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REVIEW

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Selective modulation of the cannabinoid type 1 (CB$_1$) receptor as an emerging platform for the treatment of neuropathic pain

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Neuropathic pain is caused by a lesion or dysfunction in the nervous system, and it may arise from illness, be drug-induced or caused by toxin exposure. Since the discovery of two G-protein-coupled cannabinoid receptors (CB$_1$ and CB$_2$) nearly three decades ago, there has been a rapid expansion in our understanding of cannabinoid pharmacology. This is currently one of the most active fields of neuropharmacology, and interest has emerged in developing cannabinoids and other small molecule modulators of CB$_1$ and CB$_2$ as therapeutics for neuropathic pain. This short review article provides an overview of the chemotypes currently under investigation for the development of novel neuropathic pain treatments targeting CB$_1$ receptors.

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 CB$_1$ 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 composed of two well-characterized G protein-coupled receptors (GPCRs), the cannabinoid type 1 and type 2 receptors (CB$_1$ and CB$_2$, respectively), endogenous signaling lipids such as anandamide (arachidonoylethanolamide, 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 CB$_1$ and CB$_2$ receptors is known to reduce nociceptive signaling, and nociceptive processing is topically controlled by endocannabinoids, like AEA and 2-AG.[27]

Anandamide is believed to serve as a natural pain modulator[28] 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

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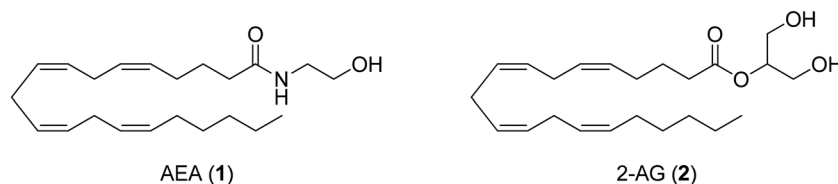*Med. Chem. Commun.*, 2019, **10**, 647–659 | 647

Fig. 1   Selected endogenous cannabinoids.

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 CB_1 and CB_2, as well as inhibitors of endocannabinoid metabolism.[32] Ligands that activate central CB_1 receptors *via* their orthosteric site produce psychoactive effects, limiting the broad utility of such drugs. Selective CB_2 agonists have demonstrated efficacy in preclinical models of neuropathic pain, but despite extensive efforts by the pharmaceutical industry, no CB_2 agonist has advanced to the 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 was 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 available,[36] 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 (CB_1) 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] CB_1 has been cloned and encodes 473 amino acid protein.[47] Like other class A G protein-coupled receptors (GPCRs), 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 re-

ceptor ligands, the identification of the signaling pathways regulated by each wave, and the underlying molecular mechanisms and physiological 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 CB_1 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 CB_1 receptor is capable of ligand- and heterodimer-directed functional selectivity.[49] The CB_2 receptor is also known to possess diverse, ligand-directed signaling bias.[50] There is also the physical and functional interplay between CB_1 receptors and other CNS targets of relevance to pain, including D_2 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 CB_1–DOR heteromeric complex in the cortex of neuropathic animals, with concomitant changes in the activity of each protein.[51]

The development of CB_1 knock-out (KO) mice has provided insights regarding the role of CB_1 receptors in nociception. CB_1 KO mice are hypoalgesic[52] and demonstrate a reduced analgesic response to cannabinoids,[53] however, CB_1 KO mice still develop neuropathic pain,[54,55] consistent with the complex pathophysiology of neuropathic pain. Spinal and supraspinal CB_1 receptors are upregulated following nerve injury, and this may account for the efficacy of CB_1 agonists in neuropathic pain.[56] Indeed, CB_1 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 CB_1 receptor as a relevant platform for the development of novel analgesics.[60] The earliest indication that modulation of the CB_1 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 2700 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, Fig. 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.

Phytocannabinoids interact non-selectively with many targets of the human endocannabinoid system beyond CB_1 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 CB_1 is confounded by their complex pharmacology.[68] For example, Δ^9-THC acts as a potent, low efficacy agonist of CB_1 and CB_2 receptors, while CBD is a negative allosteric modulator (NAM) of CB_1,[69] yet both compounds produce analgesic effects in rodent models. It is believed that non-cannabinoid targets like spinal $\alpha 3$ 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 Δ^9-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 CB_1

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 CB_1/CB_2 and CB_1-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 the cannabinoid function, such as HU-210 (8).[72]

Like Δ^9-THC, these cannabinoids are typically orthosteric CB_1 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^{-1} and 6 mg kg^{-1} (per os p.o.), respectively, whereas CP 55,940 produced similar effects at 0.05 mg kg^{-1} and 0.025
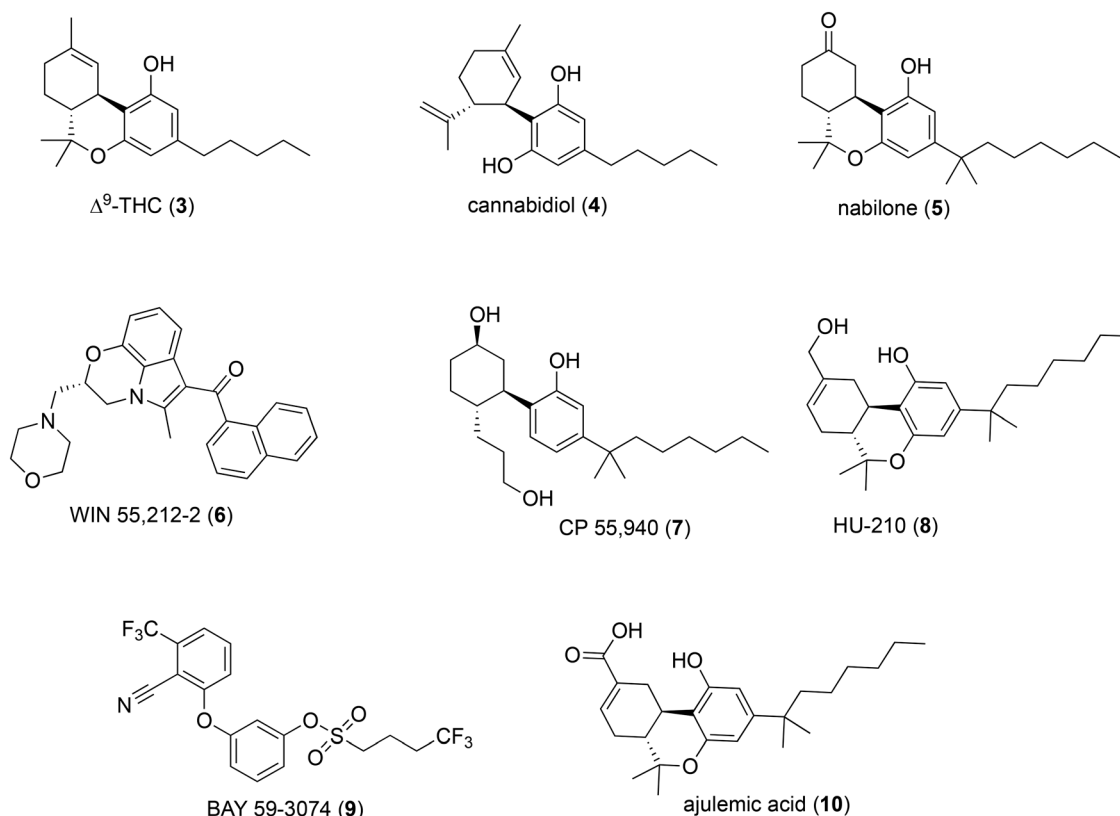

Fig. 2 Selected phytocannabinoids and analogs.

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mg kg^{-1} (intraperitoneal i.p. injection), respectively.[73] Both cannabinoids were more potent than traditional neuropathic pain treatments such as morphine (8 mg kg^{-1} and 16 mg kg^{-1} p.o., respectively) and gabapentin (50 mg kg^{-1} 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 CB$_1$ antagonist.[74] The antinociceptive and motor effects of CP 55,940 observed in wild-type mice are absent in CB$_1$ but not CB$_2$ KO mice, indicating that CP 55,940 exerts its analgesic properties *via* orthosteric activation of CB$_1$ 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 CB$_1$ orthosteric agonists limit their broad application as an analgesic agent; therefore, CB$_1$ agonists without central activity have been suggested as therapeutics.[82] Several strategies for selective modulation of CB$_1$ have been proposed, with focus on reducing adverse reactions and psychotropic activity.[83,84] In this regard, the peripherally restricted CB$_1$ agonists retain antinociceptive effects without apparent psychoactivity and are currently under development.[85] Another alternative is the development of CB$_1$ positive allosteric modulators (PAMs) or functionally selective CB$_1$ ligands that induce biased signaling.[86]

Peripherally-restricted CB$_1$ agonists

The use of centrally active CB$_1$ 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 CB$_1$ receptors mediate CB$_1$ antihyperalgesia.[76,88]

Ajulemic acid (CT-3, **10**), a synthetic analogue of 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 CB$_1$ and CB$_2$ 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 CB$_1$ antagonists are under development for metabolic disorders and provide proof-of-principle for the development of CB$_1$ agonists with reduced CNS liabilities.[95,96] Although ajulemic acid has shown
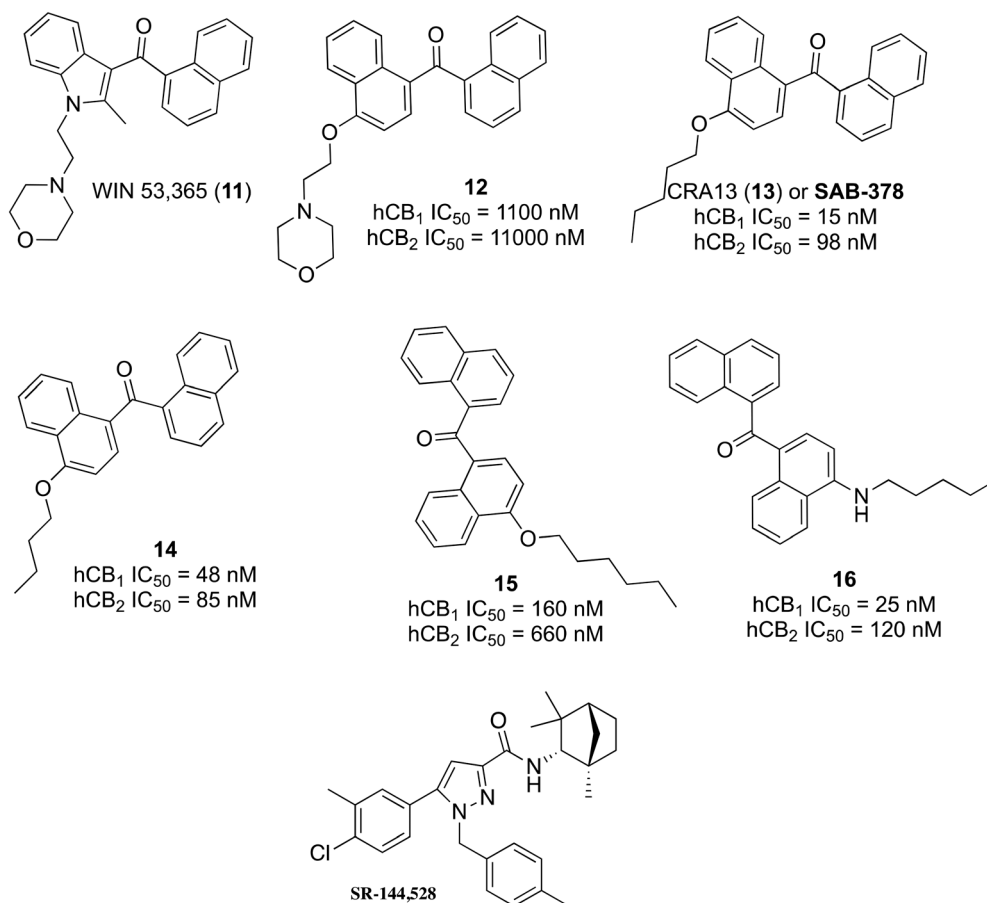

Fig. 3 Structures and activities of selected ligands reported during the discovery of CRA13.

promise in clinical trials, phytocannabinoids are not ideal drug leads for peripherally-restricted CB_1 agonists owing to their high lipophilicity and limited scope of structural modification.

The aminoalkylindole (AAI) class of CB_1 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 the generation of peripheral CB_1 agonists by Novartis.[97] The indole core was substituted with naphthalene based on previous bio-equivalency 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 AAIs,[99,100] with a pentyl ether found providing greater hCB_1 binding (CRA13, 13, IC_{50} = 15 nM) than a butyl (14, IC_{50} = 48 nM) or hexyl (15, IC_{50} = 160 nM) ether.

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 CB_1/CB_2 agonist activity with restricted CNS penetration.[101] CRA13 produced up to 90% hyperalgesia reversal when administered orally at 3 mg kg^{-1} in a rat neuropathic model of mechanical hyperalgesia with rapid onset and long duration of action and without apparent CNS-mediated side effects. The antihyperalgesic effects of CRA13 were inhibited by a CB_1-selective inverse agonist/antagonist, rimonabant, but not by a CB_2-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 were 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 CB_1 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 the 5-sulfonamide benzimidazole scaffold.

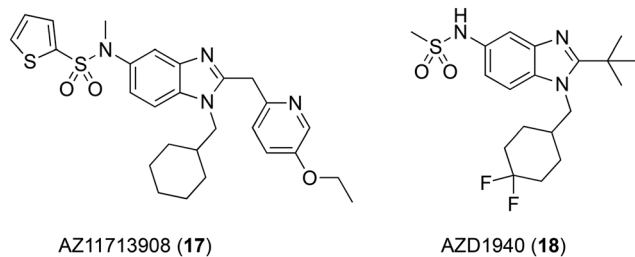AZ11713908 demonstrated high affinity for CB_1 and CB_2 receptors derived from mouse, rat, and human sources (hCB_1 pIC_{50} 8.4, hCB_2 pIC_{50} 9.0), but little selectivity between sub-types.[103] This drug showed potent, efficacious agonist activity at hCB_1 in the $GTP\gamma S$ assay (pEC_{50} 8.0, E_{max} = 115%) compared to WIN 55,212. It was noted that compared to WIN 55,212, which showed a 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 µmol kg^{-1} AZ11713908 reduced mechanical allodynia with 100% efficacy, whereas 8 µmol kg^{-1} WIN 55,212 was required to achieve the same effect.

AZD1940 is a high affinity hCB_1/hCB_2 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 its carbon-11-labeled isotopologue ([^{11}C]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 was 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 demonstrated moderate affinity for both CB receptors (hCB_1 K_i = 143 nM, hCB_2 K_i = 14 nM) and functioned suitably as an agonist (rCB_1 EC_{50} = 1440 nM, E_{max} = 47%). Additionally, 18 had reasonable aqueous solubility (>3 mg mL^{-1} at pH 7.4), unlike many classes of cannabinoids.

Starting from hit 19, with a moderate brain-to-plasma ratio of 0.74, AstraZeneca aimed to increase the 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 were explored at the

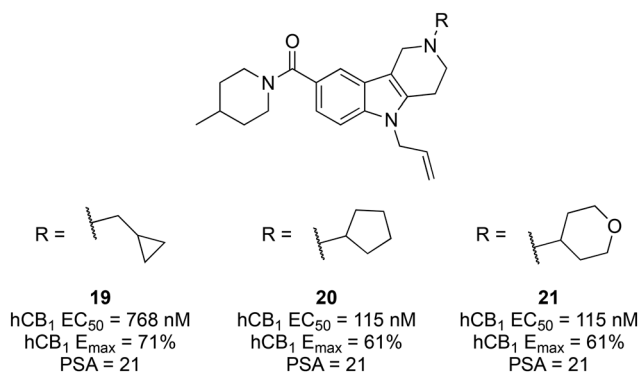Fig. 4 Peripherally-restricted CB_1 agonists under development by AstraZeneca.

Fig. 5 Effect of the terminal carboline amine substituent on CB_1 agonist activity and PSA.

terminal carboline nitrogen, with all leading to increased hCB_1 potency and comparable efficacy, except for various pyridines. The cyclopentyl (**20**, EC_{50} = 33 nM, E_{max} = 65%) and 4-tetahydropyranyl (**21**, EC_{50} = 60 nM, E_{max} = 57%) analogues showed the greatest improvement in potency without compromising metabolic stability (HLM clearance of 93 and 42 μl min^{-1} mg^{-1}, respectively) or decreasing the PSA (21 and 30, respectively) (Fig. 5).

While retaining the cyclopentyl and 4-tetrahydropyranyl groups of **20** and **21**, an array of polar substituents was introduced at the carboline central nitrogen. All alkyl substituents (methyl, ethyl, and propyl) were tolerated with minimal effects on CB_1 agonist potency, efficacy, or PSA. Compared to propyl analogue **22** (EC_{50} = 103 nM, E_{max} = 72%, PSA 30), introduction of a carbonyl spacer as in **23** increased the PSA to 48 with little effect on potency (EC_{50} = 117 nM), but the efficacy was dramatically reduced (E_{max} = 17%). The 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_{max} = 100%). Truncation of the propyl group of **24** to an ethyl group (**25**; EC_{50} = 49 nM, E_{max} = 120%, PSA 67) furnished a potent CB_1 agonist with a suitably high PSA, whereas further truncation to a methylsulfone reduced the potency 10-fold (**26**; EC_{50} = 473 nM). Attempts to further increase the PSA by introduction of a sulfonamide (*e.g.*, **27**; PSA 83) completely eliminated the activity (Fig. 6).

Compound **25** was selected as a potent, efficacious agonist at hCB_1 (EC_{50} = 49 nM, E_{max} = 120%) and rCB_1 (EC_{50} = 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 for 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%) and 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^{-1} subcutaneous, s.c.). No further reports of the translation of **25** have appeared.
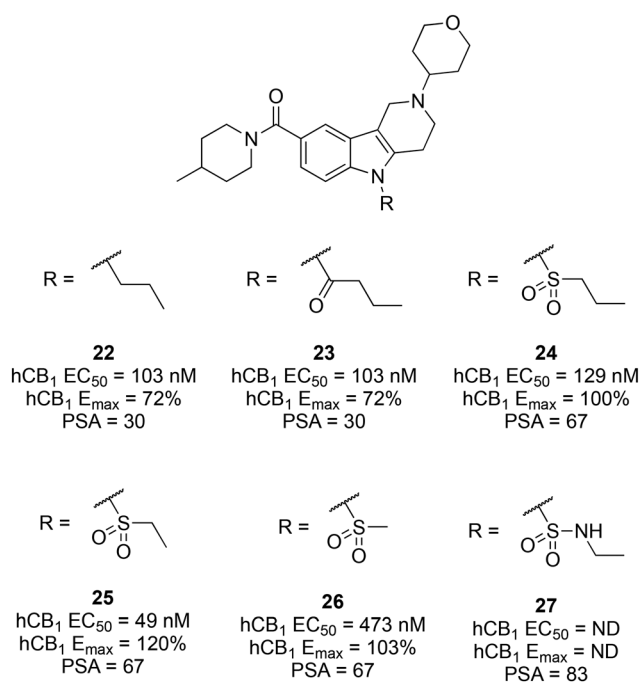
Researchers at Merck have also employed the strategy of reducing CNS penetration by increasing the PSA.[111] Using MONIKA, a web tool developed internally with Organon, Merck optimized parameters related to both oral bioavailability and CNS permeability (used as exclusion criteria), to further optimize a previously developed indole cannabinoid with desirable properties (**28**, Fig. 7). Compound **28** hydrochloride was a moderately potent CB_1 agonist (pEC_{50} = 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 the PSA but reduced the potency. Introduction of polar groups as pendant substituents of the oxadiazole ring was found to increase the potency and PSA (**31** and **32**).

Compound **32** is a moderately potent agonist of CB_1 (pK_i 7.7, pEC_{50} 7.1) with negligible selectivity over CB_2 (pK_i 7.7). LBP1 had a suitably low brain-to-plasma ratio in mouse (0.16) and good aqueous solubility (89 mg L^{-1}) and oral bioavailability (45%). **32** showed antihyperalgesic effects in the rat Chung Hagreaves model and antiallodynic effects in the rat Chung Von Frey model, while the CB_1 antagonist rimonabant blocked these effects. As with other centrally penetrant CB_1 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 μmol kg^{-1}. 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, a study with a series of 3-alkylated indenes was recently reported, where peripherally restricted cannabinoids were designed and tested for selectivity by introduction of charge to CB_1. 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 (Fig. 8).[112]

This CB_1 agonist showed repeated suppression of neuropathy symptoms with a lack of side effects mediated by activation of central CB_1 receptors. The potency, peripheral selectivity, *in vivo* efficacy, and absence of CNS side effects of the peripherally restricted cannabinoids suggest promising further development of such compounds for viable treatment for neuropathic pain states.
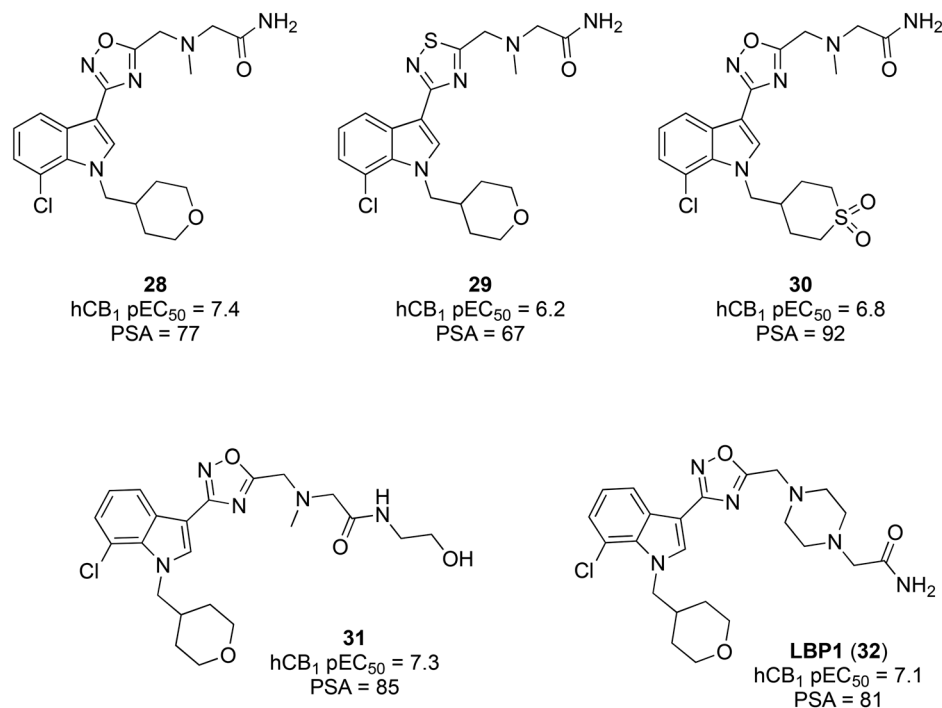

Fig. 6 Effect of the central carboline amine substituent on CB_1 agonist activity and PSA.

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Fig. 7 Peripherally-restricted indole-3-heterocycle CB$_1$ agonists.

Positive allosteric modulators (PAMs) of CB$_1$

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, CB$_1$ contains an allosteric site that can be targeted by small molecules.[116–119] Several endogenous allosteric modulators of CB$_1$ 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 CB$_1$ allosteric modulation limits their utility as lead structures for the rational design of CB$_1$ PAMs.

The first identified CB$_1$ AM was the indole-2-carboxamide Org27569 (**35**). Org27569 enhances the CB$_1$ 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

Fig. 8 Peripherally restricted CB$_1$ agonists with efficacy in rodent models of neuropathic pain.

Org27569 functions as a positive or negative allosteric modulator of CB$_1$ in rodents.[122–124] Nguyen *et al.* found that the modification of 1*H*-indole-2-carboxamides compounds related to Org27569 (**35**), *i.e.* Org27759 (**36**), and Org29647 (**37**), showed modulation potency of this series at the CB$_1$ receptor which was enhanced by the presence of a diethylamino group at the 4-position of the phenyl ring, a chloro or fluoro group at the C$_5$ position and short alkyl groups at the C$_3$ position on the indole ring.[125] The most active compound (**37**) had an IC$_{50}$ value of 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 CB$_1$ receptor.[125] Org27569 has served as a useful scaffold for the development of CB$_1$ NAMs intended to probe the CB$_1$ structure and function,[126–130] and several structurally distinct CB$_1$ NAMs have also been identified, including PSNCBAM-1 (**38**).[131,132]

Although CB$_1$ NAMs may have utility in obesity and other conditions, fewer CB$_1$ PAMs are known. By enhancing the signaling of endogenous CB$_1$ agonists, CB$_1$ PAMs may represent a relatively safe and efficacious treatment option for neuropathic pain. ZCZ-011 (**39**) was recently shown to enhance CB$_1$ signaling and reverse nociceptive behavior in neuropathic and inflammatory pain models, without apparent psychoactive effects in mice.[133,134] GAT-211 (**40**) is a CB$_1$ 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] the anomalous pharmacology of the dopamine transporter (DAT) inhibitor
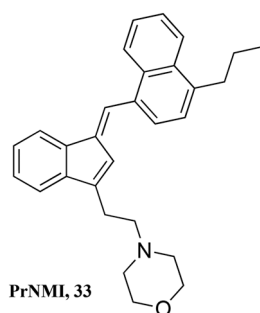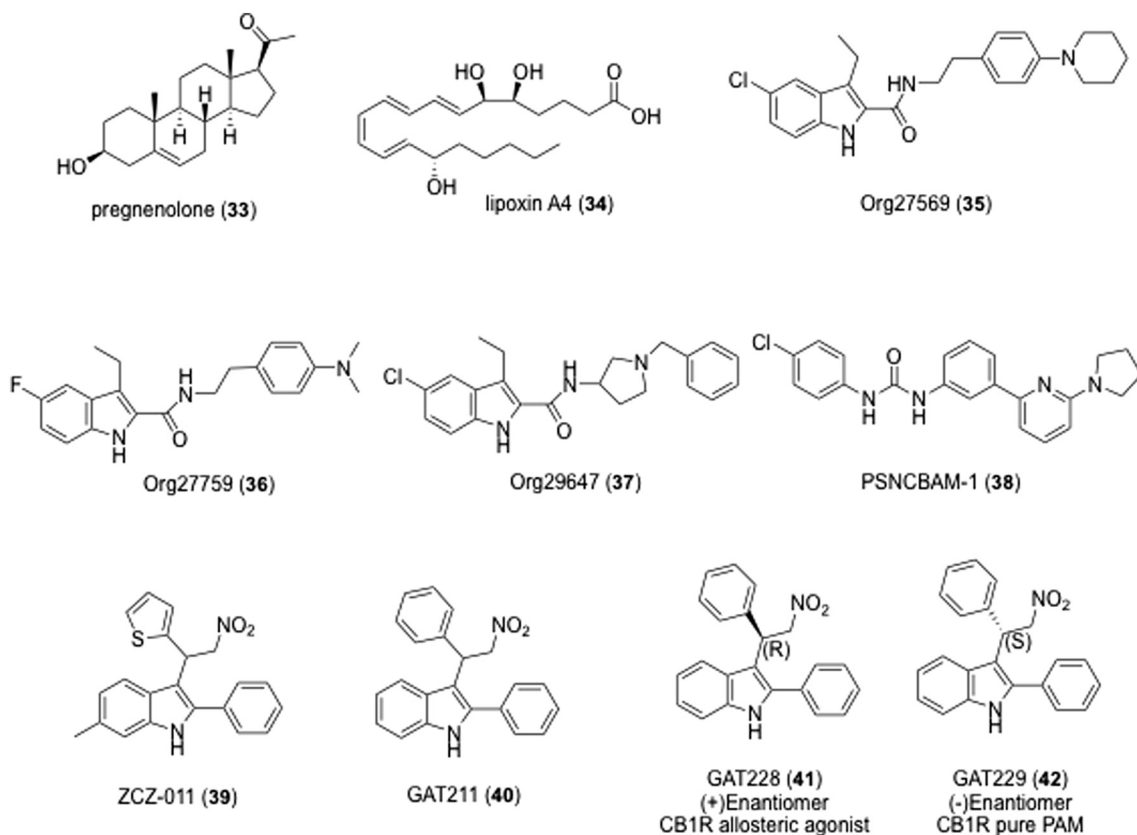
Fig. 9   Selected endogenous and exogenous allosteric modulators of CB$_1$.

JHW007 (**43**) led to the discovery of RTI-371 (**44**) as the first tropane with PAM activity at CB$_1$ receptors (Fig. 10).[137]

The potential of biased CB$_1$ ligands for pain

The cannabinoid CB$_1$ 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 (CB$_1$) 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 (ST*Hdh*Q7/Q7) or mutant huntingtin protein (ST*Hdh*Q111/Q111) showed that Gαi/o- and G$\beta\gamma$-selective CB$_1$ ligands are probably the most therapeutically useful cannabinoids in the treatment of HD. However, highly potent syn-

thetic 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 Gαi/o- and G$\beta\gamma$-dependent signaling in the ST*Hdh* 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 elevated endocannabinoid levels affects the signs and symptoms of HD *in vivo* (Fig. 11).

Cannabinoid agonists displayed distinct biased signaling profiles at CB$_1$.[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, CB$_1$ offer pharmacological approaches
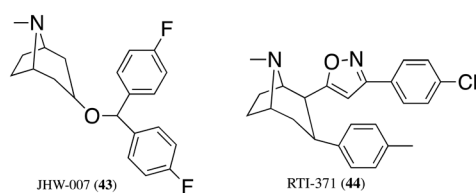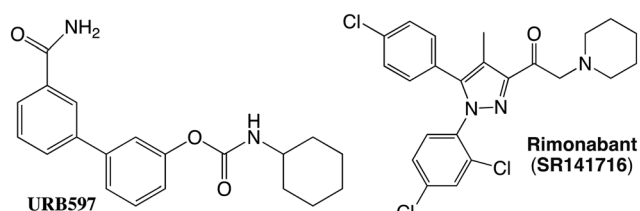

Fig. 10   Selected PAM modulator of CB$_1$.

Fig. 11   CB$_1$ ligands for the treatment of Huntington disease.

that may enable the development of improved CB_1 drugs, through modulation of only therapeutically desirable CB_1 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 CB_1 using cyclic adenosine monophosphate (cAMP)[154] signaling assay showed that cannabinoid agonists displayed distinct biased signaling of CB_1R. For instance, whereas 2-arachidonylglycerol and WIN 55,212-2 (**6**) [(*R*)-(1)-[2,3-dihydro-5-methyl-3-(4-morpholinylmethyl)pyrrolo[1,2,3-de]-1,4-benzoxazin-6-yl]-1-napthalenylmethanone] showed little preference for inhibition of cAMP and phosphorylation of extracellular signal-regulated kinase 1/2 (pERK1/2), *N*-arachidonoylethanolamine (anandamide), methanandamide, CP55940 (**7**) [2-[(1*R*,2*R*,5*R*)-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-1*H*-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, *i.e.* [5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-*N*-(piperidin-1-yl)-1*H*-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 phenomenon 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] The structural dynamic study of allosteric inactivation of CB_1R showed that a previously unidentified structure is induced in the marijuana receptor CB_1 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 CB_1 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 CB_1 receptor plays an important role in diverse processes such as pain, cognition, metabolism, *etc.* However, the psychoactive side effects of CB_1 activation in the brain have limited the use of CB_1 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 CB_1 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

Conflicts of interest

There are no conflicts to declare.

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