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Allosteric Modulation of Class A GPCRs: Targets, Agents, and Emerging Concepts

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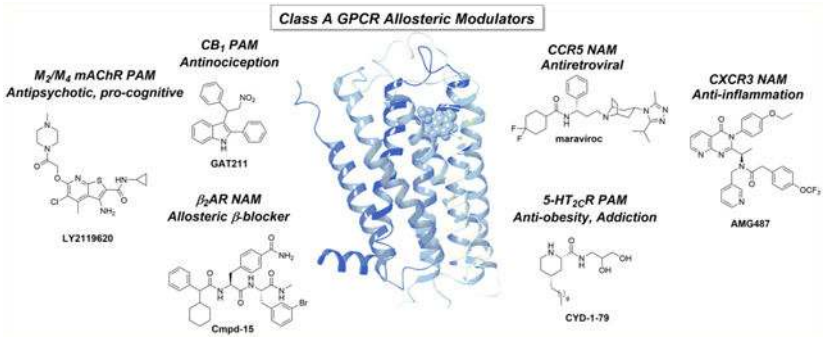
Abstract

G-protein-coupled receptors (GPCRs) have been tractable drug targets for decades with over one-third of currently marketed drugs targeting GPCRs. Of these, the class A GPCR superfamily is highly represented, and continued drug discovery for this family of receptors may provide novel therapeutics for a vast range of diseases. GPCR allosteric modulation is an innovative targeting approach that broadens the available small molecule toolbox and is proving to be a viable drug discovery strategy, as evidenced by recent FDA approvals and clinical trials. Numerous class A GPCR allosteric modulators have been discovered recently, and emerging trends such as the availability of GPCR crystal structures, diverse functional assays, and structure-based computational approaches are improving optimization and development. This Perspective provides an update on allosterically targeted class A GPCRs and their disease indications and the medicinal chemistry approaches toward novel allosteric modulators and highlights emerging trends and opportunities in the field.

Graphical abstract



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1. INTRODUCTION

G-protein-coupled receptors (GPCRs) are seven-transmembrane proteins that have been of high pharmaceutical interest for decades due to their physiological importance and accessibility for small molecule targeting. GPCRs are integral for physiological responses to a variety of stimuli, which span photons, ions, small molecules, macromolecules, peptides, and proteins. These diverse stimuli, environmental and endogenous, are in accordance with the vast functions mediated by GPCRs. Aspects of cognition, immune response, and cellular organization, among many others, are regulated by GPCR signaling. The pharmacological modulation of GPCRs provides leverage for the treatment of diseases of the central nervous system (CNS), cancer, viral infections, inflammatory disorders, metabolic disorders, and others. Additionally, the location of GPCRs in the cellular membrane allows unique pharmacological access to these proteins and the effectors and second messenger systems coupled to the receptors allow for efficient drug action. The location, topology, and physicochemical attributes of many GPCR binding pockets have resulted in the discovery of numerous small molecule drugs that have been in clinical use for decades.[1]

GPCRs represent the largest family of druggable proteins in the human genome and unsurprisingly are targeted by more than 30% of marketed drugs in the United States.[2] Despite this large percentage, recent studies highlight that a small fraction of the possible druggable GPCRome has been exploited by approved drugs.[3] Multiple reasons persist for the relatively low number of GPCRs targeted by FDA-approved drugs. Foremost, the biological functions of many potentially druggable GPCRs remain unclear, as seen in the understudied orphan GPCRs (oGPCRs), and new biological complexities remain to be added to established targets.[4] Another reason is several disease indications with established GPCR targets have not progressed drugs into the clinic when targeted by traditional agonists or antagonists, which bind at highly conserved orthosteric sites and may produce off-target effects.[5,6] Undeniably, most of GPCR-targeted FDA-approved drugs bind to orthosteric sites; however, when therapeutic efficacy or safety hinges on distinguishing between highly homologous receptor subtypes, other modes of modulation, such as allosteric modulation, confer specific advantages.[7]

Allosteric modulation of protein function was first described in enzymes and is now understood to be an integral aspect of functionality in other protein types, including GPCRs.[8] A GPCR allosteric modulator is generally defined as a modulatory ligand that does not occupy the orthosteric binding site and binds to a spatially and topologically distinct (allosteric) site on the receptor. It is now known that endogenous allosteric modulators are ubiquitous among GPCRs, the most apparent being the heterotrimeric G proteins, and many synthetic ligands may exploit these sites.[9] Other examples of allosteric sites seem to be unrelated to endogenous molecules yet display topologically favorable features due to the receptor folding and assembly. Nevertheless, allosteric site residues tend to be less conserved among receptor subtypes and can offer unparalleled subtype specific targeting. Other advantages of allosteric ligands are fundamental to their mode of action, including the ability to fine-tune the response to an orthosteric ligand in a time and spatially dependent manner, a feature that holds promise for immune and CNS targets.[10] Additionally, allosteric

modulators may also confer signaling bias and probe dependence, further contributing to the possibility for remarkably precise pharmacological modulation.[11]

The GPCR superfamily is subdivided into divergent groups (classes) based on homology and function including class A (rhodopsin-like receptors), class B (the secretin family), class C (metabotropic glutamate receptors), class D (fungal mating pheromone receptors), class E (cyclic adenosine monophosphate (cAMP) receptors), and class F (Frizzled and Smoothened receptors).[12–14] Class A represents the largest class of GPCRs and contains further classifications for members based on the type of endogenous signaling molecules (Figure 1). This Perspective will focus on recent medicinal chemistry advances for allosteric modulators across a selection of class A GPCRs with diverse signaling molecules, including small molecules, peptides, proteins, and lipids. Due to the size of class A and the historical importance of many of its members, these receptors make up most of the current receptor drug targets.[15] Likewise, industry and academic programs have been established to discover allosteric modulators of class A receptors for the treatment of mental health disorders, viral infection, inflammation, and other indications.

Mechanistically, allosteric modulators can increase the functional response to an orthosteric agonist, acting as a positive allosteric modulator (PAM), or inhibit the functional response to an orthosteric agonist, acting as a negative allosteric modulator (NAM). There have been reported neutral allosteric ligands (NALs), which do not modulate the receptor function but compete with other PAMs or NAMs at the allosteric binding site. Additionally, for many targets included in this Perspective, allosteric ligands may possess an intrinsic agonist profile in the absence of an orthosteric ligand, despite binding to the allosteric site. In such instances, allosteric ligands that potentiate agonists as well as display intrinsic efficacy are termed ago-PAMs. Further, NAMs may act directly via allosteric antagonism of an orthosteric agonist's stimulation or they may assert antagonism by binding to an allosteric site and inducing changes to the receptor's structure or "life cycle" that prohibit eventual receptor activation by an orthosteric agonist. The latter has been observed in cases where NAMs alter receptor trafficking or, in the case of the CCR5 NAM maraviroc, dimer populations of CCR5 are altered.[16] New insights have shown these allosteric alterations can be mediated by various components of the receptor, lipid membrane, orthosteric ligand(s), and effector proteins, ultimately providing a more complete, and complex, view of the potential for allosteric modulation.[8,17] These modes of allosteric modulation are conferred via diverse allosteric binding sites that include extracellular regions, the interior, and lipid-facing exterior of the transmembrane (TM) helix bundle and intracellular regions. Elegant structural studies have confirmed these interesting binding poses and provide the framework for modulating new targets with notable precision. Figure 2, containing three GPCR-allosteric modulator cocomplex crystal structures, illustrates the concept of allosteric modulators functioning in diverse modes and binding to class A GPCRs in diverse structural regions. The structural interactions between class A GPCR residues and the corresponding allosteric modulators have been reviewed by Lu and Zhang, also included in the Allosteric Modulators special issue.[18] This Perspective will review key concepts for allosteric modulator discovery and optimization, provide a thorough overview of the recent medicinal chemistry efforts for class A GPCR allosteric modulation, and highlight diverse applications of allosteric modulators across the class A GPCR family.

2. OVERVIEW OF THE ALLOSTERIC MODULATION OF CLASS A GPCR DRUG TARGETS

GPCRs are the most targeted protein class by modern pharmacotherapies due to their innate capability of transducing extracellular signals into wide-ranging cellular responses. In a growing number of molecular and structural studies, activated GPCRs are increasingly appreciated to transduce their signal through structural alterations in the TM domains that not only result in the association of effector heterotrimeric G proteins but also lead to association of β-arrestins, scaffolding proteins, and various kinases.[4] Due to the known complexities of GPCR signaling and the added intricacy of measuring allosteric modulation, targeted discovery of allosteric modulators has been enabled only in recent decades by technological improvements in ligand screening assays.[22] A common primary screening assay employed in allosteric modulator discovery is a functional assay in which the effects of allosteric modulators can be observed by alterations to orthosteric agonist or antagonist potency and/or efficacy. An orthosteric agonist or antagonist with known and reproducible activity, along with the potential allosteric ligand, is added to a system in which a functional output can be measured, as in a calcium-mobilization measurement. Since GPCR activity is not a simple on/off mechanism, it is important that the assay be able to measure the magnitude of agonist-induced functional response, as well as the magnitude of functional modulation from an allosteric modulator. The resultant concentration—response curve can provide quantitative measures of orthosteric ligand efficacy (E_{max}), potency (EC_{50}), and the potency estimate for an allosteric modulator (e.g., PAM EC_{50}). When fit to the operational model of allosterism, the unique behavior of the receptor induced by the allosteric ligand can be quantified. The allosteric modulation of orthosteric ligand affinity is denoted as the cooperativity factor (α), and the allosteric modulation of efficacy is described by the value β. These influences may be described by a composite metric of cooperativity denoted by log $\alpha\beta$. According to this model, the intrinsic efficacy of the allosteric ligand can be described by the factor τ_B and the intrinsic efficacy of the orthosteric ligand is described by the value τ_A. Excellent reviews have been published and should be consulted on this model, the contributing factors, and the importance of the quantification of allosteric modulator activities.[23–25] It is important to note that historically much of the medicinal chemistry and corresponding structure—activity relationships (SARs) in the field of allosteric modulator discovery has relied on allosteric modulator potency estimates obtained from concentration—response curves, as seen in this Perspective. While useful in screening, potency estimates have limitations and have been shown to diverge from the allosteric modulator affinity measured in binding experiments.[26]

Binding assays with radioligands are also commonly employed to understand allosteric modulation and report kinetic inhibition constants (K_i). These competition or saturation binding assays are employed on receptor-expressing membrane preparations. Membrane preparations may also be used for functional assays via the quantification of [^{35}S]-guanosine-5'-*O*-(3-thio) triphosphate (GTPγS) binding to the G protein α subunit upon receptor activation. Finally, bioluminescence resonance energy transfer (BRET) biosensors have been increasingly utilized in cells to study the protein—protein interactions that occur upon receptor activation, specifically between GPCRs and signaling proteins such as G

proteins or β-arrestins. Throughout the following Perspective, these terms will be used to describe the activity of allosteric modulators as reported in the corresponding original manuscript. Additionally, observations should be made regarding the importance of quantitatively characterizing allosteric modulators in multiple dimensions of their effect to thoroughly inform SARs.

Traditionally, allosteric modulators have displayed divergent physicochemical properties from those of class A GPCR orthosteric ligands, exemplified by higher lipophilicity, rigidity, and typically reduced affinity for their binding site.[38] Due to years of careful optimization and structurally informed design, the development of allosteric modulators has significantly improved in achieving druglikeness and is now progressing candidates forward in preclinical development and human clinical trials as well as in the market for clinical use (Table 1). A contributing factor to the optimization of allosteric ligands is the recent availability of 10 high resolution crystal structures displaying allosteric binding sites and the corresponding receptor activation states (Table 2). These structures provide information on binding sites as well as explanation toward differential allosteric modulation of downstream signaling pathways. The latter phenomenon has been termed signaling bias (also functional selectivity) and is characterized by potentiation or inhibition of a selected signaling cascade(s) over other signaling cascades activated by the GPCR.[11] Class A GPCRs translate signaling through association with effector proteins, predominately heterotrimeric G proteins, from whom the receptor class derives its name, and arrestins. Signaling bias, as reported in this Perspective, primarily reflects selective or biased modulation of either G protein-mediated signaling or β-arrestin-mediated signaling, although biased receptor interactions within the G protein and other proteins have been shown.[39,40] Selectively developing ligands that display a marked signaling bias in accordance with target-specific underlying biology may produce more efficacious drug candidates or may produce candidates with decreased adverse effects. Currently, this trend has been predominantly explored by research groups developing orthosteric ligands for class A GPCRs, but it is also a consideration for allosteric ligand development, as shown by selected allosteric modulators in this Perspective.

Interest in class A GPCR allosteric modulators initially grew because of a theoretical improvement in target selectivity, especially among receptor subtypes displaying high degrees of homology in the orthosteric site.[41] Since then, subtype selectivity has been shown to be one of many advantages, including the preservation of spatial and temporal dynamics of cell signaling. These benefits are often highlighted with respect to targeting GPCRs within the CNS in which, for example, neurotransmitter release and receptor activation are region- and circuit-specific with a high degree of temporal regulation.[10] In such a case, an allosteric modulator may preserve these important characteristics and avoid receptor desensitization by relying on endogenous activation events. Additionally, allosteric modulators have a “ceiling effect”, driven by the saturable nature of allosteric interactions and whereby the extent of their activity is dictated by the concentration of orthosteric ligand present, possibly decreasing overdose concerns. Many of these advantages have been exploited by allosteric modulators targeting receptors in the periphery. For example, the chemokine receptors are an integral component of the immune system and inflammatory response, and one receptor subtype may respond to numerous chemokine ligands (and vice

versa) in a receptor-, agonist-, tissue-, and time-dependent manner.[42–44] Thus, allosteric modulation is an attractive strategy for developing precise therapeutics for both the CNS and periphery.

3. RECENT ADVANCES IN THE DISCOVERY AND DESIGN OF CLASS A GPCR ALLOSTERIC MODULATORS

3.1. Aminergic Family Receptors.

3.1.1. Serotonin 2C Receptor (5-HT_{2C}R).—

The serotonin (5-HT) 5-HT_{2C} receptor (5-HT_{2C}R) is a member of the 5-hydroxytryptamine receptor family, which can activate phospholipase Cβ (PLCβ) via G$\alpha_{q/11}$ to result in production of intracellular inositol 1,4,5-triphosphate (IP_3) and diacylglycerol (DAG) to promote intracellular calcium release (mobilization).[45] Assays described throughout this Perspective that relay information on calcium mobilization follow a similar signaling cascade, depending on the activated G protein. The 5-HT_2 subfamily has been of pharmacological interest for decades and is implicated in neurological, psychological, and circulatory processes.[46] It is well-known that nonselective antagonists of this subfamily can alleviate symptoms of schizophrenia and anxiety, while nonselective agonists would cause cardiac abnormalities and hallucinations, among other effects.[47] However, selective stimulation of the 5-HT_{2C}R is useful for treating obesity and may be useful for treating substance use disorders (SUD), depression, and other neuropsychological disorders.[48,49] Therefore, there remains a need for developing 5-HT_{2C}R targeted ligands that display a high degree of specificity for the 5-HT_{2C}R over the highly homologous 5-HT_2 subtypes 5-HT_{2A}R and 5-HT_{2B}R. As previously mentioned, allosteric modulation holds the potential to differentiate between receptors by targeting less conserved regions and thus provides therapeutic benefit at the 5-HT_{2C}R while eliminating significant CNS (5-HT_{2A}R) and cardiovascular (5-HT_{2B}R) adverse effects. Two primary groups are engaged in projects aimed at discovering PAMs of the 5-HT_{2C}R.[50,51]

PNU-69176E (**1**; Figure 3) was discovered by Pharmacia (later acquired by Pfizer) via screening of an internal chemical library and is the first reported 5-HT_{2C}R selective positive allosteric modulator.[52] In a radioligand competition binding assay of [^{3}H]mesulergine displacement by 5-HT, it was reported that a 20 μM concentration of **1** increased the affinity of 5-HT by 25-fold to the 5-HT_{2C}R, measured at a K_i value of 6.4 nM in the presence of **1** compared to a K_i value of 159 nM for 5-HT alone. Further, **1** displays potentiation in multiple cell lines and potentiates the effects of 5-HT at multiple receptor densities (6–45 pmol/mg of protein). Binding selectivity experiments indicate that **1** is a selective 5-HT_{2C}R PAM with no appreciable binding to analogous 5-HT (5-HT_{2A}R, 5-HT_{2B}R, 5-HT_6R, and 5-HT_{7A}R) and biologically relevant dopamine receptors (D_2R and D_3R). However, unlike pure PAMs, Im and colleagues reported that introduction of 10 μM concentration of **1** alone could cause IP_3 release up to 71% of the maximal response from the same concentration of 5-HT in human embryonic kidney 293 (HEK293) cells, possibly indicating a stabilization effect of the receptor active state. This intrinsic effect was conserved to various degrees while measuring both [^{35}S]GTPγS binding and [^{3}H]IP accumulation in HEK293 cells, contributing to possibility that **1** functions as an ago-PAM. Both the long alkyl chain and polar moiety (α-D-galactopyranoside) of the chemical structure are reported to be integral to

its function and may provide anchoring to the membrane and binding with the allosteric site, respectively. Interestingly, functional characterization of PAM activity of **1** via 5-HT-evoked intracellular calcium mobilization in 5-HT_{2C}R expressing Chinese hamster ovary (CHO) cells showed **1** maintained potentiation of 5-HT_{2C}R by 5-HT activation but lacked the previously reported intrinsic agonist activity. Additionally, in the same calcium mobilization assay, its diastereomer **2** (Figure 3) did not potentiate 5-HT-mediated responses nor did **2** exhibit intrinsic agonist activity.[53] Key differences between the characterization of **1** as an ago-PAM or PAM are likely borne from the selection of cell lines and corresponding 5-HT_{2C}R protein expression levels. The HEK293 cells had expression levels of ~45 pmol/mg protein, whereas the stably expressing CHO cells were characterized with physiologically relevant expression levels of ~250 fmol/mg protein for the 5-HT_{2c}R Thus, selected cell lines and receptor protein expression levels are important considerations for ligand characterization, comparison across laboratories, and the interpretation of SAR data.

Recently, our group reported CYD-1–79 (**3**), with a 4-alkylpiperidine-2-carboxamide scaffold, as a selective 5-HT_{2C}R PAM presenting a promising in vitro and in vivo profile for the treatment of cocaine use disorder (CUD).[51] Unlike **1**, **3** (Figure 3) functions as a pure PAM to potentiate 5-HT-evoked calcium mobilization in CHO cells expressing human 5-HT_{2C}R but exhibits no intrinsic activation of calcium mobilization. When investigated in preclinical pharmacokinetic (PK) and rodent efficacy studies, **3** displays measurable blood—brain barrier permeability and significantly suppresses motor impulsivity and cue reactivity assessed as lever presses for cocaine-associated cues in a rodent cocaine self-administration assay. Excitingly, elegant work in the class A GPCR crystallography field has recently produced a high-resolution crystal structure of the 5-HT_{2C}R capable of enabling molecular docking studies.[54] Shown in Figure 3, these computational studies reveal there is a predicted bridging effect formed by **3** between the extracellular loop (EL) 2 (EL2) and the transmembrane helix (TMH) VI (TMH VI) of the 5-HT_{2c}R via a bidentate, H-bonding interaction. This predicted bridging effect is mediated by a 1,2-diol moiety on **3** to the backbone carbonyl of $Leu209^{ECL2}$ residue of ECL2, and another H-bond between the ionizable N-atom of the piperidine ring and the—OH side chain of $Ser334^{6.58}$ of TMH VI (PDB code 6BQG). Significantly, these residues are not conserved in the highly homologous 5-HT_{2A}R or 5-HT_{2B}R, and these computational studies provide a possible explanation for the selective profile of **3** among closely related receptor subtypes.[51]

Lopez-Rodriguez and colleagues highlighted 5-HT_{2C}R PAMs as potential antiobesity therapeutics and recently reported the screening hit VA024 (**4**), featuring an indole scaffold, as a 5-HT_{2c}R PAM.[50] In what is only the second reported synthetic small molecule screening hit for 5-HT_{2c}R PAMs, **4** (Figure 3) was identified in a Vivia Biotech chemical library via an innovative automated flow-cytometry-based screening system, the PharmaFlow platform (previously ExviTech platform).[50,55] A minor structural modification of the pyrimidin-5-amine side chain of **4** with pyridine results in VA012 (**5**). 5-HT concentration—response curves were assessed in cells, and the introduction of **5** at a concentration of 10 μM potentiated the 5-HT E_{max} 35% greater than the efficacy of 5-HT alone. Further in vitro studies indicate **5** does not appreciably bind to important 5-HT_2 family members (5-HT_{2A}R and 5-HT_{2B}R), displays low binding competition against the

endogenous agonist (5-HT) and other orthosteric ligands (mesulergine and clozapine), and results in no significant off-target interactions as indicated in a CEREP cellular GPCR panel. Significantly, feeding models in rodents indicate **5** reduces both food intake and body weight gain without causing taste aversion when acutely administered at 2 mg/kg (ip).

As previously discussed, proteins that interact with class A GPCRs, such as G proteins, are fundamentally allosteric in their mediation of the receptor structural state and their interference or coordination in allowing other proteins to interact. Structurally, the third intracellular loop and C-terminal tail of the 5-HT_{2C}R, like most other class A GPCRs, act as scaffolds, molecular levers, and protein recruiters with multiple protein binding and phosphorylation sites for mediating receptor function.[56–59] Phosphatase and tensin homolog (PTEN) recognize key residues on the 5-HT_{2C}R intracellular loop III (ICLIII) and mediate 5-HT_{2C}R biological responses.[60] Significantly, this interaction occurs at 5-HT_{2C}R but not at the 5-HT_{2A}R. A fragment of the 5-HT_{2C}R protein, termed 3L4F (third loop, fourth fragment of the human 5-HT_{2C}R), is a peptide derived from the protein interaction site and has been shown to disrupt the 5-HT_{2C}R–PTEN complex and promote 5-HT_{2C}R mediated downstream signaling, acting as a PAM of 5-HT_{2C}R signaling.[61] Subsequent studies revealed that 3L4F-F_1 (Pro280–Arg287), a component of the first eight amino acids of the peptide 3L4F, maintains the efficacy of the full length 3L4F peptide within the picomolar range in vitro and also functions as a PAM of 5-HT_{2C}R in rats in vivo.[61] Referencing this example as a proof-of-concept, modulation of specific protein—receptor interactions at other GPCRs may be achievable to produce PAM or NAM profiles. These examples cover two unique modes for allosterically altering signaling at the 5-HT_{2C}R, where small molecules may bind to the extracellular region of the receptor and stabilize/induce an active state, or peptides and small molecules may bind intracellularly and disrupt protein–protein interactions.

3.1.2. β_2-Adrenergic Receptor (β_2AR).—The β_2-adrenergic receptor (β_2AR) is an aminergic class A GPCR whose endogenous signaling molecule is adrenaline. The β_2AR is widely expressed in bronchial smooth muscle and plays a significant role in cardiovascular and pulmonary physiology.[18] As one of the most highly studied and characterized GPCRs, numerous studies on the β_2AR represent foundational knowledge on GPCR function, structure, and physiological importance for cell signaling.[62–67] Therapeutically, β_2AR agonists represent a large class of drugs used to treat pulmonary disorders and asthma, while β_2AR antagonists comprise selective and nonselective β-blockers (β-blockers), widely used for the treatment of hypertension, cardiac arrythmias, and other cardiovascular indications. At present, nearly all known β-adrenergic ligands act orthosterically.[68] Kobilka and colleagues have recently reported the first allosteric β-blocker, or β_2AR NAM, known as Cmpd-15 (compound-15, **6**) and, significantly, the cocrystal complex with β_2AR (Figure 4).[21,68] This recently discovered β_2AR NAM displays low micromolar affinity for β_2AR allosteric site and was identified via a DNA-encoded small-molecule library screen comprising 190 million distinct compounds.[68] SAR studies demonstrate that the formamide group in the para-formamidophenylalanine region and bromine in the meta-bromobenzyl methylbenzamide region are integral for the functional activity of **6**, and a dramatic reduction of activity was observed when removing these groups.[69] In vitro studies indicate the addition of **6** results in an inhibition of β_2AR stimulated cAMP production and fi-

arrestin recruitment. Furthermore, pharmacological studies and the β_2AR-NAM cocrystal of a polyethylene glycol—carboxylic acid derivative of **6** reveal an intracellular binding site formed by residues from helices I, II, and VI–VIII and the ICL1 of the β_2AR (PDB code 5X7D).[21] Only recently have small molecule allosteric sites been identified on the intracellular surface of GPCRs, and this finding is significant as these results could extrapolate to additional members of the class A GPCR family, opening new avenues for allosteric drug discovery.

3.1.3. Dopamine Receptors (D_1, D_2, D_3).—Dopamine receptors represent a therapeutically important subset of class A GPCRs and exist in two distinct families: (1) D_1-like family members comprise dopamine D_1 and D_5 receptors, which couple to the $G\alpha_s$ and $G\alpha_{olf}$ G proteins and stimulate cAMP production, while (2) D_2-like family members comprise D_2, D_3, and D_4 receptors, which predominantly couple to $G\alpha_{i/o}$ G proteins and attenuate cAMP production. D_2-like receptors are widely recognized as the predominant target for the treatment of schizophrenia and Parkinson's disease.[70,71] However, as a whole, dopamine receptors play a substantial role in numerous neurological and psychological disorders, including also attention deficit hyperactivity disorder (ADHD) and drug and alcohol dependence or SUD.[72] From a structural perspective of allostery, the evidence of dimerization in dopamine receptors and other GPCRs (homodimers and heterodimers) should not be overlooked, but there is still much to understand about the functional implications of GPCR dimer populations and how these populations might be therapeutically targeted.[73–79]

Due to the therapeutic potential of D_2-like receptor drugs, several allosteric modulators for D_2 and D_3 have been reported recently (Figure 5). The neuropeptide Pro-Leu-Gly-NH_2 (PLG, **7**), initially isolated from brain tissue, is an endogenous molecule that has shown potential for pharmacologically treating neurological diseases such as Parkinson's disease and tardive dyskinesia, but the peptide nature of **7** limits its development as a drug. Therefore, the rational design and modification of **7** have led to analogues containing lactam, bicyclic and spiro-bicyclic scaffolds in the search for agents with better PK properties. Subsequent studies show **7** acts as a PAM of the dopamine D_2 and D_4 receptor, and the modes of action for **7** and its peptidomimetics were validated by photoaffinity labeling peptidomimetics.[80–82] Modification of **7** in the L-proline or L-Priline and L-leucine residues led to compounds **8** and **9** displaying a similar PAM profiles as **7**, measured by increasing [^{3}H]NPA binding at concentrations between concentrations of 1pM and 1 nM in human dopamine D_2 receptors.[83] Improving upon the initial neuropeptide, analogue PAOPA (**10**) (3(*R*)-[(2(*S*)-pyroolidinylcrbony)amino]-2-oxo-1-pyrrolidine-acetamide), a dopamine D_2 recetor selective PAM, is 100- to 1000-fold more potent than **7** and significantly attenuated schizophrenia-like behavioral phenotypes in preclinical models. [84–86] Importantly, **10** displays an improved PK and toxicological profile.

Interestingly, extensive studies with the spiro-bicyclic analogues of 7 have produced both D_2 PAMS and NAMS with minor differences in the stereochemistry of the bridgehead carbon within the same series of peptidomimetics.[87]Compounds **11** and **13** are D_2 PAMS, while the corresponding distereoisomeric compounds **12** and **14,** with a difference in the 8′ a chiral

center, demonstrate D_2 NAM activity in binding experiments with the D_2 receptor agonist NPA and competitive binding with the D_2 PAM.[88] The molecular conformation is hypothesized to take either a type VI β-turn, or polyproline II helix conformation that could place the carboxamide NH_2 pharmacophore in the same topological space as that seen in the type II β-turn, which is vital for the ability to modulate dopamine receptors. Molecular modeling suggests there are two different conformations in the pucker of **14** that may result in divergent effects on the orthosteric site. The modeling results were experimentally tested by incorporating proper chemical substituents to convert the PAM to a NAM. Compounds **15** and **16** with dimethyl groups in the C2′ position of the 5.6.5 bridge were designed to display NAM activity, divergent from the parent compound PAM activity. Indeed, when compared to a control, **15** and **16** demonstrate a NAM profile, which negatively affects the binding of the dopamine receptor agonist NPA to the D_2 receptor, and the resultant shift in the EC_{50} value for [^{3}H] NPA binding to D_2 was 2.7- and 2.8-fold at concentrations of 1 μM and 10 μM, respectively.[89]

SB269652 (**17**) was found to be a negative allosteric modulator for D_2 and D_3, and the corresponding SAR shows both components of **17** possess influence to some extent on its allosteric activity (Figure 5).[90,91] The design of **17** incorporates both an orthosteric site-binding moiety and an allosteric site-binding moiety, referred to as a bitopic ligand. Modest structural modifications shifted activity in the series between competitive antagonists and compounds, such as **17**, that display allosteric pharmacology. The THIQ head group is crucial for maintaining allosteric pharmacology, while a "small" substituent in the 7-position is required, and replacement of the alkyl spacer with a linear 1,4-butylene or 1,6-hexylene spacer group conferred an increase in functional affinity. Interestingly, although the tail group is sensitive to chemical modification, an alternative 7-azaindole tail was reported and demonstrates a 30-fold increase in affinity while maintaining negative cooperativity with dopamine.[92] Further optimization of **17** led to D_3 receptor-preferring bitopic ligands, characterized by a *trans*-cyclo-propylmethyl linker replacing the *trans*-1,4-cyclohexylene linker while retaining the head and tail groups.[93] Recently, a benzothiazole scaffold compound was reported as a D_2 PAM, identified via a high-throughput screen (HTS) on 80 000 compounds, and provides another small molecule hit for the development of D_2 PAMs.[70] Although there are several identified hits coming into the arena for the allosteric modulation of D_2 and D_3, further development to provide mature clinical candidates with improved in vitro and in vivo properties, along with safer PK profiles, is still urgent.

3.1.4. Muscarinic Acetylcholine Receptors (M_1–M_5).—

Muscarinic acetylcholine (ACh) receptors contain five receptor subtypes (classified as M_1–M_5) and are involved in a wide range of biological processes and diseases, including pain, Alzheimer's disease, schizophrenia, diabetes, and obesity.[94] According to their G protein coupling preference, there are two major functional classes. The M_1, M_3, and M_5 receptors selectively couple to G proteins of the $G\alpha_{q/11}$ family, whereas the M_2 and M_4 receptors preferentially activate $G\alpha_{i/o}$ G proteins. From the perspective of biological distribution, the M_1, M_4, and M_5 receptors are predominantly expressed in the CNS, whereas the M_2 and M_3 receptor subtypes are widely distributed both in the CNS and in peripheral tissues, thus playing an important role in regulating various peripheral and central physiological functions.[95] As a

reference, a recent review by Mohr and colleagues has thoroughly summarized allosteric modulators targeting CNS muscarinic receptors.[96] Herein, we focus on the recent development of these modulators from a medicinal chemistry perspective, which is mainly reflected in M_1, M_4, and M_5 allosteric modulators.

M_1 mAChR.: Benzylquinolone carboxylic acid (BQCA, **18**), discovered through HTS efforts at Merck, is a representative scaffold for the development of highly selective M_1 mAChR PAMs and provides a basis for developing novel therapeutics to counteract the negative cognitive symptoms associated with diseases such as Alzheimer's disease and schizophrenia (Figure 6).[97] According to current ligand classifications, **18** is a PAM that lacks intrinsic activity to induce calcium mobilization at concentrations up to 10 μM but markedly increases ACh potency 129-fold at 100 μM in human M_1 mAChR expressing CHO cells. Additionally, **18** displays selectivity over the related neuronal M_2–M_5 mAChR subtypes up to >100-fold and does not modulate signaling at other examined class A GPCRs. However, **18** presents lackluster PK, resulting in high plasma protein binding and low solubility in its neutral form.

Subsequent SAR-informed optimizations to address these shortcomings were conducted. These studies suggest fluoro substituents are preferred optimizations for analogues of **18** in terms of M_1 mAChR potency in vitro,[98] which was later proven to attribute to increased intrinsic efficacy of these analogues.[99] Compounds **19** and **20** were produced based on this principle and displayed an improved inflection point (IP, also known as PAM EC_{50}) but maintained high plasma protein binding and poor brain exposure.[98,100] Amide derivative **21**, with an improved PK profile, produces higher potency binding and functional cooperativity with ACh, bearing cooperativity α and $\alpha\beta$ values of 170 and 840 in a calcium mobilization assay, respectively.[99] Modifications on the benzyl side chain and quinolin-4(1*H*)-one of **18** were examined as a means to further improve affinity, decrease plasma protein binding, and address the blood—brain barrier (BBB) permeability problem aforementioned. Aryl methyl benzoquinazolinone **22** is a resultant compound with a greater than 50-fold increase in affinity for the M_1 receptor allosteric site in comparison to **18** (K_B = 0.3 μM for **22** and 15 μM for **18**) while retaining similar positive cooperativity with ACh and relative intrinsic efficacy.[101] Mutagenesis studies and molecular modeling confirm compound **22** occupies the same allosteric binding pocket as **18**. Insights from these studies include key hydrophobic/edge-to-face π–π interactions with residues Tyr-179 in ECL2 and Trp-400$^{7.35}$ in TM7 that are critical for the increased affinity to the allosteric site and functional activity of **22**. MK-7622, a mature example in this series, advanced into a phase II clinical trial in 2013 and was terminated in 2016 for undisclosed reasons.[102]

For further optimization efforts, modifications were made to the core pharmacophore aryl ring systems. Opening of the aryl A ring of **22** furnishes 4-phenylpyridin-2-one **23**, a structure maintaining intramolecular hydrogen bonding between the carboxylic acid and ketone in **18**.[103] Compound **23** shows comparable allosteric site binding affinity to 18 with a K_B value of 43 μM in FlpIN CHO cells expressing human M_1 mAChR but interestingly improved positive cooperativity with ACh (α = 370, $\alpha\beta$ = 200), i.e., a significant 370-fold potentiation of ACh affinity, and retained high selectivity for the M_1 mAChR. Further

modification of pyridin-2(1H)-one to 6-phenylpyrimidin-4-one **24** presents an α value of 1380, a 4-fold increase in binding cooperativity with ACh, along with an 11-fold increase in intrinsic efficacy (τ_B = 2.51), suggesting further interaction with the allosteric pocket of the M_1 mAChR through the introduction of an additional tertiary nitrogen as a hydrogen bond acceptor. Ring opening of aryl B, replacing the tricyclic benzo[*h*]-quinazolin-4(3*H*)-one core with quinazolin-4(3*H*)-one, gives compound **25**.[104] Compound **25** shows improved "druglike-ness" with lower lipophilicity, topological polar surface area (tPSA), molecular weight and also reduces the toxic DNA-chelation concern for polyaromatic heterocycle scaffolds. The methyl group in the 8-position is critical to maintain affinity for the M_1 mAChR allosteric site compared to **22** with pK_B and $\alpha\beta$ values of 5.15 and 380 for **25** and 5.88 and 370 for **22**, respectively, but lower intrinsic activity (τ_B = 1.1) in radioligand binding experiments using FlpIN CHO cells. Dibenzyl-2*H*-pyrazolo[4,3-*c*]quinolin-3(5*H*)-one (**26**, DBPQ) is another example of a tricyclic scaffold similar to **22** and was discovered as a hit from a 2012 high-throughput screen of the NIH Molecular Libraries Small Molecule Repository (MLSMR).[105] A concentration—response curve of compound **26** in the presence of a submaximal concentration of ACh exhibits a PAM EC_{50} of 473 nM and limited cooperativity with ACh, resulting in a ACh maximum response of 40% measured by the calcium response assay.

Replacement of the quinolone ring system of **18** with quinolizidinone as an alternative scaffold and exchanging the side benzyl chain with a basic 4-cyanophenylpiperazine linkage led to compound **27**.[106] Compound **27** shows enhanced CNS exposure with a 13% human free fraction and improved in vivo efficacy in a mouse contextual fear conditioning model of memory, while the bioavailability is relatively low, observed as 23% in rats. Noteworthy, this modification limits the liability for efflux by the CNS efflux transporter P-glycoprotein (P-gp). Compound **28** bearing a cyano group at the 4-position of the piperidine displays increased PAM potency (M_1 mAChR EC_{50} = 135 nM) in human M_1 mAChR expressing CHO cells determined in the presence of an EC_{20} concentration of ACh by a calcium mobilization readout on a fluorometric imaging plate reader (FLIPR384).[107] The plasma free fraction value is an acceptable 30%, and oral bioavailability is greatly improved to 68% in rats with a 1.7 h half-life. Further modification by replacing the carboxylic acid with cyclic amide substituents to produce **29** significantly improved the PAM potency in functional assays (M_1 mAChR EC_{50} = 31 nM); however this led to P-gp efflux liability.[108] A replacement of the core to methoxynaphthalene led to compound **30** with a M_1 mAChR PAM EC_{50} value of 17 nM.[109] Although **30** addressed the P-gp substrate problem, **30** results in high protein binding as observed at 99.9% in rat.

VU0108370 (**31**), a hit discovered via a functional HTS of the NIH's Molecular Libraries Probe Production Centers Network (MLPCN) library, represents a second unique chemotype based on an indole core (Figure 7).[110] Compound **31** shows relatively low PAM potency for ACh at the M_1 mAChR with an EC_{50} value of 9.71 μM. Structural optimization around **31** afforded ML169 (**32**) as a selective M_1 mAChR PAM MLPCN probe, presenting a moderate yet improved potency (PAM EC_{50} = 1.38 μM). Difluoro substitution of the indole core and a pyrazine in the benzyl side chain results in VU0456940 (**33**) and further improves the PAM EC_{50} to 310 nM.[111] Azaindole (**34**) results from the replacement of the quinolone ring of **18**

with an azaindole core producing an EC_{50} value of 1.8 μM.[112–114] Modification of the substitution on a benzyl with pyrazole furnishes PF-06764427 (**35**) as a highly selective M_1 mAChR PAM and demonstrates excellent cooperativity with a 30-fold potency improvement and favorable in vivo efficacy in an amphetamine-stimulated locomotor activity model in rodents.[115] Difluoro substitution on **35**, resulting in VU6004256 (**36**), improves its safety profile and was reported as lacking severe adverse effects, such as behavioral convulsions and peripheral cholinergic adverse effects in mice, although no improvements to its activity were observed.[116]

A third unique scaffold for M_1 mAChR PAMs consists of an isatin core. VU0119498 (**37**) is a pan-M_1,M_3,M_5-PAM discovered through a HTS campaign, and subsequent SAR enabled the rational design of subtype selective PAMs.[117] Chemical optimizations on **37** led to selective M_1 mAChR PAMs ML137 (VU0366369, 38) and VU0448350 (**39**) with EC_{50} values of 0.83 μM and 2.4 μM, respectively.[117,118] Further modification on this scaffold yields VU0453595 (**40**), a highly selective M_1 mAChR PAM with improved brain exposure in mice after systemic administration.[119] Recent modification around isoindolin-1-one produces compound **41**, which displays a significantly improved PAM EC_{50} value of 47 nM, minimal intrinsic agonist activity, and oral bioavailability of 91% in rodents and 65% in canines.[120] Other work in this arena includes the benzodiazepine derivative **42**, which was recently reported by a team at Roche as a selective M_1 mAChR PAM with an PAM EC_{50} value of 80 nM in human M_1 mAChR expressing cells.[121] Overall, there are variable improvements in brain exposure and in vivo efficacy for selective M_1 mAChR allosteric modulators and development continues for multiple scaffolds presented herein.

M_5 mAChR.: The M_5 mAChR has historically been the least studied receptor of the mAChR family, primarily due to low endogenous expression levels; however there is a current upsurge in interest from drug discovery groups to identify selective PAMs that are efficacious in relevant disease models.[122] Physiological studies indicate the M_5 mAChR is enriched in the cerebrovascular system and present a potential target for the treatment of numerous CNS disorders including schizophrenia, Alzheimer's disease, ischemia, and migraine.[122] As previously highlighted, the small molecule series with an isatin core display PAM activity at the M_5 mAChR in addition to other mAChR subtypes, and thus medicinal chemistry efforts have partly focused on identifying molecular switches to convey subtype specificity in this series. VU0119498 (**43**) is the first reported hit discovered via a HTS and displays micromolar potencies as a pan-PAM for the potentiation of ACh at M_1, M_3, and M_5 mAChR subtypes in cell-based calcium mobilization assays (Figure 8).[123] Subsequent optimization led to the discovery of ML129 (VU0238429, **44**), containing a 5-trifluoromethoxy isatin scaffold, as a selective M_5 mAChR PAM with an EC_{50} of approximately 1.16 μM.[124] Further modifications included substitutions on the benzyl chain where it was shown that VU0365114 (**45**) and ML172 (VU0400265,**46**) could maintain potency, while the addition of a phenoxyethyl substituent in ML326 (**47**) achieved submicromolar level potency in a human M_5 mAChR expressing cell line (EC_{50} = 410 nM, ACh fold-shift = 20, Figure 9).[122,125,126] However, the suboptimal ionization properties of **47** preclude its utility for in vivo assessment due to low CNS exposure.[127] ML380 (**48**), a promising and divergent scaffold lacking the common isatin core, was discovered during a

HTS of the MLPCN screening deck of ~360 000 compounds against M_1, M_4, and M_5 mAChR subtypes. Compound **48** displayed selective M_5 mAChR PAM activity with submicromolar potency and markedly improved CNS penetration (EC_{50} = 190 nM, ACh fold-shift = 9.3).[127]

In regard to NAM discovery, ML375 (**49**) is the first selective M_5 mAChR NAM with submicromolar allosteric site potency, and characterization reveals an IC_{50} of300 nM at the human M_5 mAChR and an IC_{50} of 790 nM for rat M_5 mAChR (Figure 9).[128] Compound **49** was also found to exhibit favorable CNS exposure (brain/plasma K_p = 1.8).[128] However, high protein binding in both blood and brain tissue (rat f_u = 0.029, rat brain f_u = 0.003) has limited its utility as an in vivo tool compound to date.[129] Through a combination of matrix libraries and iterative parallel synthesis, optimization of **49** led to VU6000181 (**50**), which maintains potency levels but also retains an unfavorable PK profile.[129]

M_4 mAChR.: Interestingly, the analytical specialty chemical thiochrome was one of the first reported selective M_4 mAChR PAMs but was observed to exhibit low affinity for the receptor.[130] Heightened activity in this arena occurred with the discovery of LY2033298 (**51**) as a benchmark example of selective M_4 mAChR PAMs (Figure 10).[131] Compound **51** contains a 5-aminothieno[2,3-*c*]pyridine scaffold and has a measured K_B value of 200 nM at the allosteric site on the unoccupied human M_4 mAChR.[131] Chemical modification of **51** on the substitutions in the thieno[2,3-*c*]pyridazine and side chain resulted in the discovery ofVU10010 (**52**).[132] Compound **52** has a high potency with an EC_{50} value of400 nM and elicits a 47-fold leftward shift of an ACh concentration—response curve in a calcium-mobilization assay in rat M_4 mAChR expressing cells. According to follow-up manuscripts focusing on the optimization of **52**, it was stated that **52** was not centrally active in vivo and was considered to be a substrate for the P-gp efflux pump.[133] However, upon work to the scaffold, the authors concluded lack of in vivo activity of **52** was due to physicochemical properties (e.g., log *P*= 4.5) that led to a poor pharmacokinetic profile. Optimization of **52** led to ML108 (**53**) and VU0152099 (**54**), which present nearly equivalent potency while improving CNS exposure as evidenced by peak brain concentrations ranging from 3 to 5 μg/mL after 56.6 mg/ kg ip administration in rodents.[133] However, the metabolic stability of this scaffold is poor due to the hydroxylation of the 6-methyl group on the pyridine ring, resulting in less than 10% parent compound remaining after 90 min. Noteworthy, the replacement of the metabolically labile 6-methyl group with an ether linked substituent led to ML173 (**55**) and a significantly improved microsomal stability profile with greater than 90% parent remaining after 90 min, in both rat and human microsomal assays.[134] Although in vitro potency in human M_4 mAChR expressing cells was measured at an EC_{50} of 95 nM, subsequent preclinical in vivo studies demonstrated limited efficacy for **55** in the reversal of amphetamine-induced hyperlocomotion in a rat behavioral model. This result necessitates highlighting an important phenomenon that can be encountered during the preclinical phases of compound optimization: species bias. Additional data indicate that although high potency was observed at the human M_4 mAChR, **55** displays a strong species bias with an EC_{50} of only 2.4 μM for the rat M_4 mAChR, which leads to exceedingly difficult optimization and ultimately precludes preclinical development of this compound.[134] In an expert review,

Conn, Lindsley, Meiler, and Niswender advise identifying and avoiding compounds with an intractable species bias from experience in this series and others.[22]

ML293 (**56**) is characterized by a unique scaffold with a benzothiazole core and was developed through an iterative optimization effort at the Vanderbilt Center for Neuroscience Drug Discovery (Figure 10).[135] Although **56** displayed low micromolar potency at the human M_4 mAChR, the promising in vivo PK properties warrant attention with a lower iv clearance rate (11.5 mL min^{-1} kg^{-1}) than previous series and excellent brain exposure when orally administered to rats and measured as a brain to plasma ratio (10 mg/kg at 1 h, [brain] = 10 μM, *B:P* = 0.85).[135] The modest potency and half-life (EC_{50} = 1.3 μM, $t_{½}$ = 57 min) necessitates further optimization of this series. Recently reported, modification of the previously described 3-aminothieno[2,3-*b*]pyridine scaffold (as in **53**) by replacing the pyridine with a pyridazine ring results in VU0464090 (**57**).[136] Compound **57** displays a 3-fold potency improvement with an EC_{50} value of 150 nM in human M_4 mAChR expressing cells and a significant 9-fold improvement in free fraction values compared to **53** (f_u rat, human of 0.022, 0.035 for **57** and 0.015, 0.004 for **53**).[136] This relatively minor chemical modification results in large electronic changes due to the high dielectric constant of the pyridazine ring and likely led to the observed improvement in PK properties. However, the *p*-methoxybenzylamide in **57** proved to be metabolically labile via cytochrome P450 (CYP)-mediated oxidative demethylation, and alternative amides were explored. Concentrating on sulfur-containing amide moieties, Lindsley and colleagues discovered VU0467154 (**58**) through an iterative optimization campaign.[136] Although the potency of **58** for human M_4 mAChR is modest with an EC_{50} value of631 nM, **58** has proven to be an exemplary rodent M_4 mAChR PAM tool compound, due to its reported minimal off-target interactions, excellent subtype selectivity, and good PK profile.[136,137] The following optimizations to the 5-aminothieno[2,3-*c*]pyridazine class ofM_4 mAChR PAMs were recently reported and feature an azetidine substituted side chain.[138] Out of a series of examined cyclic amines, azetidine based linkers in the side chain retained activity. Chemically, compound **59** in this series maintains an amide; however utilization of the azetidine results in a rigid amide lacking H-bonding ability (Figure 10). On the basis of an interesting analysis of X-ray crystallographic data from compound **59**, the removal of the amide N–H and the introduction of conformational restraint in the side chain were hypothesized to limit intermolecular interactions between adjacent molecules due to π-stacking and H-bonding, ultimately improving upon the poor solubility of previous derivatives. Indeed, **59** maintains high potency with a human M_4 mAChR EC_{50} of 72 nM and markedly improved CNS exposure in rat (K_p = 2.6, $K_{p,uu}$ = 2.1).[138] In vivo, **59** was examined in an established rat amphetamine-induced hyperlocomotion assay to determine antipsychotic efficacy and at 30 mg/kg (oral) **59** can attenuate hyperlocomotion by up to 32%. However, the azetidine amide linker in this scaffold is a metabolic weakness with high predicted clearance resulting from both rat and human microsomal preparations (CL_{hep} = 64 (r), 20 (h) mL min^{-1} kg^{-1}). Subsequent developments detail a replacement by a 3-aminoazetidine moiety, resulting in VU6000918 (**60**).[139] Compound **60** is characterized by improved in vitro human M_4 mAChR potency (EC_{50} = 30 nM), a relatively short half-life (1–2h), and suboptimal oral bioavailability, as measured at 11% in canine (2 mg/kg). At present, this extended series of compounds has demonstrated excellent in vitro activity and preferred species selectivity;

however achieving an optimal combination of distribution, metabolism, and pharmacokinetic (DMPK) properties such as bioavailability, CNS exposure, P-gp efflux liability and metabolic stability has proven to be challenging.

M_2 mAChR.: The M_2 mAChR is distributed in both the CNS and periphery and has been predominately studied for its role in regulating parasympathetic cardiac function. In this capacity, the M_2 mAChR modulates potassium channels and its activation leads to the closing of calcium channels, necessary for heart rate reduction.[140] Early examples of M_2 mAChR allosteric modulators, discovered by Mohr and colleagues, include alkane-bisammonio-type ligands.[141] This chemotype was subjected to subsequent rounds of chemical modifications, where a switch from negative to positive cooperativity was observed and conjugation to orthosteric ligands resulted in bitopic molecules of interest.[142] The importance of the M_2 mAChR lies not in its therapeutic utility as the general consensus is that M_2 mAChR activation leads to undesirable off-target effects for mAChR modulators.[143] However, from a structural perspective, elegant work on the M_2 mAChR and its PAM LY2119620 (**61**) has enabled major leaps in the understanding of mAChR, and GPCR, allosteric modulation.[19,144] Compound **61** is reported as a high-affinity PAM of both the M_4 and M_2 mAChR, and it potentiates the activity of agonist iperoxo. The pioneering cocrystal structure of **61** bound to the M_2 mAChR shows **61** occupying a site above the orthosteric iperoxo binding site on the extracellular side ofhelices II, VI, and VII and forming extensive contacts with ECL2. and ECL3 (PDB code 4MQT, Figure 11).[19] These contacts include a charge—charge interaction between the piperidine group and residue Glu172 in ECL2, hydrogen bonds between both the amide oxygen and N–H with the side chains of $Tyr80^{2.61}$ and Asn419 in ECL3, and additional hydrophobic interactions with aromatic residues in ECL2. This structural analysis provides insight into one way a PAM can impact agonist binding and resultant receptor activity.

Further contributions from the structure of the M_2 mAChR include a recent discovery effort aimed at finding highly selective PAMs that potentiate binding of nonselective antagonists, whereby the antagonist is essentially turned selective due to PAM induced cooperativity.[145,146] These studies utilized extensive molecular libraries for ensemble docking at the M_2 mAChR and realize the potential of allosteric modulators to improve orthosteric ligand selectivity. The resultant compound '628 (**62**) features a unique triazoloquinazolinone and could enhance binding of the M_2 mAChR antagonist *N*-methyl scopolamine (NMS) with a cooperativity factor (α) of 5.5. Interestingly, **62** can markedly slow the dissociation rate of NMS from the M_2 mAChR by 50-fold. The specific PAM effect of **62** on NMS antagonism was further validated in cell-based functional assays, and the observations translated to membranes from adult rat hypothalamus and to neonatal rat cardiomyocytes.[146]

3.2. Lipid Family Receptors.

3.2.1. Cannabinoid Receptors (CB_1 and CB_2).—

The CB_1 receptor and the CB_2 receptor are key mediators of the endocannabinoid system. The cannabinoid CB_1 receptor is widely distributed throughout the CNS and endogenous agonists of the CB_1 receptor include anandamide (AEA) and 2-arachidonylglycerol (2-AG), which regulate many physiological processes related to pain, metabolism, nociception, and neurotransmission.[147] To date, CB_1

receptor orthosteric agonists and antagonists have not realized therapeutic expectations largely due to adverse effects, for example, the orthosteric antiobesity antagonist rimonabant was withdrawn from the market owing to neuropsychiatric adverse effects.[148] Alternatively, development of both NAMs and PAMs of the CB_1 receptor has been of high interest in recent years, encompassing structurally distinct synthetic, plant-derived and endogenous allosteric ligands. NAMs of the CB_1 receptor were reported first, mainly comprising two scaffolds that have been extensively characterized: the 1*H*-indole-2-carboxamide and the diarylurea analogues. The CB_2 receptor, another important member of the endocannabinoid system, is highly expressed in the periphery, especially in blood cells, and in blood-cell producing organs.[149] Additionally, recent work has highlighted the expression of CB_2 receptors in the CNS and, functionally, CB_2 receptor modulation of dopamine-mediated neural activity and cocaine self-administration in rodents.[150,151] Despite recent pharmacological advances in the characterization of cannabidiol as an allosteric modulator of the CB_2 receptor,[152] the therapeutic potential of PAMs and NAMs targeting the CB_2 receptor requires additional investigation, and thus we focus on CB_1 receptor ligand discovery herein.

1H-Indole-2-carboxamide CB_1, Receptor NAMs.: A major advance was made with the discovery of 1*H*-indole-2-carboxamide analogues as CB_1 receptor NAMs. Org27569, Org29647, and Org27759 (**63–65**, Figure 12) displayed an interesting pharmacological profile by enhancing the affinity yet reducing the efficacy of CB_1 receptor agonists and suggests the existence of an allosteric binding site at the CB_1 receptor. These allosteric ligands have proven to be an excellent series of tool compounds and provide a basis for medicinal chemistry development. When examined in a binding assay, **63–65** augment specific binding of the CB_1 receptor agonist 2-[(1*R*,2*R*,5*R*)-5-hydroxy-2-(3-hydroxypropyl)cyclohexyl]-5-(2-methyloctan-2-yl)phenol, [^{3}H]-CP55,940 in membranes from cells expressing the CB_1 receptor. However, in a luciferase reporter gene assay, guanosine 5′-O-(3-[^{35}S]thio)triphosphate binding assay and mouse vas deferens assay, **63–65** elicit a significant reduction in the E_{max} for CB_1 receptor agonists, showing a resultant CB_1 receptor NAM profile.[153]

Piscitelli et al. examined a number of 4-substitutions on the phenyl B ring and discovered that piperidinyl or dimethylamino groups at the 4-position of the phenyl ring are preferential for CB_1 receptor NAM activity (Figure 12).[154] Compound **66**, with hydrogen in the C3 position of the indole, and compound **67**, with a dimethylamino substituent in the 4-position of phenyl, show potent NAM activity with IC_{50} values of 90 nM and 50 nM, respectively. The analyses of these compounds also provide evidence that the carboxamide functionality is required, and when replaced by an ester, potency is greatly reduced. Lu et al. subsequently conducted a series of SAR studies around the 1*H*-indole-2-carboxamide scaffold by measuring two essential parameters: K_B, which reflects the binding affinity of the allosteric ligand, and α, which measures the allosterically induced effects between the orthosteric and allosteric ligands when both are bound to the receptor. These SAR studies report the indole core is essential for the binding affinity (K_B) but not for generating allostery (α) on the orthosteric site, and the C3 substituents of the indole-2-carboxamides significantly impact the cooperativity. Replacing the indole with benzofuran ring (**68**) led to a 10-fold increase in

the K_B value (2594 nM for **68** vs 217.3 nM for **63**), and masking the indole nitrogen with a methyl group as in **69** led to a significant 27-fold decrease. Substitution of the C3 position with a linear *n*-pentyl group, resulting in **70**, displayed a noteworthy cooperativity enhancement (α = 17.6 for **70** vs *a* = 11.9 for **69**) while producing only a moderate binding affinity of 496.9 nM. Additionally, replacing the phenyl B ring piperidinyl substituent in **70** with a dimethylamino moiety yields **71** with improvement to the NAM binding affinity (K_B = 167.3 nM) and maintenance of the cooperativity (α = 16.55), while functional assays displayed a potent inhibition of GTPγS binding.[155,156] Further investigations modifying the C3 position with variations of the linear alkyl moieties, such as *n*-propyl and *n*-hexyl, yield compounds **72** and **73**, with **72** displaying the highest allosteric ligand affinity observed (K_B = 89.1 nM) and a reduction in cooperativity (α = 5.1), while **73** was characterized by modest affinity (K_B = 217.3 nM) and a significant enhancement of binding cooperativity (α = 24.5).[157] In addition, modification to the substitution on the A ring shows that a 5-halogen substituent is vital to maintain allosteric activity. Functional assays on **72** and **73** demonstrate an interesting effect on CB_1 receptor signaling pathways with a NAM concentration-dependent inhibition of agonist-induced GTPγS binding, yet they have a PAM effect in a β-arrestin mediated extracellular signal-regulated kinases ½ (ERK½) phosphorylation assay. Aside from alkyl chain substituents on the C3 position, less bulky substituents such as methyl and hydrogen on the C3 position and a fluoro or chloro substitution at the C5 position yielded analogues **74** and **75**, which possess improved CB_1 receptor NAM activity in a calcium-mobilization assay with an IC_{50} of 151 nM and 79 nM, respectively, which is approximately 5- and 10-fold more potent than the parent compound **71**.[158]

Ligand-assisted protein structure (LAPS) is an approach for elucidating structure—function correlates of ligand binding to GPCRs in functionally and physiologically relevant conditions, which is useful for obtaining structural information about the allosteric site since the structures of some receptors are obscure.[159] In this approach, noncovalent, pharmacologically active ligands can be designed and synthesized to accommodate chemically reactive moieties, such as electrophilic groups (e.g., isothiocyanate, benzophenone, etc.) and photoactivatable groups (trifluoromethyl diazirine, aliphatic/aromatic azide, etc.). Thus, upon ligand binding, the reactive group forms a covalent bond at an actionable amino acid residue providing spatial resolution of the allosteric site, in this case. Modification at the 5-position of **63** with the chemically reactive electrophilic group isothiocyanate (NCS) generates CB_1 receptor NAM covalent ligand **76** that retains the affinity—efficacy profile of **63** but displays reduced inverse agonist activity.[159] Functional assays show a signaling bias toward β-arrestin with an EC_{50} of 2 nM, an 87-fold difference over cAMP-dependent signaling. To validate the ability of **76** to covalently label human CB_1 receptor, time-course experiments were executed, which showed the binding of [^{3}H]-CP55,940 to human CB_1 receptor increases in a time-dependent manner, reaching a maximum by 60 min preincubation time with **76**. Compounds **77–80** are another series of photoactivatable analogues developed by Lu et al. as allosteric modulators for the CB_1 receptor.[160] These compounds preserved the pharmacological properties of **63** negatively modulating the CB_1 receptor agonist CP55,940-induced G protein coupling in a concentration dependent manner with a complete functional inhibition at 10 μM in their

assay system. These results show that the *N*-phenylethyl-1*H*-indole-2-carboxamide scaffold is a potent representative for CB_1 receptor allosteric modulation.

Diarylurea Analogues as CB_1 Receptor NAMs.: A second substantial body of work describes the discovery and interrogation of the diarylurea scaffold as a CB_1 receptor NAM. Numerous studies have now reported chemical leads bearing this scaffold, along with thorough SAR. 1-(4-Chlorophenyl)-3-(3-(6-(pyrrolidin-1-yl)pyridine-2-yl)phenyl)urea PSNCBAM-1 (**81**, Figure 13), discovered via HTS, displays PAM-like positive cooperativity in agonist binding affinity but decreases functional response in cellular assays. As an excellent case for a thorough pharmacological workup, **81** enhanced radioligand [^{3}H]-CP55940 binding levels but decreased functional responses stimulated by orthosteric agonists in numerous assays, including intracellular calcium mobilization, [^{35}S]GTP-γ-S binding, cAMP accumulation, and β-arrestin recruitment.[161,162] Interestingly, this profile is similar to *N*-phenylethyl-1*H*-indole-2-carboxamide Org27569 (**63**) with the notable exception of intrinsic efficacy as an agonist in some assays such as ERK½ phosphorylation; **81** has not displayed marked agonist activity in similar assays thus far.[163,164]

Detailed SAR studies on **81** have been reported, focusing on various substitutions on the diphenyl rings of the urea. Zhang et al. first reported chemical modifications to the 4-chlorophenyl at the A ring and the alkyl substitution at the 6-aminopyridinyl rings of the B ring. The smaller *N,N*-dimethylamino analog **82** displays comparable potency to **81** (27.4 nM vs 32.5 nM), suggesting the pyrrolidinyl ring is not required for this parameter. Further investigation on the A ring uncovered that the key property correspondent to activity was electron density. For example, electron withdrawing groups at the 4-position provided good potency, with the fluoro (**83**, IC_{50} = 32 nM) and the cyano analogues (**84**, IC_{50} = 33 nM). Among them, the cyano analogue possesses much better potency in CB_1 receptor calcium mobilization and radioligand [^{3}H]-CP55940 binding assays with an EC_{50} of 55.2 nM. Subsequent replacement of the pyridine ring by a pyrimidine ring, resulting in **85** and **86** (Figure 13), presents comparable activity to **81** in binding assays. Functional studies suggest that these compounds now display signaling biased PAM activity, promoting the β-arrestin-1 pathway toward ERK½ phosphorylation at 10 μM without the G_i-mediated signaling activity typically displayed by the agonist CP55940.[165] Further replacing the pyridinyl group with lipophilic aromatic rings (**87** and **88**) or introducing a spacer (N–H group) between the pyridine ring and the core phenyl (**89**) maintained activity at the CB_1 receptor and agreed with other work showing the exact properties lent by the pyridinyl and the pyrrolidinyl ring were not necessary for CB_1 receptor activity.[166,167] Among this series, RTICBM-74 (**87**) displayed comparable potency to **81** with an IC_{50} of 23 nM (33 nM for **81**) in a calcium mobilization assay; however, it was not as effective in antagonizing agonist-stimulated [^{35}S]GTP-γ-S binding to mouse CB_1 receptor in mouse cerebellar membranes (**81**, IC_{50} = 89 nM; **87**, IC_{50} = 153 nM). A significant metabolic and PK improvement was afforded by the lipophilic phenyl substituent in **87**, as examined in rat liver microsomes showing a more than 22-fold improved half-life ($t_{½}$ > 300 min) and clearance (CL = 4.6 μL min^{-1} mg^{-1}). Possibly due to the improved metabolic and PK profile, **87** is more effective than **82** in vivo in attenuating the reinstatement of extinguished cocaine-seeking behavior in rats, producing an effect at 10 mg/kg equal to that of 30 mg/kg of **81**.

Finally, some noteworthy additional scaffolds have been reported as CB_1 NAMs recently. Fenofibrate (**90**), a PPARα agonist, and cannabidiol (**91**), a nonpsychoactive phytocannabinoid with therapeutic utility in numerous disorders, have both been shown to act at the CB_1 receptor as NAMs (Figure 13).[168,169] The steroid pregnenolone (**92**) is also reported acting as a NAM of CB_1 receptor-mediated ERK½ phosphorylation, devoid of effects on orthosteric agonist binding affinity or cAMP-mediated signaling.[170] Lastly, there have been increased investigations on peptide endocannabinoids (Pepcans) and their ability to allosterically modulate cannabinoid receptor signaling, where some have shown CB_1 receptor NAM activity.[171]

CB_1 Receptor PAMs.: With a greater understanding of CB_1 receptor biology and the possible therapeutic benefit of agonism, the discovery and development of CB_1 receptor PAMs is a current and evident trend. RTI-371 (**93**), a dopamine transport inhibitor with a 3-phenyltropane backbone, was first found to be a CB_1 receptor PAM via an initial functional assay screen. Compound **93** (Figure 14) at 10 mM demonstrates potentiation of the efficacy of agonist CP55940 with an E_{max} value of 36% in a human CB_1 receptor cell-based calcium mobilization assay.[172] Next, a study found the endogenous anti-inflammatory mediator, lipoxin A4 (**94**), demonstrated allosteric enhancement of CB_1 receptor signaling.[173] Interestingly, this endogenous molecule could enhance the affinity of the assayed ligands to the CB_1 receptor with 100% enhancement of [^{3}H]-CP55940 binding and nearly 30% of [^{3}H]-WIN55212–2 binding while increasing the potency of AEA in decreasing forskolin (FSK)-induced cAMP levels by 386 times at a concentration of 100 nM in HEK-CB_1 cells. When studied in vivo and administered via an intracerebroventricular route (1 pmol/2 μL), **94** could promote neuroprotection against β-amyloid (1–40) (400 pmol/2 μL, icv)-induced performance deficits in the Morris water maze assay in mice. A novel synthetic small molecule GAT211 (**95**), with a 2-phenyl-1*H*-indole scaffold, was first reported as a CB_1 receptor PAM in a patent filed by Northeastern University, and recently Thakur et al. described the synthesis and in vitro and ex vivo pharmacology of **95** (racemic) and its resolved enantiomers, GAT228 (**96**, *R*) and GAT229 (**97**, *S*).[171,174] In membranes taken from CHO cells expressing human CB_1 receptor, **95** enhances the binding of the agonist [^{3}H]-CP55,490 at 100 nM and 1 μM and maintains binding enhancement from 1 nM to 10 μM in ex vivo mouse brain membranes while markedly reducing the binding of the antagonist/inverse agonist [^{3}H]SR141716A from 1 μM to 10 μM in ex vivo mouse brain membranes. Compound **95** (1 μM) displays both PAM and intrinsic agonist activity in human CB_1 receptor expressing HEK293A and Neuro2a cells and in mouse brain membranes rich in native CB_1 receptor. However, **95** also exhibits strong PAM activity at a concentration of 1 μM in isolated mouse vas deferens endogenously expressing CB_1 receptor without displaying intrinsic activity. Upon further investigation, the R-(+)-enantiomer (**96**) is the contributing factor for the Ago-PAM property of **95** and displays allosteric agonist activity when tested alone, whereas the *S*-(—)-enan-tiomer (**97**) contributes to the PAM activity of **95**, lacking intrinsic efficacy when isolated and tested. This example highlights the importance of interrogating the activities of racemates independently of the racemic allosteric modulator hit and provides evidence that minor stereochemical differences contribute divergent activities. Also, excellently shown in this case is that the ability to detect allosteric agonism (e.g., ago-PAMs) is dependent on the receptor expression levels in

a given cell-type and sensitivity of detection may differ between in vitro and ex vivo assays. Thus, these factors should be accommodated in programs screening racemic allosteric modulators in highexpressing cell lines that are not representative of in vivo receptor expression levels.

Subsequently, a report on the 2-phenyl-1*H*-indole analogue ZCZ011 (**98**) revealed in vitro and in vivo evidence of PAM activity. In vitro, **98** (Figure 14) increases the CB_1 receptor agonists [^{3}H]-CP55,940 and [^{3}H]-WIN55212 binding affinity and results in an enhanced functional output of E_{max} value of 207% and 225%, respectively (normalized to each agonist functional response at E_{max} 100%). Compound **98** (10 nM) displays enhanced AEA-stimulated signaling via [^{35}S]GTPγS binding with a 40% increase over AEA alone in mouse brain membranes. When additional signaling pathways were investigated, **98** displays a concentration-dependent enhancement of AEA-stimulated β-arrestin recruitment with an E_{max} value of 195% at 1 μM and shows an increase in agonist (AEA and CP55,940) potency by ERK½ phosphorylation assays in human CB_1 receptor expressing cells. In vivo, **98** (40 mg/kg, ip) is brain penetrant and increases the potency of administered orthosteric agonists when examined in cannabimimetic activity behavioral assays in rodents. Therefore, due to broad PAM activity across signaling pathways and multiple agonists, **98** may be useful as a pharmacological tool for mechanistic studies as well as for exploring proof-of-concept studies and potential therapeutic applications of CB_1 receptor PAMs.[175]

Straiker and colleagues recently report a physiologically relevant neuronal model of endogenous cannabinoid signaling as an assay to test CB_1 receptor allosteric modulators. In this model, CB_1 receptor ligands are applied to cultured autaptic hippocampal neurons that exhibit depolarization-induced suppression of excitation (DSE), a form of synaptic plasticity that is mediated endogenously by the CB_1 receptor and 2-arachidonoyl glycerol (2-AG).[176,177] The aforementioned NAMs **63**, **81**, and Pepcan12 attenuate DSE and do not directly inhibit CB_1 receptors, while NAMs **92** and hemopressin as well as the PAM **94** are without effect in this model. Compounds **95** and **98** each show PAM-like responses in autaptic hippocampal neurons, representing the first PAMs to display efficacy via the 2-AG-utilizing neuronal model system. In context of the abovementioned 2-phenyl-1*H*-indole **95**, further examination of its enantiomers **96** and **97** shows that the (*S*) enantiomer **97** exhibits pure PAM-like behavior and the (*R*) enantiomer **96** appears to directly activate the CB_1 receptor as an allosteric agonist, which is in accordance with the previous report.[171]

Molecular dynamics (MD) simulations of GPCR-ligand binding can provide a unique view into the subtleties ofreceptor activation and modulation and, importantly, illuminate ligand interactions for complexes that have proven difficult to crystallize thus far. A recent MD study by Tautermann et al. proposes a mechanism of interaction for certain CB_1 receptor ago-PAMs with two points. First, the agonism may result from the ligand binding to the orthosteric binding site, and the PAM effect is the result of the ligand interacting with an adjunct, deeper binding site in the receptor. Second, the pockets may overlap, resulting in the interaction with residues in both sites simultaneously as one unified pocket and producing the activation and/or modulation effects. Interestingly, this mechanism may explain the observation that Ago-PAM **97** displays competitive binding with CP 55,940 at high concentrations. This interaction mechanism is experimentally validated by showing that

multiple binding sites of **98**, another ago-PAM, contribute to its activity, where positive modulation of the orthosteric agonist is observed until the concentration of **98** is increased above its PAM EC_{50} value and it begins to compete with CP 55,940, owing to its additional affinity for the agonist binding site.[147]

The past decade has seen the identification and characterization ofmultiple promising compounds as NAMs targeting the CB_1 receptor; however these purported NAMs are beset by moderate efficacy and some CB_1 receptor inverse agonist activity that may hinder their future development. Additionally, in vivo studies on CB_1 receptor NAMs are thus far limited and will need to progress toward proof-of-concept studies to show therapeutic utility. As for CB_1 receptor PAMs, the reported small molecules trend toward multifaceted and complicated pharmacology that is sensitive to small molecular modifications, as seen from PAMs of other targets in this Perspective. Additionally, the structural diversity remains relatively small and novel scaffold discovery may open opportunities for CB_1 receptor PAMs with tractable pharmacology. Thus, due to the physiological importance and biological abundance of the CB_1 receptor, innovative medicinal chemistry efforts are necessary to further discovery and development of chemical probes and drug-candidates with improved DMPK characteristics.

3.2.2. Free Fatty Acid Receptors (FFA1—FFA3).—

Free fatty acid receptors (FFARs) are a recently "deorphanized" family of receptors that are activated by nonesterified, or free, fatty acids (FFAs), which comprise a carboxylic acid linked to an aliphatic chain ofvarying length. The receptors are classified based on the chain length of the endogenous agonist, which are termed short chain fatty acids (SCFAs), medium chain fatty acids (MCFAs), or long chain fatty acids (LCFAs). For example, the FFA1 receptor (also GPR40) and FFA4 receptor (also GPR120) are activated by MCFAs and LCFAs, while the FFA2 (also GPR43) and FFA3 (also GPR41) receptors are activated by SCFAs. The change in name designation, e.g., GPR40 to FFA1, aligns with the discovery of endogenous signaling ligands. As key sensors for dietary and other signaling FFAs, this family of receptors has attracted elevated interest for their role in regulating metabolic and inflammatory processes and has recently been implicated as targets in metabolic disorders and type 2 diabetes. Milligan et al. recently published an extensive review of this receptor family, including FFAR biological importance and druggability.[178]

FFA1 Receptor (GPR40).: The FFA1 receptor, which has been previously designated GPR40, is predominately expressed in pancreatic β cells and intestinal enteroendocrine cells and has been validated as a potential target for the treatment of type 2 diabetes.[179] Allosteric modulator discovery for this target has produced a rich collection of pharmacologically diverse ligands, and elegant structural work has identified multiple allosteric binding sites.[180,181] In recent years, numerous full and partial allosteric agonists of FFA1 have been discovered and are described as binding to a select number of distinct allosteric sites, such as TAK 875 (**99**), AM 8182 (**100**), AM 1638 (**101**), AMG 837(**102**), MK8666 (**103**), and AP8 (**104**). Additionally, recent work in FFAR structural biology and biochemistry describes and validates this multiple-site postulation.[18,30,20]

The FFA1 partial agonist TAK-875 (**99**) was discovered as an ago-PAM, binding to a distinct allosteric site and characterized as enhancing the activity of endogenous FFAs.[182] Importantly, **99** (Figure 15) was progressed into phase III clinical trials for the treatment of type 2 diabetes mellitus, but the trial underwent early termination due to toxicity. The cocrystal complex of **99** with FFA1 reveals that the binding site for **99** is formed by helices III–V and the ECL2 and is adjacent to the exterior lipid membrane surface (PDB code 4PHU).[30] The allosteric partial agonist MK-8666 (**103**) has recently been approved to advance into a phase I clinical trial for the treatment of type 2 diabetes mellitus. Interestingly, the cocrystal complex of **103** bound to FFA1 demonstrates a binding site adjacent to the lipid membrane, similar to **99** (PDB code 5TZR).[20] Identification of membrane-adjacent binding sites for allosteric modulators is thus far uncommon; however due to the allosteric nature of GPCR interactions with membrane lipids and cholesterol, this site of action may be more common than currently appreciated or may be yet unexploited at additional receptors. A novel ago-PAM, AP8 (**104**), was subsequently discovered and displays a far higher potency for potentiating endogenous FFAs than **99** or **103**.[180] The ternary complex structure of FFA1-**103**-**104** reveals that **104** binds to a lipid-facing pocket formed by helices II–V and ICL2, which is outside the intracellular halves of the TM helical bundle, and this site is completely distinct from the allosteric binding site of **103** (PDB code 5TZY).[20] Further validation of the allosteric mechanism and signaling bias of allosteric modulators for FFA1 are still progressing.[181]

FFA2 Receptor.: FFA2 and FFA3 receptors are predominately expressed in the gut enteroendocrine cells, pancreatic β cells, and adipose tissue and have been found expressed in various cancer cells, including breast, colon, and liver.[181] The phenyl-acetamide scaffold series of FFA2 receptor allosteric modulators remain the most studied and characterized allosteric ligands for this target. 4-CMTB (4-chloro-α-(1-methylethyl)-*N*-2-thiazolylbenzeneacetamide, **105**) and its analogue **106** (Figure 16) were identified via high-throughput screening and represent the first series of synthetic small molecules that display allosteric agonism and PAM-like effects at the FFA2 receptor.[183,184] Initial characterization shows that **105** can stimulate signaling via both $G\alpha_{i/o}$ and $G\alpha_{q/11}$ promoted pathways. However, subsequent chemical modifications based on SAR around 4-CMTB result in limited improvements, and poor PK properties in male Sprague-Dawley rats have limited its further development as a preclinical candidate.[185,186] As an alternative, phenylacetamide **107** was used for in vivo proof-of-concept studies to demonstrate an FFA2-mediated reduction in plasma nonesterified fatty acids in wild-type mice.[186] Recently, AZ1729 (**108**) was discovered by introducing a phenyl linkage between the amide and thiazole,and **108** displays an interesting G_i-biased profile as a FFA2 receptor allosteric agonist and can potentiate agonist signaling as a PAM.[187]

FFA3 Receptor.: Hexahydroquinolone-3-carboxamides and derivatives thereof are the predominantly reported allosteric FFA3 receptor ligands and derive from a patent by Arena Pharmaceuticals.[188] Compound **109** (Figure 17) displays intrinsic efficacy as well as orthosteric agonist potentiation as an ago-PAM of the human FFA3 receptor with modest potency and is without activity at the FFA2 receptor.[189] Interestingly, modification of the hexahydroquinolone results in molecular switches that significantly alter the activity profile.

For example, when replaced by a 2-bromophenyl group, the modification yields **110** demonstrating a pure PAM profile for the FFA3 receptor without intrinsic agonism, while modification of the phenyl to 3-phenoxyl yields **111** demonstrating a inhibition of the agonist maximal response and an increase binding cooperativity.[189] The authors continue to describe a unique case for **111**, where there was a negative influence on signaling (log β = −21.92 ± 0.20) but a positive impact on binding cooperativity (log α = 1.20 ± 0.39), leaving the conclusion that **111** may be appropriately described as a PAM-antagonist. At present, although FFARs are considered to be an important target for drug discovery and two allosteric ligands have progressed into clinical trials, the overall body of literature remains relatively small, especially for FFA2-FFA4 receptors. Pharmacological tool compounds characterized by high selectivity for a single FFAR and high potency are needed for further evaluation and validation of mechanisms, binding sites, and in vivo tolerability. Thus, there remains high value in medicinal chemistry around these receptors and the evaluation of unique allosteric binding sites may provide insight for targeting FFAR family members as well as other GPCRs.

3.3. Nucleotide Family Receptors.

3.3.1. Adenosine Receptors (A_1R–A_3R).—

Adenosine receptors (ARs), classified as A_1R, $A_{2A}R$, $A_{2B}R$, and A_3R, have been involved in the treatment of diseases that span cardiovascular disease, CNS disorders, inflammatory and allergic disorders, and cancer.[190] Historically, both agonists and antagonists have been used to indiscriminately modulate ARs, the most well-known being the endogenous agonist adenosine and the common antagonist caffeine. Recent work has begun to identify subtype selective orthosteric ligands; however allosteric modulation may provide multiple benefits for therapeutically targeting ARs.[191] Allosteric modulators of ARs are increasingly pursued to avoid side effects caused by agonists acting through indiscriminate AR activation and the propensity for orthosteric agonists to cause receptor desensitization upon prolonged exposure. ARs interact with multiple and divergent second messenger systems, as the A_1R and A_3R reduce the production of cAMP by coupling to $G\alpha_i$ protein resulting in adenylate cyclase inhibition, while the A_{2A} and A_{2B} subtypes stimulate the production of cAMP via coupling to $G\alpha_s$ or $G\alpha_{olf}$ protein.

A_1R.: Evaluation of the A_1R allosteric modulators reviewed herein have been predominately performed in two in vitro functional assays; the first evaluates the inhibitory activity of forskolin-stimulated cAMP accumulation in CHO cells stably expressing the human A_1R, while the second measures phosphorylation of ERK½ in the same cell type. Additionally, groups have reported results on A_1Rbinding parameters (affinity K_D and density B_{max}) and radioligand binding assays that provide association and dissociation kinetics to assess the allosteric modulation of the orthosteric agonist—receptor—G-protein ternary complex. Finally, some observations of antagonist competition binding assays are reported.

In pioneering work, Bruns et al. in 1990 developed a novel series of PAMs (designated allosteric enhancers) known as the “PD” series [PD 81,723 (**112**), PD 71,605 (**113**), PD 117,975 (**114**)], and they were characterized by a 2-amino-3-benzoyl-thiophene (2A3BT) scaffold, selectively enhancing the binding of N^6-cyclopentyladenosine (CPA) to the A_1R

(Figure 18).[192–194] Analogues of PD 71,605 (**113**) led to the discovery of T-62 (**116**) and LUF 5484 (**117**) with markedly improved potency over **112**.[192,195] Among them, **116** (2-amino-4,5,6,7-tetrahydrobenzo [*b*]thiophen-3-yl-(4-chlorophenyl)-methanone) was developed by King Pharmaceuticals and advanced into clinical trials for the potential treatment of neuropathic pain associated with hyperalgesia and allodynia.[194] However, the program was terminated after failure to meet the end point for efficacy in phase IIB.[196,197] The 2-aminothiophene series of PAMs has been recognized as a representative core scaffold for the A_1R, but subsequent observations of intrinsic antagonist activity at high concentrations and moderate efficacy at lower concentrations necessitated further chemical modifications and optimization.[198]

The early SAR studies around the 2-amino-3-benzoyl thiophene core generated a preliminary principle that the 2-amino group, 3-benzoyl, and the corresponding hydrophobic *para*- and *meta*-substituents on the phenyl in the C3-position are critical to the PAM activity of these analogues.[199] Large hydrophobic groups at the 4-position of the thiophene ring and small substituents (H and CH_3) at the 5-position could improve the PAM activity, while bulky substituents at the C5-position resulted in allosteric enhancer activity with an apparent intrinsic antagonist profile.[200] The proper combination of these modifications remains a challenging task and requires achieving a high PAM potency with as minimal as possible antagonist activity. On the basis of this SAR, two functionally divergent binding pockets within the larger allosteric site are proposed, with one interacting with the 2A3BT core and a second possible lipophilic domain to accommodate the C4-/C5-substituents on the thiophene ring.[201,202]

Subsequently, detailed SAR studies on the 4- and 5-substitution of 2-amino-3-benzoyl thiophene was pursued. Modifying the C4-/C5-substituents with a fused cycloalkyl ring increased lipophilicity and led to **118**, which displays allosteric enhancer and partial agonist characteristics with >50% intrinsic activity and 98% of the maximum agonist R-PIA response at 10 μM on A_1R-mediated stimulation of ERK½ phosphorylation in vitro. However, increasing the ring size from a cyclopentyl moiety gradually to a cycloheptyl substituent in efforts to improve allosteric enhancement results in the loss of activity. Opening the C4/C5 ring furnishes a series of compounds with improved PAM potency. Among the different substitutions on C4/C5, **119** with a bromide in C5 displays higher potency than compound **112** in the kinetic binding assay (2-fold greater affinity than **112** and 3-fold less inhibition of antagonist [^{3}H]-CPXbinding).[203] Compound **120** contains a 5-phenyl substituent that increases potency (EC_{50}) 6-fold over **112**, but when no substituent is present in the 5-position (**121**), allosteric enhancement efficacy is improved (77% vs 28%) along with greater antagonist activity compared to **112**.[204] Additional modifications and SAR provided support for these results, and it was concluded that alkyl and aryl groups at the C4-position are favored for allosteric enhancer activity.[205]

Romagnoli and colleagues further modify the C4-substitution to compound **122** (Figure 18), characterized by aryl piperazine moieties linked to a methylene at the 4-position of the thiophene ring, and evaluate **122** with a cAMP functional assay in human A_1R expressing CHO cells. A maximal 87% attenuation of cAMP production is observed at 10 μM without antagonist activity, observed by negligible binding inhibition activity to displace the binding

of selective agonists to A_1R, $A_{2A}R$, and A_3R Saturation binding experiments show **122** produces a A_1R density (B_{max}) shift of [^{3}H]-CCPA binding 7.7-fold to A_1R in CHO cells and enhances the apparent affinity of CCPA approximately 6.3-fold in the A_1R CHO cell membranes by titrating the radioligand [^{3}H]-DPCPX at 10 μM concentration. The number and position of electron-withdrawing or electron-releasing groups on the phenyl attached to the piperazine moiety were determined as highly influential for the overall allosteric enhancement activity. Among them, **123–126** (Figure 18) possess 4-chloro, 3,4-difluoro, 3-chloro-4-fluoro, and 4-trifluor-omethoxy derivatives, and each has been reported to maintain improved potency in the binding (saturation and competition) and functional cAMP studies.[200,206] Subsequently, an aryl substitution at the 5-position was discovered to have a fundamental effect by contributing additively to the allosteric enhancer activity. Compounds **127–131** with a 5-aryl substituent have substantially higher activity than **112** without significantly inhibiting antagonist binding at the A_1R, A_2R, or A_3R. Saturation and competition experiments have also shown that this series of 5-aryl-substituted thiophene derivatives were more active than the corresponding 5-unsubstituted analogues with a highest 13.3-fold decreased CCPA K_i value at 10 μM in competition binding experiments (compound **128**).[207] Encouraging results are also reported on **132–136**, which possess a neopentyl and an aryl moiety at the 4- and 5-positions. Moderate to good enhancing activity of cAMP attenuation is observed (up to 64% inhibition at 10 μM) without significant inhibition of antagonist binding across AR subtypes.[208]

Further modification around this series obtained compound **137**, characterized by a common 2-amino-3-(*p*-chlorobenzoyl)-thiophene core with neopentyl substituent at the C-4 position and benzyl acetylene at the C-5 position of the thiophene ring. Attenuation of cAMP production by **137** shows a 75% inhibition in the presence of 1 pM of orthosteric agonist CCPA, which is almost 4-fold greater than the former allosteric enhancer **112** (19%).[199] Interestingly, in [^{3}H]CCPA saturation binding experiments there was no change observed in the affinity (K_D) of [^{3}H]CCPA with the inclusion of modulator **137**. However, when using [^{3}H]DPCPX as a radioligand in competition binding studies of CCPA with and without **137**, the derived apparent affinity (K_i) value for CCPA was decreased by a 10-fold shift with the inclusion of **137**. Thus, it was concluded that **137** was unable to increase the affinity (K_D) of CCPA when bound to the A_1R high affinity site but could shift the population of A_1Rs toward a high affinity state as evidenced by the increased apparent affinity of CCPA to A_1R in competition binding studies. Compound **137** also displayed a slowing of the dissociation rate of the radioagonist [^{3}H]-NECA by 2.1-fold with a corresponding 1.9-fold increase of apparent affinity. Compound **138** with *p*-chlorobenzyl at the C4-position of thiophene and *p*-chlorophenyl substituent at the C5-position showed a 4-fold increase in cAMP production attenuation compared to **112** in a functional assay (84% vs 21%) and delayed the dissociation rate constant of agonist [^{3}H]-NECA by 2.5-fold.[209] No significant binding inhibition was noted for **138** for antagonists of the A_1R, A_2R, and A_3R subtypes in competition binding assays. Additionally, significant antinociceptive effects were observed in mice at doses of 0.3 and 3 mg/kg of **138**, compared to vehicle-treated mice.

Scammells et al. reported studies interrogating the stimulus bias between cAMP and ERK½ associated pathways (i.e., biased signaling, functional selectivity) of their AR molecules,

which may provide a strategy to achieve selectivity ofsignaling at GPCRs associated with ligand directed signaling outcomes manifested as changes in rank orders ofpotency and or maximal effects relative to a reference (e.g., the endogenous) agonist.[202] This highlights how a GPCR bound with both an allosteric modulator and an orthosteric agonist should be viewed as a unique protein state that differs from those promoted by either orthosteric or allosteric agonist alone. Two novel 2A3BT derivatives **139** and **140**, differing by the absence or presence of a halogen atom in the 4-position of the benzoylthiophene ring, induce functionally biased states of the A_1R. In comparison to the orthosteric agonist response from *R*-PIA, **139** alone is biased as an allosteric agonist toward cAMP accumulation over ERK½ phosphorylation with a 45-fold bias factor. Compound **139** also allosterically shifted the biased signaling of the agonist *R*-PIA (strongly biased toward the pERK½ pathway) toward activation of the two pathways in a nonbiased manner. Conversely, **140** shows minimal bias as an allosteric agonist (with bias factor 3.5) but demonstrates a pathway-biased allosteric modulation when combined with *R*-PIA. Additionally, they also found that the well-characterized 2A3BT, T62, as well as VCP520 (**141**) and VCP333 (**142**), exhibited stimulus bias toward cAMP inhibition compared to pERK½. Finally, SCH-202676 (*N*-(2,3-diphenyl-1,2,4-thiadiazol-5-(2*H*)-ylidene)-methanamine, **143**) has also been reported as an allosteric modulator of ARs. **143** could not only selectively slow the agonist dissociation at A_1R but also accelerate agonist dissociation at A_3R and antagonist competitive dissociation of adenosine $A_{2A}R$ at a concentration of 10 μM.[210]

The understanding of A_1R pharmacology has greatly benefited from the recent reports of solved structures of both the active and inactive state A_1R.[211,212] In the study by Glukhova et al., the 3.2 Å resolution crystal structure of A_1R bound to the covalent antagonist DU172 was presented along with structural insights that potentially explain the interactions of A_1R allosteric modulators such as **121**, which was used in docking studies with the inactive A_1R structure. As shown previously, some allosteric modulators of the A_1R have displayed a complex pharmacological profile that results in PAM activity toward orthosteric agonists while inhibiting the effect of orthosteric antagonists. Although a large, extended orthosteric pocket is present, docking studies of **121** suggest that it preferentially binds to the orthosteric pocket of the inactive state A_1R.[212] The cryoelectron microscopy active state A_1R has also been reported and provides additional insight for allosteric modulator binding when considered in context ofprevious mutagenesis studies that identify ECL2 as a critical domain for PAM activity.[211,213] Taken together, the structural information shows that ECL2 maintains a similar position between the active and inactive states leading to the suggestion that A_1R PAM activity is governed by the availability of active state receptors that provide a preferential PAM binding site upon A_1R activation and subsequent collapsing inward of the TM domains.

$A_{2A}R$.: Amiloride (**144**) and analogue HMA (2,5-(*N*,*N*-hexamethylene)amiloride, **145**) are reported to bind to the sodium ion site of adenosine receptors.[214,215] Compounds **146–149** are derivatives of **144** and **145** (Figure 19) via employing varied 5′-substitutions on amiloride, showing consistent potency as $A_{2A}R$ allosteric antagonists by displacing orthosteric radioligand [^{3}H]-ZM-241,385 from the wild-type human $A_{2A}R$ (59–73%) and displaying even greater potency in the W246A sodium ion site mutant human $A_{2A}R$

(94.6%–100%).[216] Docking studies on the high resolution agonist-bound $A_{2A}R$ show that these analogues conform to similar binding poses to that of amiloride and HMA observed in previous docking studies, with hydrogen bonding and salt bridge interactions with Asp52$^{2.50}$ and Thr88$^{3.36}$ and occupation of the Trp246$^{6.48}$ position.[217] Noteworthy, the interactions with the Trp246$^{6.48}$ are predicted to be π–π stacking between Trp246$^{6.48}$ and the phenyl group of most analogues, which is not present in **145**. The phenethyl moieties and the substituents attached on the phenyl groups are predicted to reach into a part of the orthosteric binding site surrounded by hydrophobic residues of Phe168^{EL2}, Met177$^{5.38}$, Leu249$^{6.51}$, Asn253$^{6.55}$, and Ile274$^{7.39}$, suggesting that **146–149** can intrude the orthosteric site from the allosteric site and displace orthosteric ligand ZM-241,385 in a direct, competitive manner.

A_{2B}R.: The $A_{2B}R$ is the least characterized subtype in the AR family. Among ARs, the $A_{2B}R$ subtype exhibits low affinity for the endogenous agonist adenosine compared to the A_1R, $A_{2A}R$, and A_3R subtypes and is therefore suggested to be activated when local concentrations ofadenosine increase to a large extent following tissue damage. Compounds **150–156**, with small differences in the side chain of the 1-benzyl-3-ketoindole scaffold, are the only reported allosteric modulators for the $A_{2B}R$ (Figure 20) and have been characterized through binding and functional assays, including cAMP functional assays, dissociation kinetic assays, equilibrium binding assays, and [^{35}S]GTPγS binding assays in CHO cells expressing human A_1R, $A_{2A}R$, $A_{2B}R$, and A_3Rs.[218,219] The PAMs **150–152** potentiate agonist efficacy but not agonist potency (similar submicromolar potencies at $A_{2B}R$) with PAM EC_{50} values between 250 nM and 446 nM. PAM **151** demonstrates a significant reduction in the radioligand [^{3}H]NECA dissociation constant from 0.0162 min^{-1} to 0.0086 min^{-1} and increases the efficacy of agonist BAY 60–6583 to stimulate guanine nucleotide exchange with E_{max} values from 155.2% to 175.0% at 1 μM in [^{35}S]GTPγS binding assays. Slight alterations to the side chain are discovered as chemical switches and yield NAMs **153–156** that reduce agonist potency and efficacy. Compound **156** significantly increases the dissociation rate of [^{3}H]NECA from $A_{2B}R$ with K_{off} value 0.0481 and results in a pronounced attenuation of the orthosteric ligand BAY 606583 mediated stimulation of guanine nucleotide exchange. None of the compounds reported (**150–156**) display appreciable affinity for the A_1R, $A_{2A}R$, and A_3R except for **152** and **153**, which display submicromolar affinity for the A_1R(**152** K_i = 161.5 nM, **153** K_i = 343.0 nM). More work will certainly be done to elucidate the therapeutic potential of the $A_{2B}R$, especially in inflammation and injury, and there remains great potential for further interrogating allosteric modulators of the $A_{2B}R$ through medicinal chemistry. However, these current allosteric modulators comprise both PAMs and NAMs and may provide useful chemical probes to explore the biology and therapeutic potential of $A_{2B}R$ allosteric modulators.

A_3R.: The A_3R is widely expressed and displays tissue specific regulation regarding cellular energy consumption and energy deficits. Agonists and antagonists have recently been studied, and antagonists have been prime candidates for rheumatoid arthritis, glaucoma, psoriasis, and hepatocellular carcinoma, as the $A_3R$is found to be overexpressed in cancer cells.[220] Allosteric modulators of the A_3R are a recent development and may provide a unique therapeutic approach toward these disorders and others. Relative to the other

members of the AR family, modest numbers of selective allosteric compounds have been reported for the A_3R The core scaffolds of A_3R allosteric modulators predominately comprise 1H-imidazo[4,5-*c*]-quinolin-4-amine and 2,4-disubstituted quinoline analogues. Reported characterizations of A_3R allosteric ligands are mainly based on the results of radioligand displacement, kinetic dissociation experiments, and functional (cAMP-based) assays. LUF6000 (**157**) with a 1*H*-imidazo[4,5-*c*]quinolin-4-amine scaffold was discovered (Figure 21) and prioritized for optimization due to significant potentiation of agonist efficacy compared to the former discovered A_3R PAMs. However, like $A_{2B}R$ PAMs, it shows no enhancing effect at 10 μM on agonist potency for human A_3R expressed in CHO cells, observed via cAMP functional readouts.[221,222] Ring opening around **157** to LUF6096 (**158**), also bearing a 2,4-disubstituted quinoline core, is another example of A_3R PAMs and equally potentiates orthosteric agonist efficacy. Interestingly, **158** also displays allosteric effects on the agonist potency and could produce a shift in the EC_{50} value of the agonist Cl-IBMECA from 31 nM alone to 9 nM with PAM, a ~3-fold shift.[223]

3.3.2. P2Y Receptors ($P2Y_1$ and $P2Y_2$ Receptors).—

Purine and pyrimidine receptors exist in two families: P1 receptors (adenosine receptors) activated by adenosine, discussed above, and P2 receptors activated by adenosine 5′-tri- or diphosphate (ATP or ADP) and/or uridine 5′-tri- or diphosphate (UTP or UDP). P2 receptors are further divided as P2X and P2Y receptors, which are ligand gated ion channels and GPCRs, respectively.[224] The human purinergic GPCRs (P2Y) are divided into two subfamilies based on their coupling to specific G-proteins, $G\alpha_{q/11}$ coupled P2Yrlike receptors and $G\alpha_{i/o}$ coupled $P2Y_{12}$-like receptors. They are activated by ADP to trigger glutamate release, facilitating thrombus formation and are essential for platelet aggregation and thus considered promising new drug targets.[225]

$P2Y_1$ Receptor.: The $P2Y_1$ receptor is a promising therapeutic target due to its critical role in ADP-induced platelet aggregation and the potential for an improved safety profile over $P2Y_{12}$ receptor inhibitors regarding bleeding liability.[225] Early efforts in this arena essentially focused on nucleotide derivative orthosteric antagonists. However, the discovery of BPTU (**159**) by Bristol-Myers Squibb (Figure 22), a hydrophobic diarylurea derivative, as a non-nucleotide allosteric antagonist of the $P2Y_1$ receptor has provided the foundation for allosteric modulators to be considered as potential therapeutic agents of this receptor.[226] Thus, there have been recent innovative approaches to design non-nucleotide, diarylurea scaffold allosteric antagonists as antithrombotic agents with improved safety profiles.[227,228] As a relatively new target and mode of antagonism, the most extensive characterizations have been performed on **159**, which demonstrates a 68 ± 7% thrombus weight reduction in a rat arterial thrombosis model (10 mg/kg **159**, 10 mg kg^{-1} h^{-1} rate) with minor effects on overall bleeding in provoked rat bleeding time models. Important structural and pharmacological studies by Jacobson and colleagues have recently begun to illuminate the complex allosteric mechanisms of **159** and its effect on both multiple downstream signaling pathways as well as multiple agonists.[31,229] In the recent cocrystal complex, **159** is the first $P2Y_1$ receptor antagonist shown to bind to an allosteric site entirely outside of the helical bundle, not only outside of the orthosteric site. The allosteric binding site of **159** is situated on the outside of the TM domain bundle adjacent to the lipid membrane and engages **159** by

mostly hydrophobic and aromatic residues located in the TM helices I–III as well as minor involvement of ECL1. The two nitrogen atoms of the urea group in **159** promote two bidentate hydrogen bonds with the backbone carbonyl of Leu102$^{2.55}$, which result in the only polar interactions present. The pyridyl group, the benzene ring of the phenoxy group tethered to pyridine, and the *tert*-butyl substituent on the phenoxy group are responsible for forming the main hydrophobic interactions with the $P2Y_1$ receptor.[225] Insights from the cocrystal complex indicate that this interaction may reasonably stabilize the extracellular helical bundles and restrain the receptor in an inactive state. Thorough pharmacological studies of **159** $P2Y_1$ receptor allosteric antagonism provide a more nuanced view of its mode of action, including a description of probe dependence and signaling bias.[229] When provoked by structurally diverse agonists, **159** displayed varying degrees of antagonism across multiple signaling pathways. For example, allosteric antagonism of the agonists 2MeSADP and MRS2365 resulted in decreased potency for ERK½ stimulation with no effect on maximal response (E_{max}); however in [^{35}S]GTPγS binding assays and β-arrestin2 recruitment, **159** was able to significantly suppress the respective agonist-mediated responses. Antagonism of the agonist Ap4A resulted in insurmountable suppression of the maximal response across all assays tested. These studies highlight the high level of complexity for allosteric GPCR modulation but also call to attention the high degree of specificity that can be achieved if probe dependence and signaling bias are therapeutically desired outcomes based upon biological understanding. The FDA approval of ticagrelor (AZD6140), a $P2Y_{12}$ receptor allosteric antagonist discovered by AstraZeneca, has paved the way for antithrombotic drugs in this class with safer bleeding profiles to emerge as therapeutics.[230]

P2Y$_2$ Receptor.: The $P2Y_2$ receptor couples primarily to $G\alpha_{q/11}$ to activate PLC-β and has been implicated in diverse physiological processes, including platelet aggregation, immunity, lipid metabolism, gastrointestinal functions, and bone homeostasis.[231] Allosteric agonists for the $P2Y_2$ receptor have been recently reported.[224,232] Of these, **160** is characterized as a partial allosteric agonist and was discovered by the modification of 5′-methylenephosphonate, a derivative of UTP (uridine 5′-triphosphate).[224] Additionally, compound **89** (**161**), with a novel 4(1*H*)-quinolinone scaffold, is among the first non-nucleotide $P2Y_2$ receptor allosteric agonists and is selective over closely related subtypes. Initial characterizations displayed activity in calcium mobilization assays in the 1321N1 human astrocytoma cell line, induction of nuclear receptor 4A (NR4A) in a gene reporter assay, and the attenuation of isoproterenol-induced cardiac hypertrophy in neonatal rat cardiomyocytes (NRCMs).[232] These studies relay **161** (Figure 22) as a validated chemical tool compound for utilization in further proof-of-concept studies to investigate the therapeutic potential of $P2Y_2$ receptor allosteric agonists for the treatment of cardiovascular disorders. Interestingly, the $P2Y_2$ receptor serves as an attractive drug target for dry eye disease (DED) and nucleotide-derived agonists have been approved for the treatment of DED in Japan and Korea.[233] Further development of **161** and other allosteric agonists may prove advantageous for DED, cardiovascular indications, and others.

3.4. Peptide and Protein Family Receptors.

3.4.1. Chemokine Receptors (CCR5, CCR9, CXCR1, CXCR2, CXCR4).—

Chemokine GPCRs contain four families as CCR, CXCR, CX3CR, and XCR based on the relative positioning of conserved cysteine residues in the N-terminal domain of their mature ligands. At present, there are roughly 50 chemokines and at least 18 chemokine GPCRs have been identified in humans.[234,235] The development of allosteric modulators for chemokine receptors (Figure 23) represents a profound advance for allosteric modulators of class A GPCRs with marketed drugs (maraviroc, NAM of CCR5; plerixafor, NAM of CXCR4), clinical candidates (reparixin, NAM of CXCR1; ladarixin, NAM of CXCR1/CXCR2; vercirnon, allosteric antagonist of CCR9), and structural studies of allosteric modulators binding to the receptors in high resolution (for CCR2, CCR5, and CCR9).[235,18] Besides synthetic drugs, chemokine receptor allosteric sites have been shown to also bind endogenous mineral cations such as sodium, calcium, zinc, and magnesium. These studies included CCR1, CCR4, CCR5, and CCR8, and additional work has shown the metal ion Zn(II) or Cu(II) complex to be an allosteric enhancer of CCL3.[236] Herein, we describe representative allosteric modulators for seven chemokine receptors and the structural and chemical knowledge relating to their discovery.

CCR5.: The chemokine receptor CCR5 is widely implicated for its role in the process ofhuman immunodeficiency virus type 1 (HIV-1) infection. Mechanistically, CCR5 forms a co-receptor with the viral envelope glycoprotein gp120, which is required for HIV-1 cell recognition and entry leading to infection.[237,238] Maraviroc (**162**) is a marketed allosteric drug for anti-HIV (Figure 23), stabilizing CCR5 in an inactive conformation that blocks CCR5-gp120 interaction by allosterically binding to CCR5.[239,240] The cocrystal complex of CCR5 and maraviroc demonstrate that maraviroc occupies an extracellular site of the 7TM helical bundle.[27] The protonated nitrogen of the tropane group forms a salt bridge with Glu283$^{7.39}$. The carboxamide nitrogen and the amine of the triazole group of the ligand form hydrogen bonds with Tyr251$^{6.51}$ and Tyr37$^{1.39}$, and the phenyl, triazole, and cyclohexane ring are responsible for the formation of hydrophobic interaction (PDB code 4MBS). Additionally, CCR5 and the highly homologous CCR2 are promising targets for immunologic and cardiovascular diseases due to their important functions in macrophages, T-lymphocytes, and natural killer cells. Chemokine receptors are activated by more than 50 chemokine ligands in a concerted manner in response to various immunologic or inflammatory events. Thus, probe dependence may be a primary advantage for allosteric modulators of chemokine receptors. In a recent study, Wünsch and colleagues discovered the first probe dependent CCR5 PAM.[241] Through an innovative bioluminescence resonance energy transfer (BRET)-cAMP assay, the endogenous agonists CCL4 and CCL5 were screened at CCR2 and CCR5. Chemical modifications and resulting SAR were performed on a 2-benzazapine scaffold showing a sensitive 7-position where the addition of *p*-tolyl moiety led to a chemical switch toward CCR2 modulation without CCR5 activity. The parent compound displayed PAM activity at CCR5, with no activity at CCR2, and selectively modulated CCL4 versus CCL5.[241] Bipyridine and terpyridine, small molecule metal chelators, have also been shown to modulate CCR5 with prode dependency. Biochemical studies indicate that bipyridine and terpyridine are PAMs of CCL3, weakly potentiate CCL4, and compete with CCL5 binding to CCR5.[242] CCR5 remains an active

target for PAM and NAM discovery, and the identification of probe dependent ligands will broaden the biological knowledge of chemokine signaling and the therapeutic relevance.

CXCR1.: CXCR1 and CXCR2 are largely expressed on T lymphocytes and natural killer cells, playing a key role in acute and chronic inflammatory conditions.[235] Reparixin (**163**) is a noncompetitive NAM for CXCR1 (Figure 23) presenting a 400-fold higher efficacy in inhibiting CXCR1 activity versus CXCR2.[243] Compound **163** inhibits the signaling triggered by chemokine CXC ligand 8 (CXCL8) and binds CXCR1 at an allosteric site between TM I, III, and VI and has been advanced into a phase III clinical trial for pancreatic islet autotransplantaion.[243] Ladarixin (DF 2156A, **164**) is the second representative example of this series as a highly potent allosteric inhibitor of CXCR1/CXCR2 with an IC_{50} value 0.1 nM and has been advanced into clinical trials for type 1 diabetes.[235]

CXCR4.: CXCR4 is expressed by hematopoietic stem cells and progeny, as well as by over 48 different cancers types, and is essential for hematopoietic stem cell colonization of fetal bone marrow during development.[244] Interestingly, plerixafor (**165**), a NAM of CXCR4 with tetraazacyclotetradecane scaffold (Figure 23), was initially in development as an anti-HIV drug but has been repurposed and is now marketed for an indication of bone marrow transplantation for patients with non-Hodgkin's lymphoma or multiple myeloma, where upon administration **165** is efficacious in mobilizing stem cells into the peripheral blood for collection.[235]

CCR9.: CCR9 is another member of the CC chemokine receptor subfamily implicated in inflammatory bowel disease. Vercirnon (**166**) is a selective allosteric antagonist of CCR9 (Figure 23) that has entered phase III clinical trials for the treatment of Crohn's disease.[245] The cocrystal structure of CCR9 with **166** shows that **166** binds to the intracellular side of the CCR9, which is similar to that of CCR2-RA-[*R*] bound to CCR2. The sulfone group, ketone group, and pyridine-*N*-oxide group of **166** could contribute to forming multiple hydrogen bonds with intracellular side of the CCR9. The *tert*-butylphenyl and chlorophenyl group are responsible for the hydrophobic interactions with the hydrophobic cleft (PDB code 5LWE).[29] Allosteric modulation via binding intracellular allosteric sites is still uncommon for class A GPCRs; however this mode of action may have important therapeutic implications, especially for peptide and protein receptors such as chemokine receptors.

CCR2.: CCR2 is implicated in numerous inflammatory and neurodegenerative diseases.[246] CCR2-RA-[*R*] (**167**) is a NAM of CCR2 (Figure 23) with good selectivity against CCR1 and CCR5, in vitro activity characterized by an IC_{50} of 0.17 μM, and also an excellent DMPK profile.[18] The cocrystal structure of CCR2 in a ternary complex with **167** and orthosteric BMS-681 antagonist demonstrates that **167** occupies an intracellular allosteric binding site, as seen in other chemokine receptors. The pyrrolone structure is very important for forming hydrogen bonds between hydroxyl group and Glu3 1 $0^{8.48}$ and $Lys311^{8.49}$, and carbonyl group with the backbone amide of$Phe312^{8.50}$. The existence of the phenyl group is vital for hydrophobic interactions with various amino residues.[28]

CCR4.: Chemokine receptor 4 (CCR4) is mainly expressed in T helper 2 (Th2) cells and contributes to the pathogenesis of allergic diseases in inflamed tissues. Endogenous agonists chemokine ligand 17 (CCL17) and chemokine ligand 22 (CCL22) are two signaling proteins that bind the orthosteric site of CCR4 and are crucial for recruiting T cells during the inflammatory response upon exposure to allergens.[247,248] Interestingly, a functional interrogation of these signaling ligands shows that CCL22 activated CCR4 was able to couple efficiently to β-arrestin and stimulate GTPγS binding, while CCL17 activated CCR4 did not couple to β-arrestin and only partially stimulated GTPγS binding.[248] Thus, the physiological conditions under which some chemokines are released and activate their respective receptors remain an active area of research. The CCR4 has been a target for the discovery of small molecule therapeutics for many due to its central role in pathogenesis such as asthma, atopic dermatitis, cancer, and mosquito-borne tropical diseases.[247]

Indazole sulfonamide series were recent synthesized and examined as human CCR4 antagonists, and SAR studies around the C4, C5, C6, C7 and N1, N3 positions were conducted to provide compounds with a better in vivo profile. Among them, **168** with a methoxy group as C4 substituents, 5-chlorothio-phene-2-sulfonamide at N3, and a meta-substituted benzyl group possessing an α-amino-3-[(methylamino)acyl] group at N1 was the most potent, presenting a pIC_{50} of 7.4 for CCR4. Compound **168** also demonstrates a good PK profile in three species (rat, dog, and human) and was selected for further development.[247] Subsequent studies on CCL17- and CCL22-induced responses of human CCR4 expressing T cells suggest there are two additional allosteric sites to which small molecules bind. Compound **168** and its analogues bind to one of them, the intracellular allosteric binding site. Lipophilic heteroarenes possessing basic amino groups have been shown to bind to another site. Additionally, a heteroarylpyrazole arylsulfonamide scaffold was also reported as a potent lead for further development.[249]

CXCR3.: The chemokine receptor CXCR3 is mainly activated by γ-inducible chemokines CXCL11, CXCL10, and CXCL9, directing activated T cells to the sites of inflammation, and is implicated to play a role in a myriad of inflammatory diseases, such as rheumatoid arthritis, multiple sclerosis, cancer, atherosclerosis, and allograft rejection; thus, CXCR3 is viewed as a promising drug target.[250–252] 8-Azaquinazolinone derivatives (**169–173**) were characterized as promising allosteric modulators of the chemokine receptor CXCR3 and commonly demonstrate properties of signaling bias and probe-dependence.[252] Among them, **172** can inhibit CXC chemokine 11 (CXCLll)-dependent G protein activation over β-arrestin recruitment with 187-fold selectivity, and it inhibits CXCL11-over CXCL10-mediated G protein activation with 12-fold selectivity.[252]

Structure-based drug discovery (SBDD) is still in the early phases for receptors such as CXCR3 due to limited structural information and complicated interactions between receptors and chemokine signaling proteins. As an alternative, photoaffinity labeling is an effective biochemical tool to elucidate the binding pocket at the CXCR3 receptor. By this principle, photoactivatable **174** with a nanomolar affinity was synthesized based on the 2,3-disubstituted-8-azaquinazolinone scaffold. Notably, **174** could attenuate radioligand binding by 80% in [^{3}H]-RAMX3 radioligand displacement assay and proved to be a promising chemical tool for further exploration of the allosteric binding site of CXCR3.[251] Aside from

photoaffinity labeling, site-directed mutagenesis is another approach to reveal information about ligand—receptor interactions through the mutations of amino acid residues and detection of their influence on modulator binding, signaling and transmission of cooperativity. Compounds **171** and **173** are two biased NAMs and have been shown to exhibit probe-dependent inhibition of CXCR3 signaling. Homology modeling and docking provided direction for site-directed mutagenesis, and functional outcomes of the mutations were measured by a BRET-cAMP and β-arrestin recruitment assay. These studies indicated that F131$^{3.32}$, S304$^{7.39}$, and Y308$^{7.43}$ act as key residues for the compounds to modulate the chemokine response, and notably, mutations of D186$^{4.60}$, W268$^{6.48}$, and S304$^{7.39}$ led to a G-protein-active rather than β-arrestin-inactive conformation.[252]

3.4.2. Opioid Receptors (δ-OR, κ-OR, μ-OR).—Opioid receptors (ORs), specifically the μ-opioid receptor (MOR), is the therapeutic target for numerous clinically used medications, predominately analgesics. Although MOR activation produces profound analgesia, tolerance develops for opioid drugs and addiction can be severely problematic.[253,254] Additionally, side effects such as respiratory depression, nausea, constipation, and others have highlighted the urgent need for better therapeutic agents targeting the MOR. Allosteric modulators may be suitable in this scenario, as receptor desensitization is theoretically less likely and allosteric modulators have a generally safer profile, owing to the ceiling effect.[255] Both NAMs and PAMs have been explored for opioid receptors, and while most work on allosteric modulation has been directed toward the MOR, a few ligands have been reported as hits for the δ-opioid receptors (DOR).[255]

Cannabidiol and salvinorin-A are among the earliest identified NAMs for the MOR and DOR.[256,257] Recently, BMS-986121 (**175**) and BMS-986122 (**176**) are reported as the first series of selective PAMs for the MOR (Figure 24) and were identified via a high-throughput screen.[258,259] The PAM activity is further characterized by three functional assays, including β-arrestin recruitment, inhibition of adenylyl cyclase activity, and G protein activation via [^{35S}]GTPγS binding. Compound **176** was shown to shift leftward the concentration—response curve of endomorphin-I (α = 7) in β-arrestin recruitment assays, and in G protein activation assays it was revealed that **176** displayed low levels of intrinsic agonism at concentrations above those required for endomorphin-1 potentiation. Intrinsic agonist activity of **176** at high doses was possibly explained by the lack of reciprocal affinity modulation from the orthosteric agonist. Subsequent chemical SAR study of **176** around the substituents on the side phenyl led to the discovery of BMS-986124 (**177**) as a neutral allosteric ligand or NAL.[258,259] Additionally, diterpene alkaloid ignavine (**178**) demonstrates positive modulatory activity for MOR agonists DAMGO, endomorphin-1, and morphine in a cAMP assay with an analgesic effect in vivo.[260] BMS-986187 (**179**), with a chemically novel core compared to previous BMS series, was discovered as an effective PAM at the DOR and at the κ-opioid receptor (KOR) rather than the MOR with an approximately 20-to 30-fold higher affinity in the allosteric ternary complex model.[261] Recently, significant attention has been directed toward the misuse and fatalities associated with prescription OR agonists, as opioid use disorder rises to epidemic proportions. However, the MOR remains a highly effective and validated target for analgesia and OR ligands are essential therapeutics. Thus, there is great interest in the discovery and development of novel OR therapeutics that

have a safer profile without the loss of efficacious analgesia. Allosteric modulation of ORs holds promise of delivering safer analgesics and other therapeutics to the clinic; however significant optimization and development are still needed.

3.4.3. Other Peptide and Protein Family Receptors.

Melanin-Concentrating Hormone Receptor 1 (MCH_1R).: The MCH_1 receptor is activated by melanin-concentrating hormone (MCH) and is widely expressed in the human CNS and to a lesser degree in the periphery. Due to its specific expression patterns in the CNS, the MCH_1 has been postulated to be drug target in anxiety, depression, and obesity disorders.[262] Predominately explored as an antiobesity target, the MCH_1 receptor is reported to be allosterically inhibited by the small molecule MQ1 (**180**, Figure 25) in multiple signaling pathways for $G\alpha_{i/o}$, $G\alpha_{q/11}$, and β-arrestin. MQ1 has been shown to be a slowly dissociating reversible MCH1 receptor blocker in washout experiments as well as affinity selection-mass spectrometry.[263] Increased efforts in this area should provide important in vivo tool compounds to validate the therapeutic potential of this attractive target.

Neuropeptide Y Receptors (Y_1R-Y_5R).: The human neuropeptide Y receptors comprised Y_1R, Y_2R, Y_4R, and Y_5R and are activated by a set of three known endogenous peptides, neuropeptide Y (NPY), peptide YY (PYY), and pancreatic polypeptide (PP). Each of these receptors is characterized by varying affinity for the endogenous peptides as well as varying physiological distribution. While each member of this receptor family has been identified as a probable therapeutic target for diseases such as obesity, cancer, or other metabolic disorders, most studies have focused on the Y_4R. Importantly, the Y_4R has higher affinity for the endogenous ligand PP, which is secreted by pancreatic cells in proportion to caloric content intake and is thought to modulate satiety in feeding, food intake, energy homeostasis, and colon transit.[264] Niclosamide (**181**) and structurally related compounds are revealed as nonselective small molecule PAM ligands for Y_4R versus Y_1R, Y_2R, and Y_5R via HTS.[265] Further efforts yielded the small molecule *tert*-butylphenoxycyclohexanol (tBPC, **182**), a purely efficacy-driven selective Y_4R PAM, and is reported to potentiate Y_4R activation in G-protein signaling and arrestin recruitment experiments.[266] Thus, the early efforts toward this target are promising and may yield important clinical candidates for obesity in the future.

Proteinase-Activated Receptors (PAR2).: PAR2 has recently been recognized as an important modulator of coagulation and inflammatory responses.[267] PAR2 is activated upon cleavage of the extracellular amino terminus by proteinases. This cleavage results in a new amino terminus that subsequently acts as a “tethered” agonist for receptor activation.[268] Thus, allosteric modulation of this receptor may be an attractive strategy for retention of this unique mechanism of activation. AZ3451 (**183**) is an allosteric antagonist binding to a remote, lipid-facing allosteric site of PAR2, and the solved cocrystal structure represents increased success in the structural elucidation for allosteric modulators of class A GPCRs.[32]

Tachykinin Receptors (NK2).: The NK2 receptor is widely expressed throughout the human gastrointestinal system and has been shown to be a key player in intestinal motility and the high degree of motility observed upon intestinal inflammatory responses. Thus,

efforts toward discovering small molecule agents for this target have focused on antagonists and NAMs to block inflammation and motility for indications such as irritable bowel syndrome.[269] Compound **184** was discovered as a NAM for the NK2 receptor in a FRET-based binding assay. Interestingly, the NK2 receptor has been shown to be active in two distinct activation conformations, which correspond to downstream signaling biases. Compound **184** can shift the active conformations away from a cAMP-producing conformation, suppressing agonist-induced cAMP production by 30% in the presence of 10 μM of **184**.[270] This shift in conformation populations results in a slight potentiation of agonist-induced calcium response, indicating a possible increase of this particular conformation. Further modification on **184** to **185** with a butyronitrile side chain yielded a compound that retains suppression of cAMP production while increasing potentiation of agonist-induced calcium mobilization E_{max} (50% to 68%).[271] Additional efforts toward NK2 NAMs may elucidate the discrepancies between active conformations and provide evidence for NAMs as therapeutic candidates for gastrointestinal-related diseases.

4. CHALLENGES AND EMERGING CONCEPTS IN CLASS A GPCR DRUG DISCOVERY

4.1. Complexities and Nuance Observed in Screening, Optimizing, and Advancing Class A GPCR Allosteric Modulators.

The discovery of allosteric modulators for class A GPCRs has been advanced by numerous academic and industry groups over the past decade and has provided a framework for optimization and development of such ligands. This framework includes understanding the multiple facets of allosteric modulator influences on the receptor complex with orthosteric agonists and antagonists, as well as the influence on coupling to effector molecules. As previously suggested, an allosteric modulator—receptor complex can be described as a "new receptor" that results in new or differential biology compared to the native receptor.[5] Thus, it is important to quantify the allosteric modulator's effect on the receptor, orthosteric signaling molecule, and the downstream effectors. The quantification metrics (cooperativity, intrinsic efficacy, etc.) used throughout hit optimization have been shown to sometimes improve (or decrease) in tandem; however, these measures have often been shown to "uncouple" such that orthosteric agonist affinity may improve, but there may be no impact on other metrics such as agonist efficacy. Additionally, this trend is observed in which, upon chemical modification, allosteric modulators may acquire intrinsic efficacy as agonists or serve as antagonists. While these outcomes may be advantageous for a particular target, the trend likely will not translate to other orthosteric ligands of the receptor (probe dependence). This can be problematic when the endogenous signaling ligand is not amenable to screening in functional assays (peptides, proteins, etc.) and should be approached with caution. Importantly, GPCRs exist in an ensemble of states and can be stabilized by high-affinity allosteric modulators in a variety of functionally relevant states, such as active states, pathway-specific active states, or inactive states.[40] The induced receptor conformation may then be more likely to couple with some effectors (e.g., β-arrestins) than others (e.g., G proteins), leading to signaling bias. These complexities may yield therapeutically important results; however, the team must obtain a thorough characterization and SARs of the candidate molecule to effectively move through optimization and development. Allosteric

modulators exert their effects on receptor activation in numerous ways, which contribute to the nuances described above. As seen throughout this Perspective, drug discovery teams utilize various assays to quantify the effects of allosteric modulators, but the assays and assay conditions employed are likely to diverge between groups. Additionally, experts have warned against characterizing allosteric modulators with singular assays, such as calcium mobilization, to determine potency estimates that solely drive SAR.[26] Robust in vitro characterizations that lead to results that are readily compared across drug discovery groups is a continuing challenge for the field.

Advancing allosteric modulators from in vitro to in vivo is beset with challenges as well. For instance, dissection of signaling bias in animal models and behavioral paradigms is difficult to achieve and may be highly sensitive to probe dependence, especially if the endogenous signaling agonist could not be used for iterative screening and SAR.[22] Additionally, allosteric sites can display a greater divergence between species (e.g., rat vs human) in comparison to orthosteric sites, complicating interpretations of drug effect across species.[134] One possible explanation for this observation is that allosteric sites are less homologous due to decreased evolutionary pressure, a feature exploited for subtype selectivity, and may be more pronounced between species. Thus, screening preclinical candidate allosteric modulators at both human and rat GPCRs will help to alleviate this unknown when advancing compounds. Also relevant to in vivo characterization is the presence of "chemical switches" (slight chemical changes that significantly alter or reverse activity) that become apparent during chemical optimization.[272] Although standard chemical switches are likely to be addressed at an early stage, recent work has highlighted the presence of metabolic chemical switches that lead to major metabolites displaying a different or opposite activity profile.[273] Chemical switches may be identified and addressed through early core optimization and the use of the "fluorine walk" to determine scaffold positions amenable to modification, and some success has been reported in the use of halogens or deuterium to dissuade metabolism of some scaffolds.[22] Species bias and metabolic switches may complicate preclinical development and should be a key, deciding factor for the abandonment or development of select scaffold.

4.2. Emerging Strategies for Class A GPCR Small Molecule Allosteric Modulator Discovery and Development.

Structure-based drug design (SBDD) is emerging in the discovery and optimization of allosteric modulators for class A GPCRs.[274] The growing number of crystal structures available and the structural studies on allosteric modulator mechanisms are providing the material and insight into engage in SBDD for allosteric modulators.[275,276] Importantly, the resolution at allosteric "hot spots", such as ECLs, has greatly improved in recent reported crystals and is suitable for docking studies or molecular dynamics simulations. It should also be noted from a receptor structure perspective, that there is an ever-growing number of class A GPCR structures being reported via electron cryomicroscopy (cryo-EM) techniques. The resolution of these structures has significantly improved in recent years, and thus, cryo-EM structures will likely play a pivotal role in understanding receptor complexes and informing SBDD.[277] From a ligand optimization perspective, these structural studies can be combined with functional assays and site-directed mutagenesis to provide greater clarity on the

allosteric mechanism of action at the receptor. Compounds displaying chemical switches may be used in simulations and further inform the structural biology of GPCR activation and signaling enhancement. From a discovery perspective, exciting studies are emerging with de novo allosteric modulators discovered via virtual screening oflarge libraries. For example, Valant and colleagues recently published the results of an iterative molecular docking and screening project where two subtype selective M_2 mAChR NAMs and one PAM were discovered from the National Cancer Institute (NCI) compound library.[278] The success of molecular docking approaches in this example, in which validated and chemically diverse PAMs and NAMs were discovered, is encouraging, and this arena is projected to be highly important in the future. Additionally, fragment-based drug design (FBDD) may be an important tool for the discovery of novel allosteric modulator chemotypes and becomes a reality with improved crystal structures.[279,280] An in silico approach has been shown by Bian et al. for the class C GPCR metabotropic glutamate receptor 5.[281] Additionally, a more traditional approach without structural information has been shown for the class C GPCR metabotropic glutamate receptor 2 by Szabo et al.[282] Considerations of receptor activation state and the ability to predict allosteric modulation by docking to unknown sites remain to be addressed for different members ofclass A GPCRs.

Another emergent strategy is the utilization of covalent allosteric probes to identify and define the allosteric binding site. Chemically reactive groups can be adapted to allosteric modulators to afford covalent binding to the allosteric site, and subsequent peptide mass spectrometry can yield surrounding residues. Followed by site-directed mutagenesis and informed by known structural information, this may be a powerful tool for structurally informed rational design. This strategy was successfully implemented by Thakur and colleagues to map the CB_1 receptor allosteric binding site.[159] Electrophilic and photoactivatable moieties were added to CB1 receptor NAMs, which retained their activity and provided useful chemical tool compounds. Of note, the authors engaged in iterative rational design of numerous covalent derivatives based on previous knowledge of the NAM SAR and discussed modifications that abolished activity. Thus, this powerful strategy may not be suitable for scaffolds prone to chemical switches or shallow SAR.

4.3. New Concepts in Pharmacology for Class A GPCRs and Implications for Allosteric Modulator Agents.

As studies continue to shed light on the intricate pharmacology of class A GPCRs, new paradigms in drug discovery will emerge. The initial concept of allosteric modulation was developed based on the understanding that allosteric regulation was a ubiquitous and essential element for functional proteins throughout biology.[9] Likewise, biological and pharmacological studies will elucidate new mechanisms for GPCR regulation, expression, function, and modulation. There are emerging studies regarding class A GPCR dimerization/oligomerization, subcellular location of GPCRs, and temporal regulation of GPCRs that pose interesting paradigms for GPCR modulation. The dimerization, whether homodimers, heterodimers, or higher order oligomers, of class A GPCRs has been a thoroughly discussed topic in relation to its biological relevance.[283–285] A noteworthy reminder, class C GPCRs are known to form obligatory dimers; thus the discussion is centered on class A GPCRs.[286] A recent review by Gurevich and Gurevich addresses dimerization from a signaling

perspective and discusses the stoichiometry observed between class A GPCRs and their effectors: G proteins, β-arrestins, and GRKs.[287] The conclusions state that a single monomeric class A GPCR is sufficient for effector coupling and downstream signaling through multiple pathways, and this is supported by biochemical and functional assays. It is also known that class A GPCRs do indeed interact as dimers during their "life cycle" and that these interactions may be important regulators for expression, localization, and trafficking. Whether or not functional signaling dimers exist, there is evidence for receptor crosstalk that can be modulated, and allosteric modulators can play an important role in regulating the monomer—dimer equilibrium and may impart therapeutic effects in this manner. Indeed, a recent report shows how the CCR5 receptor allosteric modulator maraviroc (**162**) can influence the dimer population by inducing a third inactive dimer conformation.[16] Dimerization of CCR5 is necessary for translocation to the membrane, and the dimer induced by **162** may contribute to its efficacy in blocking HIV entry in to the cell. Much more information is needed on these facets of GPCR pharmacology, but novel chemical probes and tool compounds can provide new ways of investigating dimers and the future may hold targeted therapies for such complexes.

Additionally, as class A GPCR dimers have been visualized in the cell, so have functional intracellular class A GPCRs. Opioid receptors (ORs) were highlighted in a recent report that identified differential signaling patterns between endogenous peptide-bound ORs at the cell surface and opioid drug-bound ORs in the Golgi membrane within the cell.[288] The authors argue that this "distortion" of typical endogenous peptide activation may drive neuronal toxicity and adverse effects from OR-targeted therapeutics. This effect has been termed location bias for GPCR activation. In a review by Grundmann and Kostenis, the recent consensus on time-encoded GPCR signaling ("temporal bias") is presented, as are other important kinetic parameters for class A GPCR signaling.[289] Additional indepth reviews have highlighted signaling and ligand kinetics as an important consideration for interpreting results of receptor activation, especially in the context of allosteric ligands.[290] Current pharmacological studies have identified signaling bias and probe dependence as emerging therapeutic strategies, and the future will likely see dimer equilibrium, location bias, and temporal bias become topics of discussion in class A GPCR allosteric modulator drug discovery.

5. CONCLUSIONS AND FUTURE DIRECTIONS

Class A GPCRs hold significant clinical importance and are targeted by a high percentage of currently marketed drugs. Utilizing allosteric modulation to precisely alter the function of these receptors may enable the therapeutic targeting of previously intractable GPCRs or provide safer therapeutics for currently targeted receptors. The ability to modulate signaling in a spatial and temporal dependent manner, as well as the potential to therapeutically exploit probe dependence, moves beyond achieving subtype selectivity and toward a remarkably precise therapeutic paradigm. However, the complexities that confer these advantages also must be addressed during allosteric modulator discovery, optimization, development, and advancement into preclinical/clinical assessments. Multiple signaling pathways downstream of effectors, such as G proteins and β-arrestins, should be examined to provide informed SAR of the molecule series. Additionally, the endogenous agonist

should be assayed, when appropriate, to ensure the translation of the allosteric modulation in vivo and avoid unforeseen probe dependence. There are cases, especially in the chemokine receptor family, where probe dependence will be a desirable outcome and should be addressed early in scaffold optimization. Other in vivo considerations arise from species bias, which has been observed in mAChRPAMs and NAMs, where activity does not translate from in vitro assays employing human mAChRs to rat in vivo assays. Thus, early examination of rat and human class A GPCRs in vitro may help avoid this situation. The discovery and development of class A GPCR allosteric modulators have progressed tremendously in recent years and have provided a framework for overcoming challenges and maturing clinical candidates.

The chemical diversity of class A GPCR allosteric modulators has grown along with diversity of allosteric binding sites. Allosteric binding sites have been shown to exist in extracellular regions, transmembrane regions, and intracellular regions of receptors, all contributing unique mechanisms for modulation. Most ligand interactions with these sites are classified as hydrophobic interactions, while aromatic π–π interactions are also common. Core scaffolds for class A GPCR allosteric ligands commonly contain a nitrogen amenable to H-bond polar interactions. Druglikeness has improved and is attainable in situations where there are large amounts of SAR to dictate sites available for modification. Chemically successive, iterative ligand design and synthesis should be performed to enable informed SAR and can be aided by strategies such as the “fluorine walk”. Importantly, emergent structural information may provide information toward chemical modifications that lead to high affinity allosteric ligands and should be utilized where available.

Finally, new conceptual frames for pharmacology may direct allosteric modulator discovery toward modes of action other than simple potentiation of activation. As seen in CCR5 dimerization, maraviroc may alter dimer populations to provide antiviral efficacy. Dimer stabilization/destabilization, location bias, and temporal bias may become considerations for allosteric mechanism of action. Probe dependence can hinder development; however, it may also be used to selectively potentiate (or diminish) marketed orthosteric drugs. In this way, a promiscuous orthosteric drug can have improved selectivity at its site of action or be altered for a higher affinity at an additional site. Thus, allosteric modulators may improve marketed drugs to provide greater selectivity, or if polypharmacology is desired, as in difficult psychiatric conditions, allosteric modulators could enhance activity at other receptors for a given marketed drug that displays moderate affinity for these targets. Allosteric modulation is a fundamental mechanism in biology, and exploitation of this paradigm has delivered FDA-approved therapies, multiple drug-candidates in the pipeline and promises to provide more precise and safer small molecule therapeutics in the future.

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ABBREVIATIONS USED

GPCR	G-protein-coupled receptor
oGPCR	orphan G-protein-coupled receptor
CNS	central nervous system

PAM	positive allosteric modulator
NAM	negative allosteric modulator
SAL	silent allosteric ligand
TM	transmembrane
SAR	structure–activity relationship
5-HT	5-hydroxytryptamine
5-HT$_{2C}$R	serotonin 5-HT$_{2C}$ receptor
5-HT$_{2A}$R	serotonin 5-HT$_{2A}$ receptor
5-HT$_{2B}$R	serotonin 5-HT$_{2B}$ receptor
5-HT$_{6}$R	serotonin 5-HT$_{6}$ receptor
5-HT$_{7A}$R	serotonin 5-HT$_{7A}$ receptor
IP$_3$	inositol 1,4,5-triphosphate
SUD	substance use disorder
CHO	Chinese hamster ovary
CUD	cocaine use disorder
PK	pharmacokinetics
EL	extracellular loop
TMH	transmembrane helix
PTEN	phosphatase and tensin homolog
ICL	intracellular loop
β_2AR	β_2-adrenergic receptor
cAMP	cyclic adenosine monophosphate
DR	dopamine receptor
ADHD	attention deficit hyperactivity disorder
PLG	Pro-Leu-Gly-NH$_2$
HTS	high-throughput screen
mACh	muscarinic acetylcholine
BQCA	benzylquinolone carboxylic acid
BBB	brain–blood barrier

IP	inflection point
tPSA	topological polar surface area
P-gp	P-glycoprotein
CB	cannabinoid
GTPγS	guanosine 5′-*O*-[γ-thio]triphosphate
ERK½	extracellular signal-regulated kinase ½
FSK	forskolin
LAPS	ligand-assisted protein structure
DSE	depolarization-induced suppression of excitation
2-AG	2-arachidonoyl glycerol
MD	molecular dynamics
FFAR	free fatty acid receptor
AR	adenosine receptor
AE	allosteric enhancer
AM	allosteric modulator
UTP	uridine 5′-triphosphate
NR4A	nuclear receptor 4A
NRCM	neonatal rat cardiomyocyte
DED	dry eye disease
CCR	chemokine receptor
HIV-1	human immunodeficiency virus type 1
BRET	bioluminescence resonance energy transfer
SBDD	structure-based drug design
FBDD	fragment-based drug design
OR	opioid receptor
MOR	μ-opioid receptor
DOR	δ-opioid receptors
KOR	κ-opioid receptor
PP	pancreatic polypeptide

PAR	proteinase-activated receptor
MCH1R	melanin-concentrating hormone receptor 1
MCH	melanin-concentrating hormone

REFERENCES

(1). Wang L; Martin B; Brenneman R; Luttrell LM; Maudsley S Allosteric modulators of G protein-coupled receptors: future therapeutics for complex physiological disorders. J. Pharmacol. Exp. Ther. 2009, 331, 340–348. [PubMed: 19667132]

(2). Rask-Andersen M; Almén MS; Schiöth HB Trends in the exploitation of novel drug targets. Nat. Rev. Drug Discovery 2011, 10, 579–590. [PubMed: 21804595]

(3). Hauser AS; Attwood MM; Rask-Andersen M; Schiöth HB; Gloriam DE Trends in GPCR drug discovery: new agents, targets and indications. Nat. Rev. Drug Discovery 2017, 16, 829–842. [PubMed: 29075003]

(4). Wacker D; Stevens RC; Roth BL How ligands illuminate GPCR molecular pharmacology. Cell 2017, 170, 414–427. [PubMed: 28753422]

(5). Melancon BJ; Hopkins CR; Wood MR; Emmitte KA; Niswender CM; Christopoulos A; Conn PJ; Lindsley CW Allosteric modulation of seven transmembrane spanning receptors: theory, practice, and opportunities for central nervous system drug discovery. J. Med. Chem. 2012, 55, 1445–1464. [PubMed: 22148748]

(6). Jacoby E; Bouhelal R; Gerspacher M; Seuwen K The 7 TM G protein-coupled receptor target family. ChemMedChem 2006, 1, 760–782.

(7). Wootten D; Christopoulos A; Sexton PM Emerging paradigms in GPCR allostery: implications for drug discovery. Nat. Rev. Drug Discovery 2013, 12, 630–644. [PubMed: 23903222]

(8). Changeux J-P; Christopoulos A Allosteric modulation as a unifying mechanism for receptor function and regulation. Cell 2016, 166, 1084–1102. [PubMed: 27565340]

(9). van der Westhuizen ET; Valant C; Sexton PM; Christopoulos A Endogenous allosteric modulators of G proteincoupled receptors. J. Pharmacol. Exp. Ther. 2015, 353, 246–260. [PubMed: 25650376]

(10). Foster DJ; Conn PJ Allosteric modulation of GPCRs: new insights and potential utility for treatment of schizophrenia and other CNS disorders. Neuron 2017, 94, 431–446. [PubMed: 28472649]

(11). Kenakin TP Biased signalling and allosteric machines: new vistas and challenges for drug discovery. Br. J. Pharmacol. 2012, 165, 1659–1669. [PubMed: 22023017]

(12). Lee Y; Basith S; Choi S Recent advances in structure-based drug design targeting class A G protein-coupled receptors utilizing crystal structures and computational simulations. J. Med. Chem. 2018, 61, 1–46. [PubMed: 28657745]

(13). Foord SM; Bonner TI; Neubig RR; Rosser EM; Pin JP; Davenport AP; Spedding M; Harmar AJ International union of pharmacology. XLVI. G protein-coupled receptor list. Pharmacol. Rev. 2005, 57, 279–288. [PubMed: 15914470]

(14). Fredriksson R; Lagerstrom MC; Lundin L-G; Schiöth HB The G protein-coupled receptors in the human genome form five main families. Phylogenetic analysis, paralogon groups, and fingerprints. Mol. Pharmacol. 2003, 63, 1256–1272. [PubMed: 12761335]

(15). Roth BL; Kroeze WK Integrated approaches for genome-wide interrogation of the druggable non-olfactory G protein-coupled receptor superfamily. J. Biol. Chem. 2015, 290, 19471–19477. [PubMed: 26100629]

(16). Jin J.; Momboisse F; Boncompain G; Koensgen F; Zhou Z; Cordeiro N; Arenzana-Seisdedos F; Perez FB; Kellenberger E; Brelot A CCR5 adopts three homodimeric conformations that control cell surface delivery. Sci. Signaling 2018, 11, eaal2869.

(17). Furness SGB; Liang Y-L; Nowell CJ; Halls ML; Wookey PJ; DalMaso E; Inoue A; Christopoulos A; Wootten D; Sexton PM Ligand-dependent modulation ofG protein conformation alters drug efficacy. Cell 2016, 167, 739–749. [PubMed: 27720449]

(18). Lu S; Zhang J Small molecule allosteric modulators of G protein-coupled receptors: drug-target interactions. J. Med. Chem. [Online early access]. DOI: 10.1021/acs.jmedchem.7b01844. Publication Date (Web): February 19, 2018.

(19). Kruse AC; Ring AM; Manglik A; Hu J; Hu K; Eitel K; Hubner H; Pardon E; Valant C; Sexton PM; Christopoulos A; Felder CC; Gmeiner P; Steyaert J; Weis WI; Garcia KC; Wess J; Kobilka BK Activation and allosteric modulation of a muscarinic acetylcholine receptor. Nature 2013, 504, 101–106. [PubMed: 24256733]

(20). Lu J; Byrne N; Wang J; Bricogne G; Brown FK; Chobanian HR; Colletti SL; Di Salvo J; Thomas-Fowlkes B; Guo Y; Hall DL; Hadix J; Hastings NB; Hermes JD; Ho T; Howard AD; Josien H; Kornienko M; Lumb KJ; Miller MW; Patel SB; Pio B; Plummer CW; Sherborne BS; Sheth P; Souza S; Tummala S; Vonrhein C; Webb M; Allen SJ; Johnston JM; Weinglass AB; Sharma S; Soisson SM Structural basis for the cooperative allosteric activation of the free fatty acid receptor GPR40. Nat. Struct. Mol. Biol. 2017, 24, 570–577. [PubMed: 28581512]

(21). Liu X; Ahn S; Kahsai AW; Meng KC; Latorraca NR; Pani B; Venkatakrishnan AJ; Masoudi A; Weis WI; Dror RO; Chen X; Lefkowitz RJ; Kobilka BK Mechanism of intracellular allosteric β_{2A}R antagonist revealed by X-ray crystal structure. Nature 2017, 548, 480–484. [PubMed: 28813418]

(22). Conn PJ; Lindsley CW; Meiler J; Niswender CM Opportunities and challenges in the discovery of allosteric modulators of GPCRs for treating CNS disorders. Nat. Rev. Drug Discovery 2014, 13, 692–708. [PubMed: 25176435]

(23). Gregory KJ; Sexton PM; Christopoulos A Overview of receptor allosterism. Curr. Protoc. Pharmacol. 2010, 51, 1–34.

(24). Leach K; Sexton PM; Christopoulos A Allosteric GPCR modulators: taking advantage of permissive receptor pharmacology. Trends Pharmacol. Sci. 2007, 28, 382–389. [PubMed: 17629965]

(25). May LT; Leach K; Sexton PM; Christopoulos A Allosteric modulation of G protein-coupled receptors. Annu. Rev. Pharmacol. Toxicol. 2007, 47, 1–51. [PubMed: 17009927]

(26). Lindsley CW; Emmitte KA; Hopkins CR; Bridges TM; Gregory KJ; Niswender CM; Conn PJ Practical strategies and concepts in GPCR allosteric modulator discovery: recent advances with metabotropic glutamate receptors. Chem. Rev. 2016, 116, 6707–6741. [PubMed: 26882314]

(27). Tan Q; Zhu Y; Li J; Chen Z; Han GW; Kufareva I; Li T; Ma L; Fenalti G; Li J; Zhang W; Xie X; Yang H; Jiang H; Cherezov V; Liu H; Stevens RC; Zhao Q; Wu B Structure of the CCR5 chemokine receptor-HIV entry inhibitor maraviroc complex. Science 2013, 341, 1387–1390. [PubMed: 24030490]

(28). Zheng Y; Qin L; Zacarias NV; de Vries H; Han GW; Gustavsson M; Dabros M; Zhao C; Cherney RJ; Carter P; Stamos D; Abagyan R; Cherezov V; Stevens RC; IJzerman AP; Heitman LH; Tebben A; Kufareva I; Handel TM Structure of CC chemokine receptor 2 with orthosteric and allosteric antagonists. Nature 2016, 540, 458–461. [PubMed: 27926736]

(29). Oswald C; Rappas M; Kean J; Doré AS; Errey JC; Bennett K; Deflorian F; Christopher JA; Jazayeri A; Mason JS; Congreve M; Cooke RM; Marshall FH Intracellular allosteric antagonism of the CCR9 receptor. Nature 2016, 540, 462–465. [PubMed: 27926729]

(30). Srivastava A; Yano J; Hirozane Y; Kefala G; Gruswitz F; Snell G; Lane W; Ivetac A; Aertgeerts K; Nguyen J; Jennings A; Okada K High-resolution structure of the human GPR40 receptor bound to allosteric agonist TAK-875. Nature 2014, 513, 124–127. [PubMed: 25043059]

(31). Zhang D; Gao Z-G; Zhang K; Kiselev E; Crane S; Wang J; Paoletta S; Yi C; Ma L; Zhang W; Han GW; Liu H; Cherezov V; Katritch V; Jiang H; Stevens RC; Jacobson KA; Zhao Q; Wu B Two disparate ligand-binding sites in the human P2Y1 receptor. Nature 2015, 520, 317–321. [PubMed: 25822790]

(32). Cheng RKY; Fiez-Vandal C; Schlenker O; Edman K; Aggeler B; Brown DG; Brown GA; Cooke RM; Dumelin CE; Dore AS; Geschwindner S; Grebner C; Hermansson NO; Jazayeri A; Johansson P; Leong L; Prihandoko R; Rappas M; Soutter H; Snijder A; Sundstrom L; Tehan B; Thornton P; Troast D; Wiggin G; Zhukov A; Marshall FH; Dekker N Structural insight into allosteric modulation of protease-activated receptor 2. Nature 2017, 545, 112–115. [PubMed: 28445455]

(33). Liu H; Kim HR; Deepak RNVK; Wang L; Chung KY; Fan H; Wei Z; Zhang C Orthosteric and allosteric action of the C5a receptor antagonists. Nat. Struct. Mol. Biol. 2018, 25, 472–481. [PubMed: 29867214]

(34). Robertson N; Rappas M; Doré AS; Brown J; Bottegoni G; Koglin M; Cansfield J; Jazayeri A; Cooke RM; Marshall FH Structure of the complement C5a receptor bound to the extra-helical antagonist NDT9513727. Nature 2018, 553, 111–114. [PubMed: 29300009]

(35). Zhang K; Zhang J; Gao Z-G; Zhang D; Zhu L; Han GW; Moss SM; Paoletta S; Kiselev E; Lu W; Fenalti G; Zhang W; Muller CE; Yang H; Jiang H; Cherezov V; Katritch V; Jacobson KA; Stevens RC; Wu B; Zhao Q Structure of the human P2Y12 receptor in complex with an antithrombotic drug. Nature 2014, 509, 115–118. [PubMed: 24670650]

(36). Weinert T; Olieric N; Cheng R; Brünle S; James D; Ozerov D; Gashi D; Vera L; Marsh M; Jaeger K; Dworkowski F; Panepucci E; Basu S; Skopintsev P; Dore AS; Geng T; Cooke RM; Liang M; Prota AE; Panneels V; Nogly P; Ermler U; Schertler G; Hennig M; Steinmetz MO; Wang M; Standfuss J Serial millisecond crystallography for routine room-temperature structure determination at synchrotrons. Nat. Commun. 2017, 8, 542. [PubMed: 28912485]

(37). Fenalti G; Giguere PM; Katritch V; Huang X-P; Thompson AA; Cherezov V; Roth BL; Stevens RC Molecular control of 5-opioid receptor signalling. Nature 2014, 506, 191–196. [PubMed: 24413399]

(38). van Westen GJP; Gaulton A; Overington JP Chemical, target, and bioactive properties of allosteric modulation. PLoS Comput. Biol. 2014, 10, e1003559.

(39). Bologna Z; Teoh J-p.; Bayoumi, A. S.; Tang, Y.; Kim, I.-m. Biased G protein-coupled receptor signaling: new player in modulating physiology and pathology. Biomol Ther. 2017, 25, 12–25.

(40). Lane JR; May LT; Parton RG; Sexton PM; Christopoulos A A kinetic view of GPCR allostery and biased agonism. Nat. Chem. Biol. 2017, 13, 929–937. [PubMed: 28820879]

(41). Wild C; Cunningham KA; Zhou J Allosteric modulation of G protein-coupled receptors: an emerging approach of drug discovery. Austin J. Pharmacol. Ther. 2014, 2, 1101.

(42). Amarandi R-M; Hjort0, G. M.; Rosenkilde, M. M.; Karlsh0j, S. Probing Biased Signaling in Chemokine Receptors. In Methods in Enzymology; Handel, T. M., Ed.; Academic Press, 2016; Vol. 570, pp 155–186, DOI: 10.1016/bs.mie.2015.09.001.

(43). Steen A; Larsen O; Thiele S; Rosenkilde MM Biased and G protein-independent signaling of chemokine receptors. Front. Immunol. 2014, 5, 1–11. [PubMed: 24474949]

(44). Viola A; Luster AD Chemokines and their receptors: drug targets in immunity and inflammation. Annu. Rev. Pharmacol. Toxicol. 2008, 48, 171–197. [PubMed: 17883327]

(45). Seitz PK; Bremer NM; McGinnis AG; Cunningham KA; Watson CS Quantitative changes in intracellular calcium and extracellular-regulated kinase activation measured in parallel in CHO cells stably expressing serotonin (5-HT) 5-HT2A or 5-HT2C receptors. BMC Neurosci. 2012, 13, 25–25. [PubMed: 22397586]

(46). Leysen JE; Pauwels PJ 5-HT2 receptors, roles and regulation. Ann. N. Y. Acad. Sci 1990, 600, 183–193. [PubMed: 2252309]

(47). Quesseveur G; Nguyen HT; Gardier AM; Guiard BP 5-HT2 ligands in the treatment of anxiety and depression. Expert Opin. Invest. Drugs 2012, 21, 1701–1725.

(48). Bubar MJ; Cunningham KA Prospects for Serotonin 5-HT2R Pharmacotherapy in Psychostimulant Abuse In Progress in Brain Research; Di Giovann G, Di Matteo V, Esposito E, Eds.; Elsevier, 2008; Vol. 172, pp 319–346, DOI: 10.1016/S0079-6123(08)00916-3. [PubMed: 18772040]

(49). Smith SR; Prosser WA; Donahue DJ; Morgan ME; Anderson CM; Shanahan WR Lorcaserin (APD356), a selective 5-HT2C agonist, reduces body weight in obese men and women. Obesity 2009, 17, 494–503. [PubMed: 19057523]

(50). Garcia-Carceles J; Decara JM; Vazquez-Villa H; Rodriguez R; Codesido E; Cruces J; Brea J; Loza MI; Alen F; Botta J; McCormick PJ; Ballesteros JA; Benhamu B; Rodriguez de Fonseca F; Lopez-Rodriguez ML A positive allosteric modulator of the serotonin 5-HT2C receptor for obesity. J. Med. Chem. 2017, 60, 9575–9584 [PubMed: 29116785]

(51). Wild CT; Miszkiel JM; Wold EA; Soto CA; Ding C; Hartley RM; White MA; Anastasio NC; Cunningham KA; Zhou J Design, synthesis, and characterization of 4-undecylpiperidine-2-

carboxamides as positive allosteric modulators of the serotonin (5-HT) 5-HT2C receptor. J. Med. Chem. [Online early access]. DOI: DOI: 10.1021/acs.jmedchem.8b00401. Publication Date (Web): 4 5, 2018.

(52). Im WB; Chio CL; Alberts GL; Dinh DM Positive allosteric modulator of the human 5-HT2C receptor. Mol. Pharmacol. 2003, 64, 78–84. [PubMed: 12815163]

(53). Ding C; Bremer NM; Smith TD; Seitz PK; Anastasio NC; Cunningham KA; Zhou J Exploration of synthetic approaches and pharmacological evaluation of PNU-69176E and its stereoisomer as 5-HT2C receptor allosteric modulators. ACS Chem. Neurosci. 2012, 3, 538–545 [PubMed: 22860223]

(54). Peng Y; McCorvy JD; Harpsoe K;Lansu K; Yuan S; Popov P; Qu L; Pu M; Che T; Nikolajsen LF; Huang X-P; Wu Y; Shen L; Bjorn-Yoshimoto WE; Ding K; Wacker D; Han GW; Cheng J; Katritch V; Jensen AA; Hanson MA; Zhao S; Gloriam DE; Roth BL; Stevens RC; Liu Z-J 5-HT2C receptor structures reveal the structural basis of GPCR polypharmacology. Cell 2018, 172, 719–730. [PubMed: 29398112]

(55). Bennett TA; Montesinos P; Moscardo F; Martinez-Cuadron D; Martinez J; Sierra J; García R; de Oteyza JP; Fernandez P; Serrano J; Fernandez A; Herrera P; Gonzalez A; Bethancourt C; Rodriguez-Macias G; Alonso A; Vera JA; Navas B; Lavilla E; Lopez JA; Jimenez S; Simiele A; Vidriales B; Gonzalez BJ; Burgaleta C; Hernandez J. A. Rivas; Mascuñano RC; Bautista G; Simon J. A. Perez; Fuente A. d. l.; Rayón C; Troconiz IF; Janda A; Bosanquet AG; Hernandez-Campo P; Primo D; Lopez R; Liebana B; Rojas JL; Gorrochategui J; Sanz MA; Ballesteros J Pharmacological profiles of acute myeloid leukemia treatments in patient samples by automated flow cytometry: a bridge to individualized medicine. Clin. Lymphoma, Myeloma Leuk. 2014, 14, 305–318. [PubMed: 24468131]

(56). Tobin AB; Butcher AJ; Kong KC Location, location, location...site-specific GPCR phosphorylation offers a mechanism for cell-type-specific signalling. Trends Pharmacol. Sci. 2008, 29, 413–420. [PubMed: 18606460]

(57). Chakir K; Xiang Y; Yang D; Zhang S-J; Cheng H; Kobilka BK; Xiao R-P The third intracellular loop and the carboxyl terminus of β_2-adrenergic receptor confer spontaneous activity of the receptor. Mol.Pharmacol. 2003, 64, 1048–1058. [PubMed: 14573753]

(58). Tobin AB G-protein-coupled receptor phosphorylation: where, when and by whom. Br. J. Pharmacol. 2008, 153, S167–S176. [PubMed: 18193069]

(59). Lawson Z; Wheatley M The third extracellular loop of G-protein-coupled receptors: more than just a linker between two important transmembrane helices. Biochem. Soc. Trans. 2004, 32, 1048–1050. [PubMed: 15506960]

(60). Müller CP; Carey RJ Intracellular 5-HT2C-receptor dephosphorylation: a new target for treating drug addiction. Trends Pharmacol. Sci. 2006, 27, 455–458. [PubMed: 16876260]

(61). Anastasio NC; Gilbertson SR; Bubar MJ; Agarkov A; Stutz SJ; Jeng Y; Bremer NM; Smith TD; Fox RG; Swinford SE; Seitz PK; Charendoff MN; Craft JW Jr.; Laezza FM; Watson CS; Briggs JM; Cunningham KA Peptide inhibitors disrupt the serotonin 5-HT2C receptor interaction with phosphatase and tensin homolog to allosterically modulate cellular signaling and behavior. J. Neurosci. 2013, 33, 1615–1630. [PubMed: 23345234]

(62). Rasmussen SGF; Choi H-J; Rosenbaum DM; Kobilka TS; Thian FS; Edwards PC; Burghammer M; Ratnala VRP; Sanishvili R; Fischetti RF; Schertler GFX; Weis WI; Kobilka BK Crystal structure of the human β_2 adrenergic G-protein-coupled receptor. Nature 2007, 450, 383–387. [PubMed: 17952055]

(63). Lefkowitz RJ Seven transmembrane receptors: something old, something new. Acta Physiol. 2007, 190, 9–19.

(64). Brandt DR; Asano T; Pedersen SE; Ross EM Reconstitution of catecholamine-stimulated guanosine triphosphatase activity. Biochemistry 1983, 22, 4357–4362. [PubMed: 6138091]

(65). Cerione RA; Codina J; Benovic JL; Lefkowitz RJ; Birnbaumer L; Caron MG Mammalian β_2-adrenergic receptor: reconstitution of functional interactions between pure receptor and pure stimulatory nucleotide binding protein of the adenylate cyclase system. Biochemistry 1984, 23, 4519–4525. [PubMed: 6149763]

(66). Ross E; Maguire M; Sturgill T; Biltonen R; Gilman A Relationship between the beta-adrenergic receptor and adenylate cyclase. J. Biol. Chem. 1977, 252, 5761–5775. [PubMed: 195960]

(67). De Lean A; Stadel J; Lefkowitz R A ternary complex model explains the agonist-specific binding properties of the adenylate cyclase-coupled beta-adrenergic receptor. J. Biol. Chem. 1980,255,7108–7117. [PubMed: 6248546]

(68). Ahn S; Kahsai AW; Pani B; Wang QT; Zhao S; Wall AL; Strachan RT; Staus DP; Wingler LM; Sun LD; Sinnaeve J; Choi M; Cho T; Xu TT; Hansen GM; Burnett MB; Lamerdin JE; Bassoni DL; Gavino BJ; Husemoen G; Olsen EK; Franch T; Costanzi S; Chen X; Lefkowitz RJ Allosteric "beta-blocker" isolated from a DNA-encoded small molecule library. Proc. Natl. Acad. Sci. U. S.A. 2017, 114, 1708–1713. [PubMed: 28130548]

(69). Meng K; Shim P; Wang Q; Zhao S; Gu T; Kahsai AW; Ahn S; Chen X Design, synthesis, and functional assessment of Cmpd-15 derivatives as negative allosteric modulators for the β_2-adrenergic receptor. Bioorg. Med. Chem. 2018, 26, 2320–2330. [PubMed: 29588128]

(70). Wood M; Ates A; Andre VM; Michel A; Barnaby R; Gillard M In vitro and in vivo identification of novel positive allosteric modulators of the human dopamine D2 and D3 receptor. Mol. Pharmacol. 2016, 89, 303–312. [PubMed: 26655303]

(71). Urs NM; Peterson SM; Caron MG New concepts in dopamine D2 receptor biased signaling and implications for schizophrenia therapy. Biol. Psychiatry 2017, 81, 78–85. [PubMed: 27832841]

(72). Kumar V; Bonifazi A; Ellenberger MP; Keck TM; Pommier E; Rais R; Slusher BS; Gardner E; You Z-B; Xi Z-X; Newman AH Highly selective dopamine D3 receptor (D3R) antagonists and partial agonists based on eticlopride and the D3R crystal structure: new leads for opioid dependence treatment. J. Med. Chem. 2016, 59, 7634–7650. [PubMed: 27508895]

(73). Cheng MH; Garcia-Olivares J; Wasserman S; DiPietro J; Bahar I Allosteric modulation of human dopamine transporter activity under conditions promoting its dimerization. J. Biol. Chem. 2017, 292, 12471–12482. [PubMed: 28584050]

(74). Marsango S; Caltabiano G; Jimenez-Roses M; Millan MJ; Pediani JD; Ward RJ; Milligan G A molecular basis for selective antagonist destabilization of dopamine D3 receptor quaternary organization. Sci. Rep. 2017, 7, 2134. [PubMed: 28522847]

(75). Niewiarowska-Sendo A; Polit A; Piwowar M; Tworzydlo M; Kozik A; Guevara-Lora I Bradykinin B2 and dopamine D2 receptors form a functional dimer. Biochim. Biophys. Acta, Mol. Cell Res. 2017, 1864, 1855–1866. [PubMed: 28757212]

(76). Salmas RE; Seeman P; Aksoydan B; Erol I; Kantarcioglu I; Stein M; Yurtsever M; Durdagi S Analysis of the glutamate agonist LY404,039 binding to nonstatic dopamine receptor D2 dimer structures and consensus docking. ACS Chem. Neurosci 2017, 8, 1404–1415. [PubMed: 28272861]

(77). Carli M; Kolachalam S; Aringhieri S; Rossi M; Giovannini L; Maggio R; Scarselli M Dopamine D2 receptors dimers: how can we pharmacologically target them? Curr. Neuropharmacol 2018, 16, 222–230. [PubMed: 28521704]

(78). Kasai RS; Ito SV; Awane R M.; Fujiwara, T. K.; Kusumi, A. The class-A GPCR dopamine D2 receptor forms transient dimers stabilized by agonists: detection by single-molecule tracking. Cell Biochem. Biophys. 2018, 76, 29–37. [PubMed: 29116599]

(79). Lai CY; Liu YJ; Lai HL; Chen HM; Kuo HC; Liao YP; Chern Y The D2 dopamine receptor interferes with the protective effect of the A2A adenosine receptor on TDP-43 mislocalization in experimental models of motor neuron degeneration. Front. Neurosci. 2018, 12, 187. [PubMed: 29615863]

(80). Verma V; Mann A; Costain W; Pontoriero G; Castellano JM; Skoblenick K; Gupta SK; Pristupa Z; Niznik HB; Johnson RL; Nair VD; Mishra RK Modulation of agonist binding to human dopamine receptor subtypes by L-prolyl-L-leucyl-glycinamide and a peptidomimetic analog. J. Pharmacol. Exp. Ther. 2005, 315, 1228–1236. [PubMed: 16126839]

(81). Fisher A; Mann A; Verma V; Thomas N; Mishra RK; Johnson RL Design and synthesis of photoaffinity-labeling ligands of the L-prolyl-L-leucylglycinamide binding site involved in the allosteric modulation of the dopamine receptor. J. Med. Chem. 2006, 49, 307–317. [PubMed: 16392815]

(82). Mann A; Verma V; Basu D; Skoblenick KJ; Beyaert MG; Fisher A; Thomas N; Johnson RL; Mishra RK Specific binding of photoaffinity-labeling peptidomimetics of Pro-Leu-Gly-NH2 to the dopamine D2L receptor: evidence for the allosteric modulation of the dopamine receptor. Eur. J. Pharmacol. 2010, 641, 96–101. [PubMed: 20639138]

(83). Ferreira da Costa J; Caamano O; Fernandez F; Garcia-Mera X; Sampaio-Dias IE; Brea JM; Cadavid MI Synthesis and allosteric modulation of the dopamine receptor by peptide analogs of L-prolyl-L-leucyl-glycinamide (PLG) modified in the L-proline or L-proline and L-leucine scaffolds. Eur. J. Med. Chem. 2013, 69, 146–158. [PubMed: 24013414]

(84). Dyck B; Guest K; Sookram C; Basu D; Johnson R; Mishra RK PAOPA, a potent analogue of Pro-Leu-glycinamide and allosteric modulator of the dopamine D2 receptor, prevents NMDA receptor antagonist (MK-801)-induced deficits in social interaction in the rat: implications for the treatment of negative symptoms in schizophrenia. Schizophr. Res. 2011, 125, 88–92. [PubMed: 21036015]

(85). Beyaert MG; Daya RP; Dyck BA; Johnson RL; Mishra RK PAOPA, a potent dopamine D2 receptor allosteric modulator, prevents and reverses behavioral and biochemical abnormalities in an amphetamine-sensitized preclinical animal model of schizophrenia. Eur. Neuropsychopharmacol 2013, 23, 253–262. [PubMed: 22658400]

(86). Tan ML; Basu D; Kwiecien JM; Johnson RL; Mishra RK Preclinical pharmacokinetic and toxicological evaluation of MIF-1 peptidomimetic, PAOPA: examining the pharmacology of a selective dopamine D2 receptor allosteric modulator for the treatment of schizophrenia. Peptides 2013, 42, 89–96. [PubMed: 23416534]

(87). Bhagwanth S; Mishra RK; Johnson RL Development of peptidomimetic ligands ofPro-Leu-Gly-NH2 as allosteric modulators of the dopamine D2 receptor. Beilstein J. Org. Chem. 2013, 9, 204–214. [PubMed: 23400263]

(88). Raghavan B; Skoblenick KJ; Bhagwanth S; Argintaru N; Mishra RK; Johnson RL Allosteric modulation ofthe dopamine D2 receptor by Pro-Leu-Gly-NH2 peptidomimetics constrained in either a polyproline II helix or a type II beta-turn conformation. J. Med. Chem. 2009, 52, 2043–2051. [PubMed: 19271750]

(89). Bhagwanth S; Mishra S; Daya R; Mah J; Mishra RK; Johnson RL Transformation of Pro-Leu-Gly-NH2 peptidomimetic positive allosteric modulators of the dopamine D2 receptor into negative modulators. ACS Chem. Neurosci. 2012, 3, 274–284. [PubMed: 22860194]

(90). Silvano E; Millan MJ; la Cour C. Mannoury; Han Y; Duan L; Griffin SA; Luedtke RR; Aloisi G; Rossi M; Zazzeroni F; Javitch JA; Maggio R The tetrahydroisoquinoline derivative SB269,652 is an allosteric antagonist at dopamine D3 and D2 receptors. Mol. Pharmacol. 2010, 78, 925–934. [PubMed: 20702763]

(91). Rossi M; Fasciani I; Marampon F; Maggio R; Scarselli M The first negative allosteric modulator for dopamine D2 and D3 receptors, SB269652 may lead to a new generation of antipsychotic drugs. Mol. Pharmacol. 2017, 91, 586–594. [PubMed: 28265019]

(92). Shonberg J; Draper-Joyce C; Mistry SN; Christopoulos A; Scammells PJ; Lane JR; Capuano B Structure-activity study of N-((trans)-4-(2-(7-cyano-3,4-dihydroisoquinolin-2(1H)-yl)ethyl)-cyclohexyl)-1H-indole-2-carboxamide (SB269652), a bitopic ligand that acts as a negative allosteric modulator of the dopamine D2 receptor. J. Med. Chem. 2015, 58, 5287–5307. [PubMed: 26052807]

(93). Kumar V; Moritz AE; Keck TM; Bonifazi A; Ellenberger MP; Sibley CD; Free RB; Shi L; Lane JR; Sibley DR; Newman AH Synthesis and pharmacological characterization of novel trans-cyclopropylmethyl-linked bivalent ligands that exhibit selectivity and allosteric pharmacology at the dopamine D3 receptor (D3R). J. Med. Chem. 2017, 60, 1478–1494. [PubMed: 28186762]

(94). Conn PJ; Christopoulos A; Lindsley CW Allosteric modulators of GPCRs: a novel approach for the treatment of CNS disorders. Nat. Rev. Drug Discovery 2009, 8, 41–54. [PubMed: 19116626]

(95). Wess J; Eglen RM; Gautam D Muscarinic acetylcholine receptors: mutant mice provide new insights for drug development. Nat. Rev. Drug Discovery 2007, 6, 721–733. [PubMed: 17762886]

(96). Bock A; Schrage R; Mohr K Allosteric modulators targeting CNS muscarinic receptors. Neuropharmacology 2018, 136, 427–437. [PubMed: 28935216]

(97). Ma L; Seager MA; Wittmann M; Jacobson M; Bickel D; Burno M; Jones K; Graufelds VK; Xu G; Pearson M; McCampbell A; Gaspar R; Shughrue P; Danziger A; Regan C; Flick R; Pascarella D; Garson S; Doran S; Kreatsoulas C; Veng L; Lindsley CW; Shipe W; Kuduk S; Sur C; Kinney G; Seabrook GR; Ray WJ Selective activation of the muscarinic acetylcholine receptor achieved

by allosteric potentiation. Proc. Natl. Acad. Sci. U. S. A. 2009, 106, 15950–15955. [PubMed: 19717450]

(98). Yang FV; Shipe WD; Bunda JL; Nolt MB; Wisnoski DD; Zhao Z; Barrow JC; Ray WJ; Ma L; Wittmann M; Seager MA; Koeplinger KA; Hartman GD; Lindsley CW Parallel synthesis of N-biaryl quinolone carboxylic acids as selective M1 positive allosteric modulators. Bioorg. Med. Chem. Lett. 2010, 20, 531–536. [PubMed: 20004574]

(99). Mistry SN; Valant C; Sexton PM; Capuano B; Christopoulos A; Scammells PJ Synthesis and pharmacological profiling of analogues of benzyl quinolone carboxylic acid (BQCA) as allosteric modulators of the M1 muscarinic receptor. J. Med. Chem. 2013, 56, 5151–5172. [PubMed: 23718562]

(100). Kuduk SD; Di Marco CN; Cofre V; Ray WJ; Ma L; Wittmann M; Seager MA; Koeplinger KA; Thompson CD; Hartman GD; Bilodeau MT Fused heterocyclic M1 positive allosteric modulators. Bioorg. Med. Chem. Lett. 2011, 21, 2769–2772. [PubMed: 21055928]

(101). Abdul-Ridha A; Lane JR; Mistry SN; Lopez L; Sexton PM; Scammells PJ; Christopoulos A; Canals M Mechanistic insights into allosteric structure-function relationships at the M1 muscarinic acetylcholine receptor. J. Biol. Chem. 2014, 289, 33701–33711. [PubMed: 25326383]

(102). Kuduk SD; Beshore DC; Di M. C. Ng; Greshock TJ Aryl Methyl Benzoquinazolinone M1 Receptor Positive Allosteric Modulators. W02010059773, 2010.

(103). Mistry SN; Jorg M; Lim H; Vinh NB; Sexton PM; Capuano B; Christopoulos A; Lane JR; Scammells PJ 4-Phenylpyridin-2-one derivatives: a novel class of positive allosteric modulator of the M1 muscarinic acetylcholine receptor. J. Med. Chem. 2016, 59, 388–409. [PubMed: 26624844]

(104). Mistry SN; Lim H; Jorg M; Capuano B; Christopoulos A; Lane JR; Scammells PJ Novel fused arylpyrimidinone based allosteric modulators of the M1 muscarinic acetylcholine receptor. ACS Chem. Neurosci. 2016, 7, 647–661. [PubMed: 26891194]

(105). Han C; Chatterjee A; Noetzel MJ; Panarese JD; Smith E; Chase P; Hodder P; Niswender C; Conn PJ; Lindsley CW; Stauffer SR Discovery and SAR of muscarinic receptor subtype 1 (M1) allosteric activators from a molecular libraries high throughput screen. Part 1: 2,5-dibenzyl-2H-pyrazolo[4,3-c]quinolin-3(5H)-ones as positive allosteric modulators. Bioorg. Med. Chem. Lett. 2015, 25, 384–388. [PubMed: 25435150]

(106). Kuduk SD; Chang RK; Di Marco CN; Ray WJ; Ma L; Wittmann M; Seager MA; Koeplinger KA; Thompson CD; Hartman GD; Bilodeau MT Quinolizidinone carboxylic acids as CNS penetrant, selective m1 allosteric muscarinic receptor modulators. ACSMed. Chem. Lett. 2010, 1, 263–267.

(107). Kuduk SD; Chang RK; Di Marco CN; Pitts DR; Greshock TJ; Ma L; Wittmann M; Seager MA; Koeplinger KA; Thompson CD; Hartman GD; Bilodeau MT; Ray WJ Discovery of a selective allosteric M1 receptor modulator with suitable development properties based on a quinolizidinone carboxylic acid scaffold. J. Med. Chem. 2011, 54, 4773–4780. [PubMed: 21682298]

(108). Kuduk SD; Chang RK; Greshock TJ; Ray WJ; Ma L; Wittmann M; Seager MA; Koeplinger KA; Thompson CD; Hartman GD; Bilodeau MT Identification of amides as carboxylic Acid surrogates for quinolizidinone-based M1 positive allosteric modulators. ACS Med. Chem. Lett. 2012, 3, 1070–1074. [PubMed: 24900430]

(109). Kuduk SD; Di Marco CN; Saffold JR; Ray WJ; Ma L; Wittmann M; Koeplinger KA; Thompson CD; Hartman GD; Bilodeau MT; Beshore DC Identification of a methoxynaphthalene scaffold as a core replacement in quinolizidinone amide M1 positive allosteric modulators. Bioorg. Med. Chem. Lett. 2014, 24, 1417–1420. [PubMed: 24485781]

(110). Reid PR; Bridges TM; Sheffler DJ; Cho HP; Lewis LM; Days E; Daniels JS; Jones CK; Niswender CM; Weaver CD; Conn PJ; Lindsley CW; Wood MR Discovery and optimization of a novel, selective and brain penetrant M1 positive allosteric modulator (PAM): the development of ML169, an MLPCN probe. Bioorg. Med. Chem. Lett. 2011, 21, 2697–2701. [PubMed: 21194936]

(111). Tarr JC; Turlington ML; Reid PR; Utley TJ; Sheffler DJ; Cho HP; Klar R; Pancani T; Klein MT; Bridges TM; Morrison RD; Blobaum AL; Xiang Z; Daniels JS; Niswender CM; Conn PJ; Wood MR; Lindsley CW Targeting selective activation of M1 for the treatment of Alzheimer's disease:

further chemical optimization and pharmacological characterization of the M1 positive allosteric modulator ML169. ACS Chem. Neurosci. 2012, 3, 884–895. [PubMed: 23173069]

(112). Ballard TM; Flohr A; Groebke-Zbinden K; Pinard E; Rychmans T; Schaffhauser H Preparation of Pyrrolopyridine and Pyrazolopyridine Derivatives as Muscarinic M1 Receptor Modulators. W015028483, 2015.

(113). Payne A; Castro-Pineiro JL; Birch LM; Khan A; Braunton AJ; Kitulagoda JE; Soejima M Preparation of 4-Azaindole Derivatives as Muscarinic M1 Receptor Modulators for Treatment of Cognitive Deficits. W015049574, 2015.

(114). Ballard TM; Groebke-Zbinden K; Pinard E; Rychmans T; Schaffhauser H Preparation of Indole and Indazole Derivatives as Muscarinic M1 Receptor Pos. Allosteric Modulators. WO15044072, 2015

(115). Davoren JE; O'Neil SV; Anderson DP; Brodney MA; Chenard L; Dlugolenski K; Edgerton JR; Green M; Garnsey M; Grimwood S; Harris AR; Kauffman GW; LaChapelle E; Lazzaro JT; Lee CW; Lotarski SM; Nason DM; Obach RS; Reinhart V; Salomon-Ferrer R; Steyn SJ; Webb D; Yan J; Zhang L Design and optimization of selective azaindole amide M1 positive allosteric modulators. Bioorg. Med. Chem. Lett. 2016, 26, 650–655. [PubMed: 26631313]

(116). Rook JM; Abe M; Cho HP; Nance KD; Luscombe VB; Adams JJ; Dickerson JW; Remke DH; Garcia-Barrantes PM; Engers DW; Engers JL; Chang S; Foster JJ; Blobaum AL; Niswender CM; Jones CK; Conn PJ; Lindsley CW Diverse effects on M1 signaling and adverse effect liability within a series of M1 ago-PAMs. ACS Chem. Neurosci. 2017, 8, 866–883. [PubMed: 28001356]

(117). Bridges TM; Kennedy JP; Noetzel MJ; Breininger ML; Gentry PR; Conn PJ; Lindsley CW Chemical lead optimization of a pan Gq mAChRM1, M3, M5 positive allosteric modulator (PAM) lead. Part II: development of a potent and highly selective M1 PAM. Bioorg. Med. Chem. Lett. 2010, 20, 1972–1975. [PubMed: 20156687]

(118). Melancon BJ; Poslusney MS; Gentry PR; Tarr JC; Sheffler DJ; Mattmann ME; Bridges TM; Utley TJ; Daniels JS; Niswender CM; Conn PJ; Lindsley CW; Wood MR Isatin replacements applied to the highly selective, muscarinic M1 PAM ML137: continued optimization of an MLPCN probe molecule. Bioorg. Med. Chem. Lett. 2013, 23, 412–416. [PubMed: 23237839]

(119). Ghoshal A; Rook JM; Dickerson JW; Roop GN; Morrison RD; Jalan-Sakrikar N; Lamsal A; Noetzel MJ; Poslusney MS; Wood MR; Melancon BJ; Stauffer SR; Xiang Z; Daniels JS; Niswender CM; Jones CK; Lindsley CW; Conn PJ Potentiation of M1 muscarinic receptor reverses plasticity deficits and negative and cognitive symptoms in a schizophrenia mouse model. Neuropsychopharmacology 2016, 41, 598–610. [PubMed: 26108886]

(120). Davoren JE; Garnsey M; Pettersen B; Brodney MA; Edgerton JR; Fortin JP; Grimwood S; Harris AR; Jenkinson S; Kenakin T; Lazzaro JT; Lee CW; Lotarski SM; Nottebaum L; O'Neil SV; Popiolek M; Ramsey S; Steyn SJ; Thorn CA; Zhang L; Webb D Design and synthesis of gamma- and delta-lactam M1 positive allosteric modulators (PAMs): convulsion and cholinergic toxicity of an M1-selective PAM with weak agonist activity. J. Med. Chem. 2017, 60, 6649–6663. [PubMed: 28598634]

(121). Flohr A; Hutter R; Mueller B; Bohnert C; Pellisson M; Schaffhauser H Discovery of the first low-shift positive allosteric modulators for the muscarinic M1 receptor. Bioorg. Med. Chem. Lett. 2017, 27,5415–5419. [PubMed: 29146472]

(122). Gentry PR; Bridges TM; Lamsal A; Vinson PN; Smith E; Chase P; Hodder PS; Engers JL; Niswender CM; Daniels JS; Conn PJ; Wood MR; Lindsley CW Discovery ofML326: The first sub-micromolar, selective M5 PAM. Bioorg. Med. Chem. Lett. 2013, 23, 2996–3000. [PubMed: 23562060]

(123). Marlo JE; Niswender CM; Days EL; Bridges TM; Xiang Y; Rodriguez AL; Shirey JK; Brady AE; Nalywajko T; Luo Q; Austin CA; Williams MB; Kim K; Williams R; Orton D; Brown HA; Lindsley CW; Weaver CD; Conn PJ Discovery and characterization of novel allosteric potentiators of Mi muscarinic receptors reveals multiple modes of activity. Mol. Pharmacol. 2009, 75, 577–588. [PubMed: 19047481]

(124). Bridges TM; Marlo JE; Niswender CM; Jones CK; Jadhav SB; Gentry PR; Plumley HC; Weaver CD; Conn PJ; Lindsley CW Discovery of the first highly M5-preferring muscarinic acetylcholine receptor ligand, an M5 positive allosteric modulator derived from a series of 5-trifluoromethoxy N-benzyl isatins. J. Med. Chem. 2009, 52, 3445–3448. [PubMed: 19438238]

(125). Bridges TM; Kennedy JP; Hopkins CR; Conn PJ; Lindsley CW Heterobiaryl and heterobiaryl ether derived M5 positive allosteric modulators. Bioorg. Med. Chem. Lett. 2010, 20, 5617–5622. [PubMed: 20801651]

(126). Bridges TM; Kennedy JP; Cho HP; Breininger ML; Gentry PR; Hopkins CR; Conn PJ; Lindsley CW Chemical lead optimization of a pan Gq mAChR M1, M3, M5 positive allosteric modulator (PAM) lead. Part I: Development of the first highly selective M5 PAM. Bioorg. Med. Chem. Lett. 2010, 20, 558–562. [PubMed: 20004578]

(127). Gentry PR; Kokubo M; Bridges TM; Daniels JS; Niswender CM; Smith E; Chase P; Hodder PS; Rosen H; Conn PJ; Engers J; Brewer KA; Lindsley CW; Wood MR Development of the First CNS Penetrant M5 Positive Allosteric Modulator (PAM) Based on a Novel, Non-Isatin Core In Probe Reports from the NIH Molecular Libraries Program; National Center for Biotechnology Information (U.S.): Bethesda, MD, 2010.

(128). Gentry PR; Kokubo M; Bridges TM; Kett NR; Harp JM; Cho HP; Smith E; Chase P; Hodder PS; Niswender CM; Daniels JS; Conn PJ; Wood MR; Lindsley CW Discovery of the first M5-selective and CNS penetrant negative allosteric modulator (NAM) of a muscarinic acetylcholine receptor: (S)-9b-(4-chlorophen-yl)-1-(3,4-difluorobenzoyl)-2,3-dihydro-1H-imidazo[2,1-a]isoi ndol-5(9bH)-one (ML375). J. Med. Chem. 2013, 56, 9351–9355. [PubMed: 24164599]

(129). Kurata H; Gentry PR; Kokubo M; Cho HP; Bridges TM; Niswender CM; Byers FW; Wood MR; Daniels JS; Conn PJ; Lindsley CW Further optimization of the M5 NAM MLPCN probe ML375: tactics and challenges. Bioorg. Med. Chem. Lett. 2015, 25, 690–694. [PubMed: 25542588]

(130). Lazareno S; Dolezal V; Popham A; Birdsall NJ Thiochrome enhances acetylcholine affinity at muscarinic M4 receptors: receptor subtype selectivity via cooperativity rather than affinity. Mol. Pharmacol. 2004, 65, 257–266. [PubMed: 14722259]

(131). Chan WY; McKinzie DL; Bose S; Mitchell SN; Witkin JM; Thompson RC; Christopoulos A; Lazareno S; Birdsall NJ; Bymaster FP; Felder CC Allosteric modulation of the muscarinic M4 receptor as an approach to treating schizophrenia. Proc. Natl. Acad. Sci. U. S.A. 2008, 105, 10978–10983. [PubMed: 18678919]

(132). Shirey JK; Xiang Z; Orton D; Brady AE; Johnson KA; Williams R; Ayala JE; Rodriguez AL; Wess J; Weaver D; Niswender CM; Conn PJ An allosteric potentiator of M4 mAChR modulates hippocampal synaptic transmission. Nat. Chem. Biol. 2008, 4, 42–50. [PubMed: 18059262]

(133). Brady AE; Jones CK; Bridges TM; Kennedy JP; Thompson AD; Heiman JU; Breininger ML; Gentry PR; Yin H; Jadhav SB; Shirey JK; Conn PJ; Lindsley CW Centrally active allosteric potentiators of the M4 muscarinic acetylcholine receptor reverse amphetamine-induced hyperlocomotor activity in rats. J. Pharmacol. Exp. Ther. 2008, 327, 941–953. [PubMed: 18772318]

(134). Kennedy JP; Bridges TM; Gentry PR; Brogan JT; Kane AS; Jones CK; Brady AE; Shirey JK; Conn PJ; Lindsley CW Synthesis and structure-activity relationships of allosteric potentiators of the M4 muscarinic acetylcholine receptor. ChemMed-Chem 2009, 4, 1600–1607.

(135). Salovich JM; Vinson PN; Sheffler DJ; Lamsal A; Utley TJ; Blobaum AL; Bridges TM; Le U; Jones CK; Wood MR; Daniels JS; Conn PJ; Niswender CM; Lindsley CW; Hopkins CR Discovery of N-(4-methoxy-7-methylbenzo[d]thiazol-2-yl)-isonicatinamide, ML293, as a novel, selective and brain penetrant positive allosteric modulator of the muscarinic 4 (M4) receptor. Bioorg. Med. Chem. Lett. 2012, 22, 5084–5088. [PubMed: 22738637]

(136). Wood MR; Noetzel MJ; Poslusney MS; Melancon BJ; Tarr JC; Lamsal A; Chang S; Luscombe VB; Weiner RL; Cho HP; Bubser M; Jones CK; Niswender CM; Wood MW; Engers DW; Brandon NJ; Duggan ME; Conn PJ; Bridges TM; Lindsley CW Challenges in the development of an M4 PAM in vivo tool compound: The discovery of VU0467154 and unexpected DMPK profiles of close analogs. Bioorg. Med. Chem. Lett. 2017, 27, 171–175. [PubMed: 27939174]

(137). Bubser M; Bridges TM; Dencker D; Gould RW; Grannan M; Noetzel MJ; Lamsal A; Niswender CM; Daniels JS; Poslusney MS; Melancon BJ; Tarr JC; Byers FW; Wess J; Duggan ME; Dunlop J; Wood MW; Brandon NJ; Wood MR; Lindsley CW; Conn PJ; Jones CK Selective activation of M4 muscarinic acetylcholine receptors reverses MK-801-induced behavioral impairments and enhances associative learning in rodents. ACS Chem. Neurosci. 2014, 5, 920–942. [PubMed: 25137629]

(138). Tarr JC; Wood MR; Noetzel MJ; Melancon BJ; Lamsal A; Luscombe VB; Rodriguez AL; Byers FW; Chang S; Cho HP; Engers DW; Jones CK; Niswender CM; Wood MW; Brandon NJ; Duggan ME; Conn PJ; Bridges TM; Lindsley CW Challenges in the development of an M4 PAM preclinical candidate: The discovery, SAR, and biological characterization of a series of azetidine-derived tertiary amides. Bioorg. Med. Chem. Lett. 2017, 27, 5179–5184. [PubMed: 29089231]

(139). Tarr JC; Wood MR; Noetzel MJ; Bertron JL; Weiner RL; Rodriguez AL; Lamsal A; Byers FW; Chang S; Cho HP; Jones CK; Niswender CM; Wood MW; Brandon NJ; Duggan ME; Conn PJ; Bridges TM; Lindsley CW Challenges in the development of an M4 PAM preclinical candidate: The discovery, SAR, and in vivo characterization of a series of 3-aminoazetidine-derived amides. Bioorg. Med. Chem. Lett. 2017, 27, 2990–2995. [PubMed: 28522253]

(140). Li Q; Chen H-F Synergistic regulation mechanism ofiperoxo and LY2119620 for muscarinic acetylcholine M2 receptor. RSC Adv. 2018, 8, 13067–13074.

(141). Raasch A; Scharfenstein O; Trankle C; Holzgrabe U; Mohr K Elevation ofligand binding to muscarinic M2 acetylcholine receptors by bis(ammonio)alkane-type allosteric modulators. J. Med. Chem. 2002, 45, 3809–3812. [PubMed: 12166953]

(142). Holzgrabe U; De Amici M; Mohr K Allosteric modulators and selective agonists of muscarinic receptors. J. Mol. Neurosci. 2006, 30, 165–168. [PubMed: 17192667]

(143). Moulton BC; Fryer AD Muscarinic receptor antagonists, from folklore to pharmacology; finding drugs that actually work in asthma and COPD. Br. J. Pharmacol. 2011, 163, 44–52. [PubMed: 21198547]

(144). Croy CH; Schober DA; Xiao H; Quets A; Christopoulos A; Felder CC Characterization of the novel positive allosteric modulator, LY2119620, at the muscarinic M2 and M4 receptors. Mol. Pharmacol. 2014, 86, 106–115. [PubMed: 24807965]

(145). Miao Y; Goldfeld DA; Moo EV; Sexton PM; Christopoulos A; McCammon JA; Valant C Accelerated structure-based design of chemically diverse allosteric modulators of a muscarinic G protein-coupled receptor. Proc. Natl. Acad. Sci. U. S. A. 2016, 113, E5675–E5684. [PubMed: 27601651]

(146). Korczynska M; Clark MJ; Valant C; Xu J; Moo EV; Albold S; Weiss DR; Torosyan H; Huang W; Kruse AC; Lyda BR; May LT; Baltos JA; Sexton PM; Kobilka BK; Christopoulos A; Shoichet BK; Sunahara RK Structure-based discovery of selective positive allosteric modulators of antagonists for the M2 muscarinic acetylcholine receptor. Proc. Natl. Acad. Sci. U. S. A. 2018, 115, E2419–E2428. [PubMed: 29453275]

(147). Saleh N; Hucke O; Kramer G; Schmidt E; Montel F; Lipinski R; Ferger B; Clark T; Hildebrand PW; Tautermann CS Multiple binding sites contribute to the mechanism of mixed agonistic and positive allosteric modulators of the cannabinoid CB1 receptor. Angew. Chem., Int. Ed. 2018, 57, 2580–2585.

(148). Di Marzo V CB1 receptor antagonism: biological basis for metabolic effects. Drug Discovery Today 2008, 13, 1026–1041. [PubMed: 18824122]

(149). Navarro G; Morales P; Rodriguez-Cueto C; Fernandez-Ruiz J; Jagerovic N; Franco R Targeting cannabinoid CB2 receptors in the central nervous system. Medicinal chemistry approaches with focus on neurodegenerative disorders. Front. Neurosci. 2016, 10, 406. [PubMed: 27679556]

(150). Zhang H-Y; Gao M; Shen H; Bi G-H; Yang H-J; Liu QR; Wu J; Gardner EL; Bonci A; Xi Z-X Expression of functional cannabinoid CB2 receptor in VTA dopamine neurons in rats. Addict. Biol. 2017, 22, 752–765. [PubMed: 26833913]

(151). Zhang H-Y; Gao M; Liu Q-R; Bi G-H; Li X; Yang H-J; Gardner EL; Wu J; Xi Z-X Cannabinoid CB2 receptors modulate midbrain dopamine neuronal activity and dopamine-related behavior in mice. Proc. Natl. Acad. Sci U. S. A. 2014, 111, E5007–E5015. [PubMed: 25368177]

(152). Martinez-Pinilla E; Varani K; Reyes-Resina I; Angelats E; Vincenzi F; Ferreiro-Vera C; Oyarzabal J; Canela EI; Lanciego JL; Nadal X; Navarro G; Borea PA; Franco R Binding and signaling studies disclose a potential allosteric site for cannabidiol in cannabinoid CB2 receptors. Front. Pharmacol. 2017, 8, 744. [PubMed: 29109685]

(153). Price MR; Baillie GL; Thomas A; Stevenson LA; Easson M; Goodwin R; McLean A; McIntosh L; Goodwin G; Walker G; Westwood P; Marrs J; Thomson F; Cowley P; Christopoulos A;

Pertwee RG; Ross RA Allosteric modulation of the cannabinoid CB1 receptor. Mol. Pharmacol. 2005, 68, 1484–1495. [PubMed: 16113085]

(154). Piscitelli F; Ligresti A; La Regina G; Coluccia A; Morera L; Allara M; Novellino E; Di Marzo V; Silvestri R Indole-2-carboxamides as allosteric modulators of the cannabinoid CB1 receptor. J. Med. Chem. 2012, 55, 5627–5631. [PubMed: 22571451]

(155). Ahn KH; Mahmoud MM; Samala S; Lu D; Kendall DA Profiling two indole-2-carboxamides for allosteric modulation of the CB1 receptor. J. Neurochem. 2013, 124, 584–589. [PubMed: 23205875]

(156). Mahmoud MM; Ali HI; Ahn KH; Damaraju A; Samala S; Pulipati VK; Kolluru S; Kendall DA; Lu D Structure-activity relationship study of indole-2-carboxamides identifies a potent allosteric modulator for the cannabinoid receptor 1 (CB1). J. Med. Chem. 2013, 56, 7965–7975. [PubMed: 24053617]

(157). Khurana L; Ali HI; Olszewska T; Ahn KH; Damaraju A; Kendall DA; Lu D Optimization of chemical functionalities of indole-2-carboxamides to improve allosteric parameters for the cannabinoid receptor 1 (CB1). J. Med. Chem. 2014, 57, 3040–3052. [PubMed: 24635495]

(158). Nguyen T; German N; Decker AM; Li JX; Wiley JL; Thomas BF; Kenakin TP; Zhang Y Structure-activity relationships of substituted 1H-indole-2-carboxamides as CB1 receptor allosteric modulators. Bioorg. Med. Chem. 2015, 23, 2195–2203. [PubMed: 25797163]

(159). Kulkarni PM; Kulkarni AR; Korde A; Tichkule R; Laprairie RB; Denovan-Wright EM; Zhou H; Janero DR; Zvonok N; Makriyannis A; Cascio MG; Pertwee RG; Thakur GA Novel electrophilic and photoaffinity covalent probes for mapping the cannabinoid 1 receptor allosteric site(s). J. Med. Chem. 2016, 59, 44–60. [PubMed: 26529344]

(160). Qiao CJ; Ali HI; Ahn KH; Kolluru S; Kendall DA; Lu D Synthesis and biological evaluation of indole-2-carboxamides bearing photoactivatable functionalities as novel allosteric modulators for the cannabinoid CB1 receptor. Eur. J. Med. Chem. 2016, 121, 517–529. [PubMed: 27318976]

(161). Horswill JG; Bali U; Shaaban S; Keily JF; Jeevaratnam P; Babbs AJ; Reynet C; Wong P. Kai In PSNCBAM-1, a novel allosteric antagonist at cannabinoid CB1 receptors with hypophagic effects in rats. Br. J. Pharmacol. 2007, 152, 805–814. [PubMed: 17592509]

(162). Nguyen T; Li JX; Thomas BF; Wiley JL; Kenakin TP; Zhang Y Allosteric modulation: an alternate approach targeting the cannabinoid CB1 receptor. Med. Res. Rev. 2017, 37, 441–474. [PubMed: 27879006]

(163). Baillie GL; Horswill JG; Anavi-Goffer S; Reggio PH; Bolognini D; Abood ME; McAllister S; Strange PG; Stephens GJ; Pertwee RG; Ross RA CB1 receptor allosteric modulators display both agonist and signaling pathway specificity. Mol. Pharmacol. 2013, 83, 322–338. [PubMed: 23160940]

(164). Khajehali E; Malone DT; Glass M; Sexton PM; Christopoulos A; Leach K Biased agonism and biased allosteric modulation at the CB1 cannabinoid receptor. Mol. Pharmacol. 2015, 88, 368–379. [PubMed: 26044547]

(165). Khurana L; Fu BQ; Duddupudi AL; Liao YH; Immadi SS; Kendall DA; Lu D Pyrimidinyl biphenylureas: identification of new lead compounds as allosteric modulators of the cannabinoid receptor CB1. J. Med. Chem. 2017, 60, 1089–1104. [PubMed: 28059509]

(166). Nguyen T; German N; Decker AM; Langston TL; Gamage TF; Farquhar CE; Li JX; Wiley JL; Thomas BF; Zhang Y Novel diarylurea based allosteric modulators of the cannabinoid CB1 receptor: evaluation of importance of 6-pyrrolidi-nylpyridinyl substitution. J. Med. Chem. 2017, 60, 7410–7424. [PubMed: 28792219]

(167). Bertini S; Chicca A; Gado F; Arena C; Nieri D; Digiacomo M; Saccomanni G; Zhao P; Abood ME; Macchia M; Gertsch J; Manera C Novel analogs of PSNCBAM-1 as allosteric modulators of cannabinoid CB1 receptor. Bioorg. Med. Chem. 2017, 25, 6427–6434. [PubMed: 29079014]

(168). Priestley RS; Nickolls SA; Alexander SP; Kendall DA A potential role for cannabinoid receptors in the therapeutic action of fenofibrate. FASEB J. 2015, 29, 1446–1455. [PubMed: 25550466]

(169). Laprairie RB; Bagher AM; Kelly ME; Denovan-Wright EM Cannabidiol is a negative allosteric modulator of the cannabinoid CB1 receptor. Br. J. Pharmacol. 2015, 172, 4790–4805. [PubMed: 26218440]

(170). Vallee M; Vitiello S; Bellocchio L; Hebert-Chatelain E; Monlezun S; Martin-Garcia E; Kasanetz F; Baillie GL; Panin F; Cathala A; Roullot-Lacarriere V; Fabre S; Hurst DP; Lynch DL; Shore DM; Deroche-Gamonet V; Spampinato U; Revest JM; Maldonado R; Reggio PH; Ross RA; Marsicano G; Piazza PV Pregnenolone can protect the brain from cannabis intoxication. Science 2014, 343, 94–98. [PubMed: 24385629]

(171). Laprairie RB; Kulkarni PM; Deschamps JR; Kelly MEM; Janero DR; Cascio MG; Stevenson LA; Pertwee RG; Kenakin TP; Denovan-Wright EM; Thakur GA Enantiospecific allosteric modulation of cannabinoid 1 receptor. ACS Chem. Neurosci. 2017, 8, 1188–1203. [PubMed: 28103441]

(172). Navarro HA; Howard JL; Pollard GT; Carroll FI Positive allosteric modulation of the human cannabinoid (CB) receptor by RTI-371, a selective inhibitor of the dopamine transporter. Br. J. Pharmacol. 2009, 156, 1178–1184. [PubMed: 19226282]

(173). Pamplona FA; Ferreira J; Menezes de Lima O Jr.; Duarte FS; Bento AF; Forner S; Villarinho JG; Bellocchio L; Wotjak CT; Lerner R; Monory K; Lutz B; Canetti C; Matias I; Calixto JB; Marsicano G; Guimaraes MZ; Takahashi RN Anti-inflammatory lipoxin A4 is an endogenous allosteric enhancer of CB1 cannabinoid receptor. Proc. Natl. Acad. Sci. U. S. A. 2012, 109, 21134–21139. [PubMed: 23150578]

(174). Ganeshsingh A; Thakur PM K. Allosteric Modulators of CB1 Cannabinoid Receptors. WO2013103967 A1, 2013.

(175). Ignatowska-Jankowska BM; Baillie GL; Kinsey S; Crowe M; Ghosh S; Owens RA; Damaj IM; Poklis J; Wiley JL; Zanda M; Zanato C; Greig IR; Lichtman AH; Ross RA A cannabinoid CB1 receptor-positive allosteric modulator reduces neuropathic pain in the mouse with no psychoactive effects. Neuropsychopharmacology 2015, 40, 2948–2959. [PubMed: 26052038]

(176). Straiker A; Mitjavila J; Yin D; Gibson A; Mackie K Aiming for allosterism: evaluation of allosteric modulators of CB1 in a neuronal model. Pharmacol. Res. 2015, 99, 370–376. [PubMed: 26211948]

(177). Mitjavila J; Yin D; Kulkarni PM; Zanato C; Thakur GA; Ross R; Greig I; Mackie K; Straiker A Enantiomer-specific positive allosteric modulation of CB1 signaling in autaptic hippocampal neurons. Pharmacol. Res. 2018, 129, 475–481. [PubMed: 29158048]

(178). Milligan G; Shimpukade B; Ulven T; Hudson BD Complex pharmacology of free fatty acid receptors. Chem. Rev. 2017, 117,67–110. [PubMed: 27299848]

(179). Tikhonova IG; Sum CS; Neumann S; Thomas CJ; Raaka BM; Costanzi S; Gershengorn MC Bidirectional, iterative approach to the structural delineation of the functional "chemoprint" in GPR40 for agonist recognition. J. Med. Chem. 2007, 50, 2981–2989. [PubMed: 17552505]

(180). Lin DC; Guo Q; Luo J; Zhang J; Nguyen K; Chen M; Tran T; Dransfield PJ; Brown SP; Houze J; Vimolratana M; Jiao XY; Wang Y; Birdsall NJ; Swaminath G Identification and pharmacological characterization of multiple allosteric binding sites on the free fatty acid 1 receptor. Mol. Pharmacol. 2012, 82, 843–859. [PubMed: 22859723]

(181). Milligan G; Shimpukade B; Ulven T; Hudson BD Complex Pharmacology of Free Fatty Acid Receptors. Chem. Rev. 2017, 117, 67–110. [PubMed: 27299848]

(182). Yabuki C; Komatsu H; Tsujihata Y; Maeda R; Ito R; Matsuda-Nagasumi K; Sakuma K; Miyawaki K; Kikuchi N; Takeuchi K; Habata Y; Mori M A novel antidiabetic drug, fasiglifam/TAK-875, acts as an ago-allosteric modulator of FFAR1. PLoS One 2013, 8, e76280.

(183). Bolognini D; Tobin AB; Milligan G; Moss CE The pharmacology and function of receptors for short-chain fatty acids. Mol. Pharmacol. 2016, 89, 388–398. [PubMed: 26719580]

(184). Lee T; Schwandner R; Swaminath G; Weiszmann J; Cardozo M; Greenberg J; Jaeckel P; Ge H; Wang Y; Jiao X; Liu J; Kayser F; Tian H; Li Y Identification and functional characterization of allosteric agonists for the G protein-coupled receptor FFA2. Mol. Pharmacol. 2008, 74, 1599–609. [PubMed: 18818303]

(185). Smith NJ; Ward R; Stoddart LA; Hudson BD; Kostenis E; Ulven T; Morris JC; Trankle C; Tikhonova IG; Adams DR; Milligan G Extracellular loop 2 of the free fatty acid receptor 2 mediates allosterism of a phenylacetamide ago-allosteric modulator. Mol. Pharmacol. 2011, 80, 163–173. [PubMed: 21498659]

(186). Wang Y; Jiao X; Kayser F; Liu J; Wang Z; Wanska M; Greenberg J; Weiszmann J; Ge H; Tian H; Wong S; Schwandner R; Lee T; Li Y The first synthetic agonists of FFA2: discovery and SAR of phenylacetamides as allosteric modulators. Bioorg. Med. Chem. Lett. 2010, 20, 493–498. [PubMed: 20005104]

(187). Bolognini D; Moss CE; Nilsson K; Petersson AU; Donnelly I; Sergeev E; Konig GM; Kostenis E; Kurowska-Stolarska M; Miller A; Dekker N; Tobin AB; Milligan G A novel allosteric activator of free fatty acid 2 receptor displays unique Gi-functional bias. J. Biol. Chem. 2016, 291, 18915–18931. [PubMed: 27385588]

(188). Leonard JN; Chu Z; Bruce MA; Boatman PD GPR41 and Modulators Thereof for the Treatment of Insulin-Related Disorders. WO2006052566 A3, 2006.

(189). Hudson BD; Christiansen E; Murdoch H; Jenkins L; Hansen AH; Madsen O; Ulven T; Milligan G Complex pharmacology of novel allosteric free fatty acid 3 receptor ligands. Mol. Pharmacol. 2014, 86, 200–210. [PubMed: 24870406]

(190). Giovannoni MP; Ciciani G; Cilibrizzi A; Crocetti L; Daniele S; Di Cesare Mannelli L; Ghelardini C; Giacomelli C; Guerrini G; Martini C; Trincavelli ML; Vergelli C Further studies on pyrazolo[1′,5′:1,6]pyrimido[4,5-d]pyridazin-4(3H)-ones as potent and selective human A1 adenosine receptor antagonists. Eur. J. Med. Chem. 2015, 89, 32–41. [PubMed: 25462223]

(191). Knight A; Hemmings JL; Winfield I; Leuenberger M; Frattini E; Frenguelli BG; Dowell SJ; Lochner M; Ladds G Discovery of novel adenosine receptor agonists that exhibit subtype selectivity. J. Med. Chem. 2016, 59, 947–964. [PubMed: 26756468]

(192). van der Klein PA; Kourounakis AP; IJzerman AP Allosteric modulation of the adenosine A1 receptor. Synthesis and biological evaluation of novel 2-amino-3-benzoylthiophenes as allosteric enhancers of agonist binding. J. Med. Chem. 1999, 42, 3629–3635. [PubMed: 10479294]

(193). Bruns RF; Fergus JH Allosteric enhancement of adenosine A1 receptor binding and function by 2-amino-3-benzoylthiophenes. Mol. Pharmacol. 1990, 38, 939–949. [PubMed: 2174510]

(194). Bruns RF; Fergus JH; Coughenour LLGG; Pugsley TA; Dodd JH; Tinney FJ Structure-activity relationships for enhancement of adenosine A1 receptor binding by 2-amino-3-benzoylthiophenes. Mol. Pharmacol. 1990, 38, 950–958. [PubMed: 2250667]

(195). Baraldi PG; Iaconinoto MA; Moorman AR; Carrion MD; Cara CL; Preti D; Lopez OC; Fruttarolo F; Tabrizi MA; Romagnoli R Allosteric enhancers for A1 adenosine receptor. Mini-Rev. Med. Chem. 2007, 7, 559–569. 1 [PubMed: 17584155]

(196). Childers SR; Li X; Xiao R; Eisenach JC Allosteric modulation of adenosine A1 receptor coupling to G-proteins in brain. J. Neurochem. 2005, 93, 715–723. [PubMed: 15836630]

(197). Kimatrai-Salvador M; Baraldi PG; Romagnoli R Allosteric modulation of A1-adenosine receptor: a review. Drug Discovery Today: Technol. 2013, 10, e285–e296.

(198). Ferguson GN; Valant C; Horne J; Figler H; Flynn BL; Linden J; Chalmers DK; Sexton PM; Christopoulos A; Scammells PJ 2-aminothienopyridazines as novel adenosine A1 receptor allosteric modulators and antagonists. J. Med. Chem. 2008, 51,6165–6172. [PubMed: 18771255]

(199). Romagnoli R; Baraldi PG; IJzerman AP; Massink A; Cruz-Lopez O; Lopez-Cara LC; Saponaro G; Preti D; Tabrizi M. Aghazadeh; Baraldi S; Moorman AR; Vincenzi F; Borea PA; Varani K Synthesis and biological evaluation of novel allosteric enhancers of the A1 adenosine receptor based on 2-amino-3-(4′-chlorobenzoyl)-4-substituted-5-arylethynyl thiophene. J. Med. Chem. 2014, 57, 7673–7686. [PubMed: 25181013]

(200). Romagnoli R; Baraldi PG; Carrion MD; Cara CL; Cruz-Lopez O; Iaconinoto MA; Preti D; Shryock JC; Moorman AR; Vincenzi F; Varani K; Andrea Borea P Synthesis and biological evaluation of 2-amino-3-(4-chlorobenzoyl)-4-[N-(substituted) piper-azin-1-yl]thiophenes as potent allosteric enhancers of the A1 adenosine receptor. J. Med. Chem. 2008, 51, 5875–5879. [PubMed: 18729349]

(201). Goblyos A; Ijzerman AP Allosteric modulation of adenosine receptors. Purinergic Signalling 2009, 5, 51–61. [PubMed: 18615273]

(202). Valant C; Aurelio L; Devine SM; Ashton TD; White JM; Sexton PM; Christopoulos A; Scammells PJ Synthesis and characterization of novel 2-amino-3-benzoylthiophene derivatives as biased allosteric agonists and modulators of the adenosine A1 receptor. J. Med. Chem. 2012, 55, 2367–2375. [PubMed: 22315963]

(203). Lutjens H; Zickgraf A; Figler H; Linden J; Olsson RA; Scammells PJ 2-Amino-3-benzoylthiophene allosteric enhancers of A1 adenosine agonist binding: new 3, 4-, and 5-modifications. J. Med. Chem. 2003, 46, 1870–1877. [PubMed: 12723950]

(204). Aurelio L; Figler H; Flynn BL; Linden J; Scammells PJ 5-Substituted 2-aminothiophenes as A1 adenosine receptor allosteric enhancers. Bioorg. Med. Chem. 2008, 16, 1319–1327. [PubMed: 17980606]

(205). Aurelio L; Valant C; Flynn BL; Sexton PM; Christopoulos A; Scammells PJ Allosteric modulators of the adenosine A1 receptor: synthesis and pharmacological evaluation of 4-substituted 2-amino-3-benzoylthiophenes. J. Med. Chem. 2009, 52, 4543–4547. [PubMed: 19514747]

(206). Romagnoli R; Baraldi PG; Carrion MD; Cara CL; Cruz-Lopez O; Salvador MK; Preti D; Tabrizi MA; Shryock JC; Moorman AR; Vincenzi F; Varani K; Borea PA Structure-activity relationships of 2-amino-3-aroyl-4-[(4-arylpiperazin-1-yl)methyl]-thiophenes. Part 2: Probing the influence of diverse substituents at the phenyl of the arylpiperazine moiety on allosteric enhancer activity at the A1 adenosine receptor. Bioorg. Med. Chem. 2012, 20, 996–1007. [PubMed: 22182575]

(207). Romagnoli R; Baraldi PG; Carrion MD; Cara CL; Cruz-Lopez O; Salvador MK; Preti D; Tabrizi MA; Moorman AR; Vincenzi F; Borea PA; Varani K Synthesis and biological evaluation of 2-amino-3-(4-chlorobenzoyl)-4-[(4-arylpiperazin-1-yl)methyl]-5-substituted-thioph enes. effect of the 5-modification on allosteric enhancer activity at the A1 adenosine receptor. J. Med. Chem. 2012, 55, 7719–7735. [PubMed: 22889387]

(208). Romagnoli R; Baraldi PG; Carrion MD; Cruz-Lopez O; Cara CL; Saponaro G; Preti D; Tabrizi MA; Baraldi S; Moorman AR; Vincenzi F; Borea PA; Varani K Synthesis and biological evaluation of novel 2-amino-3-aroyl-4-neopentyl-5-substi-tuted thiophene derivatives as allosteric enhancers of the A1 adenosine receptor. Bioorg. Med. Chem. 2014, 22, 148–166. [PubMed: 24332652]

(209). Romagnoli R; Baraldi PG; Lopez-Cara C; Cruz-Lopez O; Moorman AR; Massink A; IJzerman AP; Vincenzi F; Borea PA; Varani K Synthesis and biological evaluation of a new series of 2-amino-3-aroyl thiophene derivatives as agonist allosteric modulators of the A1 adenosine receptor. A position-dependent effect study. Eur. J. Med. Chem. 2015, 101, 185–204. [PubMed: 26141910]

(210). Gao ZG; Gross AS; Jacobson KA Effects of the allosteric modulator SCH-202676 on adenosine and P2Y receptors. Life Sci. 2004, 74,3173–3180. [PubMed: 15081581]

(211). Draper-Joyce CJ; Khoshouei M; Thal DM; Liang Y-L; Nguyen ATN; Furness SGB; Venugopal H; Baltos J-A; Plitzko JM; Danev R; Baumeister W; May LT; Wootten D; Sexton PM; Glukhova A; Christopoulos A Structure of the adenosine-bound human adenosine A1 receptor—Gi complex. Nature 2018, 558, 559–563. [PubMed: 29925945]

(212). Glukhova A; Thal DM; Nguyen AT; Vecchio EA; Jorg M; Scammells PJ; May LT; Sexton PM; Christopoulos A Structure of the adenosine A1 receptor reveals the basis for subtype selectivity. Cell 2017, 168, 867–877. [PubMed: 28235198]

(213). Nguyen ATN; Vecchio EA; Thomas T; Nguyen TD; Aurelio L; Scammells PJ; White PJ; Sexton PM; Gregory KJ; May LT; Christopoulos A Role of the second extracellular loop of the adenosine A1 receptor on allosteric modulator binding, signaling, and cooperativity. Mot. Pharmacol. 2016, 90, 715–725.

(214). Gao ZG; Ijzerman AP Allosteric modulation of A2A adenosine receptors by amiloride analogues and sodium ions. Biochem. Pharmacol. 2000, 60, 669–676. [PubMed: 10927025]

(215). Gao ZG; Melman N; Erdmann A; Kim SG; Muller CE; IJzerman AP; Jacobson KA Differential allosteric modulation by amiloride analogues of agonist and antagonist binding at A1 and A3 adenosine receptors. Biochem. Pharmacol. 2003, 65, 525–534. [PubMed: 12566079]

(216). Massink A; Louvel J; Adlere I; van Veen C; Huisman BJ; Dijksteel GS; Guo D; Lenselink EB; Buckley BJ; Matthews H; Ranson M; Kelso M; IJzerman AP 5/-Substituted amiloride derivatives as allosteric modulators binding in the sodium ion pocket of the adenosine A2A receptor. J. Med. Chem. 2016, 59, 4769–4777. [PubMed: 27124340]

(217). Gutierrez-de-Teran H; Massink A; Rodriguez D; Liu W; Han GW; Joseph JS; Katritch I; Heitman LH; Xia L; Ijzerman AP; Cherezov V; Katritch V; Stevens RC The role of a sodium ion

binding site in the allosteric modulation of the A2A. adenosine G proteincoupled receptor. Structure 2013, 21, 2175–2185. [PubMed: 24210756]

(218). Taliani S; Trincavelli ML; Cosimelli B; Laneri S; Severi E; Barresi E; Pugliesi I; Daniele S; Giacomelli C; Greco G; Novellino E; Martini C; Da Settimo F Modulation of A2B adenosine receptor by 1-Benzyl-3-ketoindole derivatives. Eur.J.Med. Chem. 2013, 69, 331–337. [PubMed: 24077183]

(219). Trincavelli ML; Giacomelli C; Daniele S; Taliani S; Cosimelli B; Laneri S; Severi E; Barresi E; Pugliesi I; Greco G; Novellino E; Da Settimo F; Martini C Allosteric modulators of human A2B adenosine receptor. Biochim. Biophys.Acta, Gen. Subj. 2014, 1840, 1194–1203.

(220). Borea PA; Varani K; Vincenzi F; Baraldi PG; Tabrizi MA; Merighi S; Gessi S The A3 adenosine receptor: history and perspectives. Pharmacol. Rev. 2015, 67, 74–102. [PubMed: 25387804]

(221). Goblyos A; Gao ZG; Brussee J; Connestari R; Santiago SN; Ye K; Ijzerman AP; Jacobson KA Structure-activity relationships of new 1H-imidazo[4,5-c]quinolin-4-amine derivatives as allosteric enhancers of the A3 adenosine receptor. J. Med. Chem. 2006, 49, 3354–3361 [PubMed: 16722654]

(222). Gao ZG; Verzijl D; Zweemer A; Ye K; Goblyos A; Ijzerman AP; Jacobson KA Functionally biased modulation of A3 adenosine receptor agonist efficacy and potency by imidazoquinolin-amine allosteric enhancers. Biochem. Pharmacol. 2011, 82, 658–668. [PubMed: 21718691]

(223). Heitman LH; Goblyos A; Zweemer AM; Bakker R; Mulder-Krieger T; van Veldhoven JP; de Vries H; Brussee J; Ijzerman AP A series of 2,4-disubstituted quinolines as a new class of allosteric enhancers of the adenosine A3 receptor. J. Med. Chem. 2009, 52, 926–931. [PubMed: 19161279]

(224). Van Poecke S; Barrett MO; Kumar T. Santhosh; Sinnaeve D; Martins JC; Jacobson KA; Kendall Harden T; Van Calenbergh S Synthesis and P2Y2 receptor agonist activities of uridine 5′-phosphonate analogues. Bioorg. Med. Chem. 2012, 20, 2304–2315. [PubMed: 22386981]

(225). Yuan S; Chan HC; Vogel H; Filipek S; Stevens RC; Palczewski K The molecular mechanism of P2Y1 receptor activation. Angew. Chem., Int. Ed. 2016, 55, 10331–10335.

(226). Chao H; Turdi H; Herpin TF; Roberge JY; Liu Y; Schnur DM; Poss MA; RehfUss R; Hua J; Wu Q; Price LA; Abell LM; Schumacher WA; Bostwick JS; Steinbacher TE; Stewart AB; Ogletree ML; Huang CS; Chang M; Cacace AM; Arcuri MJ; Celani D; Wexler RR; Lawrence RM Discovery of 2-(phenoxypyridine)-3-phenylureas as small molecule P2Y1 antagonists. J. Med. Chem. 2013, 56, 1704–1714. [PubMed: 23368907]

(227). Qiao JX; Wang TC; Ruel R; Thibeault C; L'Heureux A; Schumacher WA; Spronk SA; Hiebert S; Bouthillier G; Lloyd J; Pi Z; Schnur DM; Abell LM; Hua J; Price LA; Liu E; Wu Q; Steinbacher TE; Bostwick JS; Chang M; Zheng J; Gao Q; Ma B; McDonnell PA; Huang CS; Rehfuss R; Wexler RR; Lam PYS Conformationally constrained ortho-anilino diaryl ureas: discovery of 1-(2-(1/-neopentylspiro[indoline-3,4′-piperidine]-1-yl)-phenyl)-3-(4-(trifluoromethoxy)phenyl)urea, a potent, selective, and bioavailable P2Y1 antagonist. J. Med. Chem. 2013, 56, 9275–9295. [PubMed: 24164581]

(228). Yang W; Wang Y; Lai A; Qiao JX; Wang TC; Hua J; Price LA; Shen H; Chen X. -q.; Wong P; Crain E; Watson C; Huang CS; Seiffert DA; Rehfuss R; Wexler RR; Lam PYS Discovery of 4-aryl-7-hydroxyindoline-based P2Y1 antagonists as novel antiplatelet agents. J. Med. Chem. 2014, 57, 6150–6164. [PubMed: 24931384]

(229). Gao ZG; Jacobson KA Distinct signaling patterns of allosteric antagonism at the P2Y1 receptor. Mol. Pharmacol. 2017, 92, 613–626. [PubMed: 28864555]

(230). Springthorpe B; Bailey A; Barton P; Birkinshaw TN; Bonnert RV; Brown RC; Chapman D; Dixon J; Guile SD; Humphries RG; Hunt SF; Ince F; Ingall AH; Kirk IP; Leeson PD; Leff P; Lewis RJ; Martin BP; McGinnity DF; Mortimore MP; Paine SW; Pairaudeau G; Patel A; Rigby AJ; Riley RJ; Teobald BJ; Tomlinson W; Webborn PJH; Willis PA From ATP to AZD6140: the discovery of an orally active reversible P2Y12 receptor antagonist for the prevention of thrombosis. Bioorg. Med. Chem. Lett. 2007, 17, 6013–6018. [PubMed: 17827008]

(231). Lau OC; Samarawickrama C; Skalicky SE P2Y2 receptor agonists for the treatment of dry eye disease: a review. Clin. Ophthalmol. 2014, 8, 327–334. [PubMed: 24511227]

(232). Sakuma K; Nakagawa H; Oikawa T; Noda M; Ikeda S Effects of 4(lH)-quinolinone derivative, a novel non-nucleotide allosteric purinergic P2Y2 agonist, on cardiomyocytes in neonatal rats. Sci. Rep. 2017, 7, 6050. [PubMed: 28729619]

(233). Lau OCF; Samarawickrama C; Skalicky SE P2Y2 receptor agonists for the treatment of dry eye disease: a review. Clin. Ophthalmol. 2014, 8, 327–334. [PubMed: 24511227]

(234). Bachelerie F; Ben-Baruch A; Burkhardt AM; Combadiere C;Farber JM; Graham GJ; Horuk R; Sparre-Ulrich AH; Locati M; Luster AD; Mantovani A; Matsushima K; Murphy PM; Nibbs R; Nomiyama H; Power CA; Proudfoot AE; Rosenkilde MM; Rot A; Sozzani S; Thelen M; Yoshie O; Zlotnik A Update on the extended family of chemokine receptors and introducing a new nomenclature for atypical chemokine receptors. Pharmacol. Rev. 2014, 66, 1–79. [PubMed: 24218476]

(235). Allegretti M; Cesta MC; Locati M Allosteric modulation of chemoattractant receptors. Front. Immunol. 2016, 7, 170. [PubMed: 27199992]

(236). Thiele S; Malmgaard-Clausen M; Engel-Andreasen J; Steen A; Rummel PC; Nielsen MC; Gloriam DE; Frimurer TM; Ulven T; Rosenkilde MM Modulation in selectivity and allosteric properties of small-molecule ligands for CC-chemokine receptors. J. Med. Chem. 2012, 55, 8164–8177. [PubMed: 22957890]

(237). Berger EA; Murphy PM; Farber JM Chemokine receptors as HIV-1 coreceptors: roles in viral entry, tropism, and disease. Annu. Rev. Immunol. 1999, 17, 657–700. [PubMed: 10358771]

(238). Schwehm C; Kellam B; Garces AE; Hill SJ; Kindon ND;Bradshaw TD; Li J; Macdonald SJ; Rowedder JE; Stoddart LA; Stocks MJ Design and elaboration of a tractable tricyclic scaffold to synthesize druglike inhibitors of dipeptidyl peptidase-4 (DPP-4), antagonists of the C-C chemokine receptor type 5 (CCR5), and highly potent and selective phosphoinositol-3 kinase delta (PI3Kdelta) inhibitors. J. Med. Chem. 2017, 60, 1534–1554. [PubMed: 28128944]

(239). Maeda K; Das D; Nakata H; Mitsuya H CCR inhibitors: emergence, success, and challenges. Expert Opin. Emerging Drugs 2012, 17, 135–145.

(240). Scholten DJ; Canals M; Maussang D; Roumen L; Smit MJ; Wijtmans M; de Graaf C; Vischer HF; Leurs R Pharmacological modulation of chemokine receptor function. Br. J. Pharmacol. 2012, 165, 1617–1643. [PubMed: 21699506]

(241). Thum S; Kokornaczyk AK; Seki T; De Maria M; Zacarias N. V. Ortiz; de Vries H; Weiss C; Koch M; Schepmann D; Kitamura M; Tschammer N; Heitman LH; Junker A; Wunsch B Synthesis and biological evaluation of chemokine receptor ligands with 2-benzazepine scaffold. Eur. J. Med. Chem. 2017, 135, 401–413. [PubMed: 28463783]

(242). Karlshoj S; Amarandi RM; Larsen O; Daugvilaite V; Steen A; Brvar M; Pui A; Frimurer TM; Ulven T; Rosenkilde MM Molecular mechanism of action for allosteric modulators and agonists in CC-chemokine receptor 5 (CCR5). J. Biol. Chem. 2016, 291,26860–26874. [PubMed: 27834679]

(243). Bertini R; Allegretti M; Bizzarri C; Moriconi A; Locati M; Zampella G; Cervellera MN; Di Cioccio V; Cesta MC; Galliera E; Martinez FO; Di Bitondo R; Troiani G; Sabbatini V; D'Anniballe G; Anacardio R; Cutrin JC; Cavalieri B; Mainiero F; Strippoli R; Villa P; Di Girolamo M; Martin F; Gentile M; Santoni A; Corda D; Poli G; Mantovani A; Ghezzi P; Colotta F Noncompetitive allosteric inhibitors of the inflammatory chemokine receptors CXCR1 and CXCR2: prevention of reperfusion injury. Proc. Natl. Acad. Sci. U. S. A. 2004, 101, 11791–11796. [PubMed: 15282370]

(244). Miller EJ; Jecs E; Truax VM; Katzman BM; Tahirovic YA; Wilson RJ; Kuo KM; Kim MB; Nguyen HH; Saindane MT; Zhao H; Wang T; Sum CS; Cvijic ME; Schroeder GM; Wilson LJ; Liotta DC Discovery of tetrahydroisoquinoline-containing CXCR4 antagonists with improved in vitro ADMET properties. J. Med. Chem. 2018, 61, 946–979. [PubMed: 29350534]

(245). Wendt E; Keshav S CCR9 antagonism: potential in the treatment of Inflammatory Bowel Disease. Clin. Exp. Gastroenterol. 2015, 8, 119–130. [PubMed: 25897254]

(246). O'Connor T; Borsig L; Heikenwalder M CCL2-CCR2 signaling in disease pathogenesis. Endocr., Metab. Immune Disord.: Drug Targets 2015, 15, 105–118. [PubMed: 25772168]

(247). Procopiou PA; Barrett JW; Barton NP; Begg M; Clapham D; Copley RC; Ford AJ; Graves RH; Hall DA; Hancock AP; Hill AP; Hobbs H; Hodgson ST; Jumeaux C; Lacroix YM; Miah AH; Morriss KM; Needham D; Sheriff EB; Slack RJ; Smith CE; Sollis SL; Staton H Synthesis and

structure-activity relationships of indazole arylsulfonamides as allosteric CC-chemokine receptor 4 (CCR4) antagonists. J. Med. Chem. 2013, 56, 1946–1960. [PubMed: 23409871]

(248). Ajram L; Begg M; Slack R; Cryan J; Hall D; Hodgson S; Ford A; Barnes A; Swieboda D; Mousnier A; Solari R Internalization of the chemokine receptor CCR4 can be evoked by orthosteric and allosteric receptor antagonists. Eur. J. Pharmacol. 2014, 729, 75–85. [PubMed: 24534492]

(249). Miah AH; Copley RC; O'Flynn D; Percy JM; Procopiou PA Lead identification and structure-activity relationships of heteroarylpyrazole arylsulfonamides as allosteric CC-chemokine receptor 4 (CCR4) antagonists. Org. Biomol Chem. 2014, 12, 1779–1792. [PubMed: 24515101]

(250). Brox R; Milanos L; Saleh N; Baumeister P; Buschauer A; Hofmann D; Heinrich MR; Clark T; Tschammer N Molecular mechanisms of biased and probe-dependent signaling at CXC-motif chemokine receptor CXCR3 induced by negative allosteric modulators. Mol. Pharmacol. 2018, 93, 309–322. [PubMed: 29343553]

(251). Admas TH; Bernat V; Heinrich MR; Tschammer N Development of photoactivatable allosteric modulators for the chemokine receptor CXCR3. ChemMedChem 2016, 11, 575–584. [PubMed: 26880380]

(252). Bernat V; Brox R; Heinrich MR; Auberson YP; Tschammer N Ligand-biased and probe-dependent modulation of chemokine receptor CXCR3 signaling by negative allosteric modulators. ChemMedChem 2015, 10, 566–574. [PubMed: 25655398]

(253). Neelakantan H; Holliday ED; Fox RG; Stutz SJ; Comer SD; Haney M; Anastasio NC; Moeller FG; Cunningham KA Lorcaserin suppresses oxycodone self-administration and relapse vulnerability in rats. ACS Chem. Neurosci. 2017, 8, 1065–1073. [PubMed: 28107783]

(254). Morgan MM; Christie MJ Analysis of opioid efficacy, tolerance, addiction and dependence from cell culture to human. Br. J. Pharmacol. 2011, 164, 1322–1334. [PubMed: 21434879]

(255). Remesic M; Hruby VJ; Porreca F; Lee YS Recent advances in the realm of allosteric modulators for opioid receptors for future therapeutics. ACS Chem. Neurosci. 2017, 8, 1147–1158. [PubMed: 28368571]

(256). Kathmann M; Flau K; Redmer A; Trankle C; Schlicker E Cannabidiol is an allosteric modulator at μ- and δ-opioid receptors. Naunyn-Schmiedeberg's Arch. Pharmacol. 2006, 372, 354–361. [PubMed: 16489449]

(257). Rothman RB; Murphy DL; Xu H; Godin JA; Dersch CM; Partilla JS; Tidgewell K; Schmidt M; Prisinzano TE Salvinorin A: allosteric interactions at the μ-opioid receptor. J. Pharmacol. Exp. Ther. 2007, 320, 801–810. [PubMed: 17060492]

(258). Burford NT; Clark MJ; Wehrman TS; Gerritz SW; Banks M; O'Connell J; Traynor JR; Alt A Discovery of positive allosteric modulators and silent allosteric modulators of the μ-opioid receptor. Proc. Natl. Acad. Sci. U. S. A. 2013, 110, 10830–10835. [PubMed: 23754417]

(259). Burford NT; Traynor JR; Alt A Positive allosteric modulators of the μ-opioid receptor: a novel approach for future pain medications. Br. J. Pharmacol. 2015, 172, 277–286. [PubMed: 24460691]

(260). Ohbuchi K; Miyagi C; Suzuki Y; Mizuhara Y; Mizuno K; Omiya Y; Yamamoto M; Warabi E; Sudo Y; Yokoyama A; Miyano K; Hirokawa T; Uezono Y Ignavine: a novel allosteric modulator of the μ opioid receptor. Sci. Rep. 2016, 6, 31748. [PubMed: 27530869]

(261). Livingston KE; Stanczyk MA; Burford NT; Alt A; Canals M; Traynor J R Pharmacologic evidence for a putative conserved allosteric site on opioid receptors. Mol. Pharmacol. 2018, 93, 157–167. [PubMed: 29233847]

(262). Nagasaki H; Chung S; Dooley CT; Wang Z; Li C; Saito Y; Clark SD; Houghten RA; Civelli O The pharmacological properties of a novel MCH1 receptor antagonist isolated from combinatorial libraries. Eur. J. Pharmacol. 2009, 602, 194–202. [PubMed: 19041642]

(263). Sakurai T; Ogawa K; Ishihara Y; Kasai S; Nakayama M The MCH1 receptor, an anti-obesity target, is allosterically inhibited by 8-methylquinoline derivatives possessing subnanomolar binding and long residence times. Br. J. Pharmacol. 2014, 171, 1287–1298. [PubMed: 24670150]

(264). Pedragosa Badia X; Stichel J; Beck-Sickinger A Neuropeptide Y receptors: how to get subtype selectivity. Front. Endocrinol. 2013, 4, 5.

(265). Sliwoski G; Schubert M; Stichel J; Weaver D; Beck-Sickinger AG; Meiler J Discovery of small-molecule modulators of the human Y4 receptor. PLoS One 2016, 11, e0157146.

(266). Schubert M; Stichel J; Du Y; Tough IR; Sliwoski G; Meiler J; Cox HM; Weaver CD; Beck-Sickinger AG Identification and characterization of the first selective Y4 receptor positive allosteric modulator. J. Med. Chem. 2017, 60, 7605–7612. [PubMed: 28795803]

(267). Rothmeier AS; Ruf W Protease-activated receptor 2 signaling in inflammation. Semin. Immunopathol 2012, 34, 133–149. [PubMed: 21971685]

(268). Cottrell GS; Amadesi S; Schmidlin F; Bunnett N Protease-activated receptor 2: activation, signalling and function. Biochem. Soc. Trans. 2003, 31, 1191–1197. [PubMed: 14641024]

(269). Lecci A; Capriati A; Maggi CA Tachykinin NK2 receptor antagonists for the treatment of irritable bowel syndrome. Br. J. Pharmacol. 2004, 141, 1249–1263. [PubMed: 15037522]

(270). Maillet EL; Pellegrini N; Valant C; Bucher B; Hibert M; Bourguignon JJ; Galzi JL A novel, conformation-specific allosteric inhibitor of the tachykinin NK2 receptor (NK2R) with functionally selective properties. FASEB J. 2007, 21, 2124–2134. [PubMed: 17371796]

(271). Valant C; Maillet E; Bourguignon JJ; Bucher B; Utard V; Galzi JL; Hibert M Allosteric functional switch of neurokinin A-mediated signaling at the neurokinin NK2 receptor: structural exploration. J. Med. Chem. 2009, 52, 5999–6011. [PubMed: 19746979]

(272). Wood MR; Hopkins CR; Brogan JT; Conn PJ; Lindsley CW "Molecular switches" on mGluR allosteric ligands that modulate modes of pharmacology. Biochemistry 2011, 50, 2403–2410. [PubMed: 21341760]

(273). Rook JM; Noetzel MJ; Pouliot WA; Bridges TM; Vinson PN; Cho HP; Zhou Y; Gogliotti RD; Manka JT; Gregory KJ; Stauffer SR; Dudek FE; Xiang Z; Niswender CM; Daniels JS; Jones CK; Lindsley CW; Conn PJ Unique signaling profiles of positive allosteric modulators of metabotropic glutamate receptor subtype 5 determine differences in in vivo activity. Biol. Psychiatry 2013, 73, 501–509. [PubMed: 23140665]

(274). Congreve M; Oswald C; Marshall FH Applying structure-based drug design approaches to allosteric modulators of GPCRs. Trends Pharmacol. Sci. 2017, 38, 837–847. [PubMed: 28648526]

(275). Dror RO; Green HF; Valant C; Borhani DW; Valcourt JR; Pan AC; Arlow DH; Canals M; Lane JR; Rahmani R; Baell JB; Sexton PM; Christopoulos A; Shaw DE Structural basis for modulation of a G-protein-coupled receptor by allosteric drugs. Nature 2013, 503, 295–299. [PubMed: 24121438]

(276). Basith S; Cui M; Macalino SJY; Park J; Clavio NAB; Kang S; Choi S Exploring G protein-coupled receptors (GPCRs) ligand space via cheminformatics approaches: impact on rational drug design. Front. Pharmacol. 2018, 9, 128. [PubMed: 29593527]

(277). Safdari HA; Pandey S; Shukla AK; Dutta S Illuminating GPCR signaling by cryo-EM. Trends Cell Biol. 2018, 28, 591–594. [PubMed: 29945844]

(278). Miao Y; Goldfeld DA; Moo EV; Sexton PM; Christopoulos A; McCammon JA; Valant C Accelerated structure-based design of chemically diverse allosteric modulators of a muscarinic G protein-coupled receptor. Proc. Natl. Acad. Sci. U. S. A. 2016, 113, E5675–E5684. [PubMed: 27601651]

(279). Chen H; Zhou X; Wang A; Zheng Y; Gao Y; Zhou J Evolutions in fragment-based drug design: the deconstruction—reconstruction approach. Drug Discovery Today 2015, 20, 105–113. [PubMed: 25263697]

(280). Chen H; Wang CZ; Ding C; Wild C; Copits B; Swanson GT; Johnson KM; Zhou J A combined bioinformatics and chemoinformatics approach for developing asymmetric bivalent AMPA receptor positive allosteric modulators as neuroprotective agents. ChemMedChem 2013, 8, 226–230. [PubMed: 23281122]

(281). Bian Y; Feng Z; Yang P; Xie X-Q Integrated in silico fragment-based drug design: case study with allosteric modulators on metabotropic glutamate receptor 5. AAPS J. 2017, 19, 1235–1248. [PubMed: 28560482]

(282). Szabó G; Túrós GI; Kolok S; Vastag M; Sánta Z; Dékány M; Lévay GI; Greiner I; Natsumi M; Tatsuya W; Keserű GM Fragment based optimization of metabotropic glutamate receptor 2

(mGluR2) positive allosteric modulators in the absence of structural information. J. Med. Chem. 2018, DOI: 10.1021/acs.jmed-chem.8b00161.

(283). Lambert NA; Javitch JA CrossTalk opposing view: Weighing the evidence for class A GPCR dimers, the jury is still out. J. Physiol. 2014, 592, 2443–2445. [PubMed: 24931945]

(284). Bouvier M; Hébert TE CrossTalk proposal: weighing the evidence for class A GPCR dimers, the evidence favours dimers. J. Physiol. 2014, 592, 2439–2441. [PubMed: 24931944]

(285). Meral D; Provasi D; Prada-Gracia D; Möller J; Marino K; Lohse MJ; Filizola M Molecular details of dimerization kinetics reveal negligible populations of transient μ-opioid receptor homodimers at physiological concentrations. Sci. Rep. 2018, 8, 7705. [PubMed: 29769636]

(286). Pin J-P; Bettler B Organization and functions of mGlu and GABAB receptor complexes. Nature 2016, 540, 60–68. [PubMed: 27905440]

(287). Gurevich VV; Gurevich EV GPCRs and signal transducers: interaction stoichiometry. Trends Pharmacol. Sci. 2018, 39, 672–684. [PubMed: 29739625]

(288). Stoeber M; Jullie D; Lobingier BT; Laeremans T; Steyaert J; Schiller PW; Manglik A; von Zastrow M A genetically encoded biosensor reveals location bias of opioid drug action. Neuron 2018, 98, 963–976. [PubMed: 29754753]

(289). Grundmann M; Kostenis E Temporal bias: time-encoded dynamic GPCR signaling. Trends Pharmacol. Sci. 2017, 38, 1110–1124. [PubMed: 29074251]

(290). Lane JR; May LT; Parton RG; Sexton PM; Christopoulos A Akinetic view of GPCRallostery and biased agonism. Nat. Chem. Biol. 2017, 13, 929–937. [PubMed: 28820879]

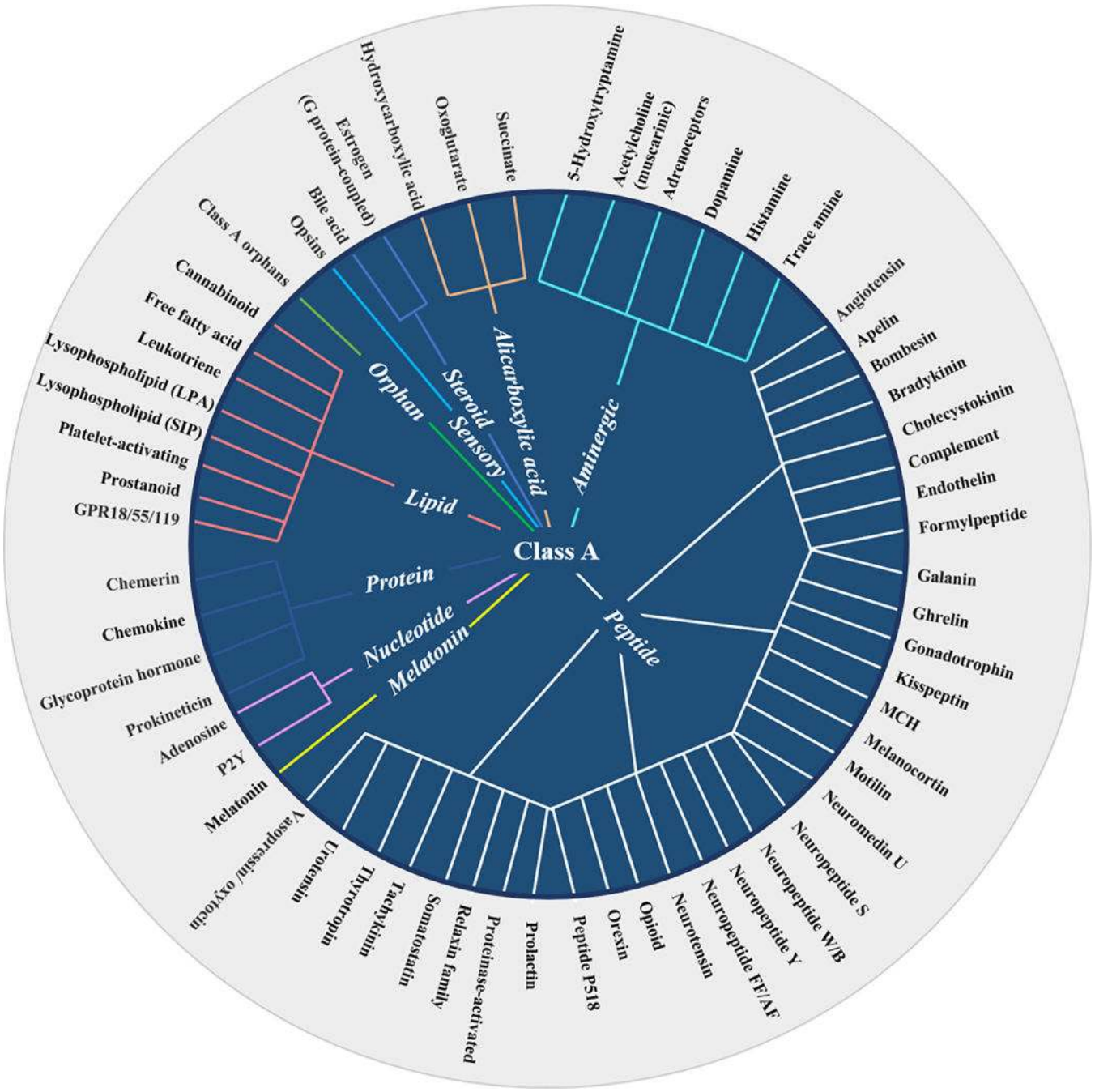


Figure 1.

Signaling molecule diversity and classification for class A GPCRs. The inner blue region highlights the diverse endogenous signaling molecules that activate class A GPCRs. Data were retrieved from Web site http://www.gpcrdb.org/drugs/drugbrowser.

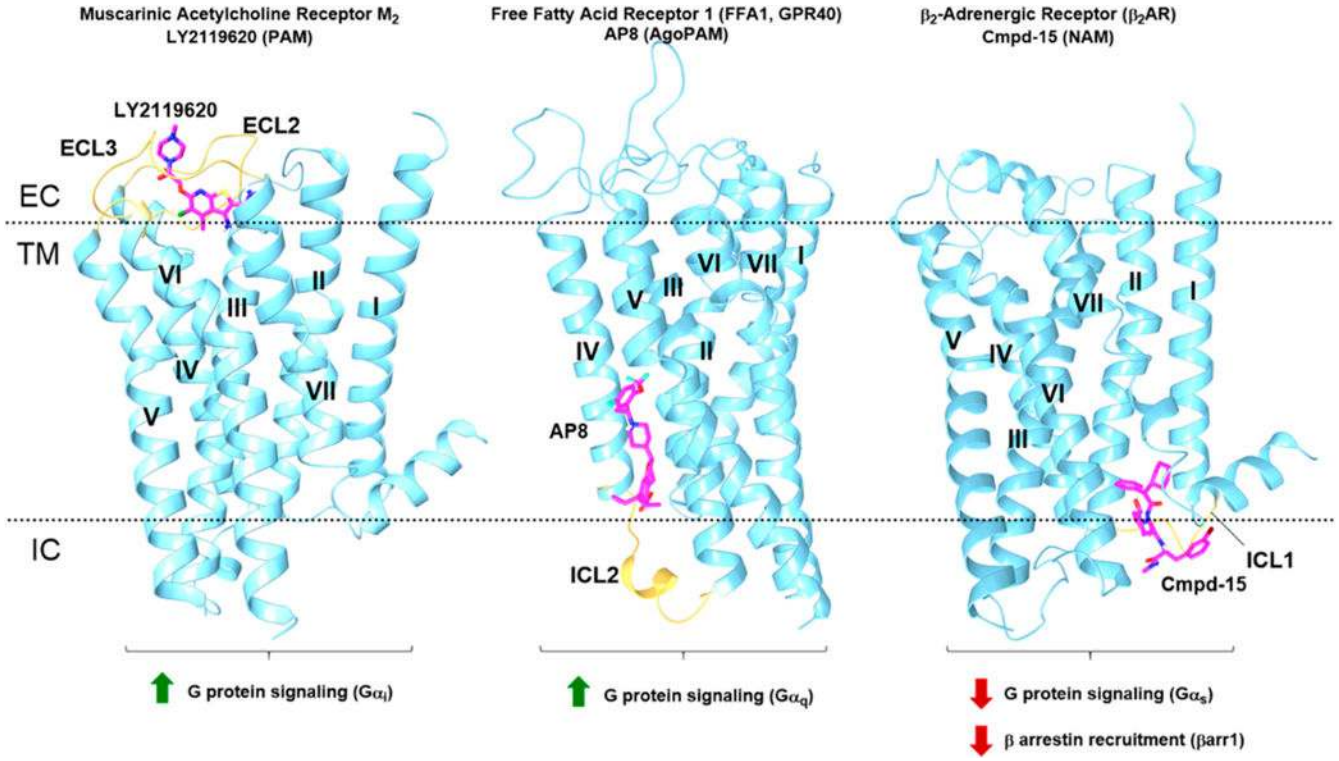


Figure 2.

Diverse allosteric binding sites reported across class A GPCRs. The representative class A allosteric modulators (stick representation,magenta) are shown bound in the corresponding cocrystal structure at distinct and diverse sites. The M2 mAChR-shows an extracellular (EC) site LY2119620 (left, PDB code 4MQT);19 FFA1-AP8 cocrystal shows AP8 in the transmembrane (TM) region adjacent to the lipid membrane (center, PDB: 5TZY);20 β_2ARCmpd-15 cocrystal shows an intracellular (IC) binding site (right, PDB code 5X7D)21 Bottom: the diverse signaling outcomes on orthosteric agonism by class A GPCR allosteric modulators.

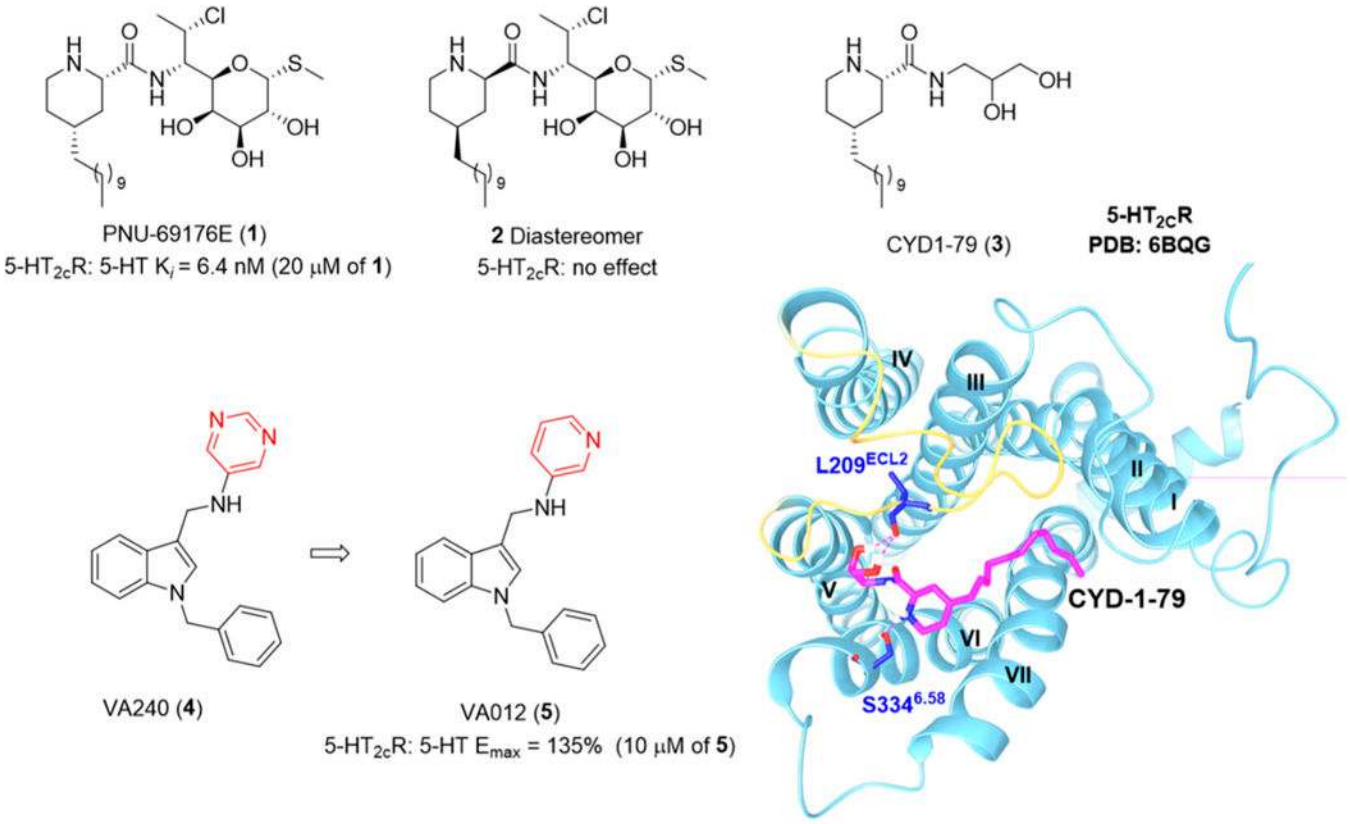


Figure 3.

Representative 5-HT_{2C}R PAMs **1–5**. Right: CYD-1–79 (stick representation, magenta, **3**) predicted binding pose from molecular docking on the recently solved 5-HT2CR crystal structure (PDB code 6BQG) interacting with L209ECL2 and $S334^{6.58}$.[51,54]

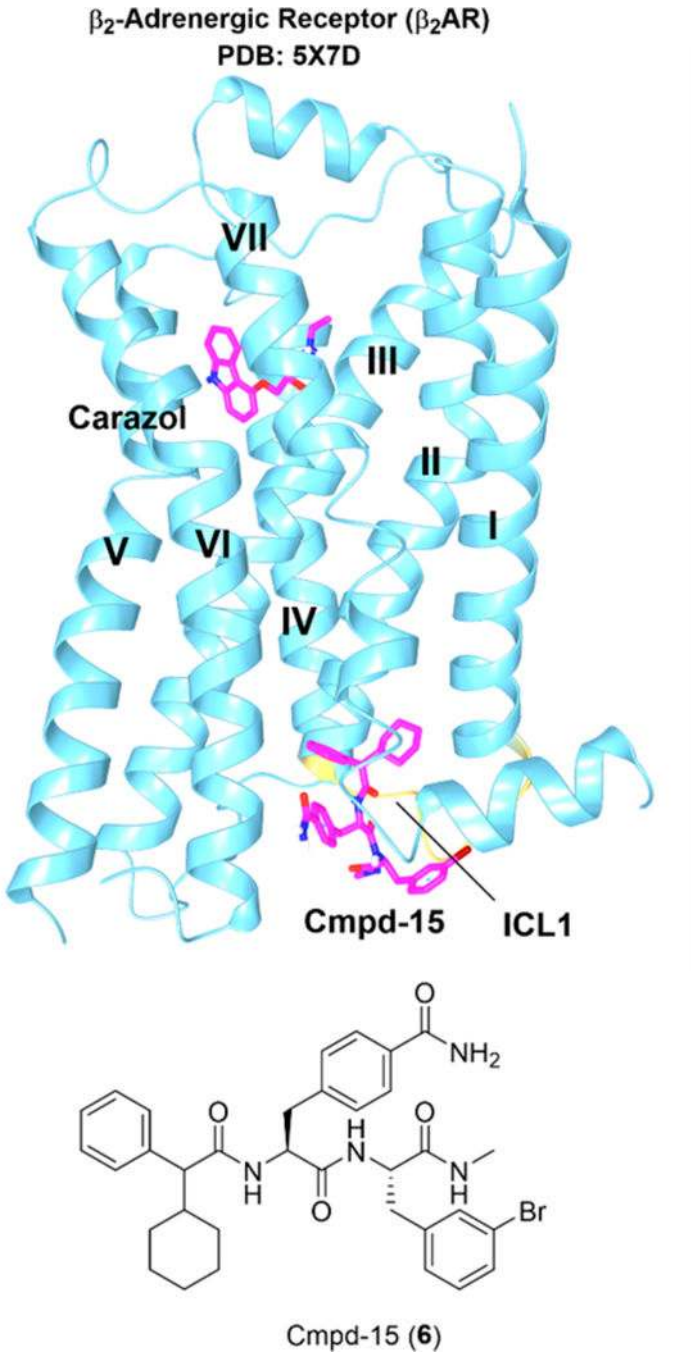


Figure 4.

Top: β_2AR cocrystal structure with Cmpd-15 (**6**) and antagonist carazol (PDB code 6X7D)[21] Bottom: structure of β_2AR NAM Cmpd-15 (**6**).

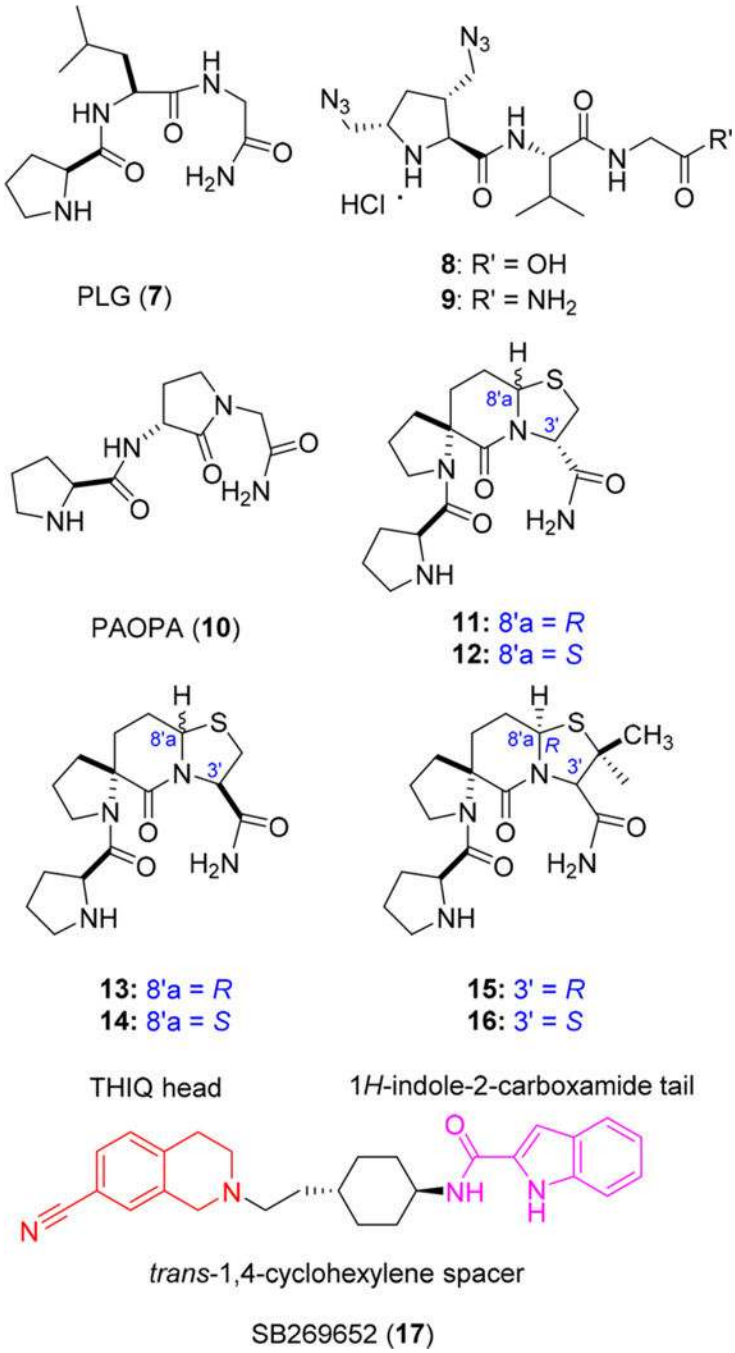


Figure 5.

Representative allosteric modulators of the dopamine D_2 and D_3 receptors with chemical modifications at selected positions.

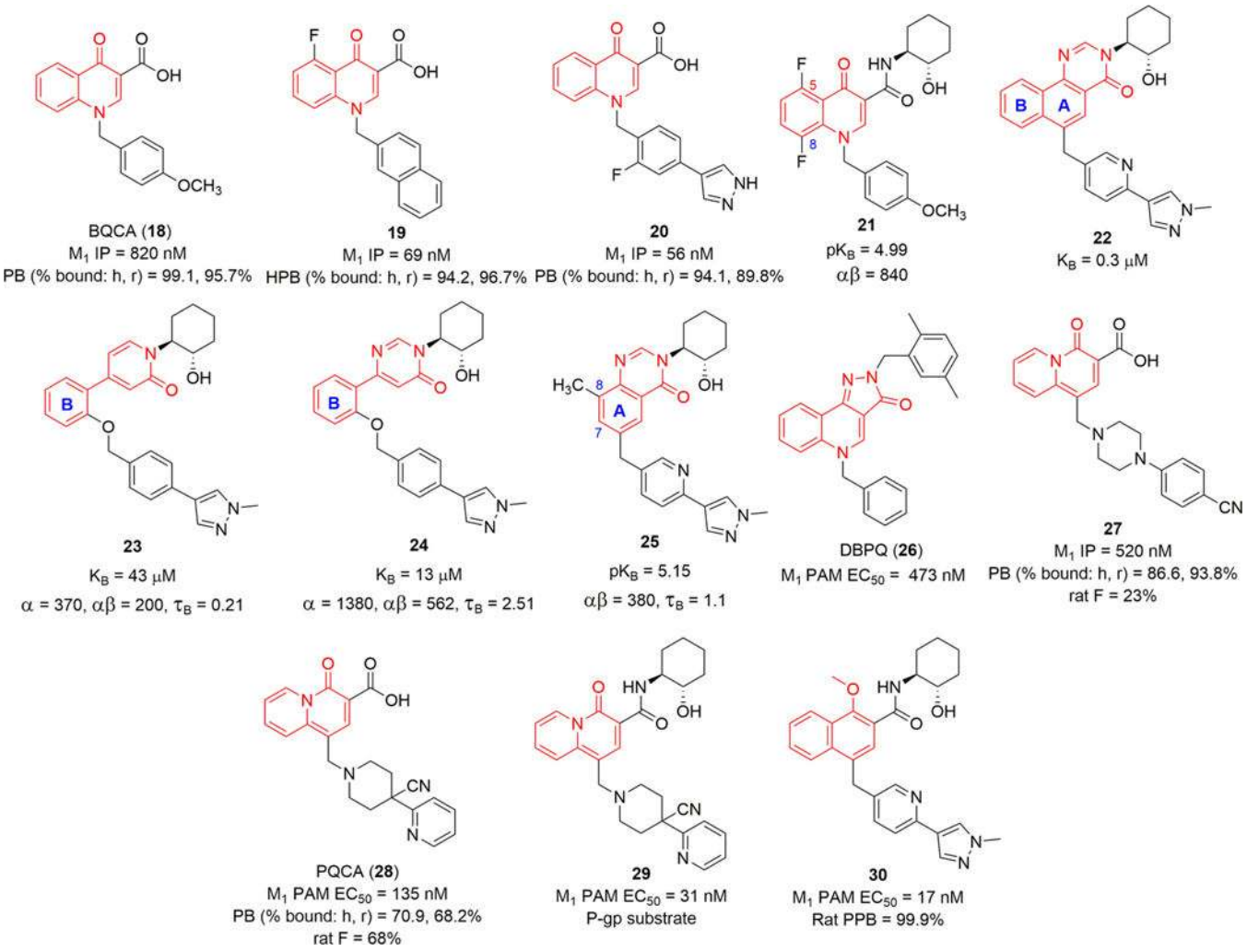


Figure 6.

M_1 mAChR PAM BQCA (**18**) and derivatives (**19–30**) with corresponding SARs.

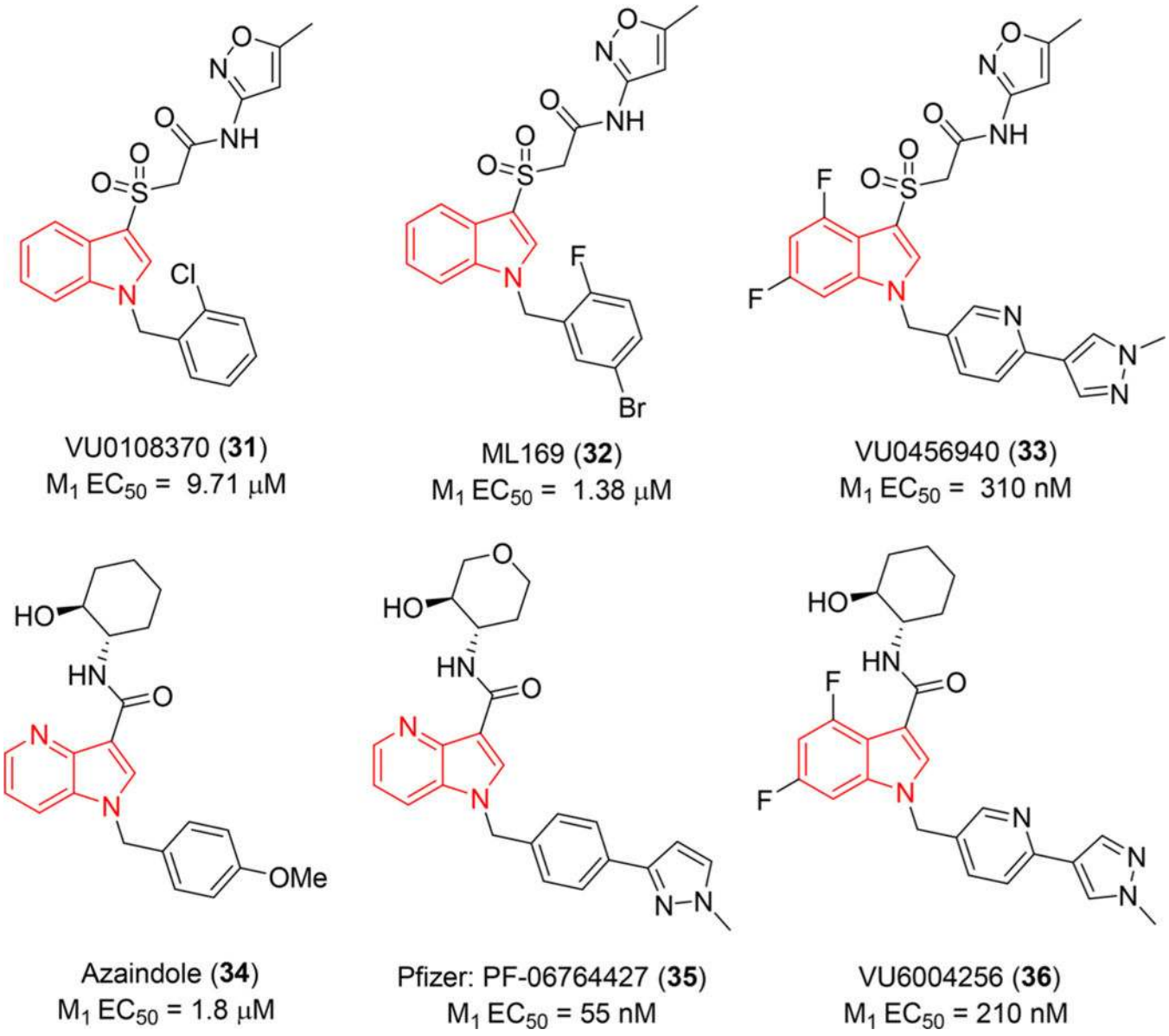


Figure 7.
Representative M_1 mAChR PAMs with an indole core (red) and SAR (compounds **31–36**).

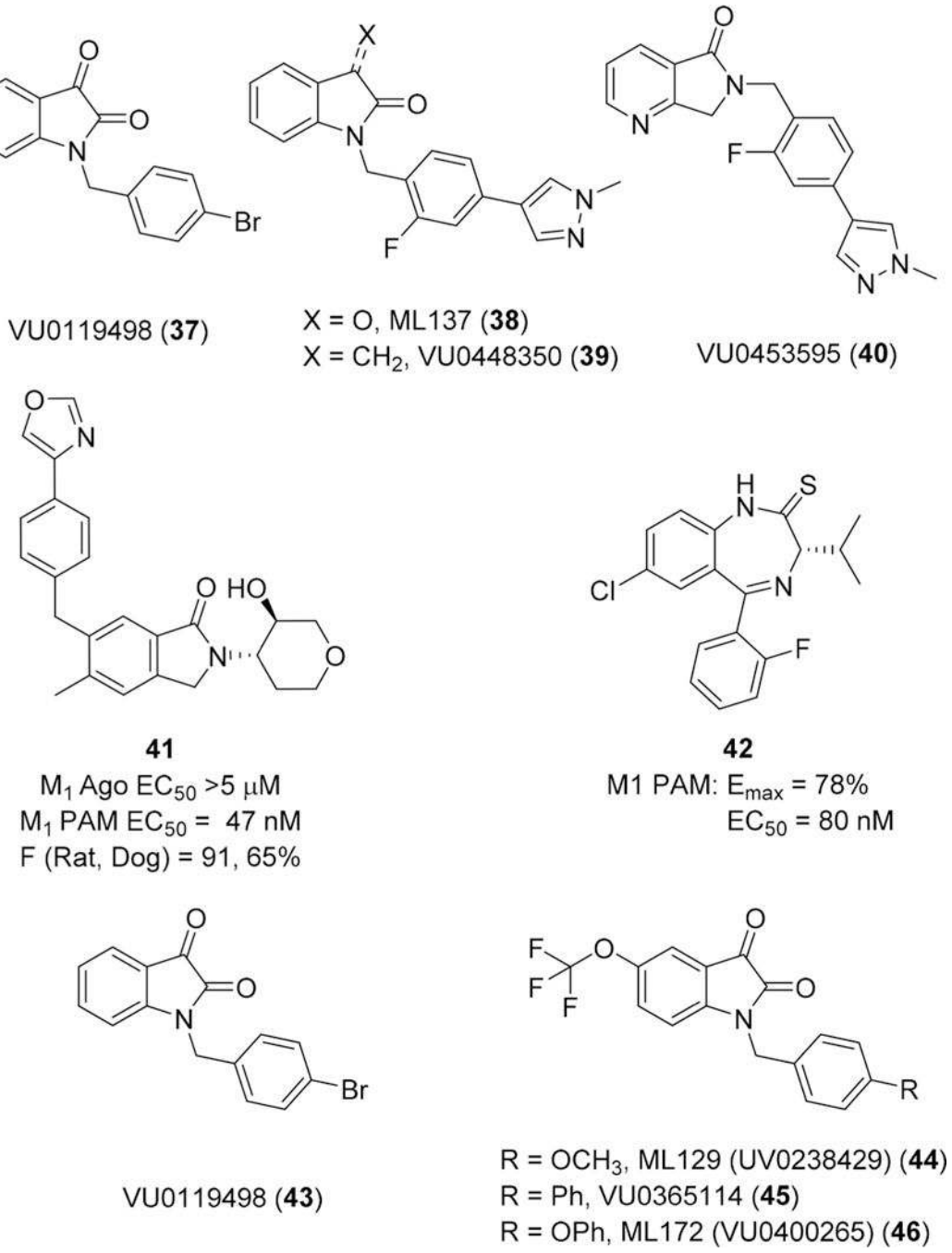


Figure 8.
Chemically diverse M_1 mAChR PAMs with key modifications (compounds **37–46**).

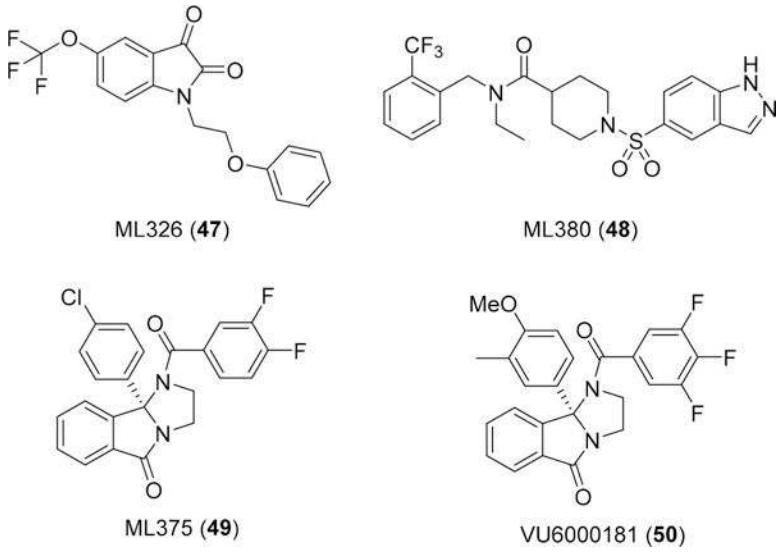


Figure 9.
Representative M_5 mAChR PAMs (**47, 48**) and NAMs (**49, 50**).

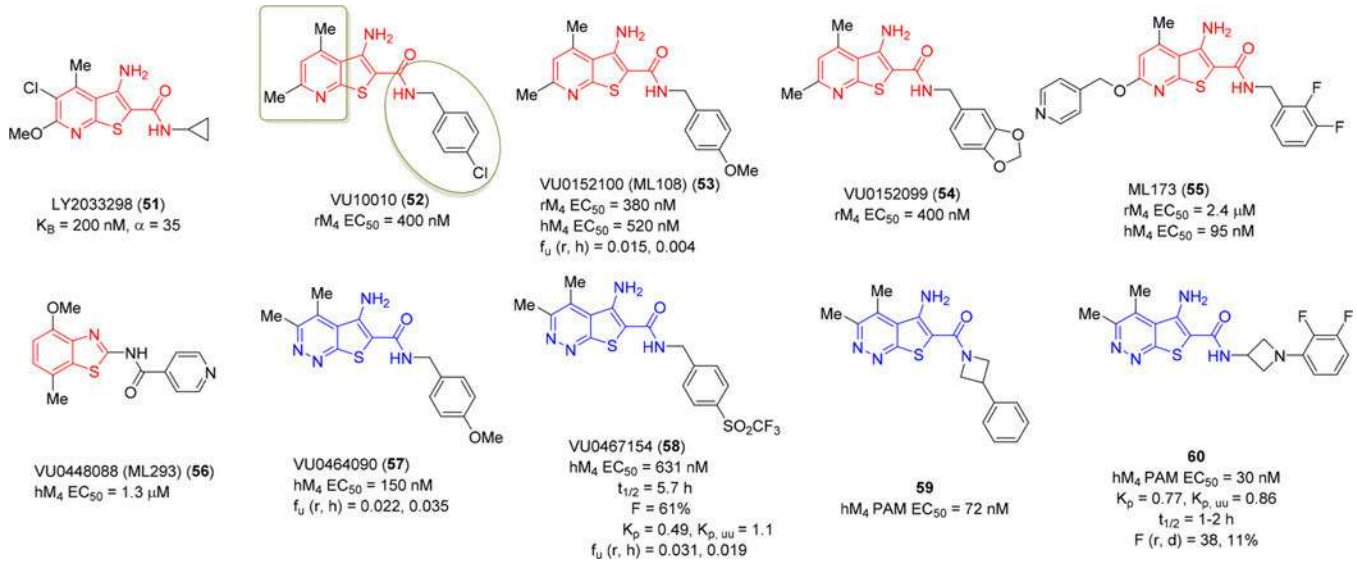


Figure 10.

M_4 mAChR PAM derivatives around the 5-aminothieno[2,3-*c*]pyridine scaffold and corresponding SARs (**51–60**).

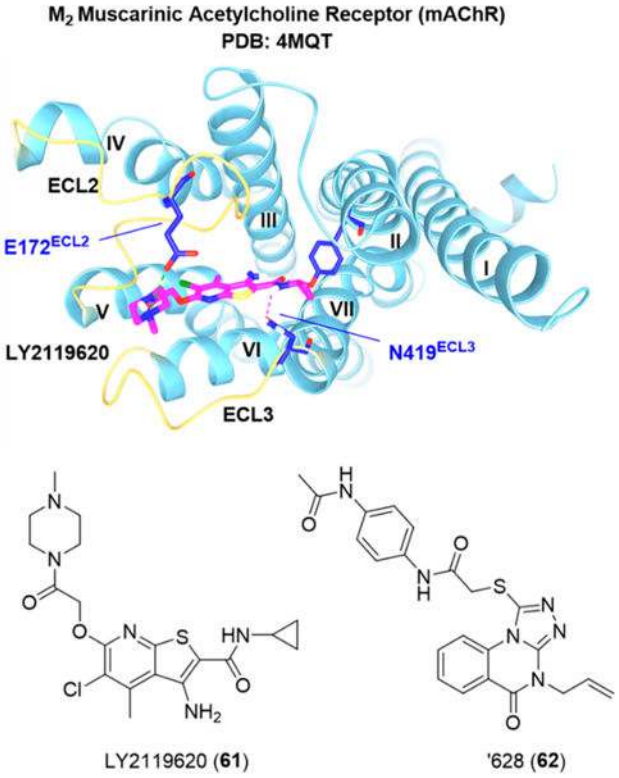


Figure 11.

Top: M_2 mAChR-LY2119620 (stick representation, magenta, **61**) cocrystal extracellular view highlighting interactions with ECL residues $E172^{ECL2}$ and $N419^{ECL3}$ (PDB code 4MQT).[19] Bottom: representative M_2 mAChR PAMs (**61, 62**).

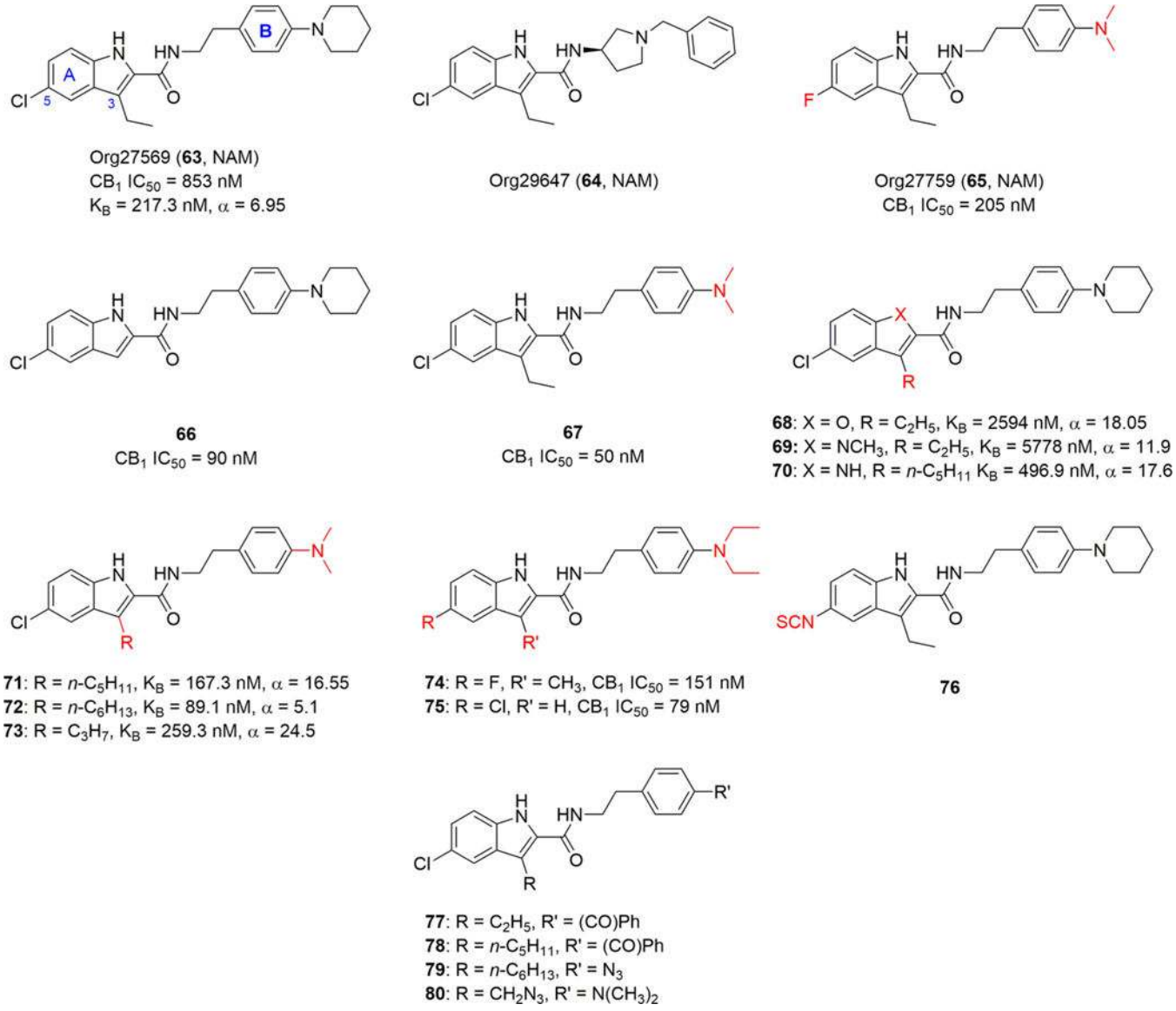


Figure 12.

CB_1 receptor NAMs (**63–80**) based on the 1*H*-indole-2-carboxamide scaffold and corresponding SAR.

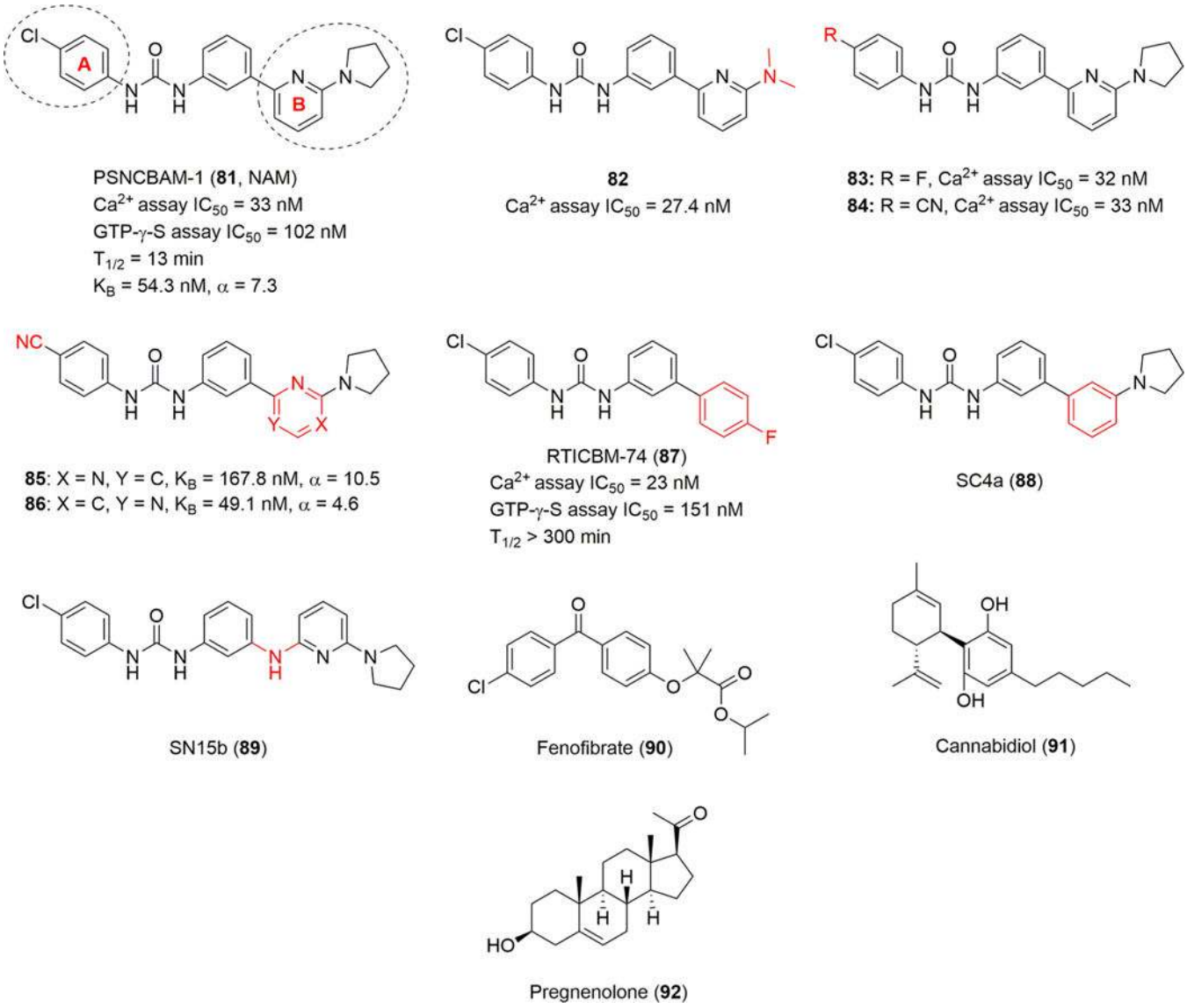


Figure 13.

CB_1 receptor NAMs based on the diarylurea scaffold (**81–89**) and corresponding SAR; additional CB_1 receptor NAMs (**90–92**).

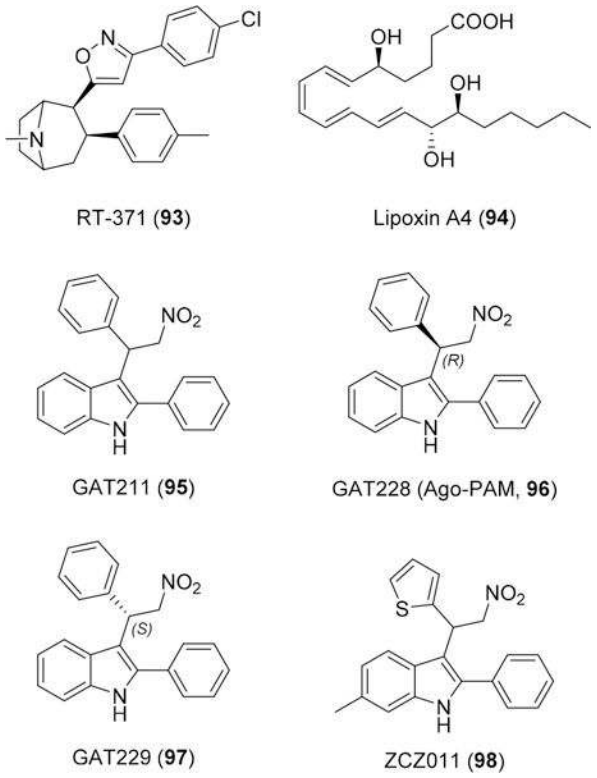


Figure 14.

Representative CB_1 receptor PAMs (**93, 94**) and 2-phenyl-1*H*-indole scaffold derivative CB_1 receptor PAMs (**95–98**).

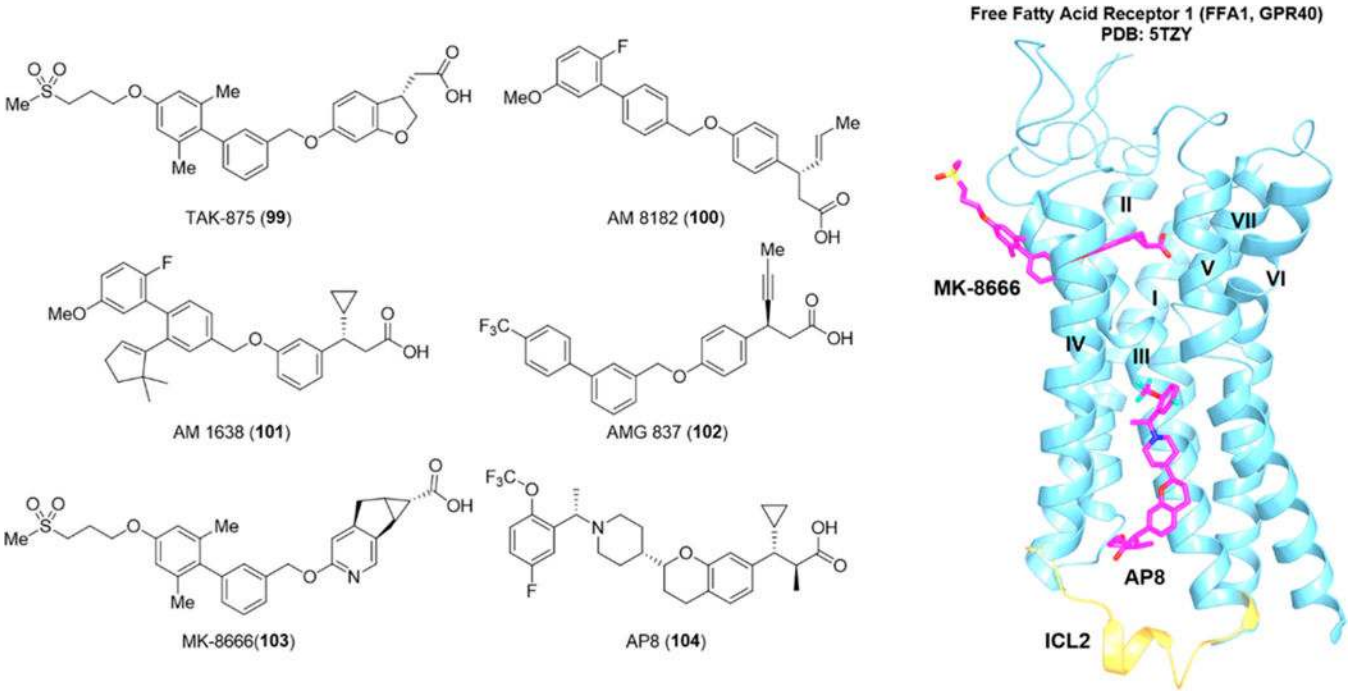


Figure 15.

Left: representative FFA1 receptor PAMs (**99–104**). Right: cocrystal complex of FFA1-MK-8666-AP8 showing distinct allosteric sites for both PAMs (PDB code 5TZY).[20]

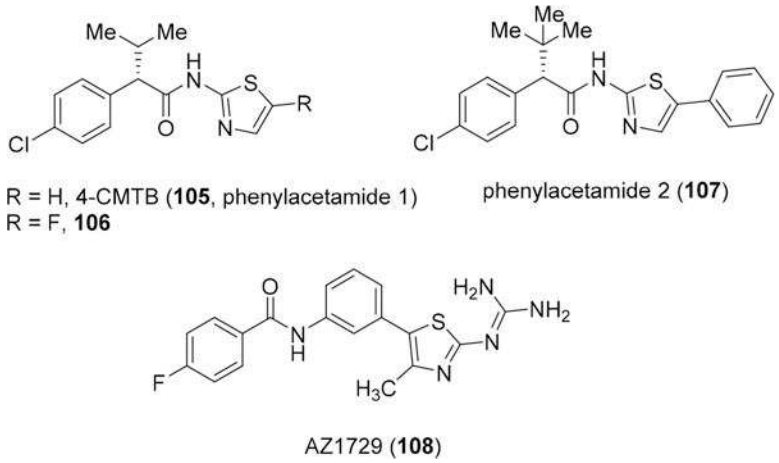
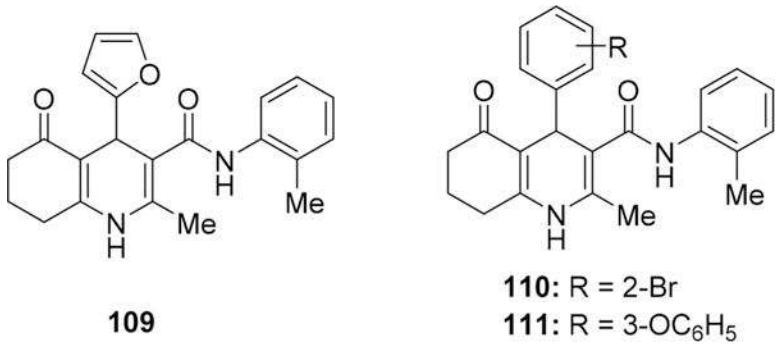


Figure 16.
Representative FFA2 receptor PAMs (**105–108**).



109

110: R = 2-Br
111: R = 3-OC_6H_5

Figure 17.
Representative FFA3 receptor PAMs (**109–111**).

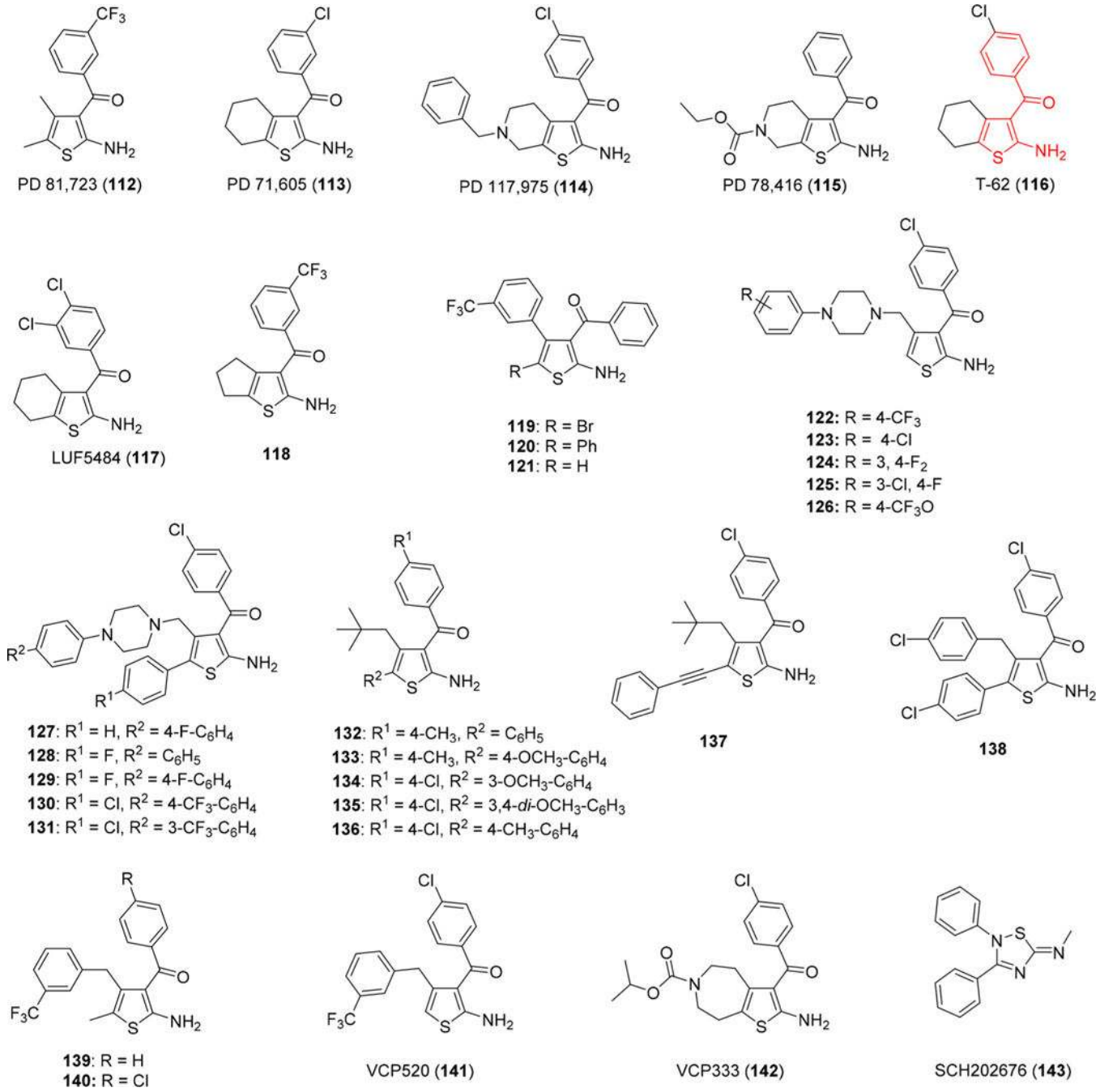


Figure 18.

A_1RPAM derivatives designed around the 2-amino-3-benzoylthiophene scaffold (**112–143**).

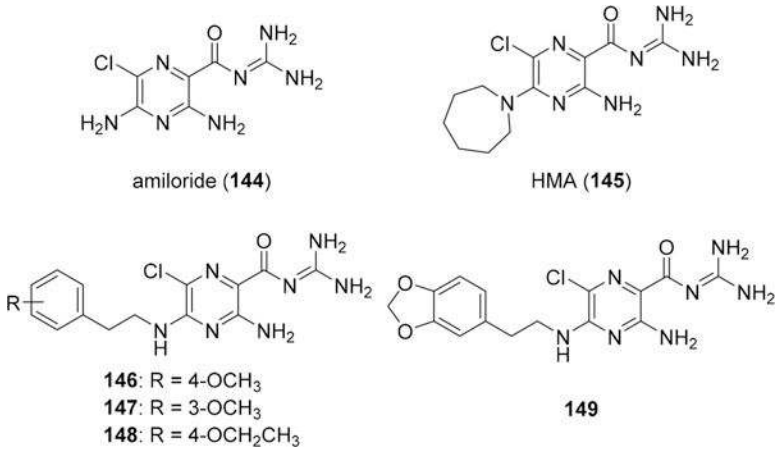


Figure 19.

Representative $A_{2A}R$ PAMs with centric pyrazine core (**144–149**).

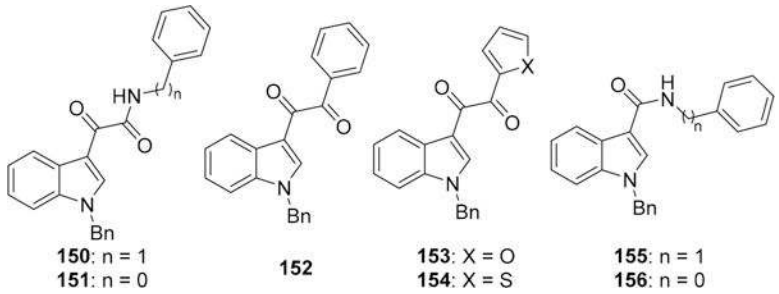


Figure 20.

$A_{2B}R$ PAM derivatives on the 1-benzyl-3-ketoindole scaffold side chain (**150–156**).

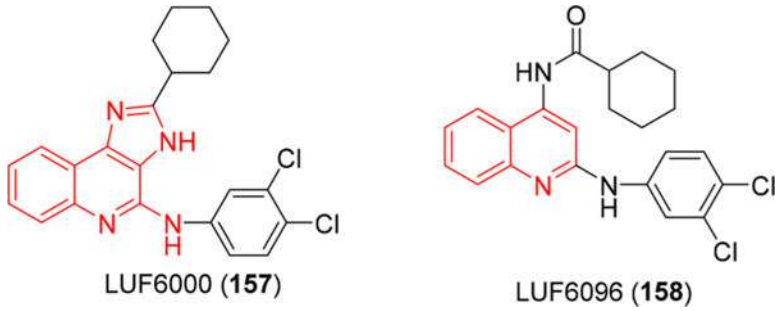


Figure 21.

Representative A_3R PAMs displaying a ring opening on the 1*H*-imidazo[4,5-*c*]quinolin-4-amine scaffold (**157, 158**).

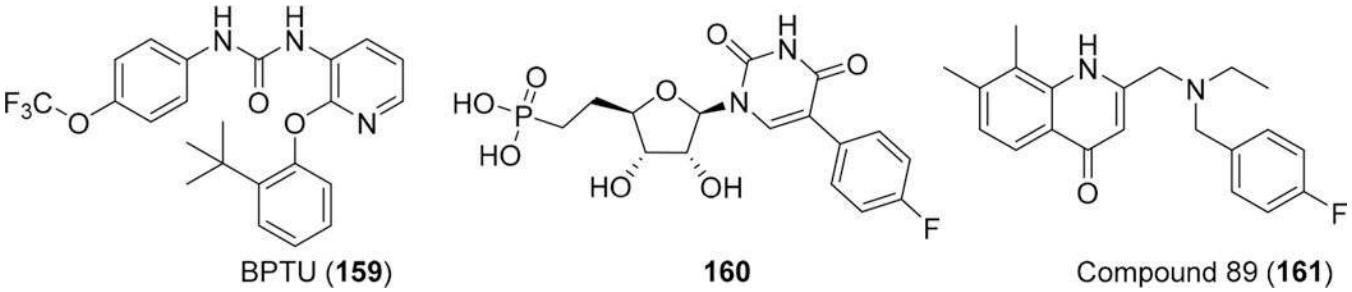


Figure 22.

$P2Y_1$ receptor allosteric antagonist BPTU (**159**) and $P2Y_2$ receptor allosteric agonists (**160, 161**).

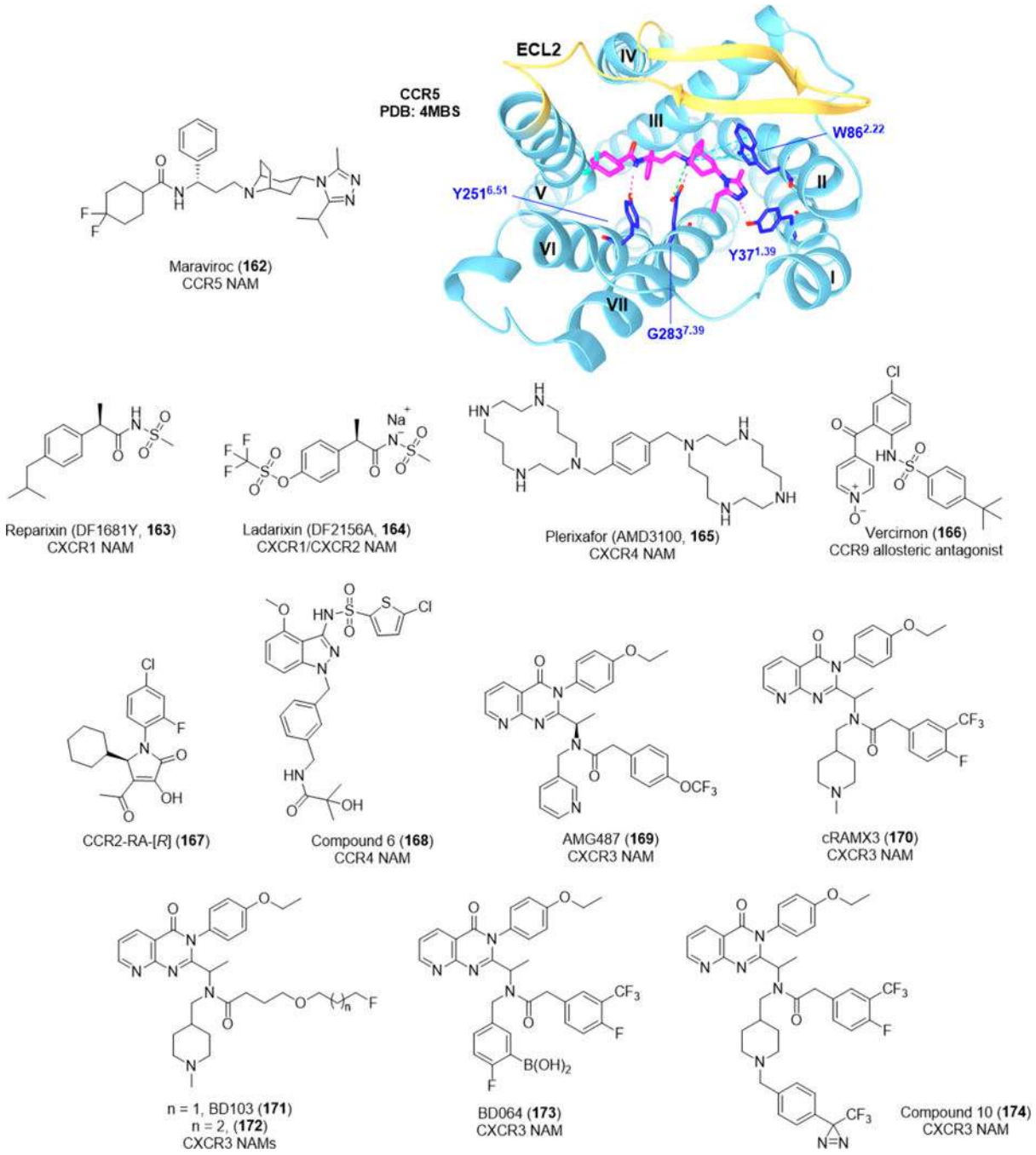


Figure 23.

Top: chemical structure of maraviroc (**162**), an FDA-approved CCR5 NAM with the CCR5-maraviroc cocrystal displaying numerous interactions with residues in the TM bundle (PDB code 4MBS).[27] Bottom: representative allosteric antagonists and NAMs discovered for chemokine receptors (**163–174**).

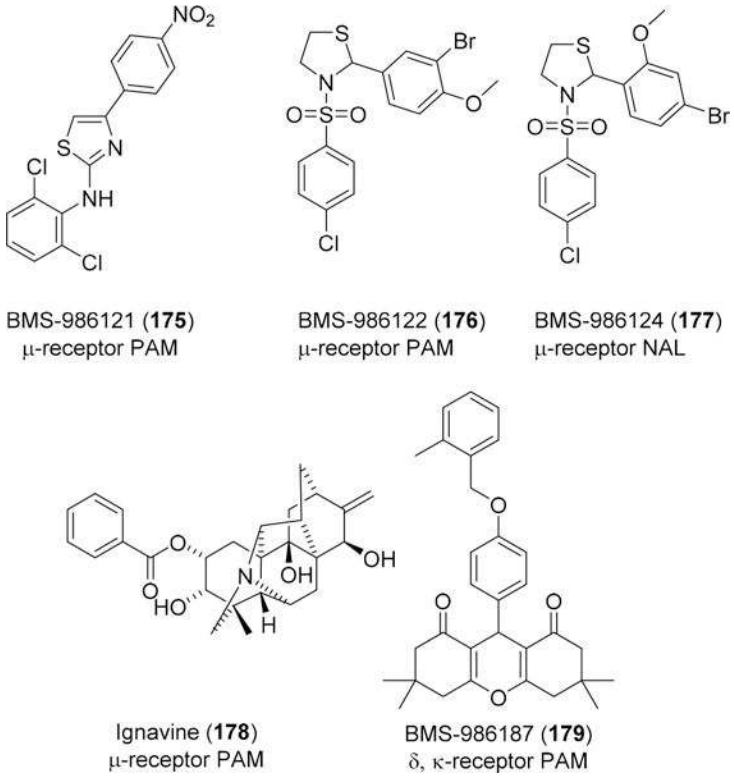


Figure 24.
Chemically diverse allosteric modulators for ORs (**175–179**).

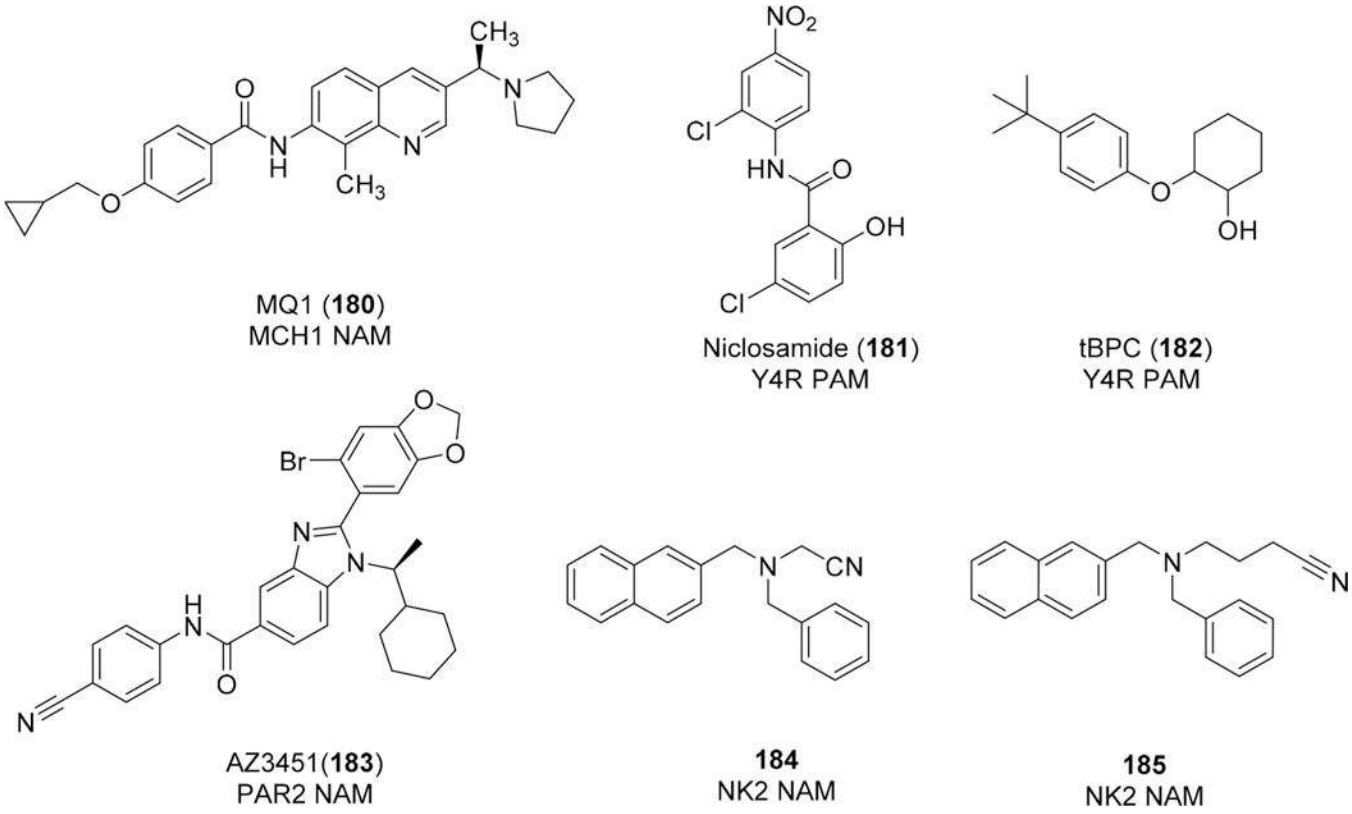


Figure 25.

NAMs and PAMs for additional peptide and protein family receptors, as described (**180–185**).

Table 1.

Selected Allosteric Modulators of Class A GPCRs Currently in Clinical Trials or Approved for Clinical Use[a]

family	target	name	indication	mechanism of action	phase
acetylcholine receptors	M1	VU319	cognitive impairment	PAM	phase I
free fatty acid receptors	FFAR1	MK-8666	type 2 diabetes mellitus	partial allosteric agonists	phase I
chemokine receptors	CXCR1, CXCR2	ladarixin (DF2156A)	onset type 1 diabetes	NAM	phase II
free fatty acid receptors	FFAR1	TAK-875	type 2 diabetes mellitus	partial allosteric agonists	phase III
chemokine receptors	CCR9	vercirnon	Crohn's disease	NAM	phase III
chemokine receptors	CXCR1	reparixin (DF1681Y)	β-cell transplantation	NAM	phase III
P2Y receptors	$P2Y_{12}$	ticagrelor	anti-thrombosis	allosteric antagonist	approved
chemokine receptors	CCR5	maraviroc	HIV infection	NAM	approved
chemokine receptors	CXCR4	plerixafor	bone marrow transplantation	NAM	approved

[a]Data retrieved from (a) Cortellis database, https://www.cortellis.com/intelligence/login.do, (b) ref 3, (c) http://www.gpcrdb.org/drugs/ drugbrowser, and d) https://www.drugbank.ca.

Table 2.

Allosteric Modulator and Class A GPCR Cocrystal Structures[a]

GPCR	allosteric modulator	mechanism of action	allosteric site distribution	PDB code	ref
CCR5	maraviroc	NAM	extracellular side	4MBS	27
CCR2	CCR2-RA-[*R*]	NAM	intracellular side	5T1A	28
CCR9	vercirnon	allosteric antagonist	intracellular side	5LWE	29
M_2	LY2119620	PAM	extracellular side	4MQT	19
FFA1 (GPR40)	TAK-875	partial allosteric agonist	extracellular side	4PHU	30
FFA1 (GPR40)	MK-8666	partial allosteric agonist	extracellular side	5TZR	20
FFA1 (GPR40)	MK-8666, AP8	Ago-PAM	intracellular side	5TZY	20
$P2Y_1$	BPTU	allosteric antagonist	intracellular side	4XNY	31
β_2AR	Cmpd-15PA	NAM	intracellular side	5X7D	21
PAR2	AZ3451	allosteric antagonist	extracellular side	5NDZ	32
C5aR1 (CD88)	NDT9513727	allosteric antagonist	transmembrane	6C1Q	33
C5aR1 (CD88)	NDT9513727	allosteric antagonist	transmembrane	6C1R	34
$P2Y_{12}$	AZD1283	allosteric antagonist	extracellular side	4NTJ	35
$A_{2A}R$	Na^+	NAM	sodium ion site	5NLX	36
DOR	Na^+	NAM	sodium ion site	4N6H	37

[a]Data as of July 31, 2D18.