REVIEW ARTICLE

Translational potential of allosteric modulators targeting the cannabinoid CB$_1$ receptor

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The cannabinoid type-1 (CB$_1$) receptor, a G-protein-coupled receptor, is an attractive target for drug discovery due to its involvement in many physiological processes. Historically, drug discovery efforts targeting the CB$_1$ receptor have focused on the development of orthosteric ligands that interact with the active site to which endogenous cannabinoids bind. Research performed over the last several decades has revealed substantial difficulties in translating CB$_1$ orthosteric ligands into druggable candidates. The difficulty is mainly due to the adverse effects associated with orthosteric CB$_1$ ligands. Recent discoveries of allosteric CB$_1$ modulators provide tremendous opportunities to develop CB$_1$ ligands with novel mechanisms of action; these ligands may potentially improve the pharmacological effects and enhance drug safety in treating the disorders by regulating the functions of the CB$_1$ receptor. In this paper, we review and summarize the complex pharmacological profiles of each class of CB$_1$ allosteric modulators, the development of new classes of CB$_1$ allosteric modulators and the results from in vivo assessments of their therapeutic value.

Keywords: allosteric modulator; cannabinoid CB$_1$ receptor; G-protein-coupled receptor; biased signaling; functional selectivity; therapeutic potential; drug discovery

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INTRODUCTION

The cannabinoid CB$_1$ receptor is a member of the endocannabinoid system that comprises at least two G-protein-coupled receptors (GPCRs), the cannabinoid type-1 receptor (CB$_1$) and the cannabinoid type-2 receptor (CB$_2$). The CB$_1$ receptor is the most abundant GPCR expressed in the central nervous system (CNS) and is widely distributed at lower concentrations in a variety of peripheral tissues [1, 2]. In contrast, the CB$_2$ receptor is primarily located in the periphery, with high concentrations in the tonsil, spleen and immune-related cells [3]. Similar to Δ^9-tetrahydrocannabinol (Δ^9-THC), the main active ingredient of cannabis, a group of lipid-derived molecules represented by *N*-arachidonoyl ethanolamide (AEA) and 2-arachidonoyl glycerol (2-AG) functions as endogenous agonists of the two cannabinoid receptors.

Several catabolic enzymes, including fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MGL), are responsible for promptly degrading the endogenous cannabinoid ligands to prevent hyperactivity of the cannabinoid receptors under physiological conditions [1–3]. The CB$_1$ receptor is linked to several disease states, including chemotherapy-induced nausea, wasting syndromes associated with cancer and AIDS, obesity, neurodegenerative disorders, pain, and substance abuse disorders [1, 4]. Traditionally, drug discovery efforts aimed at the CB$_1$ receptors have focused on ligands targeting the CB$_1$ orthosteric sites to which the endogenous cannabinoids bind. However, ligands targeting the orthosteric sites of CB$_1$ receptors exert either psychotropic side effects, such as CB$_1$ agonists [5], or psychiatric side effects, such as globally active CB$_1$ antagonists/inverse agonists [6]. These untoward side effects have hampered drug discovery efforts aimed at regulating CB$_1$ receptors for therapeutic benefits. On the other hand, the CB$_2$ receptor has attracted considerable attention as a therapeutic target for pain, cancer, inflammation-derived neurodegenerative diseases, and osteoporosis [7, 8]. A large number of preclinical models have been developed and employed to evaluate CB$_2$-selective agonists [7–10]. Some CB$_2$-selective agonists were assessed in clinical trials. Unfortunately, the outcomes were disappointing and failed to meet the primary endpoints. The possible causes for the disappointing clinical outcomes were recently discussed by other groups [7, 8]. Briefly, the failures are likely due to several factors: (1) inadequate preclinical studies to precisely predict clinical efficacies in humans; (2) the lack of CB$_2$ specific ligands to validate the target in preclinical investigations; and (3) insufficient engagement of the drug with its target and possible off-target side effects. Therefore, additional in-depth investigations are needed to address these issues before CB$_2$ selective ligands are translated into efficacious therapeutics.

The clinical failures of centrally active CB$_1$ inverse agonists and the psychoactive liability of CB$_1$ agonists have promoted the pursuit of new approaches to develop safer molecules targeting the cannabinoid CB$_1$ receptor for drug discovery. These approaches include (1) neutral antagonists of the CB$_1$ receptors, which spare the drugs from antagonizing the constitutive activity of CB$_1$ receptor while antagonizing the effects of the endocannabinoids [11, 12], (2) peripherally acting CB$_1$ ligands that prevent the ligands from interacting with central CB$_1$ receptors to abolish

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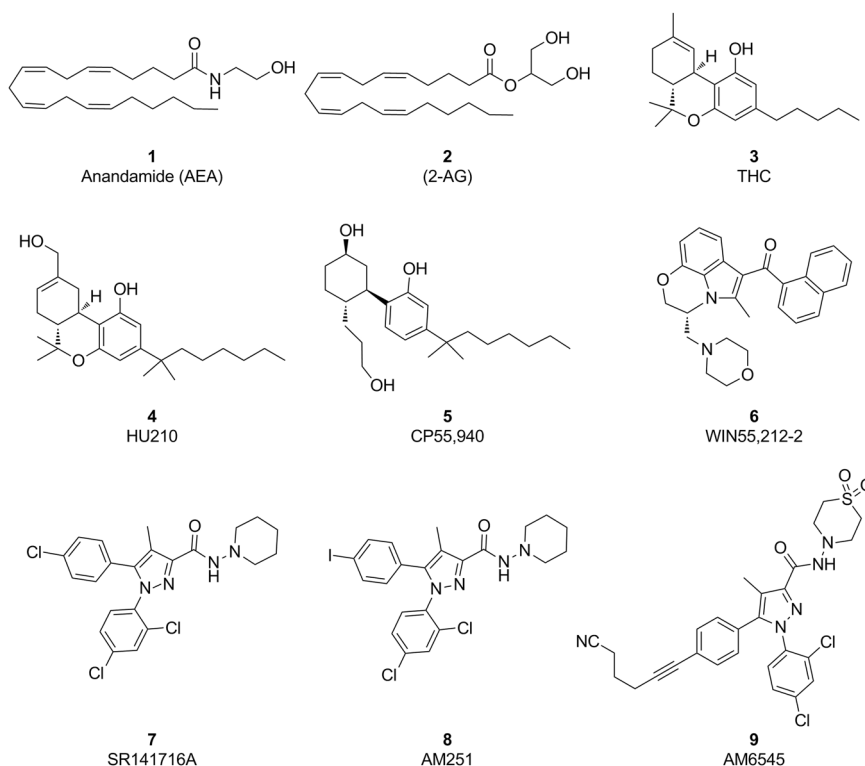D Lu et al.

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Fig. 1 Structures of representative orthosteric ligands of the CB$_1$ receptor

CNS side effects [13], and (3) allosteric modulators of the CB$_1$ receptor that display a novel mechanism of action to improve the drug safety profile of CB$_1$ ligands [14, 15]. To date, approximately nine types of small molecules that allosterically modulate the CB$_1$ receptors have been reported. These molecules offer researchers new opportunities to intervene in the actions of physiologically and pathologically important CB$_1$ receptors. This review provides basic knowledge to improve our understanding of promising CB$_1$ allosteric modulators and their potential therapeutic utility.

THE CANNABINOID CB$_1$ RECEPTOR AND ITS COMPLEX SIGNALING NETWORK

The cannabinoid CB$_1$ receptor was identified in the early 1990s in a study that revealed the mechanism of cannabis addiction and characterized a class 1A rhodopsin-like GPCR [3]. CB$_1$ receptors are widely distributed in the human body, and are primarily expressed in the CNS and to a lesser but functional extent in the periphery [16, 17]. The CB$_1$ receptor is the most abundant GPCR in the brain, with particularly high levels observed in the neocortex, hippocampus, basal ganglia, cerebellum, and brainstem [18]. CB$_1$ receptors play important roles in a variety of physiological conditions, including neuronal development, neuronal plasticity, food intake and energy balance, perception processes, immunomodulation, cell apoptosis, and cardiovascular and reproductive functions [1, 19, 20]. The multiple functions of the CB$_1$ receptor are apparently due to its complex cellular signaling pathways. Traditionally, the functions of CB$_1$ receptor are regulated by (1) endogenous cannabinoid agonists (e.g., 1, AEA and 2, 2-AG, Fig. 1), (2) exogenous agonists from phytocannabinoids (e.g., 3, Δ^9-THC, Fig. 1) and synthetic cannabimimetics (e.g., 4, HU210; 5, CP55,940; and 6, WIN55,212-2; Fig. 1), and (3) synthetic inverse agonists/antagonists (e.g., 7, SR141716A, and 8, AM251, Fig. 1) and neutral antagonists (e.g., 9, AM6545, Fig. 1). Typically, a CB$_1$ orthosteric inverse agonist binds to the same receptor site as an orthosteric agonist but induces a biological response opposite to an agonist. In contrast, a neutral antagonist binds to the receptor in the orthosteric site, does not change the equilibrium of the receptor, and only antagonizes the endogenously released endocannabinoids, but not by modulating the constitutive activity of the CB$_1$ receptor [12, 21].

Upon activation, the CB$_1$ receptor transduces signals through its interaction with G$_{i/o}$-, G$_s$-, and G$_{q/11}$-proteins, of which G$_{i/o}$ is preferentially coupled. Stimulation of the CB$_1$ receptor typically results in activation and dissociation of the coupled G$_{i/o}$-protein heterotrimers (i.e., α and βγ subunits). The released G$_{iα}$ subunits interact with adenylyl cyclase (AC) and inhibit its catalysis of cAMP production. The reduction in cAMP production down-regulates protein kinase A (PKA), which in turn suppresses PKA-mediated signaling events. The dissociated βγ subunits stimulate the phosphatidylinositide 3-kinase (PI3K) and protein kinase B (PKB) pathways, which induces the phosphorylation of mitogen-activated protein kinases (MAPKs). Along with the G-protein-mediated signal transduction, the CB$_1$ receptor also interacts with a variety of non-G-protein partners, including β-arrestins, the adaptor protein AP-3, GPCR-associated sorting proteins (GASP), the factor associated with neutral sphingomyelinase (FAN) and the cannabinoid receptor-interacting protein 1a (CRIP1a), to regulate its downstream effectors that include some MAPKs, multiple receptor tyrosine kinases and extracellular signal-regulated kinases (ERK) [22–25]. Of these non-G-protein partners, CRIP1a is interesting since it is involved in CB$_1$ desensitization and intracellular trafficking processes in which β-arrestins play critical roles. CRIP1a expression modulates ERK1/2 phosphorylation and the coupling selectivity among different G-protein α subunits [26], which are related to the functional selectivity of CB$_1$ ligands.

CB$_1$ receptors also transduce signals through other G-protein independent pathways that employ ceramide as the second messenger [27]. In addition to regulating the activities of the
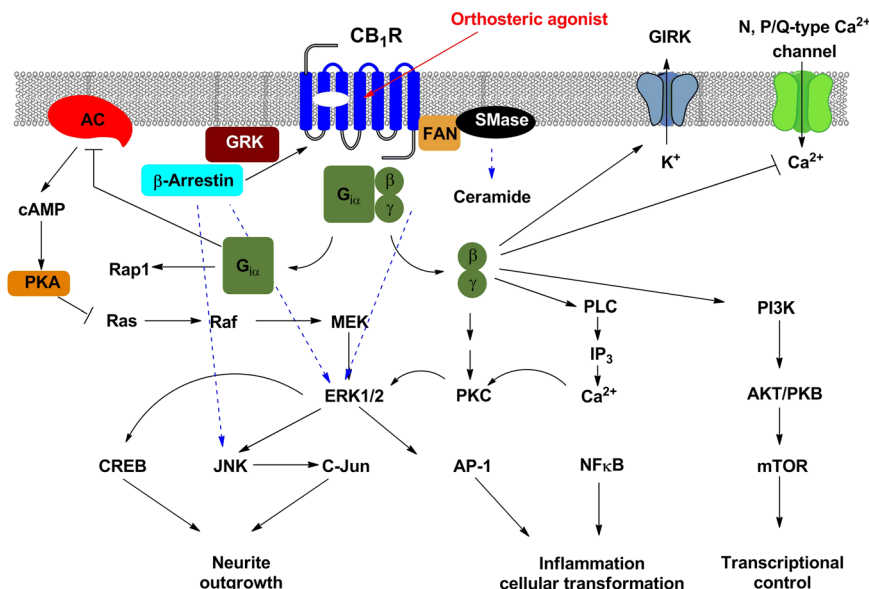
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Fig. 2 Signaling network of the CB$_1$ receptor. Upon activation, the CB$_1$ receptor elicits G-protein-dependent signal transduction, mainly through G$_{\alpha i/o}$-dependent inhibition of adenylyl cyclase (AC), G$_{\beta\gamma}$-dependent activation of PLC and PI3K, and G$_{\beta\gamma}$-dependent regulation of K$^+$ and Ca^{2+} channels. Following the CB$_1$-induced phosphorylation of GPCR kinases (GRKs), β-arrestins bind to the CB$_1$ receptor and mediate G-protein independent signal transduction. CB$_1$ also signals through other non-G protein partners, such as the adaptor protein, the factor associated with neutral sphingomyelinase (FAN). The CB$_1$ receptor may induce ERK activation through several different routes, including cAMP-dependent pathways, β-arrestin-dependent pathways, the activation of PI3K/PKB, transactivation of tyrosine receptor kinases, or the use of ceramide as the second messenger

protein kinases, CB$_1$ activation inhibits voltage-gated N- and P/Q-type calcium channels and activates A-type, and G-protein-coupled inwardly rectifying potassium channels (GIRK) [2]. Figure 2 illustrates the signaling network of the CB$_1$ receptor. Signal transduction mediated by the CB$_1$ receptor exhibits pluridimensional and spatiotemporal features.

Based on published data, researchers have postulated that CB$_1$-mediated signal transduction may occur in three different waves. The first is transient and mediated by heterotrimeric G-proteins (G$_{\alpha\beta\gamma}$). Afterwards, the second wave is mediated by β-arrestins. The third and final wave occurs in intracellular compartments and is elicited either by G-proteins or β-arrestins [28]. Without activation by agonists, the CB$_1$ receptor induces certain cellular responses through its constitutive activity, which is attenuated by CB$_1$ inverse agonists [12]. Under certain circumstances, CB$_1$ receptors exhibit functional selectivity between different cellular signaling pathways [29–32]. Functional selectivity (also known as biased agonism) refers to the ability of a ligand to stabilize a GPCR in a confirmation that preferentially binds to one or a subset of intracellular signaling proteins and selectively evokes certain response pathways over others [33–35]. For instance, the CB$_1$ ligands HU210, CP55,940, WIN55, 212-2, 2-AG and AEA elicit preferential interactions with different subtypes of G-proteins (i.e., G$_i$, G$_o$, G$_s$ and G$_{q/11}$, respectively) with varying efficacies and produce ligand-dependent functional selectivity [36–38]. Additionally, the CB$_1$ partial agonist Δ^9-THC and full agonist CP55,940 induce the internalization of CB$_1$ receptors through biased β-arrestin-2 recruitment [37]. Collectively, this evidence suggested that different CB$_1$ agonists stabilize different receptor conformations to induce functional selectivity in cellular responses. The complex signaling network of the CB$_1$ receptor indicates a necessity for special ligands that selectively transduce the signals required for the desired therapeutic effects while sparing the pathways linked to side effects. Although some orthosteric ligands have achieved ligand-dependent functional selectivity, allosteric ligands have been widely recognized and accepted as offering greater opportunities to produce functional selectivity than

orthosteric ligands [39, 40]. Allosteric modulators of a given receptor induce various receptor conformations that are distinct from the conformations stabilized by orthosteric ligands [35, 41]. Different conformations of a receptor impact the ability of the receptor to interact with its downstream effectors, leading to functional selectivity. Recently, the X-ray crystallographic structures of CB$_1$ receptors bound to agonists [42] and antagonists [43, 44] have been revealed. These ligand-bound CB$_1$ receptor structures are critical in providing insights into the molecular conformation of the active and inactive states of the CB$_1$ receptor while assisting the rational design of new CB$_1$ ligands.

ALLOSTERIC MODULATORS OF THE CANNABINOID CB$_1$ RECEPTORS

Historically, drug discovery programs aimed at regulating GPCR functions have been dominated by the identification of ligands to compete with endogenous ligands at the orthosteric sites. Recently, tremendous advances have been achieved in the discovery of ligands that regulate GPCR functions by binding to receptor sites that are topographically distinct from orthosteric sites, defining these compounds as allosteric modulators. Allosteric ligands of GPCRs induce and stabilize unique conformations of GPCRs and therefore provide fundamentally different receptors that are capable of exerting novel pharmacological effects. During the last 12 years, several structurally distinct molecules serving as allosteric modulators of the CB$_1$ receptor have been discovered. These compounds (shown in Fig. 3) include the small molecules 5-chloro-3-ethyl-N-(4-(piperidin-1-yl)phenethyl)-1H-indole-2-carboxamide (**10**, Org27569) [45], 1-(4-chlorophenyl)-3-(3-(6-(pyrrolidin-1-yl)pyridin-2-yl)phenyl)urea (**11**, PSNCBAM-1) [46], 3-(4-chlorophenyl)-5-(8-methyl-3-p-tolyl-8-azabicyclo[3.2.1]octan-2-yl)-isoxazole (**12**, RTI-371) [47], 6-methyl-3-(2-nitro-1-(thiophen-2-yl)ethyl)-2-phenyl-1H-indole (**13**, ZCZ011) [48], cannabidiol (**14**), and fenofibrate (**15**), as well as the endogenous molecules (5 S,6 R,9E,11Z,13E,15 S)-5,6,15-trihydroxyicosa-9,11,13-trienoic acid (**16**, lipoxin A$_4$) [49], pregnenolone (**17**) [50], and pepcan-12 (**18**) [51].
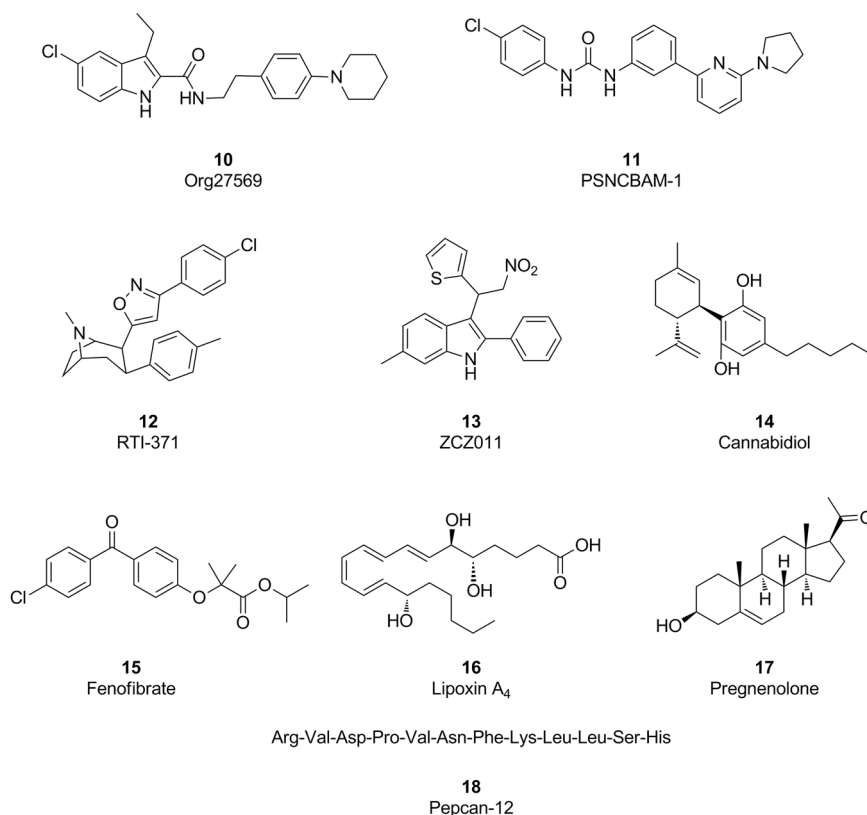
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Fig. 3 Structures of some representative CB$_1$ allosteric modulators

REPRESENTATIVE CB$_1$ ALLOSTERIC MODULATORS AND THEIR ALLOSTERIC PROPERTIES AT THE CELLULAR LEVEL

Exogenous allosteric modulators of the CB$_1$ receptor

Org27569. Org27569 (**10**, Fig. 3) is the first-in-its-class of CB$_1$ allosteric modulators. Ross and colleagues described its allosteric modulatory properties in 2005 [45]. In equilibrium binding assays, Org27569 significantly increased the binding of the CB$_1$ receptor agonist [^{3}H]CP55,940, suggesting positive binding cooperativity with the orthosteric ligand CP55,940. Simultaneously, Org27569 significantly, but not completely, reduced the specific binding of the CB$_1$ receptor inverse agonist [^{3}H] SR141716A, indicating a limited negative binding cooperativity. Its allosteric nature was further verified by studies of [^{3}H]CP55,940 dissociation kinetics. Paradoxically, its potentiation of agonist binding did not lead to an augmentation of agonistic effects induced by the same agonist. In contrast, Org27569 behaved as an insurmountable inhibitor of some CB$_1$ receptor functions and induced a significant reduction in the Emax values for some properties of CB$_1$ agonists (i.e., CP55,940 and WIN55,212-2). According to the results from several functional assays, including the reporter gene assay (i.e., the luciferase reporter assay, which determines the increase in luciferase expression induced by CB$_1$ agonist), the GTPγS binding assay and the mouse vas deferens assay, Org27569 functions as an inhibitor of some activities, and these findings identified the presence of allosteric sites on the CB$_1$ receptor for the first time [45]. One significant finding in the study of Org27569 is that this molecule induces a CB$_1$ conformation that promotes functional selectivity. Org27569 suppressed the CB$_1$ agonist (CP55,940)-induced G-protein-mediated phosphorylation of JNK while it promoted β-arrestin-1-mediated phosphorylation of ERK1/2 in HEK-hCB$_1$ cells either alone or after cotreatment with CP55,940 [52, 53]. In other words, Org27569 is a positive allosteric modulator (PAM) of certain

β-arrestin-mediated CB$_1$ signal transduction pathways, and inhibits G-protein-mediated CB$_1$ signal transduction.

PSNCBAM-1. Soon after the discovery of Org27569, Prosidon Ltd. (Oxford, UK) identified another CB$_1$ receptor allosteric modulator, PSNCBAM-1 (**11**, Fig. 3). PSNCBAM-1 is structurally unrelated to Org27569 and exhibits a diaryl urea scaffold [46]. The initial profile of this compound revealed that PSNCBAM-1 dose-dependently increased the binding of the CB$_1$ agonist [^{3}H]CP55,940 and significantly, but incompletely, decreased the binding of the CB$_1$ inverse agonist [^{3}H]SR141716A. Similar to Org27569, PSNCBAM-1 inhibited [^{35}S]GTPγS binding induced by the CB$_1$ agonist [^{3}H] CP55,940 [53]. It also inhibited Δ^9-THC-induced [^{35}S]-GTPγS binding [54]. In yeast reporter assays using the CB$_1$ receptor, PSNCBAM-1 blocked the agonistic effects of several CB$_1$ orthosteric agonists, including CP55,940, WIN55,212-2, AEA and 2-AG. In the constitutive yeast reporter assay, PSNCBAM-1 exhibited no intrinsic negative regulation of the constitutive activity of CB$_1$ receptor and did not behave as an inverse agonist like SR141716A. Similar to Org27569, PSNCBAM-1 promotes functional selectivity via the β-arrestin-1 mediated pathway, as evidenced in the study of its analogs in the ERK1/2 phosphorylation assay [55].

Following the identification of Org27569 and PSNCBAM-1, some functional assays have been employed to characterize the two compounds at molecular, receptor and cellular levels [53, 54, 56–60]. The assays employed in the characterization of these compounds include equilibrium binding, CB$_1$ agonist binding kinetics, CB$_1$ agonist-induced [^{35}S]GTPγS binding, the G$_i$-mediated inhibition of cAMP production and G$_s$-mediated stimulation of cAMP production, phosphorylation of ERK1/2, and β-arrestin recruitment, cellular hyperpolarization and receptor-internalization and desensitization. The allosteric modulation of the CB$_1$ receptor by Org27569 and PSNCBAM-1 showed a certain

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dependence on the orthosteric ligands, receptor sources, cell types, pathways and time frame over which the signaling responses occurred. Generally, Org27569 and PSNCBAM-1 show a similar pharmacological profile and behave as PAMs in potentiating the binding of the orthosteric CB$_1$ agonist (i.e., CP55,940) and function as inhibitors by antagonizing the CB$_1$ activity induced by various CB$_1$ agonists. Recent reviews have reported the multifaceted biochemical and pharmacological effects of these CB$_1$ allosteric modulators [61]. The data from those investigations confirmed that CB$_1$ allosteric modulators promote functional selectivity, an effect that is highly relevant for the discovery of new drugs with more defined pharmacology and improved drug safety.

ZCZ011. Immediately after the discovery of Org27569 and PSNCBAM-1, ZCZ011 was identified as a CB$_1$ allosteric modulator that behaves differently from Org27569 and PSNCBAM-1 [48]. This compound not only potentiates the binding of the CB$_1$ agonist CP55,940 but also enhances the binding of WIN55,212-2, a CB$_1$ orthosteric agonist with which Org27569 and PSNCBAM-1 did not show any binding cooperativity. ZCZ011 decreased the binding of the CB$_1$ inverse agonist SR141716A, similar to Org27569 and PSNCBAM-1. Unlike the Org27569- and PSNCBAM-1-mediated inhibition of CP55,940-induced [^{35}S]GTPγS binding to the CB$_1$ receptor, ZCZ011 enhanced [^{35}S]GTPγS binding to the CB$_1$ receptors stimulated by the CB$_1$ agonists CP55,940 and AEA in mouse brain membranes. This compound also increased AEA-induced β-arrestin recruitment and ERK1/2 phosphorylation induced by AEA and CP55,940 in CHO-hCB$_1$ cells. These properties established that ZCZ011 is a PAM of the CB$_1$ receptor at a functional level. Notably, ZCZ011 alone acted as an agonist and inhibited forskolin-stimulated cAMP production, while it did not significantly enhance the CP55,940- and AEA-induced inhibition of cAMP production at a test concentration of 1 μM.

Endogenous allosteric modulators of the CB$_1$ receptor
Following the discovery of small synthetic molecules that allosterically regulate the CB$_1$ receptor, several endogenous molecules were identified as CB$_1$ allosteric modulators. These molecules include pepcan-12 [51], lipoxin A$_4$ [49] and pregnenolone [50].

Pepcan-12. Pepcan-12 is an α-hemoglobin-derived peptide (pepcan) [51]. Within this group, pepcan-12 (a dodecapeptide) exhibits the properties of a negative allosteric modulator (NAM) of the CB$_1$ receptor, although previous reports indicated that it is a partial agonist of the CB$_1$ receptor [62]. In equilibrium binding studies, pepcan-12 showed saturable but incomplete displacement of the CB$_1$ orthosteric agonists [^{3}H]CP55,950 and [^{3}H]WIN55,212–2. In dissociation kinetic studies, pepcan-12 increased the dissociation constant of the CB$_1$ orthosteric agonist CP55,940. These results are consistent with the negative allosteric modulation of orthosteric agonist binding. Compared with the synthetic CB$_1$ allosteric modulators, endogenous pepcan-12 exhibited relatively high binding affinity for the CB$_1$ receptor (K$_i$ < 29 nM) [51, 62]. In functional assays, pepcan-12 reduced the efficacy of CB$_1$ receptor agonist-induced cAMP accumulation, [^{35}S]GTPγS binding, and CB$_1$ receptor internalization. The employed CB$_1$ agonists included WIN55,212–2, 2-AG and HU210. The negative modulation induced by pepcan-12 did not depend on the probe. Probe-dependence is one of the benefits of allosteric modulation, which allows a GPCR such as the CB$_1$ receptor to respond differently to the same allosteric modulator when different orthosteric ligands are bound. Pepcan-12 strongly decreases the efficacy, but not potency, of the endogenous CB$_1$ agonist 2-AG.

Lipoxin A$_4$. Following the identification of an endogenous NAM of the CB$_1$ receptor, an endogenous PAM of the CB$_1$ receptor (i.e., lipoxin A$_4$) was reported [49]. Lipoxin A$_4$ enhances the binding affinity of the CB$_1$ agonists [^{3}H]CP55,940, AEA and [^{3}H]WIN55,212–2. Lipoxin A$_4$ increased [^{3}H]CP55,940 binding by 100% and [^{3}H]WIN55,212–2 binding by approximately 30%, suggesting a probe dependence of the lipoxin A$_4$ effects. Based on the results of kinetic dissociation-binding studies, lipoxin A$_4$ slowed the rate of [^{3}H]CP55,940 displacement by competing with WIN55,212–2. This finding confirms the nature of lipoxin A$_4$ as an allosteric modulator. In CB$_1$ functional assays, lipoxin A$_4$ reduced [^{35}S]GTPγS binding induced by the CB$_1$ agonist AEA and yet strongly augmented the AEA-elicited inhibition of forskolin-stimulated cAMP production. Additionally, lipoxin A$_4$ potentiated the cannabinergic effects of AEA on mice. Lipoxin A$_4$ increased the AEA-induced cannabinergic effects (e.g., catalepsy) on wild-type (CB$_1$$^{+/+}$) mice, but not CB$_1$ knockout mice, indicating a CB$_1$-dependent mechanism of allosteric modulation. Thus, Lipoxin A$_4$ was characterized as a PAM of CB$_1$ at the molecular, receptor, cellular and whole animal levels.

Pregnenolone. In a study of the impact of the major classes of drugs of abuse on the production of neurosteroids in the rat and mouse brains, the production of pregnenolone was upregulated by Δ^9-THC. Moreover, pregnenolone functions as a signaling-specific CB$_1$ NAM [50]. Pregnenolone (at a concentration up to 100 μM) did not alter the equilibrium binding of the CB$_1$ agonists [^{3}H]CP55,940 and [^{3}H]WIN55,212–2, indicating a lack of binding cooperativity with orthosteric agonists. Pregnenolone binds to a distinct site located at the lipid face of the CB$_1$ receptor in the transmembrane TMH1/TMH7/Hx8 region. Binding to this region was validated by a mutant CB$_1$ receptor. Additionally, the binding of pregnenolone to the CB$_1$ receptor was supported by a concentration-dependent but incomplete displacement of [^{3}H]SR141716A. The TMH7/Hx8 region is located in the C-terminal region of the CB$_1$ receptor, the site at which CRIP1a has been proposed to interact with the CB$_1$ receptor and β-arrestins [26]. Pregnenolone may modulate the CB$_1$ receptor by interacting with CRIP1a. In CHO-CB$_1$ cells, pregnenolone decreased Δ^9-THC-induced ERK1/2 phosphorylation. Based on these findings, pregnenolone is characterized as an endogenous NAM of the CB$_1$ receptor. Its negative allosteric modulation was further confirmed through in vivo studies [50].

Recently, a follow-up investigation of pregnenolone and lipoxin A$_4$ was unable to validate their allosteric natures, except that the partial displacement of [^{3}H]SR141716A by pregnenolone was reproducible [57]. Therefore, the in vitro allosteric effects of pregnenolone and lipoxin A$_4$ require further investigation.

Miscellaneous allosteric modulators of the CB$_1$ receptor
Cannabidiol. Cannabidiol, a major ingredient of cannabis without psychoactive properties, exerts a variety of pharmacological effects [63, 64]. Unlike Δ^9-THC, cannabidiol does not bind to the orthosteric binding sites of the cannabinoid CB$_1$ and CB$_2$ receptors. However, it possesses receptor-dependent and receptor-independent pharmacological effects [63, 65, 66]. Recently, cannabidiol was shown to behave as a non-competitive NAM of CB$_1$ receptors [67]. It reduced the efficacy and potency of CB$_1$ agonists 2-AG and Δ^9-THC at inducing the phosphorylation of PLCβ3- and ERK1/2 in HEK293A cells (heterologously expressing CB$_1$) and in STHdh$^{Q7/Q7}$ cells (endogenously expressing CB$_1$). Cannabidiol reduced β-arrestin-2 recruitment, leading to a reduced efficacy and potency of Δ^9-THC and 2-AG in stimulating the internalization of CB$_1$ receptors. These data supported the hypothesis that cannabidiol functions as a NAM of the CB$_1$ receptor.

Fenofibrate. Cannabinoid compounds have been shown to interact with peroxisome proliferator-activated receptors (PPARs), members of the nuclear hormone receptor family [68]. For
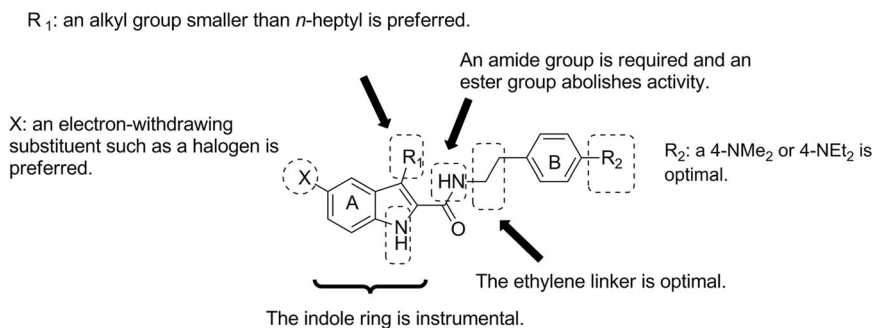
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Fig. 4 Summary of SARs for indole-2-carboxamides represented by Org27569

instance, AEA, Δ^9-THC, and WIN55,212–2 all function as PPAR-α agonists in vitro at concentrations higher than those required to activate cannabinoid receptors. Interestingly, one of the known PPAR-α receptor agonists, fenofibrate, exhibits allosteric modulation properties at high concentrations. It displaced the binding of the CB$_1$ agonist [^{3}H]-CP55,940 in an incomplete manner in CHO-hCB$_1$ cells. In functional assays, fenofibrate (at concentrations greater than 3.1 μM) dose-dependently reduces [^{35}S]GTPγS binding, ERK1/2 phosphorylation, and β-arrestin recruitment induced by the CB$_1$ agonist CP55,940, whereas it potentiates these effects at a concentration less than 3.1 μM [69]. Thus, fenofibrate functions as a bitopic ligand of the CB$_1$ receptor. At low concentrations, it functions as a partial agonist, whereas it acts as a NAM at high concentration.

RTI-371. In a study of a group of dopamine transporter inhibitors derived from the tropane scaffold, several compounds represented by RTI-371 showed the capability to potentiate induced calcium mobilization in CHO-hCB$_1$ cells induced by the CB$_1$ agonist CP55,940. Hence, RTI-371 was categorized as a PAM of CB$_1$ receptor. However, no other evidence was provided to support the claim and characterization of this compound as a CB$_1$ allosteric modulator.

RECENT DEVELOPMENTS IN CB$_1$ ALLOSTERIC MODULATORS
To date, approximately nine classes of CB$_1$ allosteric modulators have been identified. By far the best-characterized allosteric modulators of CB$_1$ receptors include the NAMs Org27569 and PSNCBAM-1, as well as the PAMs ZCZ011 and lipoxin A$_4$. Since the discovery of the nine scaffolds of CB$_1$ allosteric modulators (Fig. 2), studies in medicinal chemistry attempting to optimize these leads have mainly focused on the scaffolds of Org27569 and PSNCBAM-1. The results of their structure–activity-relationship (SAR) studies are summarized below, and representative molecules are illustrated.

SARs of indole-2-carboxamides represented by Org27569
Since the discovery of Org27569, several SAR studies have been performed on the indole-2-carboxamide scaffold [70–76]. The identified key structural requirements of indole-2-carboxamides for allosteric modulation of CB$_1$ receptors include several factors, as listed below. (1) The carboxamide functional group is required. When replaced by an ester group, the allosteric modulation of agonist binding is drastically altered, or the molecule becomes inhibitory rather than potentiating [71]. (2) The indole ring is critical. The replacement of this ring with other heteroaromatic rings, such as benzofuran or benzimidazole, abolishes or reduces the allosteric activity [72, 77]. (3) The length of the linker between the amide group and the aminophenyl ring is instrumental. Only an ethylene linker is tolerated, and any alteration in the linker length results in a complete loss of allosteric activity [74]. (4) The C3 alkyl group on the indole ring has a fairly substantial influence
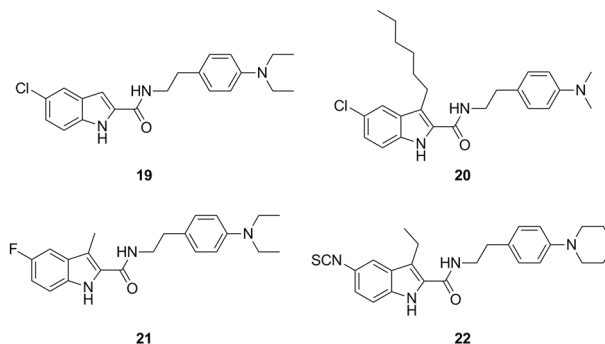

Fig. 5 Representative indole-2-carboxamide analogs of Org27569 with improved or comparable activities

on allostery, with a lower linear alkyl group preferred [72, 74]. (5) The NH of indole ring must be unsubstituted [70]. (6) The amino substituent on the phenyl ring impacts both the affinity and efficacy of allosteric modulation, with an *N,N*-dimethyl amino group showing improved allostery compared to a piperidinyl moiety [71, 74]. (7) The 5-position of the indole ring favors an electron-withdrawing group, with a preference for a halogen or an isothiocyanate group [71, 74, 76]. Figure 4 summarizes the SARs of this class of compounds. The representative members that emerged from SAR studies are presented in Fig. 5. These novel allosteric modulators include compounds **19** [73], **20** [72], **21** [73], and **22** [76].

SARs of diaryl ureas represented by PSNCBAM-1
Several SAR investigations have focused on the substituents, the major linker bond and the heteroaromatic ring of the molecule to optimize the scaffold of diaryl ureas represented by PSNCBAM-1 [55, 76, 78, 79]. The key findings are listed below. (1) An electron-withdrawing substituent is optimal at the 4-chlorophenyl position, with a cyano group being more potent than other substituents. (2) The urea skeleton is instrumental, and its replacement with a carbamate, methylated urea, or amide group abolishes the allosteric properties. (3) The pyridine ring is not necessary and can be replaced by a phenyl ring. (4) The pyrrolidinyl ring position can tolerate replacement by tertiary amino groups, such as dialkylamino, piperidinyl groups or other cyclic rings, with some size limitation. The SARs of this class of compounds are summarized in Fig. 6. The representative members that emerged from SAR studies are presented in Fig. 7. These novel allosteric modulators include compounds **23** [78], **24** [79], **25** [55], and **26** [55].

Development of new ZCZ011 analogs
Recently, a close analog of ZCZ011, GAT211 (**27**, Fig. 8), was synthesized and reported to exert pharmacologic effects similar to ZCZ011 at the molecular, receptor and cellular levels [80]. ZCZ011

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and GAT211 are racemates. Chiral resolution of GAT211 led to a pair of optically pure enantiomers of GAT211. Interestingly, the (R)-enantiomer GAT228 (**28**, Fig. 8) behaves as an allosteric partial agonist of the CB$_1$ receptor, while the (S)-enantiomer GAT229 (**29**, Fig. 8) functions as a pure PAM [80, 81]. GAT229 functions
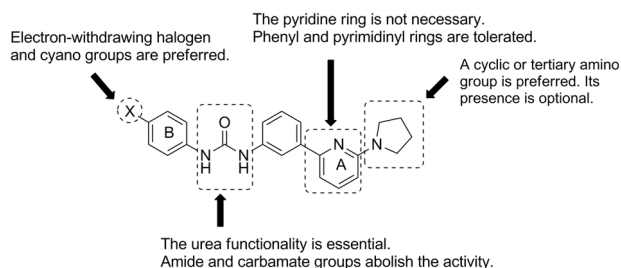

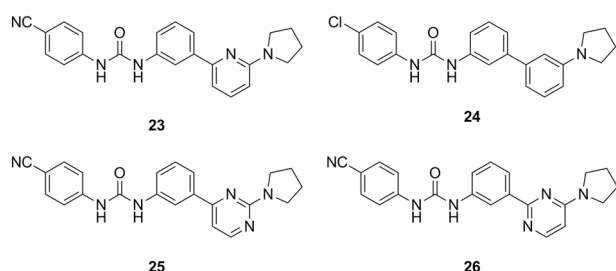**Fig. 6** Summary of SARs for diaryl ureas represented by PSNCBAM-1

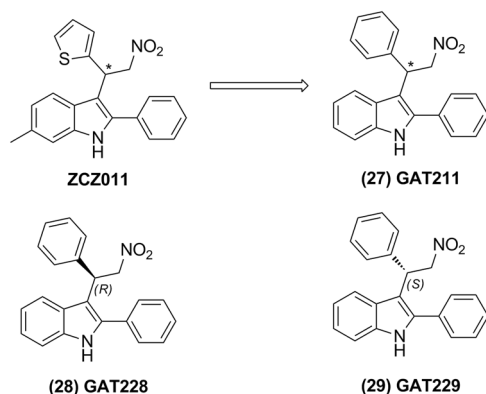**Fig. 7** Representative diaryl urea analogs of PSNCBAM-1 with improved or comparable activities

Fig. 8 Representative analogs of the CB$_1$ allosteric modulator ZCZ011

as a biphasic ligand: at high concentrations, it competes with orthosteric agonists, while at low concentrations, it behaves as an allosteric modulator [80]. A recent communication describing the binding sites of this class of compounds reported a similar biphasic mechanism for the allosteric ligands GAT228 and ZCZ011 [82]. Based on these data, 2-phenylindole analogs are likely able to bind to multiple sites on the CB$_1$ receptor. The mechanism of allosteric modulation may depend on the drug concentration and the presence of an orthosteric agonist. When an orthosteric agonist is not bound to the receptor, these compounds function as an orthosteric ligand, while they function as allosteric modulators by binding to a distinct site when an orthosteric agonist binds to the receptor [82].

Currently, the reports of ZCZ011 analogs are fairly limited [83, 84]. However, a preliminary SAR can be inferred. The SARs of ZCZ011 analogs are summarized below and illustrated in Fig. 9. The key SAR includes the factors listed below. (1) The 2-aryl indole skeleton is essential for allosteric modulation effects. (2) The 6-methyl group is influential, and its replacement with a chloro group or a hydrogen is tolerated, but the compound exhibits reduced activity. (3) The disubstituted ethyl group at the 3-position of the indole ring is instrumental. Its thiophene group can be replaced by a pyridine ring, while its replacement with a phenyl ring reduces the activity. The 2-nitro group is fairly resistant to modification. Only a cyano group (CN) or a trifluoromethyl group (CF$_3$) is tolerated. The replacement of the nitro group with a carboxylic acid (COOH), an ester (COOR), an amide (CONH$_2$) or an amino group (NH$_2$) abolishes the activity. (4) The 2-phenyl moiety can be substituted at the para-position with halogens. The racemic nature of this class of compounds becomes challenging for SAR investigations since the (R)- and (S)-isomers of GAT211 function differently in allosterically modulating the CB$_1$ receptor [80, 81].

THERAPEUTIC RELEVANCE OF CB$_1$ ALLOSTERIC MODULATORS
While CB$_1$ orthosteric agonists and antagonists have traditionally been pursued to target cannabinoid receptors, findings show untoward side effects that are unacceptable for chronic applications in clinical settings. Generally, allosteric modulators provide several mechanism-based advantages to overcome the on- and off-target side effects and increase drug safety and specificity. First, they offer the potential for better receptor subtype selectivity because of the greater structural variance in allosteric sites than the orthosteric sites, which generally are highly conserved. Second, allosteric modulators without intrinsic activity selectively exert biological responses only in tissues where the endogenous ligands are present and function. Third, once completely occupied, the allosteric sites produce a saturation effect and subsequently limit the effect of the allosteric modulator on the response induced by the orthosteric ligand. This "ceiling" action prevents
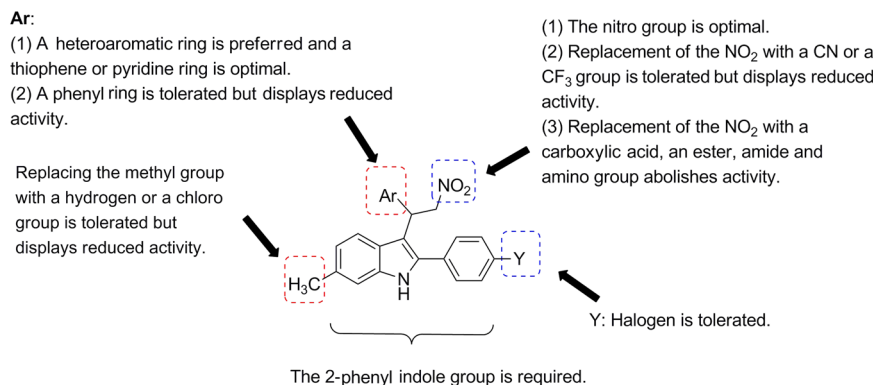

Fig. 9 Summary of SARs for 2-phenyl indoles represented by ZCZ011

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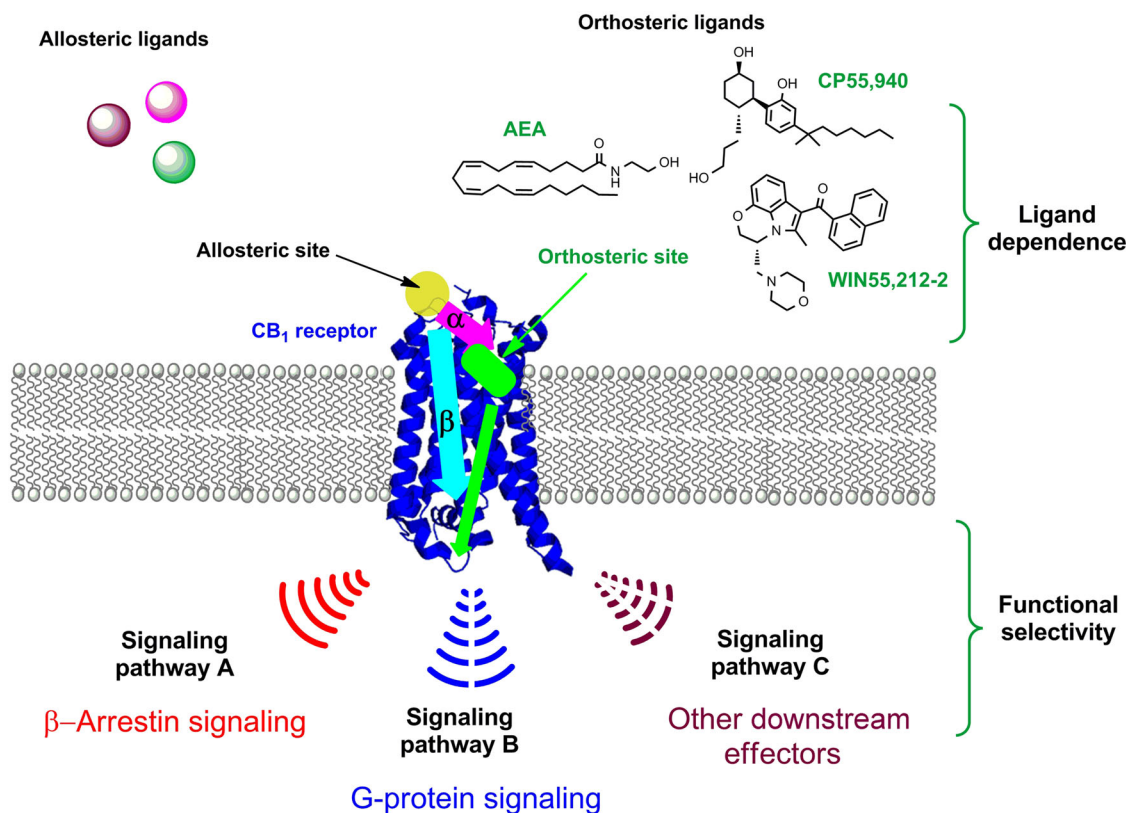D Lu et al.

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Fig. 10 Schematic illustrating GPCR allosteric modulation and functional selectivity. Allosteric modulators alter the binding affinity (**a**: pink arrow) and/or efficacy (**b**: cyan arrow) of orthosteric ligands in a positive (PAM) or negative manner (NAM). Allosteric modulation often shows ligand dependence (i.e., different orthosteric ligands induce different forms of allostery). An allosteric modulator induces the receptor to adopt various conformations that preferentially activate either G-protein-mediated or a β-arrestin-mediated signal transduction. Other downstream effectors may also be preferentially involved in signaling pathways at the expense of others (functional selectivity). The resulting functional selectivity might generate a pharmacologically improved therapeutic effect, with reduced on-target adverse effects compared to the orthosteric ligand

drug overdose. More importantly, allosteric modulators enable the fine-tuning of receptor pharmacology through ligand-dependent signaling and functional selectivity. This approach may facilitate signal transduction through the pathways that are more therapeutically relevant while failing to activate pathways involved in the untoward effects. The discovery of CB$_1$ allosteric modulators has increased the number of approaches by which the functions of the CB$_1$ receptor are manipulated for potential therapeutic benefits with the aim of improving the pharmacology and drug safety compared with traditional orthosteric CB$_1$ ligands. Figure 10 illustrates the possible allostery-engendered functional selectivity of the CB$_1$ receptor. The in vitro characterization of CB$_1$ allosteric modulators has provided the foundation for the investigation of the therapeutic values of positive and negative allosteric modulators of the CB$_1$ receptor.

The evidence from CB$_1$ positive allosteric modulators
Theoretically, PAMs function through permissive augmentation of the tone from the endogenous agonists and/or by stabilization of novel receptor conformations that have a preference for certain endogenous ligands. These effects may lead to biased signaling toward the upregulation of the preferred response induced by the endogenous agonists that cooperate with the allosteric modulator.

The first evidence of some therapeutic value of a CB$_1$ allosteric modulator was obtained from the in vivo studies of the CB$_1$ PAM lipoxin A$_4$. Intracerebroventricular (i.c.v.) injections of lipoxin A$_4$ in wild-type mice potentiate the cataleptic effects of the endogenous CB$_1$ agonist AEA. This potentiation was not observed in CB$_1$

knockout mice. Hence, the response is a CB$_1$-dependent allosteric modulatory effect. Because AEA exerts protective effects against pathogenic amyloid-β induced neurotoxicity [85, 86], the neuroprotective effects of lipoxin A$_4$ on amyloid-β induced spatial memory impairments were assessed using the Morris water maze test. Lipoxin A$_4$ provides the CB$_1$ receptor-dependent protection against pathogenic β-amyloid-induced memory impairments [49].

More convincing evidence was provided by the investigation of the effects of the CB$_1$ PAM ZCZ011 on wild-type and CB$_1$ knockout mice [48]. The administration of ZCZ011 alone does not produce any cannabinergic effects, such as catalepsy, hypothermia, antinociception and decreased locomotion. In contrast, ZCZ011 significantly potentiated the antinociceptive, cataleptic and hypothermic effects elicited by the CB$_1$ orthosteric agonist CP55,940. It also augmented the AEA-induced hypothermia, but did not alter the AEA-induced antinociceptive and cataleptic effects on FAAH knockout mice. These findings suggested a functional selectivity mediated by the allosteric modulator. In a drug discrimination assay, ZCZ011 significantly enhanced the potency of the discriminative stimulus effects of AEA on FAAH knockout mice. Additionally, ZCZ011 reverses nociceptive behaviors in well-established murine models of neuropathic and inflammatory pain. ZCZ011 blocks mechanical and cold allodynia for a fairly long period, without the development of tolerance. The compound does not produce conditioned place preference or aversion. The antiallodynic effects of ZCZ011 depend on the CB$_1$ receptor.

Recently, another PAM of the CB$_1$ receptor, GAT211, was shown to suppress the inflammatory nociception induced by complete

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Freund's adjuvant (CFA) and the neuropathic pain evoked by the cancer chemotherapeutic agent paclitaxel [87]. Antiallodynic effects of GAT211 were observed on wild-type but not CB$_1$ knockout mice, suggesting that the allosteric modulation depended on the CB$_1$ receptor. GAT211 produced synergistic antiallodynic effects with the inhibitors of FAAH and MGL on paclitaxel-treated mice. It also synergized with the orthosteric agonist WIN55,212–2 to reduce CFA-induced mechanical allodynia. Its therapeutic efficacy persisted for 19 days of chronic dosing with GAT211, whereas it was not preserved with the MGL inhibitor tested in the same study. Cannabimimetic withdrawal precipitated by the CB$_1$ antagonist SR141716A was observed in mice chronically treated with the orthosteric agonist WIN55,212–2, but not in mice treated with GAT211. The PAM GAT211 alone did not produce cannabinergic effects or other cardinal signs of direct CB$_1$ activation in the presence or absence of pathological pain. Similar to ZCZ011, GAT211 did not induce conditioned place preference or aversion in the test animals.

The positive CB$_1$ allosteric modulator GAT229 was effective at decreasing intraocular pressure (IOP) in ocular normotensive and ocular hypertensive nee mice [88]. In normotensive mice, the topical administration of GAT229 alone did not appear to exert any effect on reducing the IOP. This finding probably was due to insufficient local endocannabinoid concentrations at the site of action. However, the effect of a subthreshold concentration of WIN55,212–2 on decreasing IOP was potentiated by GAT229. In contrast, the topical administration of GAT229 alone in nee mice with ocular hypertension was sufficient to reduce the IOP. The authors did not clearly determine whether the levels of endocannabinoids at the site of action in nee mice were due to pathological conditions.

The results from recent in vivo studies of the effects of lipoxin A$_4$, ZCZ011, GAT211, and GAT229 provided compelling evidence that CB$_1$ PAMs show promise as potential therapeutics for neurodegenerative diseases, neuropathic and inflammatory pain, and glaucoma. In particular, the outcomes from studies of ZCZ011 and GAT211 suggested that CB$_1$ PAMs may lack significant abuse liability and other CNS side effects associated with orthosteric CB$_1$ agonists.

The evidence from CB$_1$ NAMs
Theoretically, the NAMs of the CB$_1$ receptor should be capable of downregulating the endocannabinoid tone or inducing a conformation that decreases or abolishes the constitutive activity of the CB$_1$ receptor to produce pharmacological effects. CB$_1$ antagonism or inverse agonism shows promising therapeutic effects on obesity, obesity-related metabolic syndromes, and substance abuse [11, 89]. Several preclinical studies have assessed the therapeutic potential of the CB$_1$ NAMs Org27569, PSNCBAM-1, and pregnenolone.

As expected for a negative CB$_1$ allosteric modulator, the administration of Org27569 alone does not elicit cannabimimetic effects [90]. However, this compound does not exhibit efficacy in attenuating the antinociceptive, cataleptic, and hypothermic effects produced by the orthosteric CB$_1$ agonists AEA, CP55,940, and Δ^9-THC, indicating a lack of negative allosteric modulation. In the drug discrimination paradigm, Org27569 was not able to substitute for either CB$_1$ agonist AEA or Δ^9-THC and did not modify the discriminative stimulus effects of any of the two CB$_1$ orthosteric agonists. Notably, Org27569 produced small but statistically significant increases in the potency of AEA-elicited catalepsy and antinociception in FAAH knockout mice [90], suggesting weak positive allosteric modulation. However, this potentiation was not observed for AEA-induced hypothermia. The aforementioned results indicated the ligand-dependent allostery and functional selectivity of a CB$_1$ allosteric modulator. In the same study, Org27569 reduced food intake by both wild-type and CB$_1$ knockout mice, while the orthosteric CB$_1$ antagonist

SR141716A (rimonabant) only reduced food intake by wild-type mice [90]. Thus, the anorectic effects of Org27569 most likely were not mediated by a CB$_1$-dependent mechanism. The results from this array of studies implied that the well-characterized CB$_1$ negative allosteric properties of Org27569 obtained from in vitro studies were not translated into the pharmacological effects on the downregulation of the CB$_1$ receptors in mice.

In a preclinical study of Org27569 using rats, the compound showed mixed effects [91]. It did not markedly alter the body temperature alone, but significantly attenuated the hypothermic effect induced by the CB$_1$ agonists CP55,940 and AEA, suggesting negative modulation. Pretreatment with Org27569 did not significantly alter the cataleptic and antinociceptive effects induced by CP55,940, whereas the CB$_1$ antagonist/inverse agonist SR141716A significantly decreased CP55,940-induced catalepsy and nociception, suggesting a lack of modulation on these CB$_1$ mediated cannabinergic effects. SR141716A precipitates Δ^9-THC withdrawal in mice and rats [91]. Typical withdrawal behavior was observed, including an increase in paw tremors and head shakes, accompanied by a decrease in normal behaviors such as grooming and scratching. Org27569 alone did not elicit increased grooming and scratching behaviors and did not significantly alter the grooming and scratching behaviors induced by SR141716A, which was shown to significantly increase the frequency of scratching and grooming. Based on these results, Org27569 does not show the pharmacological effects associated with the downregulation of CB$_1$ receptor, and it functions differently from the orthosteric CB$_1$ antagonist/inverse agonist SR141716A (rimonabant). Surprisingly, Org27569 produced hypophagic effects similar to the CB$_1$ antagonist SR141716A. It reduced food intake and body weight in rats, with outcomes similar to SR141716A. However, whether the hypophagic effects of Org27569 depend on the CB$_1$ receptor mediation remains unknown due to lack of CB$_1$ knockout rat models [91]. A follow-up investigation of Org27569 in rats found that pretreatment with Org27569 dose-dependently attenuated both cue- and drug-induced reinstatement of cocaine- and methamphetamine-seeking behaviors. SR141716A also exerted similar inhibitory effects on the reinstatement of drug-seeking behaviors [92]. Thus, Org27569 downregulates the CB$_1$ receptor in rats by functioning as a CB$_1$ inverse agonist. In the study described above, Org27569 was screened at a concentration of 10 μM to evaluate its binding selectivity toward more than 40 GPCRs, including some receptors associated with drug addiction. Org27569 showed high selectivity for CB$_1$ compared with the screened panel. The highly selective binding profile of Org27569 suggested that its effects on relapse to psychostimulant seeking behaviors are likely mediated by negative modulation of CB$_1$ receptors. The results warrant further investigations of the utility of CB$_1$ NAMs to treat substance addiction.

Similarly, another allosteric modulator of the CB$_1$ receptor, PSNCBAM-1, exerted anorectic effects on rats, without any obvious adverse effects on animal behaviors or signs of toxicity under the assessment conditions [46]. However, in a recent study, the anorectic effects of PSNCBAM-1 were not observed in wild-type mice [54]. In the same study, the compound was ineffective at attenuating the reduced locomotion, catalepsy, and hypothermia induced by CB$_1$ agonist Δ^9-THC. Similar to Org27569, PSNCBAM-1 exhibited a modest but statistically significant reduction in the antinociceptive effects induced by the CB$_1$ agonist Δ^9-THC [54]. The above results again revealed the functional selectivity of a CB$_1$ NAM. In addition to the anorectic effects, other potential therapeutic effects of PSNCBAM-1 are emerging, such as possible neuroprotective effects mediated by the inhibition of 2-AG-mediated depolarization-induced suppression of excitation (DSE) [93], and its effects on altering neuronal excitability [58].

The unexpected anorectic efficacy of Org27569 in CB$_1$ knockout mice should not discourage the investigation of the effects of CB$_1$

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allosteric modulators on feeding behaviors. A careful interpretation of the results is necesssssary. First, the two compounds exhibited a relatively weaker ability to interact with CB$_1$ receptors than the CB$_1$ inverse agonist SR141716A, as reflected by their equilibrium dissociation constants (i.e., K_B, 217 nM for Org27569 and 54 nM for PSNCBAM-1) [55, 72], and the binding affinity of SR141716A (i.e., $K_i = 1.98$ nM). Some CB$_1$-independent anorectic response may be triggered in wild-type and CB$_1^{(-/-)}$ mice at the tested doses. Second, the two compounds have very low solubility. Further studies are needed to determine whether the two CB$_1$ allosteric modulators are able to be sufficiently delivered to their sites of action in animals. In in vivo experiments, the potency at which the drug binds the receptor, as well as the absorption, distribution, metabolism, and excretion (ADME), impact the pharmacological effects. The investigations of feeding behaviors in mice treated with Org27569 and PSNCBAM-1 indicated that more potent CB$_1$ allosteric modulators and preclinical studies of their ADME properties are needed to validate the target.

Unlike Org27569 and PSNCBAM-1, which did not attenuate the complete spectrum of the cannabinergic effects induced by CB$_1$ agonists, the CB$_1$ NAM pregnenolone exhibited the capability to attenuate the locomotor suppression, hypothermia, catalepsy, and antinociception induced by Δ^9-THC [50]. Furthermore, pregnenolone suppressed the Δ^9-THC-induced increase in food consumption in rats and mice. This NAM ameliorates the Δ^9-THC-induced memory impairments in mice and blocks the Δ^9-THC-induced release of glutamate and dopamine. The compound was also capable of blocking and reinforcing the effects of cannabimimetic drugs [50]. Very recently, pregnenolone was shown to block a wide spectrum of Δ^9-THC-induced endophenotypes in mice that are typically associated with psychotic-like states [94]. Collectively, the results from preclinical studies of pregnenolone suggested that pregnenolone-based NAMs of CB$_1$ receptor represent promising new approaches for the treatment of disease states associated with overactive CB$_1$ receptors.

The results from recent in vitro and in vivo studies of the allosteric modulators Org27569, PSNCBAM-1 and pregnenolone revealed new avenues to downregulate the endocannabinoid tone and achieve therapeutic benefits in the areas of substance addiction, neurological disorders and obesity-related diseases.

CLOSING REMARKS
Since the discovery of the first CB$_1$ allosteric modulator Org27569 in 2005, nine classes of structurally diverse chemical entities have been identified as CB$_1$ allosteric modulators. Accumulating evidence from in vitro and preclinical studies have confirmed their capabilities to either positively or negatively regulate the functions of the cannabinoid CB$_1$ receptor. Some of the CB$_1$ allosteric modulators selectively manipulate signaling pathways downstream of cannabinoid CB$_1$ receptors through functional selectivity. These properties of allosteric modulators suggest that they possess tremendous potential to separate the therapy-relevant CB$_1$ responses from CB$_1$ responses that cause untoward side effects. This goal has been difficult or impossible to achieve using orthosteric CB$_1$ ligands, but may be achievable with CB$_1$ allosteric modulators, as evidenced for other GPCRs, such as the angiotensin II receptor [95]. However, the links between the specific pathways of the CB$_1$ receptor and the untoward side effects have not yet been elucidated. Therefore, advancements in the discovery of novel and more potent CB$_1$ allosteric modulators are needed; these molecules represent potentially powerful tools for studies aiming to identify the signaling factors that cause the side effects mediated by activation or downregulation of the CB$_1$ receptor. By employing the advantages of the well-established therapeutic benefits obtained from CB$_1$ orthosteric agonists

and antagonists, positive and negative allosteric modulators hold stronger promise to deliver cannabinoid-based medications that exhibit improved specificity, efficacy and drug safety.

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