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Recent Advances in the Realm of Allosteric Modulators for Opioid Receptors for Future Therapeutics

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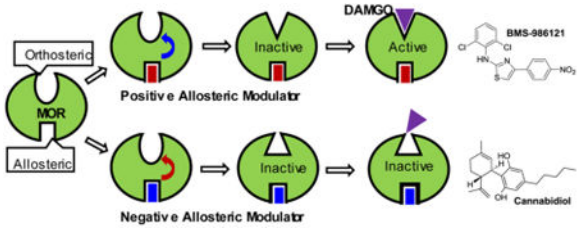
Abstract

Opioids, and more specifically μ-opioid receptor (MOR) agonists such as morphine, have long been clinically used as therapeutics for severe pain states but often come with serious side effects such as addiction and tolerance. Many studies have focused on bringing about analgesia from the MOR with attenuated side effects, but its underlying mechanism is not fully understood. Recently, focus has been geared toward the design and elucidation of the orthosteric site with ligands of various biological profiles and mixed subtype opioid activities and selectivities, but targeting the allosteric site is an area of increasing interest. It has been shown that allosteric modulators play key roles in influencing receptor function such as its tolerance to a ligand and affect downstream pathways. There has been a high variance of chemical structures that provide allosteric modulation at a given receptor, but recent studies and reviews tend to focus on the altered cellular mechanisms instead of providing a more rigorous description of the allosteric ligand's structure–function relationship. In this review, we aim to explore recent developments in the structural motifs that potentiate orthosteric binding and their influences on cellular pathways in an effort to present novel approaches to opioid therapeutic design.

Graphical abstract



Keywords

Allosteric modulation; Opioid receptors; Cannabinoid receptors; Sigma receptors; Heterodimers; Pain therapeutics; Opioid side effects

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Introduction

The μ-opioid receptor (MOR) is the target for most clinically available opioid drugs of which the majority are small molecules (morphine, codeine, fentanyl, etc.) that bind to the orthosteric site. It is a prominent pharmacological target for both acute and chronic pain states but has also been implicated as being a viable treatment for mood disorders.[1] These agonists bind to the orthosteric site of the MOR, bringing about analgesia, but also come with serious side effects such as tolerance, respiratory depression, nausea, constipation, allodynia, and addiction through direct receptor activation in acute and/or chronic administration scenarios.[2–4] Because of the presence of these detrimental effects, the opioid receptors, and more specifically the MOR, has been scrutinized to better effectively bring about pain modulation with the idea of adverse effect attenuation or ablation. Various approaches have been underway to circumvent this issue: (i) biased ligands,[5] (ii) multifunctional (or multivalent) ligands,[6,7] (iii) partial agonists due to having reduced efficacy relative to full agonists,[8] and (iv) coadministration of multiple agonistic and/or antagonistic compounds.[9] Although these approaches have afforded ligands with improved side effect profiles, they all interact with the orthosteric site. As of late, momentum has been shifting toward generating allosteric and bitopic ligands as opposed to orthosteric ligands.[10]

The opioid receptors are class A G-protein-coupled receptors (GPCRs) that contain seven hydrophobic transmembrane domains and elicit their signaling through heterotrimeric G-proteins that reside on the intracellular surface to bring about a plethora of signaling cascades and responses.[11] This receptor type is targeted by approximately 30–40% of currently marketed drugs attributed to their direct involvement in disease states and high potential druggability, for both cellular location and distribution, and receptor variance, allowing for selective therapeutics.[12] A wide array of stimuli interact with GPCRs including small molecules, lipids, amino acids, and peptides, which upon binding induce a ligand-dependent conformational change in the receptor, and depending on the nature of the ligand, recruit certain proteins or activate others. Opioid receptors and other GPCRs can also bring about cellular responses through pathways independent of G-protein-related signaling such as those originating from β-arrestin. This protein plays an important role in receptor desensitization and internalization, and it is theorized that biased signaling toward the G-protein-mediated pathway as opposed to the β-arrestin route may be clinically beneficial considering it was shown that β-arrestins negatively impact antinociception and are correlated with MOR-related side effects.[13–16] The three classic opioid receptor types are the μ-opioid receptor, δ-opioid receptor (DOR), and κ-opioid receptor (KOR). These receptors share approximately 60% sequence homology, mostly within the receptor's transmembrane domains, and signal through $G_{i/o}$, which leads to the inhibition of cyclic adenosine mono-phosphate (cAMP) formation from ATP, a reaction catalyzed by the enzyme adenylyl cyclase, and ultimately leads to the inhibition of cyclic adenosine, impacting the activities of ion channels and gene transcription.[17]

Numerous drugs target the orthosteric site (the location at which the endogenous ligand binds) and activate (agonist) or inhibit (antagonist) the receptor's activity. However, focus has been shifting toward the development of therapeutic compounds that target the allosteric site(s) of a receptor and potentiate the desired effect of the orthosteric ligand without

eliciting activity on its own.[18] The allosteric site can be found within the nonconserved regions of a receptor, allowing for enhanced selectivity among GPCR receptor subtypes and thus restricting off-target activity, giving attenuated side effects compared to their orthosteric ligands. Because of there being a lack of evolutionary similarity, these allosteric sites can be species- and receptor-dependent, and thus a compound that shows great promise in mouse models may not be translatable to humans. It is important to understand that the allosteric site does not interact with the orthosteric ligands. This characteristic enables drug discovery programs to bypass some of the major hurdles when dealing with selectivity. When it comes to dealing with multiple receptors that interact with the same endogenous ligand(s), it is typically found that the orthosteric sites between the receptors share high sequence homology in the region(s) deemed critical for ligand–receptor interaction and to which additional detrimental effects are often attributed. Furthermore, this provides a fresh start in exploring chemical space/scaffolds that target this site. In addition to this benefit, allosteric ligands can preserve the temporal and spatial fidelity of receptor activity in vivo by elucidating an effect only in the presence of an orthosteric ligand in a desired region. Particularly when dealing with opioids in which the central nervous system (CNS) is primarily targeted as opposed to the periphery, neuronal circuitry is tightly regulated with the triggered release of neurotransmitters in an exact and timely manner. Systemic administration of a therapeutic orthosteric compound can cause broadened site activation in extraneous tissues, which can dramatically hinder the therapeutic potential. Furthermore, orthosteric ligands constitutively activate a receptor enabling receptor desensitization and internalization, which correlate with tolerance and dependence, not to mention increased toxicity. Off-target side effects could be avoided as the allosteric ligand would only act in cells where innate opioid signaling occurs with the respective endogenous ligand.

Functional selectivity, or bias signaling, has recently drawn significant attention as a plethora of research cohorts have attempted to elucidate the various ligand-initiated signaling pathways, especially in the opioid arena, and yet there is a paucity of definitiveness when it comes to the underlying mechanisms. It has been shown that agonists can influence β-arrestin pathways, which have a tendency to result in receptor internalization and recyclization, receptor phosphorylation, impact the actions of ion channels, and mediate adenylyl cyclase.[19–25] Allosteric compounds can produce bias signaling by differentially controlling signaling pathways.[24] Allosteric modulators can mediate signaling away from pathways that control unwanted side effects, and considering that these influences are restricted by their ceiling cooperativity threshold with the orthosteric ligand, can further decrease overdose liability.[26,27] In particular, in circumstances where a receptor's endogenous ligand is not yet known, allosteric modulation can be particularly effective. Allosteric modulators do exhibit probe dependence, meaning that they show different activities depending on the orthosteric agonist.[28,29] In receptor systems such as the opioid receptors, where multiple endogenous ligands have been discovered, along with biologically active metabolites of the endogenous ligand(s) and where marked variance of orthosteric ligand scaffolds are observed (small molecule alkaloids to peptides of various length such as the enkephalins to β-endorphin), the complexity of modulator activity elucidation can pile up quickly as the same allosteric modulator can have a range of activities for multiple ligands. (Figure 1)

Considering allosteric modulators can result in a range of actions at the targeted receptor, they are divided into categories depending on their ability to modulate activity. Negative allosteric modulators (NAMs) may have no standard activity, but when bound to the target receptor can inhibit the binding of orthosteric ligands and/or lessen the efficacy of the receptor's orthosteric ligand (Figure 2). This reduction is typically seen in finite rightward shifts of the orthosteric ligand's potency/efficacy.[30] At the point at which modulator concentration has saturated the allosteric sites on all of the receptors, no decrease in functional potency or efficacy by the orthosteric ligand is seen despite increasing the modulator's concentration. Silent allosteric modulators (SAMs), also referred to as neutral allosteric ligands, do not interfere with the orthosteric ligand's ability to bind or produce a signal but competitively antagonize the allosteric pocket, inhibiting other positive allosteric modulator (PAM) or NAM activity. Lastly, allosteric ligands can generate receptor signaling in the presence of the orthosteric ligand. PAMs have garnered the most attention in the literature as they potentiate the orthosteric ligand's binding affinity and/or efficacy but can have no inherent effect on its own in the absence of the orthosteric ligand. These results can be achieved by promoting specific conformations for orthosteric binding or reducing the dissociation rate of the ligand. This usually shows as a finite leftward shift in the functional potency/efficacy of the orthosteric ligand. Similar to the NAMs' effect, this finite shift can be taken advantage of by designing PAMs that are unable to shift potency and/or efficacy past the desired range. This has significant implications for enabling orthosteric ligands to have decreased toxicity and patient risk of overdosing. Because of the nature of PAMs, the temporal and spatial fidelity of cell signaling is maintained in vivo and receptor downregulation can be circumvented along with other mechanisms that are triggered upon constitutive activation from orthosteric agonists. This can potentially lead to decreased tolerance and addiction compared to when only the orthosteric agonist is administered. It has been surmised that coadministration of a receptor agonist with a PAM at a lower dose rather than just administration of orthosteric agonist can result in the same functional activity. This has been partly verified by coadministration of an agonist with a PAM for the $GABA_B$ receptor resulting in a decreased level of receptor desensitization but the same level of functional activity compared to those of a higher dose of orthosteric agonist and thus may potentially be applied to opioid systems.[31]

Herein, we scrutinize the recent literature regarding opioid allosteric modulators from a structural perspective and their ability to affect the affinity of orthosteric ligands and influence signaling pathways. Furthermore, the arena of opioid receptor heteromers is traversed, and the additional complexities of allosteric modulation that is inherent in heteromeric complexes is scrutinized. The hallmark benefits of targeting the allosteric site for novel therapeutic approaches is quite evident in the numerous studies explored and is a testament to the growing consideration and interest in this field.

MOR

The MOR has been the classic therapeutic target for pain treatment. Mice that have had the MOR gene ablated show a loss of morphine-induced antinociception.[32] Clinically available MOR agonists such as morphine, codeine, and fentanyl bind to the orthosteric site and produce antinociception but also result in serious side effects such as tolerance, respiratory

depression, and addiction.[2–4] These side effects are reversed when MOR antagonists are administered and are not observed in MOR-knockout animals, showing that these effects indeed originate from the MOR.[32] Thus, the current paradigm is the development of powerful analgesics at the MOR but in such a way that the side effects are greatly attenuated or nonexistent. Multifunctional ligands that target multiple opioid receptors and the use of partial agonists are examples of ways that this can be achieved, but targeting the allosteric site may provide an additional approach and is inherently more advantageous to the biological system.

Before the MOR PAMs, two opioid NAMs were discovered. Cannabidiol (Figure 3), a cannabinoid type-1 receptor (CB1) agonist, was identified as a NAM of MOR and DOR agonists.[33] Salvinorin A, a potent KOR agonist, was also identified as a NAM of MOR in a high-throughput screen using a β-arrestin recruitment assay.[34] Cannabidiol accelerated the dissociation of [^{3}H][D-Ala^2,*N*-Me-Phe^4,Gly-ol^5]-enkephalin (DAMGO) from the MOR by at least a factor of 12 and also [^{3}H]naltrindole from the DOR by at least a factor of 2.[33] This was shown in both saturation binding studies and time-dependent alteration of equilibrium studies. Cannabidiol exhibited a Hill coefficient indicative of positive homotropic cooperativity, suggesting that it binds to an allosteric site. In addition, a 10 μM concentration of cannabidiol rightward shifted the dose–response curve of DAMGO in its inhibitory effect in the electrically induced twitch response test in the mouse vas deferens.[35] Despite the NAM properties exerted by cannabidiol, its allosteric effects occur at much higher concentrations than its activity in vivo.

Salvinorin A, a KOR agonist, was found to partially inhibit the binding of opioid ligands DAMGO, diprenorphine, and 6β-iodo-3,14-dihydroxy-17-cyclopropylmethyl-4,5α-epoxymorphinan (IOXY), shifting their inhibition curves to the right while giving off a lower E_{max}, which is a profile that is parallel to that of NAMs (Figure 4).[34] Salvinorin A also impacts the K_d and B_{max} of the MOR in a way that is diverse from competitive binding patterns. This compound first augmented the MOR B_{max} followed by a significant decrease at higher concentrations and affected the K_d of the MOR in a dose-dependent manner instead of in a linear fashion that was observed with a competitive inhibitor.[36] Salvinorin A also affects the disassociation kinetics of MOR radioligands by increasing the disassociation rate of DAMGO and slowing the disassociation rate of diprenorphine. Lastly, Salvinorin A was shown to be an uncompetitive inhibitor of DAMGO-stimulated [^{35}S]GTPγS binding by decreasing the E_{max} in a dose-dependent manner and not being able to increase the ED_{50} appreciably upon increasing its concentration as was observed with the competitive inhibitor naloxone (Figure 4).[34] Interestingly, Salvinorin A may also act as a partial agonist at the MOR considering that it exhibits an E_{max} of 42% relative to DAMGO in the [^{35}S]GTPγS assay and is able to inhibit forskolin-stimulated cAMP accumulation. Additionally, it may interact with a site that is unique from typical MOR agonists seeing as naloxone noncompetitively inhibits Salvinorin A-stimulated [^{35}S]GTPγS binding.

MOR PAMs and SAMs have recently been identified that show varying abilities to shift the orthosteric agonist's potency, affording different efficacies.[37] The MOR SAMs exhibited neutral cooperativity with the orthosteric ligands, but the PAMs that were identified, more specifically thiazol-2-amine analogue BMS-986121 (Figure 3) and a sulfonyl thiazolidine

analogue BMS-986122, potentiated the effects of DAMGO and the endogenous agonists endomorphin-1 (EM-1) and Leu-enkephalin (Leu-ENK), morphine's recruitment of β-arrestin 2, the inhibition of adenylyl cyclase, and G-protein activation. They did not appreciably recruit β-arrestin 2 in the absence of the orthosteric agonist but substantially enhanced the β-arrestin 2 recruitment response with low concentrations of EM-1. BMS-986121 potentiated recruitment of β-arrestin 2 brought about by 20 nM EM-1 to an E_{max} of 76% relative to the maximally effective response observed at 1 μM EM-1 with an EC_{50} of 1.0 μM. BMS-986122 exhibited similar results as it augmented the effect of 20 nM EM-1 to 83% relative to the maximal EM-1 response with an EC_{50} of 3.0 μM.

Further characterization of BMS-986122 was performed to determine the compound's probe dependence and mechanistic basis of activity by employing different kinds of orthosteric agonists for the MOR including both peptides and small molecules.[38] It was found that the degree of allosteric modulation was dependent upon the efficacy of the orthosteric ligand. All endogenous opioid peptides and the small molecules R-methadone and loperamide showed increased affinity and potency for the MOR, but more so for the small molecules, and none elicited any augmentation of maximal G-protein activation. Overall, variability was observed in the case of small molecule MOR agonists, which falls in line with probe dependence. Morphine, buprenorphine, and fentanyl augmented the maximal level of [^{35}S]GTPγS stimulation, but their affinities and potencies were not appreciably affected when administered in the presence of BMS-986122.

It has been previously established that physiological concentrations of Na^+ decrease an agonist's ability to bind to the MOR while antagonist binding is unaffected.[39] This phenomenon was then extended to the other opioid receptors, and it was found that Na^+ inhibits agonist binding by 65% for the MOR and the DOR and only 20% for the KOR.[40] Evidence suggested that BMS-986122 allosterically inhibited the binding of Na^+ to the MOR by disallowing stabilization of the inactive state of the MOR, pushing it toward an active conformation, which explains its marked PAM abilities.[38] The crystal structure of the human DOR revealed the presence and fundamental role of Na^+ in mediating allosteric modulation of receptor selectivity and activity.[41] The Na^+ site is shown to be centrally located in the seven-transmembrane bundle core to stabilize a reduced agonist affinity state and thereby inhibit signal transduction. Changing the allosteric site from an Asn^{131} residue to an Ala or Val residue increases β-arrestin-mediated signaling.

The use of BMS-986121 and BMS-986122 as therapeutic agents may be restricted due to the difficulty of their preparation. In an additional study, MOR PAMs and SAMs were identified using a novel chemotype with an easier scaffold to explore.[42] Sulfonamido acetamide analogue MS1 (Figure 3) was determined as the most promising compound to be functionally selective toward β-arrestin recruitment as opposed to G-protein activation in the presence of EM-1 and displayed probe dependence. MS1's most prominent effects were observed with R-methadone, which were similar to those of BMS-986122's action.[38] MS1 also pushed the threshold of maximal activation of the partial agonist morphine to stimulate the G-protein, again in accordance with BMS-986122's ability to enhance the efficacy of partial agonists. However, it was not determined if MS1's mechanism of action was the same

as that of BMS-986122, which is through allosterically inhibiting/disrupting Na^+ binding to the MOR.

It was recently discovered that the compound ignavine (Figure 3), one of the main alkaloids in Aconite root, interacts with the MOR, exhibiting an IC_{50} of 2.0 μM.[43] A receptor internalization assay determined that ignavine can modulate MOR activity in such a way that is dependent upon its concentration. GFP-tagged MOR expressing HEK-293 cells that were treated with 1 μM DAMGO showed vesicle internalization at 20 min. Upon cotreatment with 1 μM ignavine, enhanced vesicle internalization was observed at 10 min. Co-treatment of 1 μM DAMGO with 10 μM ignavine completely blocked the internalization induced by 1 μM DAMGO, and 10 μM of ignavine alone did not affect receptor internalization. Ignavine's effects on intracellular cAMP levels were parallel to those of the receptor internalization assay. Administration of ignavine alone did not influence cAMP levels; however, 1 μM concentration enhanced DAMGO activity at 7.5 min, whereas 10 μM ignavine was found to be inhibitory at 23.5 min. The same results were achieved upon cotreatment of EM-1 and morphine. In addition, ignavine produced a leftward shift in response to DAMGO at 1 μM at 7.5 min and a rightward shift at 23.5 min at a concentration of 10 μM. A concentration of 0.1 mg/kg of ignavine showed maximum response in the tail-flick test, whereas higher concentrations weakened the analgesic activity. The same trend was seen with the tail-pressure test.

Computational and docking studies elucidated that ignavine could bind to the MOR in its orthosteric pocket in a manner that is unique from that of morphine or β-funaltrexamine (β-FNA) and that the binding pocket cannot accommodate both ignavine and an agonist compound simultaneously. It was previously shown that ignavine inhibited the binding of [^{3}H]diprenorphine, which has a similar structure to that of morphine and β-FNA. In comparison to these compounds for the MOR, ignavine also has a quaternary amine structure, a motif that has been shown to interact with Asp147 of the rat MOR, an interaction that is thought to be significant in morphine's pharmacological profile.[44,45] Taking these results as a whole, it was hypothesized that, in a MOR homodimer, ignavine at a low concentration can employ positive modulatory action on an agonist bound to the other orthosteric site but at higher concentrations would bind both orthosteric sites and provide antagonistic activity.

DOR

The DOR may be a therapeutically relevant target for its antinociceptive and antidepressive properties, but much of this receptor's pharmacological behavior is still unclear. For example, contradictory evidence has been collected on the DOR's role in bringing about respiratory depression. High doses of [DPen^2,DPen^5]-enkephalin (DPDPE) brought about respiratory depression in sheep, and similarly, the small molecule SNC-80 had the same effect at 40 mg/kg.[46,47] However, deltorphin II (Del II) and (+)-BW373U86 enhanced respiratory function and inhibited the MOR agonist alfentanil's respiratory depressant effect.[48] Previous studies have also shown that it is implicated in mediating mood states and producing analgesia in chronic pain models, but agonists have also been the cause of convulsive/seizurogenic behavior.[49–52]

A structure–activity relationship study was performed on what became the first reported DOR-PAMs as they were shown to increase the affinity and/or efficacy of DOR orthosteric agonists Leu-Enk, SNC80, and TAN67 as evidenced by binding affinities, G-protein stimulation, recruitment of β-arrestin 2, inhibition of adenylyl cyclase, and stimulation of extracellular signal-regulated kinases (ERK).[53] The hexahydro-1*H*-xanthene-1,8(2*H*)-dione analogues generated (Figure 5) did not produce an appreciable amount of agonist activity, but all provided PAM actions and a range of selectivities (up to >200 fold) for the DOR over the MOR No substitutions on the benzyl (R^1, R^2, R^3 = H) afforded a DOR-PAM with an EC_{50} of 0.2 μM, showing 30-fold β-arrestin recruitment selectivity over that of the MOR Incorporation of a methyl substituent (R^1 = Me) showed an order of magnitude increase (EC_{50} = 0.03 μM) in PAM response for the DOR with negligible impact on the MOR Halogen substitutions about the ring with either fluorine or chlorine had little impact on DOR-PAM activity, but the dichloro adduct in the ortho and meta positions showed a marked improvement in DOR selectivity by decreasing MOR activity (EC_{50} > 10 μM). It appeared that CF_3 substitution in the ortho position resulted in the loss of DOR-PAM activity (EC_{50} > 10 μM). Bromination of the ortho position afforded an EC_{50} of 0.05 μM for the DOR and >200-fold selectivity over that of the MOR.

The most promising compound, BMS-986187 (R^1 = Me), did not show any agonist function and provided an EC_{50} of 48 nM and 2 μM in the company of EC_{20} concentrations of Leu-Enk or EM-1, respectively. BMS-986187 also afforded 100-fold selectivity for the DOR over the MOR and an EC_{50} of 33 nM in the β-arrestin recruitment assay when run with an EC_20 concentration of Leu-Enk (18-fold increase in Leu-Enk's potency along with a small increase in maximal response). In an inhibition of forskolin-stimulated cAMP assay, BMS-986187 exhibited complete inhibition of cAMP propagation when administered >3 μM, showed robust PAM activities in ERK1/2 phosphorylation and [^{35}S]GTPγS assays, and potentiated the affinities of Leu-Enk, SNC80, and TAN67 (3–32-fold).

Interestingly, some of the BMS-986122 analogues, MOR PAMs, also showed some DOR PAM activity, suggesting that MOR and DOR may share a similar allosteric site, and thus selectivity between two receptors can be engineered into the compounds.

Sigma-1 Receptor

The sigma receptor was originally proposed as a subtype of opioid receptors but is now confirmed as a nonopioid receptor that binds various psychotropic drugs.[54,55] The sigma receptor has two pharmacologically distinct subtypes, sigma-1 and sigma-2, in which the former is emerging as a novel CNS drug target. It is expressed in the periphery, but primarily in the CNS, and is involved in neuronal plasticity, which is a hallmark of the pathophysiology of neurological diseases, such as Alzheimer's, schizophrenia, and major depressive disorders, along with being implicated in pain and addiction.[56–58] Sigma receptor ligands were previously shown to influence opioid receptor-induced analgesic properties without modulating additional opioid action and later found to physically complex with the MOR.[59–63] Despite this association, it was determined that the sigma-1 ligands do not interfere with the binding of ligands to the MOR but rather impact opioid receptor transduction.[59] When administered alone, sigma-1 ligands were unable to stimulate

[^{35}S]GTPγS binding, but the selective sigma-1 receptor ligand BD-1047 was able to enhance DAMGO-induced signaling 3–10-fold without changing the maximal response nor DAMGO binding to the MOR. Additionally, sigma-1 receptor downregulation mimics the effect of the sigma-1 receptor antagonists. In lieu of these results, it may be worthwhile to explore the realm of sigma-1 receptors and their potential as allosteric targets.

Presently, compounds of the racetam family (pyrrolidone analogues, Figure 6) and ligands containing an *N*,*N*-dialkyl or *N*-alkyl-*N*-cycloalkyl motif are the paradigm for treating such neurological disease states, serving as nootropic drugs.[64] Sigma-1 has a been shown to mediate a wide variety of biological functions such as Na^+, Ca^{2+}, and K^+ ion channels, neuro-transmitter release, and inflammation.[56,65–67] Typically, the ideal choice of ligand partners for allosteric modulators is the endogenous ligand for a given receptor, but this is arduous in the case of the sigma-1 receptor considering the obscurity of the endogenous ligands.[68]

Chiral resolution of a racemic 4,5-disubstituted piracetam analogue, 2-(5-methyl-4-phenyl-2-oxopyrrolidin-1-yl)-acetamide (Figure 7), at the sigma-1 receptor was investigated using the selective sigma-1 receptor agonist PRE-084 (Figure 8) in an electrically stimulated rat vas deferens model.[69] Permutations of chirality at C4 and C5 of the pyrrolidin-2-one ring were explored. Serendipitously, the (4*R*,5*S*)-isomer (E1R) was discovered to interact at the sigma-1 receptor by increasing the binding affinity of an orthosteric ligand, which is characteristic of a PAM. The set of enantiomers did not change the height of contractions of the electrically stimulated vas deferens, but when co-administered with PRE-084, an increase in activity was observed for all compounds. E1R and (4*R*,5*R*)-isomer yielded contractions that were at least twice as high compared to when PRE-084 was administered alone, showing the importance of the *R*-configuration at the C4 position.

E1R augmented the binding of [^{3}H]ditolylguanidine, a nonselective sigma receptor radioligand, but was not able to show any allosteric effect on sigma-1 receptors using [^{3}H](+)-pentazocine, a sigma-1 receptor agonist[70] (Figure 8). E1R did not increase contractions in the vas deferens assay when co-administered with PB-28, a sigma-2 receptor agonist, but did potentiate the effect of PRE-084 upon bradykinin-induced intracellular Ca^{2+} increase in vitro. The PAM attributes of E1R were confirmed in vivo by protecting against scopolamine-induced cognitive deficit effects that were antagonized by NE-100, a sigma-1 receptor-selective antagonist.[71] In addition, E1R was observed to show no locomotion impairment up to 100 mg/kg nor interact with Na^+ and K^+ channels.

SKF83959, SCH23390, and SKF38393, which possess an *N*,*N*-dialkyl moiety (Figure 9), potentiated the binding of [^{3}H](+)-pentazocine in allosteric fashion selectively to the sigma-1 receptor.[72] SKF83959 afforded improved binding and an increased PAM effect relative to that of phenytoin, a classic sigma-1 receptor PAM.[73,74] In an additional study, it was demonstrated that SFK83959 enhanced the binding activity of dehydroepiandrosterone, an endogenous agonist for the sigma-1 receptor.[75] Unlike E1R, which is selective for the sigma-1 receptor, SKF83959 has inherent D_1 dopamine receptor affinity among other activities.[76–80] In an effort to ablate these off-target interactions, a series of modifications

were performed, bringing to fruition SOMCL668, which exhibited no appreciable activity at the dopamine 1(D_1), D_2, D_3, 5-hydroxytryptamine$_{1A}$ (HT), or 5-HT_{2A} receptors.[81,82]

Heterodimers

GPCR homodimers and heterodimers have been observed in a variety of systems including tissues, cells that endogenously express both receptors, and in primary cell cultures. In some cases, it is critical for receptor function to form heteromeric pairs.[83–85] Considerable evidence has shown that the opioid receptors can form homodimers or heterodimers with each other and other receptor families such as the cannabinoids.[86–88] This offers an additional layer of intricacy to the allosteric regime due to individual protomers causing allosteric interactions or novel allosteric binding pockets that can impact affinity and/or efficacy for either one or both of the protomers.[89–91] Orthosteric binding at one protomer can influence the conformation of its paired receptor and thus influence the response of orthosteric binding to that paired receptor. Because of the varying nature of probe dependence, it would be paramount to recognize pairs of ligands that work well together so that low doses of compound may be administered, reducing the severity of side effects.

MOR/DOR

MOR/DOR heterodimer levels are increased in vital areas of the CNS that are thought to play major roles in processing the pain response after chronic administration of morphine and thus serve as viable targets for chronic pain states, addiction, and tolerance.[92] Studies have also shown that MOR/DOR upregulation occurs via chronic administration of morphine and various protein chaperones, which leads to a modification of binding characteristics and functional signaling of the heteromer.[93,94] Therapeutic design could be geared toward making a PAM of the DOR capable of augmenting MOR agonist signaling, affording better analgesia with less severe adverse effects. The benefit of this would be that such a ligand would not interact with the MOR or MOR homodimers, allowing for increased selectivity in which only MOR/DOR heterodimers are present.

The binding equilibrium and association/dissociation kinetics of the DOR agonist Del II and the MOR agonist DAMGO were scrutinized in the presence of MOR-selective ligands and DOR-selective compounds, respectively, in an established model system for MOR/DOR heterodimers.[95] It was indeed found that MOR ligands are capable of allosterically potentiating binding to the DOR and vice versa, suggesting that orthosteric binding of the ligands to the protomer modulates the receptor pair's activity. In the case of MOR compounds, morphine was able to enhance DOR activity by 5 orders of magnitude, whereas other ligands ranged from 3 to 4 orders of magnitude. The rate at which the orthosteric ligands dissociated from their respective receptors was also found to be decreased when coadministered with the heterodimeric partner's orthosteric ligand, again falling in line with positive allostery between the two protomers. It was determined that there was no correlation between the binding affinities of the MOR and DOR ligands for their respective receptor's orthosteric sites and their efficacy in ligand binding to the DOR or MOR, respectively, delineating that it is not essential for the ligand to remain bound to the protomer to maintain the conformational change.

DOR/KOR

Because of the adverse effects that come with targeting the opioid receptors in the CNS, the periphery has also been garnering attention. Opioid receptors are expressed in peripheral primary sensory neurons that are involved in relaying pain signaling. These peripheral receptors have not shown an analgesic response when a peripherally limited opioid is administered but provide analgesia to tissues under a pathological state.[96,97] Previous studies have revealed that the DOR and KOR can form heterodimers, which allow for the development of compounds that are heterodimer-selective, enabling enhanced tissue specificity considering that these ligands would only interact where heteromeric pairs are expressed.[98–100] KDN21, a bivalent ligand in which KOR selective antagonist pharmacophore 5′-guanidinonaltrindole (GNTI) tethered through a spacer to the DOR-selective antagonist naltrindole I, was found to enhance binding to the DOR/KOR heterodimer using [^{3}H]diprenorphine.[100]

Recently, more evidence has shown that DOR/KOR heterodimers in peripheral sensory neurons exist and that KOR antagonists modulate DOR agonist function in a manner likely to be through allosteric interplay between the protomers.[101] Nor-binaltorphimine (Nor-BNI), a KOR antagonist, increased the potency of DPDPE, decreased the potency of [DAla^2,DLeu^5]-enkephalin (DADLE), and decreased the potency and efficacy of SNC80. Dissimilar to Nor-BNI, 6′-GNTI weakened DPDPE's potency, as both of the KOR antagonists had these results confirmed in a behavioral model of thermal allodynia. Although a possible mechanistic explanation could be mediated through downstream interactions of the two DOR and KOR signaling pathways, the ligand's effects show probe dependence, a trademark of allosteric modulation.

DOR/CB1

Cannabinoid receptors, like the opioid receptors, are codistributed in both the peripheral and CNS and are implicated in both ascending and descending pain pathways and emotional/mood processing such as anxiety and depression.[102–106] Both cannabinoid and opioid receptors can bring about analgesia in pain states and suppress anxiety and depression, more specifically through the DOR and CB1, and thus have been considered therapeutic targets.[107–109] Synergistic interplay between the cannabinoid and opioid receptors has been well-documented.[110] A prominent coinhabitation of CB1 and MOR has been seen in lamina II interneurons.[111] Furthermore, naltrindole, a DOR-selective antagonist, can inhibit anxiolytic responses resulting from the CB1 agonist Δ^9-tetrahydrocannabinol (THC).[112] (Figure 9)

Further studies have shown that the DOR and CB1 can be coimmunoprecipitated when coexpressed in cells, providing evidence that the DOR and CB1 do form heterodimeric complexes.[88] Furthermore, CB1 stimulation is augmented in DOR-knockout mice, and CB1 stimulation is reduced in cells that are transfected with the DOR and in the presence of DOR-selective ligands showing an allosteric inhibitory response. CB1 undergoes upregulation in chronic pain states and shows a marked enhancement of analgesic response when an agonist such as WIN 55,212-2 (Figure 10) is administered.[113–115] Ablation of the DOR heightens anxiety and depressive behavior, whereas administration of a CB1 agonist

results in antidepressive activity, but in low doses can produce anxiety.[108,116,117] Because of the DOR and CB1's roles in emotional processing and pain states, the DOR/CB1 heterodimer is a viable clinical target for neuropathic pain-associated adverse emotional states. DOR and CB1 expression both show an upregulation in brain regions 2 weeks after a peripheral nerve lesion, but only CB1 activity is increased as DOR activity is decreased.[118] This observation suggested that CB1 lowers DOR action. However, nonsignaling doses of CB1 agonists or antagonists were found to potentiate DOR ligand affinities and signaling but were inhibited by a heterodimer-targeting antibody. Ultimately, this indicates that the decreased levels of DOR activity that are observed in neuropathic pain states can be remedied through allosteric mediation of the CB1-DOR heterodimer, whether it is by a bivalent partial agonist/antagonist at CB1 and an agonist at DOR or a CB1-DOR-selective ligand that inhibits heterodimeric activity.

Concluding Remarks

The push to develop allosteric modulators for the opioid receptors has resulted in exciting times in opioid therapeutics. The discovery of novel PAMs, SAMs, and NAMs that can fine-tune cellular transduction in a positive or negative way has resulted in new scaffolds of opioid receptor interactions to explore along with interesting biological profiles. With new iterations of modulator design, these compounds now possess greater affinity for their respective receptors with improved pharmacokinetic and safety profiles. Because of this, more of these modulators can be tested for their efficacy toward pain and other physiological models, and they can be administered alone to determine their effect on the endogenous opioids or in conjunction with an exogenous opioid in the hope of attenuating opioid-related side effects such as tolerance and addiction that are established during chronic receptor activation.

There are still a number of hurdles to circumvent that have proven to be challenging. The probe dependency that comes with allosteric modulation is a major feat to overcome and requires the testing of numerous orthosteric compounds through both binding and functional assays. Furthermore, allosteric modulators will need to be tested against multiple receptor subtypes considering the nonselectivity of many endogenous and opioid drugs, including biologically active metabolites. Additionally, the synthetic approach should be performed in a high-throughput manner instead of using a single compound approach considering the vast number of SARs that have been observed. Subtle changes in substitution patterns can drastically change the effect on the orthosteric ligand, such as going from a PAM to a SAM.

There is a paucity of literature regarding allosteric modulators for the KOR or a KOR-containing heteromeric pair. It has been well-documented that stimulation of the KOR brings about dysphoria and has a lesser maximal analgesic potential than that of the MOR, but KOR agonists have been shown to attenuate the rewarding effect of coadministered addictive drugs and can be an alternative approach to pain relief in those that possess a risk of drug abuse by providing analgesia mediated through the KOR.[119]

All things considered, because allosteric modulators are able to maintain temporal and spatial therapeutic control, improve physiochemical properties, and modulate biased

signaling, the future appears to be bright for the allosteric paradigm in GPCR drug discovery for developing potent analgesics that lack the serious side effects of traditional opioids.

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Abbreviations

cAMP	cyclic adenosine monophosphate
CB1	cannabinoid type-1 receptor
CNS	central nervous system
D	dopamine
DADLE	[DAla^2,DLeu^5]-enkephalin
DAMGO	[D-Ala^2, *N*-Me-Phe^4,Gly-ol^5]enkephalin
Del II	deltorphin II
DOR	δ-opioid receptor
DPDPE	[DPen^2,DPen^5]-enkephalin
Dyn A	dynorphin A
EM-1	endomorphin-1
ERK	extracellular signal-regulated kinases
β-FNA	β-funaltrexamine
GNTI	guanidinonaltrindole
GPCRs	G-protein-coupled receptors
HT	hydroxytryptamine
IOXY	6β-iodo-3,14-dihydroxy-17-cyclopropyl-methyl-4,5α-epoxymorphinan
KOR	κ-opioid receptor

Leu-Enk	Leu-enkephalin
MOR	μ-opioid receptor
NAMs	negative allosteric modulators
Nor-BNI	nor-binaltorphimine
PAM	positive allosteric modulator
SAMs	silent allosteric modulators
THC	tetrahydrocannabinol

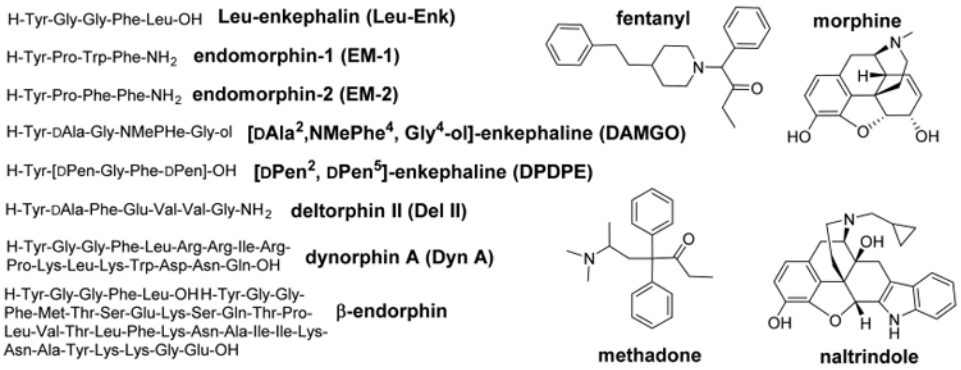


Figure 1.
Structures of orthosteric opioids.

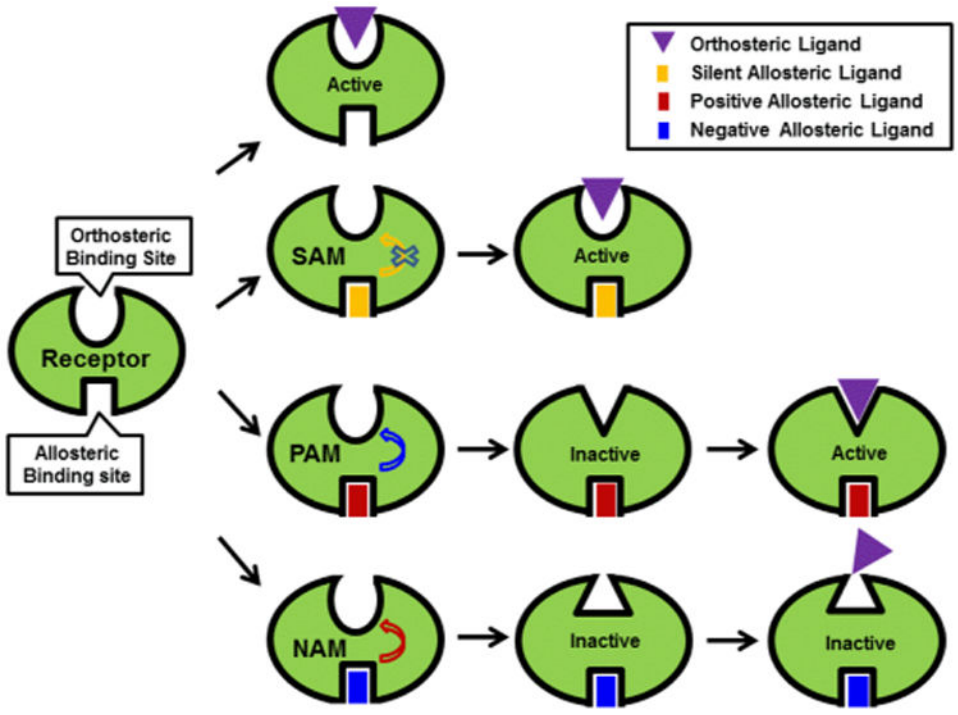
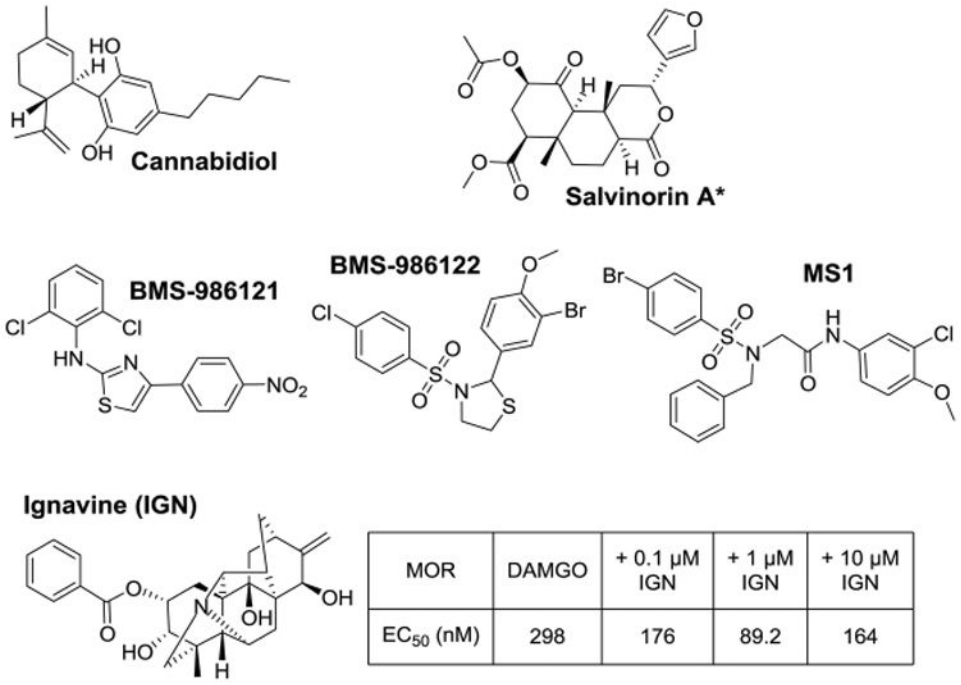


Figure 2.

Receptor binding modes: (row 1) orthosteric interaction; (row 2) silent allosteric modulator (SAM); (row 3) positive allosteric modulator (PAM), and (row 4) negative allosteric modulator (NAM).



MOR	DAMGO	+ 0.1 μM IGN	+ 1 μM IGN	+ 10 μM IGN
EC_{50} (nM)	298	176	89.2	164

Figure 3.

Allosteric ligands of MOR: (upper row) NAMs, (middle row) PAMs, and (lower row) concentration-dependent AM. *MOR and DOR.

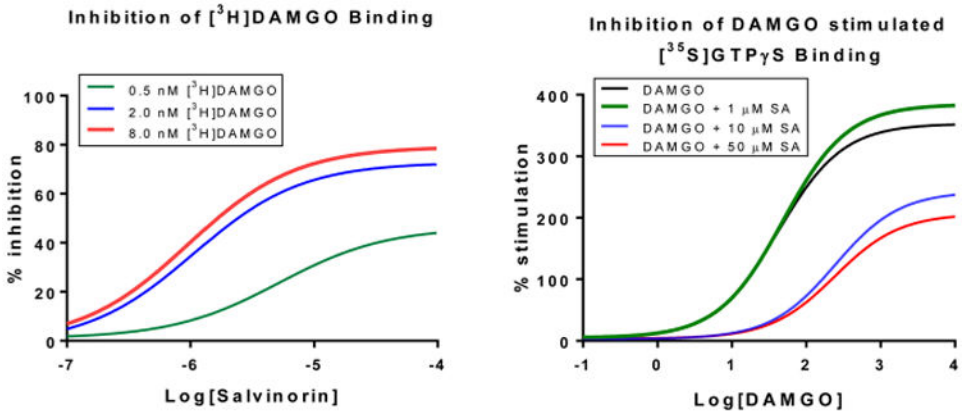


Figure 4.

Inhibition of (left) [^{3}H]DAMGO binding and (right) DAMGO-stimulated [^{35}S]GTPγS binding by Salvinorin A (SA) to hMOR-CHO cells.

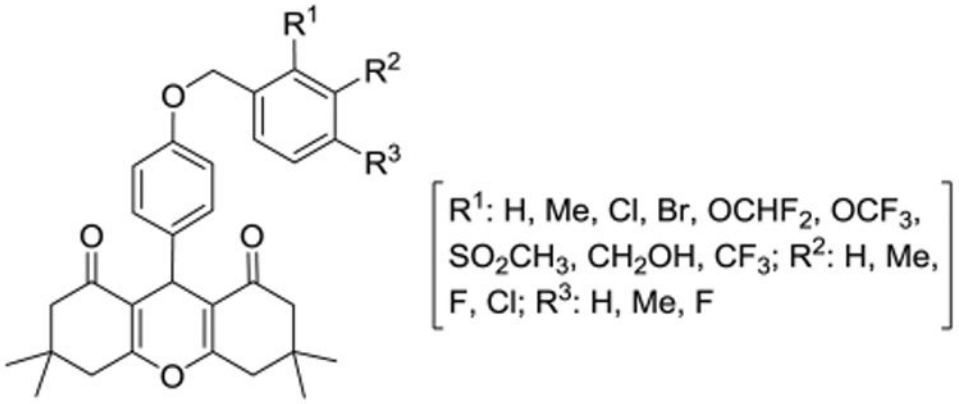
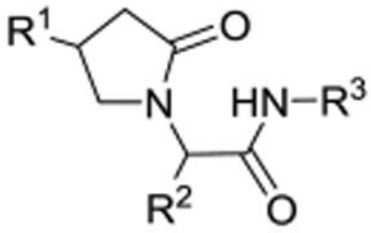
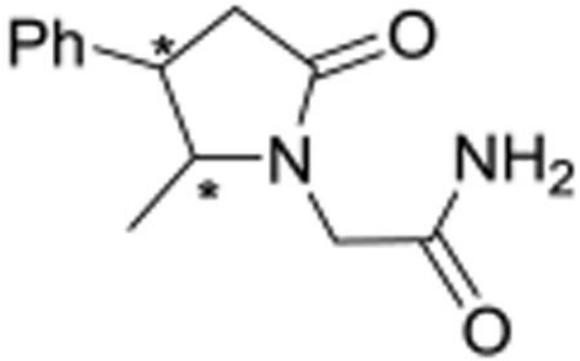


Figure 5.
Structures of DOR-PAM chemotype.



R^1 & R^2 & R^3 = H: **piracetam**
R^1 & R^2 = H; R^3 = $(CH_2)_2N(i\text{-}Pr)_2$: **pramiracetam**
R^1 & R^3 = H; R^2 = Et: **etiracetam**
R^1 & R^3 = H; R^2 = 2,6-dimethylphenyl: **nefiracetam**
R^1 = OH; R^2 & R^3 = H: **oxiracetam**
R^1 = Ph; R^2 & R^3 = H: **phenylpiracetam**

Figure 6.
Structures of the racetam family.



(4R,5R)
(4R,5S)
(4S,5R)
(4S,5S)

Figure 7.
4,5-Disubstituted piracetam analogues.

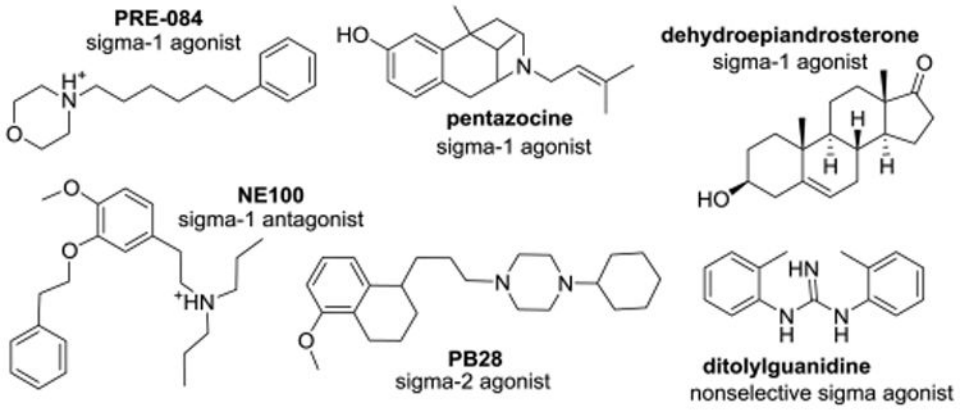


Figure 8.
Sigma receptor ligands.

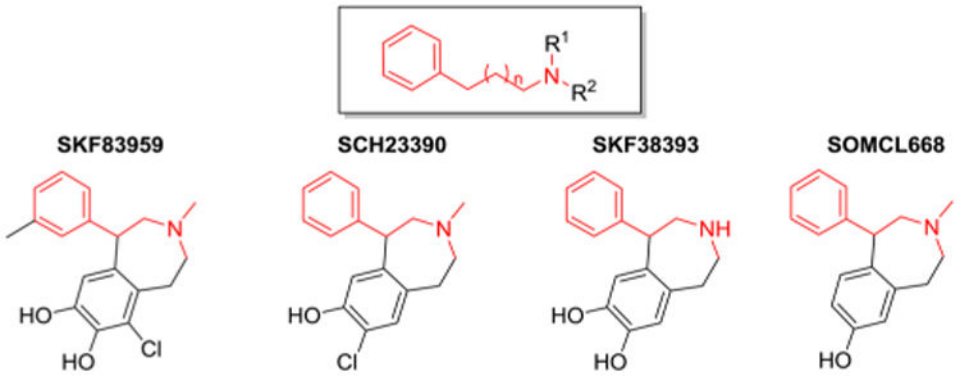


Figure 9.
N,N-Dialkyl analogues for the sigma-1 receptor.

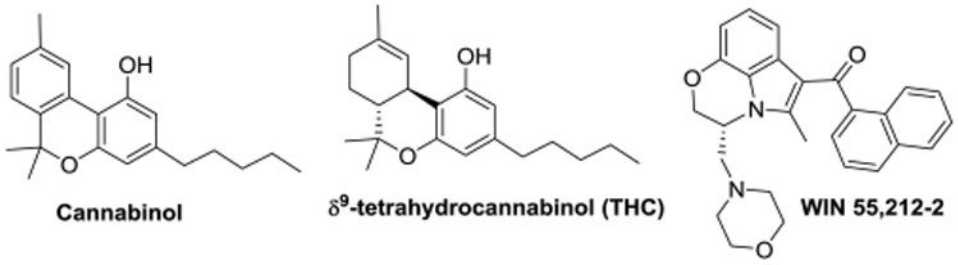


Figure 10.
Structures of CB1 ligands.