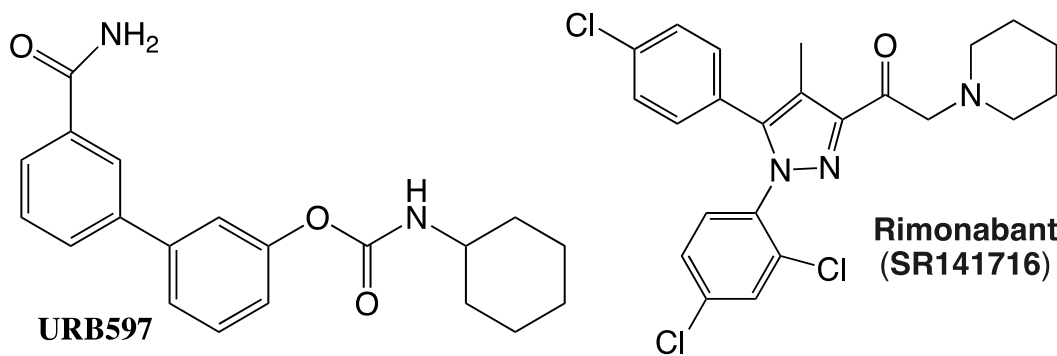


Figure 11: CB₁ ligands for the treatment of Huntington disease

Cannabinoid agonists displayed distinct biased signaling profiles at CB₁.¹⁵² Therefore, the clinical application of cannabinoid ligands has been hampered owing to their adverse on-target effects. Ligand-biased signaling from, and allosteric modulation of, CB₁ offer pharmacological approaches that may enable the development of improved CB₁ drugs, through modulation of only therapeutically desirable CB₁ signaling pathways. There is growing evidence that CB₁Rs are subject to ligand-biased signaling and allosterism.¹⁵³ Quantification of ligand-biased signaling and allosteric modulation at CB₁ using Cyclic Adenosine Monophosphate (cAMP)¹⁵⁴ Signaling assay showed that Cannabinoid agonists displayed distinct biased signaling of CB₁R. For instance, whereas 2-arachidonylglycerol and WIN55,212-2 (**6**) [(R)-(1)-[2,3-dihydro-5-methyl-3-(4-morpholinylmethyl)pyrrolo[1,2,3-de]-1,4-benzoxazin-6-yl]-1-naphthalenylmethanone] showed little preference for inhibition of cAMP and phosphorylation of extracellular signal-regulated kinase 1/2 (pERK1/2), N-arachidonylethanolamine (anandamide), methanandamide, CP55940 (**7**) [2-[(1R,2R,5R)-5-hydroxy-2-(3-hydroxy propyl)cyclohexyl]-5-(2-methyloctan-2-yl)phenol] and HU-210 (**8**) [11-hydroxy-D-THC-dimethylheptyl] were biased

toward cAMP inhibition. The small-molecule allosteric modulator Org27569 [5-chloro-3-ethyl-1H-indole-2-carboxylic acid [2-(4-piperidin-1-yl-phenyl)ethyl]amide] displayed biased allosteric effects by blocking cAMP inhibition mediated by all cannabinoid ligands tested, at the same time having little or no effect on ERK1/2 phosphorylation mediated by a subset of these ligands. Org27569 also displayed negative binding cooperativity with [H] SR141716 (A [5-(4-chlorophenyl)-1-(2,4-dichloro-phenyl)-4-methyl-N-(piperidin-1-yl)-1H-pyrazole-3-carboxamide]); however, it had minimal effects on binding of cannabinoid agonists. Furthermore, we highlight the need to validate the reported allosteric effects of the endogenous ligands lipoxin A4 and pregnenolone at CBRs. Pregnenolone but not lipoxin A4 displaced [H] SR141716A, but there was no functional interaction between either of these ligands and cannabinoid agonists. This study demonstrates an approach to validating and quantifying ligand-biased signaling and allosteric modulation at CBRs, revealing ligand-biased “fingerprints” that may ultimately allow the development of improved CBR-targeted therapies.

Evidence suggests that GPCRs can adopt multiple conformations and these might explain biased signaling—the phenomena where different drugs binding to the same orthosteric site on the receptor can cause activation of different signaling pathways, such as β -arrestin signaling.¹⁵⁵ Structural dynamic study of allosteric inactivation of the CB₁R showed that a previously unidentified structure is induced in the marijuana receptor CB₁ by an unusual allosteric ligand that blocks G-protein signaling but increases agonist binding and elicits biased signaling, which suggests that a common structural state may exist for β -arrestin biased signaling, one that can also be attained by allosteric ligand binding.¹⁵⁶ Together all these studies constitute a comprehensive description of signaling from CB₁ and suggest modulation of receptor endocytic trafficking as a therapeutic approach.¹⁵⁷ Therefore, to effectively match cannabinoids with therapeutic goals, these compounds must be screened for their signaling bias.

Overall, the CB₁ receptor plays an important role in diverse processes such as pain, cognition, metabolism, etc. However, the psychoactive side effects of CB₁ activation in the brain have limited the use of CB₁ ligands as drugs. The endocannabinoid system is ubiquitously expressed throughout the body and is responsible for the homeostatic control of many basic physiological processes. Thus, a great opportunity exists for the development of cannabinoid-based drugs for a

wide range of therapeutic applications. When new strategies are developed to mitigate the side effects, tremendous potential exists for future development of efficacious drugs targeting CB₁ for a variety of disease states.

Abbreviations

AAI: aminoalkylindole

CBD: cannabidiol

CCI: chronic constriction injury

GPCR: G protein-coupled receptor

SNL: spinal nerve ligation

THC: tetrahydrocannabinol

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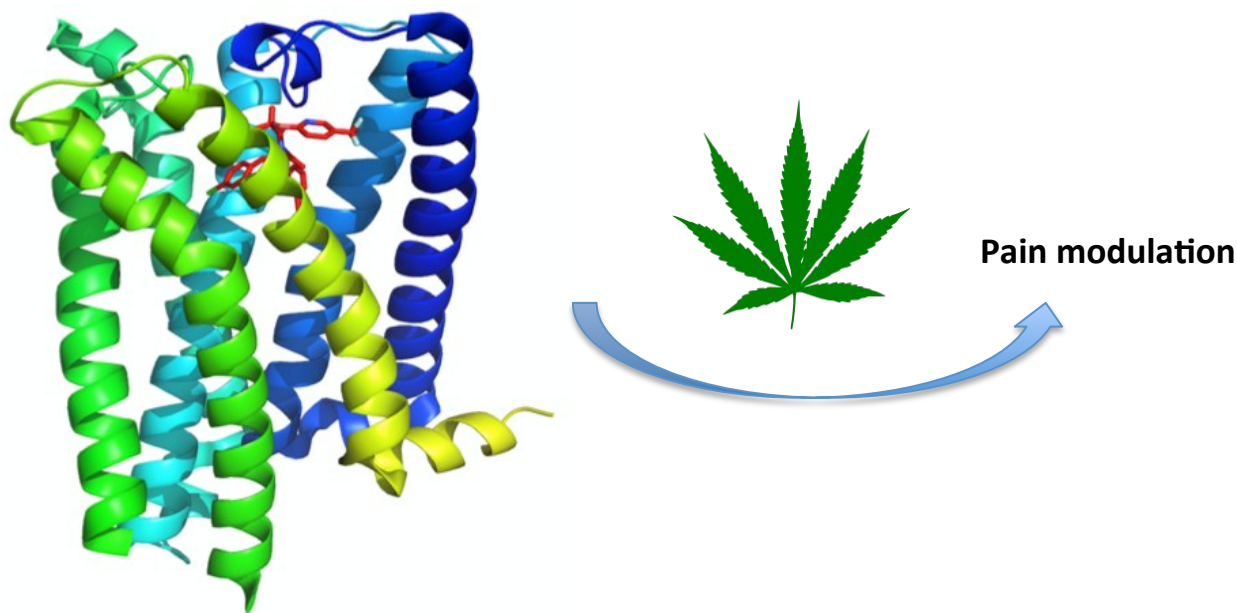
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Selective modulation of the cannabinoid type 1 (CB₁) receptor as an emerging platform for the treatment of neuropathic pain

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This article provides an overview of the chemotypes that have been investigated or under investigation for the development of novel neuropathic pain treatments targeting CB₁ receptor.



Cannabinoid 1 Receptor (CB₁)