Chapter 11

Allosteric modulators targeting GPCRs

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List of abbreviations:

7TM	**Transmembrane Helix Bundle**
AM	**Allosteric Modulator**
CNS	**Central Nervous System**
CRF	**Corticotropin-Releasing Factor**
CT	**Calcitonin**
ECL	**Extracellular loop**
FDA	**Food and Drug Administration**
GCG	**Glucagon**
HIV	**Human Immunodeficiency Virus**
HTS	**High Throughput Screening**
ICL	**Intracellular Loop**
NAM	**Negative Allosteric Modulator**
NMDA	***N*-methyl-D-aspartate**
PACAP	**Pituitary Adenylate Cyclase-Activating Polypeptide**
PAM	**Positive Allosteric Modulator**
PK	**Pharmacokinetic**
SAM	**Silent Allosteric Modulator**
VIP	**Vasoactive Intestinal Peptide**
Wnt	**Wingless/Integrated**

11.1. INTRODUCTION

Over one-third of currently marketed drugs modulate G-protein coupled receptors (GPCRs), though these exploited therapeutic targets represent a small fraction of the possible druggable GPCRs in the human genome (Hauser et al., 2017; Rask-Andersen et al., 2011). One of the multiple reasons for this is that several GPCR-targeted candidates have not progressed into the clinic due to efficacy and/or safety issues related to off-target effects. In this context, GPCR allosteric modulation appears to be an innovative targeting approach that confers specific advantages on distinguishing between highly homologous receptor subtypes and is proving to be a viable drug discovery strategy, as evidenced by recent FDA approvals and clinical trials (Lutjens and Rocher, 2017; Wootten et al., 2013a).

The term *allostery* was first coined to describe a newly identified protein function observed in enzymes composed of various subunits (Monod et al., 1965). Nowadays, allosteric modulation is understood as a unifying mechanism for functionality in many other protein types, including GPCRs (Canals et al., 2011, 2012; Changeux and Christopoulos, 2016). A GPCR allosteric modulator (AM) is defined as a ligand that binds to a spatially and topologically distinct (allosteric) site, and does not occupy the orthosteric binding site of the endogenous ligand. Hence, AMs do not compete with the endogenous ligand; the binding to the allosteric site produces conformational changes in the orthosteric site that result in the modulation of the endogenous ligand response. Mechanistically, AMs can either increase or inhibit the

GPCRs:Structure, Function, and Drug Discovery. https://doi.org/10.1016/B978-0-12-816228-6.00011-8

functional response to an orthosteric agonist, acting as positive or negative AMs (PAMs or NAMs), respectively. Silent AMs (SAMs) bind to an allosteric site but do not modulate the receptor function (Fig. 11.1). There have also been reports of other ligands that bind to an allosteric site: ago-PAMs, which possess an intrinsic agonist profile even in the absence of an orthosteric ligand, and allosteric agonists or antagonists that activate or block, respectively, the receptor but do not potentiate or decrease responses to the orthosteric agonist (Christopoulos et al., 2014; Gentry et al., 2015).

The mode of action of GPCR AMs include: (i) fine-tuning the cellular response to the orthosteric ligand in a time and spatially dependent manner, which mimics the physiological effect of the endogenous ligand (Conn et al., 2009); (ii) "ceiling effect" or saturable nature of the binding, whereby the extent of their activity is dictated by the orthosteric ligand concentration, potentially protecting against overdose concerns (Soudijn et al., 2004); (iii) receptor specificity due to the reduced residue conservation in allosteric binding sites compared to that in highly homologous orthosteric sites (Jacoby et al., 2006; Melancon et al., 2012); (iv) signaling bias (also termed functional selectivity), differentially modulating a selected intracellular pathway(s) over other signaling cascades activated by the GPCR (Kenakin, 2012; Lane et al., 2013); and (v) probe dependence, inducing different effects depending on the nature of the orthosteric ligand (Canals et al., 2012). Altogether, the remarkably precise pharmacological modulation confers to GPCR AMs unique advantages over orthosteric classical ligands (Wootten et al., 2013a), contributing to hold promise for central nervous system (CNS) and periphery targets (Conn et al., 2009; Foster and Conn, 2017).

Allosteric drugs work by manipulating binding (allosteric) pockets away from (orthosteric) active sites to effect global conformational changes, making them harder to predict. This brings a number of intense challenges to the discovery of AMs from the standpoint of standard drug discovery programs, including, in particular, rational design (Wagner et al., 2016). The recent breakthroughs in structural biology have made several structures of GPCRs with small-molecule AMs available (Lu and Zhang, 2018). The complex crystal structures have revealed, indeed, the high diversity of allosteric binding sites in extracellular loops (ECLs), the transmembrane helix bundle (7TM), and intracellular loops (ICLs) of receptors. Knowledge of the key receptor−modulator interactions at the allosteric sites and the complementary computational and biological approaches are expected to contribute to the structure-based design of GPCR allosteric drugs.

Allosteric sites on GPCRs represent novel drug targets because theoretical advantages over classic orthosteric ligands confer an improved therapeutic action to AMs. Very recently the first AMs of GPCRs have been approved as drugs (Fig. 11.2). These are ticagrelor (Brilinta, Brilique, Possia), an allosteric antagonist of the purinergic $P2Y_{12}$ receptor (a class A GPCR) that is used as a platelet aggregation inhibitor in antithrombotic therapy; maraviroc (Celsentry), an NAM of the chemokine receptor CCR5 (a class A GPCR) used as a virus entry inhibitor in human immunodeficiency virus (HIV) therapy; plerixafor (Mozobil), an NAM of the chemokine receptor CXCR4 (a class A GPCR) used for stem cell mobilization for transplantation in patients with lymphoma and multiple myeloma; cinacalcet (Sensipar, Mimpara), a PAM of the calcium-sensing receptor (a class C GPCR) used for the treatment of hyperparathyroidism; and vismodegib

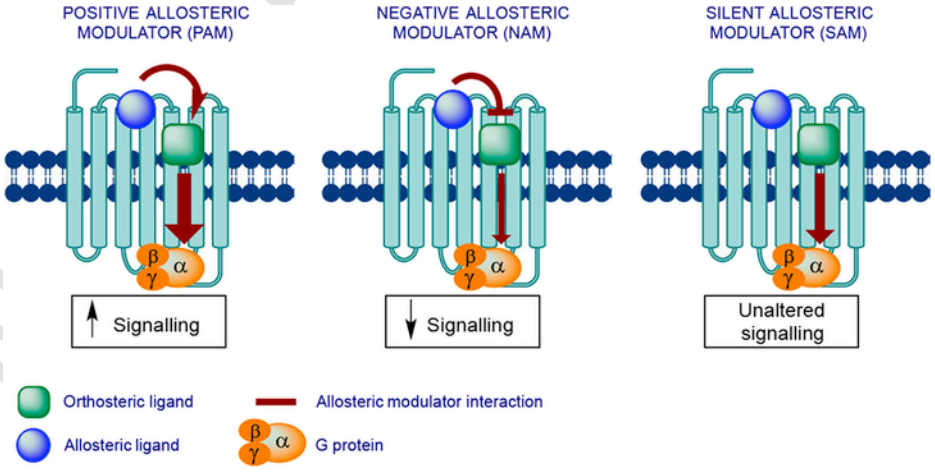

FIGURE 11.1 Influence of AMs on orthosteric agonist function. The binding of orthosteric agonist results in the conformational changes of GPCRs, which result in the activation of downstream signaling cascades. AMs bind to a topographically distinct site and induce conformational changes to a receptor, which influence the orthosteric agonist function. Negative AM (NAM) decreases and positive AM (PAM) increases the affinity and/or efficacy of orthosteric agonist. Silent AMs (SAMs) have no influence on the function of orthosteric agonist. *Credit: Reprinted with permission from J. Med. Chem. 2019, doi: org/10.1021acs.jmedchem.8b00368. Copyright (2019) American Chemical Society.*

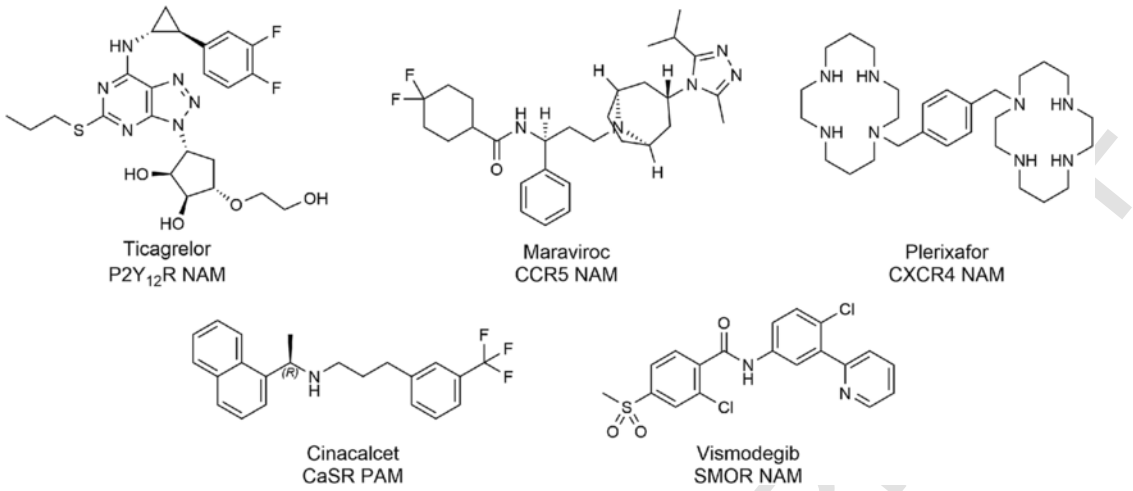

FIGURE 11.2 AMs of GPCRs approved by the FDA as drugs. Ticagrelor (Brilinta, Brilique, Possia), used as a platelet aggregation inhibitor in antithrombotic therapy; maraviroc (Celsentry), used as a virus entry inhibitor in HIV therapy; plerixafor (Mozobil), used for stem cell mobilization for transplantation in patients with lymphoma and multiple myeloma; cinacalcet (Sensipar, Mimpara), used for the treatment of hyperparathyroidism; and vismodegib (Erivedge), used for the treatment of basal cell carcinoma.

(Erivedge), a NAM of the smoothened receptor (a class F GPCR), used for the treatment of basal cell carcinoma (Drugbank; Gpcrdb).

This chapter will focus on relevant medicinal chemistry advances for small-molecule AMs targeting GPCRs of class A —rhodopsin-like receptors—, class B —the secretin family—, class C —metabotropic glutamate receptors—, and class F —frizzled and smoothened receptors— (Foord et al., 2005; Lin et al., 2013). Herein, we provide an update on reported AMs, their therapeutic relevance, and their binding sites.

11.2. ALLOSTERIC MODULATORS TARGETING CLASS A GPCRS

Class A represents the largest class of human GPCRs and makes up most of the current receptor drug targets (Roth and Kroeze, 2015). Interest in class A GPCR AMs initially grew because of a theoretical improvement in target selectivity, especially among receptor subtypes displaying high degrees of homology in the orthosteric site (Wild et al., 2014). Since then, the development of AMs in class A has significantly improved and is now progressing candidates forward in preclinical development and human clinical trials (Table 11.1) as well as in the market for clinical use (Fig. 11.2) (Wold et al., 2018). 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 ligand-receptor interactions (Lu and Zhang, 2018).

TABLE 11.1 Selected AMs of GPCRs currently in clinical trials.

GPCR	Compound	Indication (phase)
D_1	LY3154207 (PAM)	Parkinson disease, dementia (II)
M_1	VU319 (PAM)	Cognitive impairment (I)
FFAR1	MK-8666 (ago-PAM)	Type 2 diabetes mellitus (I)
CXCR1	Reparixin (NAM)	β-cell transplantation (III)
CXCR1/CXCR2	Ladarixin (NAM)	Onset type 1 diabetes (II)
$GABA_BR$	ADX71441 (PAM)	Addiction (I)
$mGlu_4R$	Foliglurax (PAM)	Parkinson disease (II)
$mGlu_5R$	Mavoglurant (NAM)	Fragile X syndrome, alcohol drinking, cocaine-related disorder (II)

Allosterically targeted class A GPCRs belong to β-adrenergic, serotonin, dopamine, muscarinic acetylcholine, cannabinoid, free fatty acid, adenosine, purinergic P2Y, chemokine, opioid, and proteinase-activated receptor families.

11.2.1 β_2-adrenergic receptor (β_2AR)

The β_2AR is the most extensively characterized member of adrenergic receptors or adrenoceptors, an aminergic family of class A GPCRs whose endogenous signaling molecules are catecholamines. It is largely expressed in bronchial smooth muscle and triggers bronchodilation upon activation (Lefkowitz, 2007). 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, widely used for the treatment of hypertension, cardiac arrhythmias, and other cardiovascular indications. At present, nearly all known β-adrenergic ligands act orthosterically.

Kobilka and Lefkowitz (Ahn et al., 2017) have reported the first allosteric β-blocker, or β_2AR NAM, compound **1** (Fig. 11.3), identified via a DNA-encoded small-molecule library screen comprising 190 million distinct compounds. In vitro studies indicate the addition of compound **1** results in an inhibition of β_2AR stimulated cAMP production and β-arrestin recruitment. The cocrystal complex of this recently discovered NAM with β_2AR (Fig. 11.4) reveals an intracellular binding site formed by residues from helices I, II, and VI−VIII and the ICL1 of the β_2AR, which is spatially distant from the orthosteric site for binding of inverse agonist carazolol (Liu et al., 2017). This significant finding of a small-molecule allosteric site on the intracellular surface of β_2AR could extrapolate to additional class A GPCRs, opening new avenues for allosteric drug discovery.

11.2.2 Serotonin 5-HT_{2C} receptor (5-HT_{2C}R)

The 5-HT_{2C}R belongs to the aminergic family of class A GPCRs that are activated by the endogenous neurotransmitter serotonin throughout the brain and the periphery. This subtype is critical for the anorectic effect of serotoninergic activation (Sargent and Henderson, 2011) and several research efforts support the interest of a selective 5-HT_{2C}R PAM as a safer antiobesity drug devoid of potential hallucinogenic effects and cardiac valvulopathy associated to activation of highly homologous 5-HT_{2A} and 5-HT_{2B} subtypes (Astrup, 2010; Dinicolantonio et al., 2014).

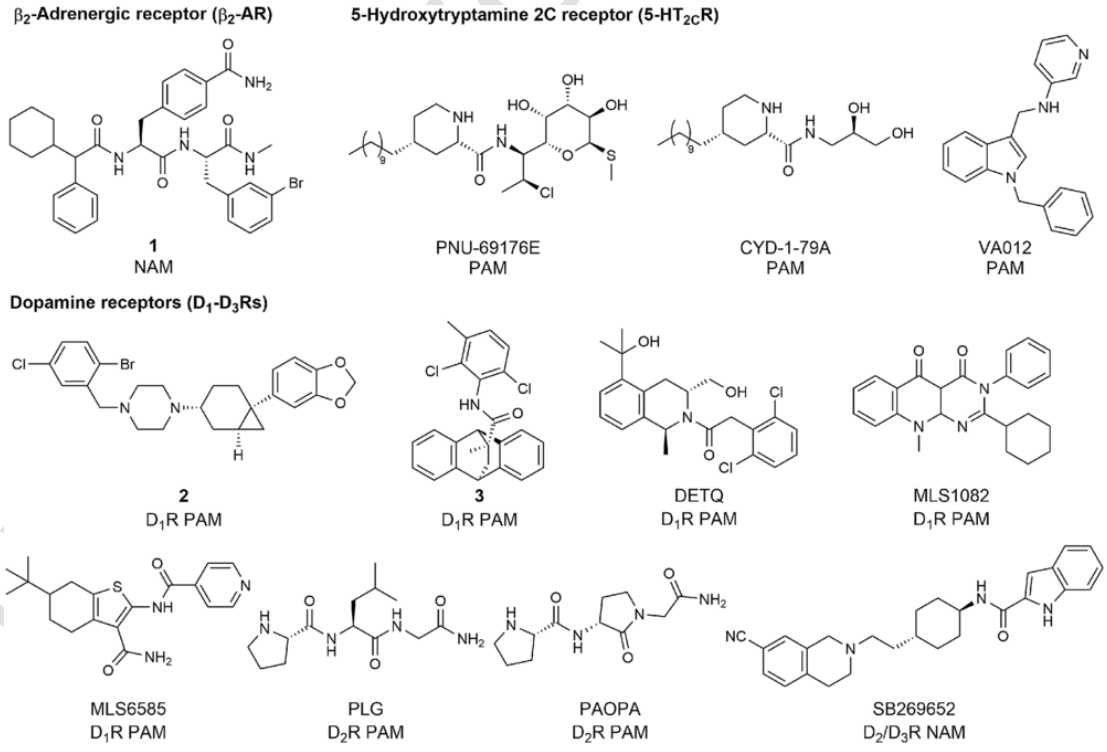

FIGURE 11.3 Representative allosteric ligands targeting aminergic β-adrenergic, serotonin, and dopamine GPCRs of class A.

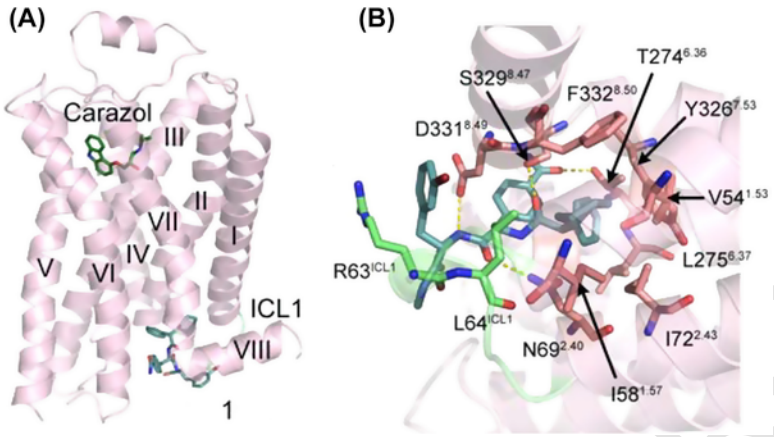

FIGURE 11.4 Cocrystal structure of the β_2AR in a ternary complex with orthosteric inverse agonist carazolol and NAM compound **1** (see Fig. 11.3). (A) Compound **1** is located in an intracellular binding site formed by residues from helices I, II, and VI−VIII and the ICL1 of the β_2AR, which is spatially distant from the orthosteric site for binding of carazolol. (B) In the allosteric binding site, compound **1** forms hydrogen bonding interactions with the side chains of Asn69$^{2.40}$, Thr274$^{6.36}$, Ser329$^{8.47}$, and Asp331$^{8.49}$ and a cation−π interaction with Arg63^{ICL1} via the bromobenzyl ring. The cyclohexylmethylphenyl group is located inside a hydrophobic pocket surrounded by residues Val54$^{1.53}$, Ile58$^{1.57}$, Leu64^{ICL1}, Ile72$^{2.43}$, Leu275$^{6.37}$, Tyr326$^{7.53}$, and Phe332$^{8.50}$ (PDB code 5X7D). Superscript represents residue number in accordance with Ballesteros−Weinstein nomenclature (Ballesteros and Weinstein, 1995). *Credit: Adapted with permission from J. Med. Chem. 2019, https://doi.org/10.1021/acs.jmedchem.7b01844. Copyright (2019) American Chemical Society.*

PNU-69176E (Fig. 11.3) was the first reported 5-HT$_{2C}$R selective PAM, which was identified via screening of a chemical library of Pharmacia (now Pfizer) (Ding et al., 2012; Im et al., 2003). However, the authors described the behavior as an ago-PAM showing intrinsic activity in inositol phosphate release in HEK293 cells. Both the long alkyl chain and polar moiety (α-D-galactopyranoside) in PNU-69176E are claimed to be essential to its function and may explain anchoring to the membrane and binding to the allosteric site, respectively. CYD-1-79A (Fig. 11.3), also containing a 4-alkylpiperidine-2-carboxamide scaffold, has recently been reported to function as a pure 5-HT$_{2C}$R PAM with no intrinsic activity and a good preclinical pharmacokinetic (PK) profile, and to potentiate signaling in vivo in drug discrimination assay (Wild et al., 2018), though no antiobesity properties have been described to our knowledge. Using the recent high-resolution crystal structure of the 5-HT$_{2C}$R (Peng et al., 2018), the authors performed molecular docking studies that predict a bidentate hydrogen-bonding interaction between the 1,2-diol moiety of CYD-1-79A and the backbone carbonyl of Leu209^{ECL2} residue in ECL 2, and between the ionizable N atom of the piperidine ring of the ligand and the –OH side chain of Ser334$^{6.58}$ in helix VI. These computational studies provide a possible explanation for the selective profile of CYD-1-79A versus closely related 5-HT$_{2A}$R and 5-HT$_{2B}$R, since these residues are not conserved in the highly homologous receptor subtypes (Wild et al., 2018).

Our group has recently contributed to highlight the promise of 5-HT$_{2C}$R PAMs as antiobesity therapeutics (García-Cárceles et al., 2017). In what is only the second reported synthetic small-molecule screening for 5-HT$_{2C}$R PAMs, an indole scaffold hit of Vivia Biotech chemical library was identified via an innovative automated flow-cytometry-based screening system, the PharmaFlow platform (previously ExviTech platform) (Bennett et al., 2014). Further structural modification led to the discovery of VA012 (Fig. 11.3), which exhibited dose-dependent enhancement of serotonin efficacy, no significant off-target activities including 5-HT$_2$ family members (5-HT$_{2A}$R and 5-HT$_{2B}$R), and low binding competition against the endogenous agonist (5-HT) and other orthosteric ligands (mesulergine and clozapine). Significantly, feeding rodent models indicate that PAM VA012 reduces both food intake and body weight gain without causing taste aversion when acutely administered at 2 mg/kg (ip) (García-Cárceles et al., 2017).

11.2.3 Dopamine receptors (D_1-D_3Rs)

Two distinct families are distinguished among dopamine receptors: D_1-like family —D_1 and D_5 subtypes— stimulate cAMP production, and D_2-like family —D_2, D_3, and D_4 receptors— attenuate cAMP production. As a whole, dopamine receptors play a substantial role in numerous neuropsychiatric disorders, including schizophrenia, Parkinson disease, attention deficit hyperactivity disorder, and drug and alcohol dependence (Arnsten et al., 2017; Beaulieu et al., 2015; Beaulieu and Gainetdinov, 2011).

Stimulation of the D_1-like receptors is challenging; despite the promise in preclinical studies, attempts to develop D_1R agonists for clinical use have so far not been successful. To date, two pharmaceutical companies have reported their efforts toward a selective D_1R activation by using the allosteric approach. Following a high-throughput screening (HTS) of the Bristol-Myers Squibb chemical library, compounds **2** and **3** (Fig. 11.3) were identified as two distinct D_1R PAM chemotypes designated as piperazines and ethanoanthracenes, respectively, and represented the first described D_1R PAMs (Lewis et al., 2015).

At the same time, Lilly discovered the tetrahydroisoquinoline DETQ (Fig. 11.3) and demonstrated its activity in a broad array of behavioral and neurochemical animal models that are thought to be predictive of therapeutic utility in Parkinson disease, Alzheimer disease, schizophrenia, major depressive disorder, attention deficit disorder, and narcolepsy (Beadle et al., 2014; Bruns et al., 2018; Svensson et al., 2017). At present, Lilly's molecule LY3154207, directly related analogue of DETQ (Fig.11.3), is the first D_1R potentiator that is being clinically studied (phase II) for the treatment of dementia associated with Parkinson disease (Hall et al., 2019).

More recently, the discovery and characterization of two novel PAMs of D_1R signaling, MLS1082 and MLS6585 (Fig. 11.3), have been reported (Luderman et al., 2018). Both compounds have no inherent agonist activity, but potentiate receptor signaling stimulated by both the endogenous ligand dopamine and other D_1R agonists. Using functional additivity as well as mutational approaches, the authors suggest that MLS1082 and MLS6585 likely bind to diverse receptor sites.

Our group has also contributed with the development of biphenylsulfoximines as a new class of D_1R PAMs as promising safe agents for the treatment of CNS-related pathologies (López Rodríguez et al., 2018).

Due to the therapeutic potential of D_2-like receptors, several AMs have been reported. The neuropeptide Pro-Leu-Gly-NH_2 (PLG, Fig. 11.3), initially isolated from brain tissue, was first characterized as an endogenous PAM of the D_2 and D_4 receptors with potential for Parkinson disease treatment (Khan et al., 2010). However, the peptide nature of PLG limited its development as a drug, and the rational design and modification strategies in the search for agents with better PK properties have led to peptidomimetic analogs containing lactam, bicyclic and spiro-bicyclic scaffolds (Mann et al., 2010; Verma et al., 2005). Interestingly, these extensive studies have produced both D_2R PAMs and NAMs with minor structural differences within the same series of peptidomimetics (Bhagwanth et al., 2013). Among them, analog PAOPA (Fig. 11.3) has been characterized as a selective D_2R PAM displaying 100- to 1000-fold higher potency than the parent neuropeptide, a significantly improved PK and toxicological profile, and activity in preclinical models of schizophrenia (Beyaert et al., 2013; Dyck et al., 2011; Tan et al., 2013a).

The atypical, allosteric antagonism of the tetrahydroisoquinoline derivative SB269652 (Fig. 11.3) at D_2 and D_3 receptors supported its characterization as the first small-molecule NAM of dopamine receptors (Silvano et al., 2010). Further SAR studies revealed that SB269652 is a bitopic ligand that incorporates both an orthosteric site-binding moiety (the tetrahydroisoquinoline “head”) and an allosteric site-binding moiety (“the indole tail”) separated by an alkyl chain linker (Rossi et al., 2017; Shonberg et al., 2015). Interestingly, a *trans*-cyclopropylmethyl linker replacing the *trans*-1,4-cyclohexylene linker while retaining the head and tail groups led to D_3R preferring bitopic ligands (Kumar et al., 2017). Fragmentation of bitopic ligand SB269652 was approached to target the putative allosteric site, and indole-2-carboxamide derivatives of the allosteric fragment moiety were identified as new NAMs of the D_2R (Mistry et al., 2015).

Recently, a benzothiazole scaffold compound was reported as a D_2R PAM both in vitro and in vivo, identified via an HTS of 80,000 compounds, and provides another small-molecule hit for the development of AMs useful to treat hypodopaminergic function (Wood et al., 2016).

A crystal structure of an AM in complex with its targeted dopamine receptor is certainly awaited; meanwhile, a recent study of the structural basis for the allosteric mechanism of SB269652 by combining site-directed mutagenesis with molecular modeling and simulations of D_2R in complex with either SB269652 or its derivatives (Draper-Joyce et al., 2018b) may aid the rational development to provide clinical candidates with improved in vitro and in vivo properties.

11.2.4 Muscarinic acetylcholine receptors (M_1, M_2, M_4, and M_5Rs)

Muscarinic acetylcholine receptors (mAChRs) are aminergic class A GPCRs activated by endogenous acetylcholine, and they are broadly expressed in the CNS and other tissues in the periphery. They regulate a large body of peripheral and central physiological functions in the human body and represent important drug targets for a number of diseases including pain, Alzheimer disease, Parkinson disease, schizophrenia, diabetes, and obesity (Wess et al., 2007).

The muscarinic family of receptors is divided into five subtypes (M_1–M_5Rs) of which M_1–M_4Rs have been crystallized. All subtypes possess at least one allosteric binding site, which is located in the extracellular region of the

receptor on top of the ACh (i.e., orthosteric) binding site. The former can be specifically targeted by chemical compounds (mostly small molecules); notably, the concept of allosteric modulation at GPCRs was initially described at muscarinic receptors several decades ago (Mohr et al., 2013; Stockton et al., 1983). Today, a plethora of AMs exist for all five muscarinic receptor subtypes (Bock et al., 2018).

mACh M_1R. Benzylquinolone carboxylic acid BQCA (Ma et al., 2009), indole VU0108370 (Reid et al., 2011), and isatin VU0119498 (Bridges et al., 2010) are the three initial hits identified (Fig. 11.5) by HTS campaigns that have been used as representative scaffolds for optimization. Thus, highly selective M_1R PAMs have been developed with improved in vivo efficacy, in the search for novel therapeutics to counteract the negative cognitive symptoms associated with diseases such as Alzheimer disease and schizophrenia. Structural modification of the quinolone ring of BQCA discovered by Merck afforded new M_1R PAMs with alternative scaffolds such as phenylpyridinone, phenylpyrimidinone, quinolizidinone, or methoxynaphthalene (Kuduk et al., 2010b, 2011a, 2012, 2014; Mistry et al., 2016a, 2016b). Further optimization of the quinolone core system was conducted (Abdul-Ridha et al., 2014; Kuduk et al., 2011b; Mistry et al., 2013; Yang et al., 2010) to provide benzoquinazolinone MK-7622 (Fig. 11.5), which advanced into a phase II clinical trial in 2013 and was terminated in 2016 for undisclosed reasons (Kuduk et al., 2010a). Interestingly, dibenzylpyrazoloquinolinone DBPQ (Fig. 11.5) is a tricyclic scaffold related to MK-7622 that was discovered as a hit from an HTS of the NIH (Han et al., 2015).

Indole and azaindole analogs of hit VU0108370 have been also described exhibiting improved nanomolar potency (Davoren et al., 2016; Rook et al., 2017).

Among modifications around the third chemotype VU0119498 (Ghoshal et al., 2016; Melancon et al., 2013; Swahn et al., 2010), the most recent is isoindolinone PF-06827443 (Fig. 11.5), a potent, low-clearance, orally bioavailable, and CNS-penetrant M_1R-selective PAM with minimal intrinsic agonist activity (Davoren et al., 2017).

Overall, preclinical studies have revealed significant improvements in PK and in vivo efficacy for selective mACh M_1R AMs. Importantly, PAM VU319 (undisclosed structure), discovered by a team of scientists at the Vanderbilt Center for Neuroscience Drug Discovery, has recently entered phase I clinical trials for cognitive impairment associated with Alzheimer disease.

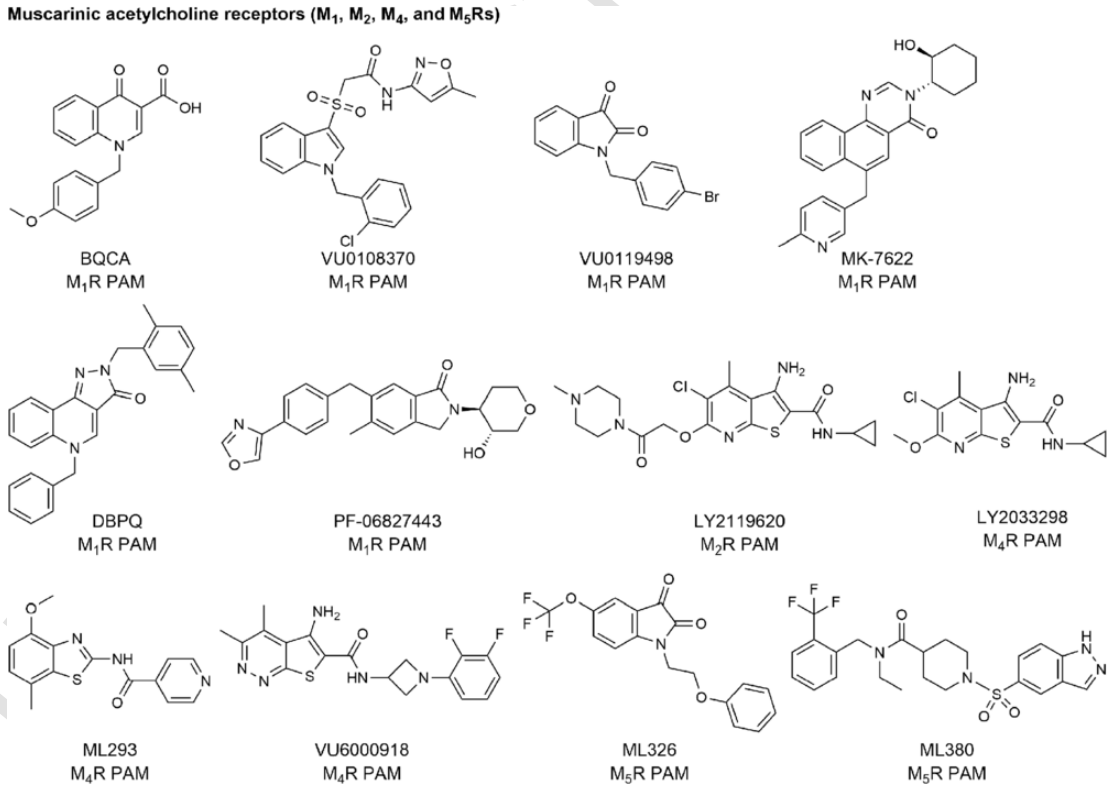

FIGURE 11.5 Representative allosteric ligands targeting aminergic muscarinic GPCRs of class A.

mACh M_2R. The mACh M_2R is distributed in both the CNS and periphery and has been predominately studied for its role in regulating parasympathetic cardiac function. However, the general consensus is that M_2R activation leads to undesirable off-target effects for mAChR modulators (Moulton and Fryer, 2011). The importance of the M_2R lies on the pioneering cocrystal structure bound to PAM LY2119620 (Figs 11.5 and 11.6) that has contributed to the understanding of mAChR, and GPCR, allosteric modulation (Croy et al., 2014; Kruse et al., 2013). LY2119620, a high-affinity PAM of both M_4R and M_2R that potentiates the activity of agonist iperoxo, occupies a site above the orthosteric iperoxo binding site on the extracellular side of helices II, VI, and VII forming extensive contacts with ECL2 and ECL3 (Kruse et al., 2013).

mACh M_4R. Several aminothienopyridine derivatives of LY2033298 (Fig. 11.5) identified as a selective M_4R PAM have been developed (Chan et al., 2008). However, a poor PK profile and/or a strong species bias have precluded their preclinical development (Brady et al., 2008; Kennedy et al., 2009). An iterative optimization effort from Vanderbilt Center for Neuroscience Drug Discovery improved in vivo PK properties in benzothiazole series such as ML293 (Fig. 11.5), though lowering allosteric potency (Salovich et al., 2012). Further optimization resulted in the recently reported aminothienopyridazine VU6000918 (Fig. 11.5) that maintains high potency, good in vivo PK profile, and antipsychotic efficacy in an established hyperlocomotion assay (Tarr et al., 2017).

mACh M_5R. The therapeutic interest of drugs targeting the M_5R subtype has currently upsurged with the discovery of selective PAMs that are efficacious in relevant disease models including schizophrenia, Alzheimer disease, ischemia, and migraine (Bock et al., 2018). Starting from nonselective mAChR PAM VU0119498 (Fig. 11.5), isatin scaffold was used to optimize potency and selectivity that were achieved in ML326 (Fig. 11.5) (Gentry et al., 2013). However, a low CNS exposure precluded its in vivo assessment. Non-isatin ML380, discovered during an HTS, displays selective M_5R PAM activity with submicromolar potency and markedly improved CNS penetration, representing a promising hit (Berizzi et al., 2016).

With muscarinic receptor crystal structures at hand, many new scaffolds of AMs should be discovered by structure-based drug design and virtual screening. First evidence comes from a very recent study at the M_2R, which used accelerated molecular dynamics simulations and has led to AMs with novel chemical scaffolds (Miao et al., 2016).

11.2.5 Cannabinoid CB_1 receptor (CB_1R)

Cannabinoid receptors (CB_1 and CB_2Rs) belong to the endocannabinoid system that is a ubiquitous neuromodulatory system with wide-ranging actions (Pacher and Kunos, 2013). CB_1R is widely distributed throughout the CNS and is involved in the regulation of many physiological processes related to pain, metabolism, nociception, and

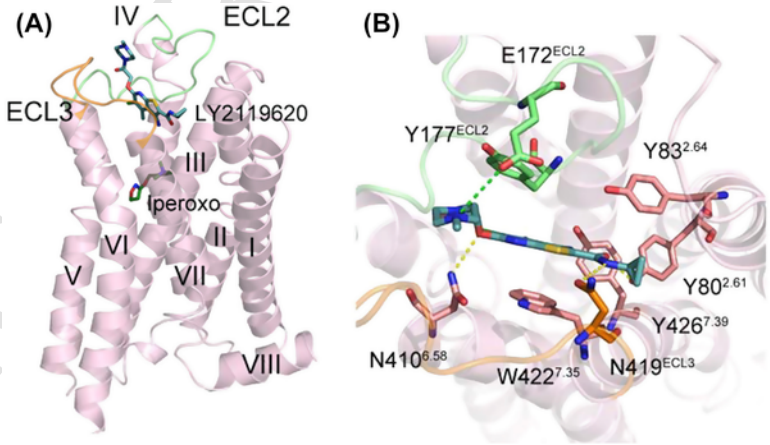

FIGURE 11.6 Cocrystal structure of the M_2R in a ternary complex with orthosteric agonist iperoxo and PAM LY2119620 (see Fig. 11.5). (A) The allosteric LY2119620 agonist binds to the extracellular vestibule of the receptor, which is directly above the orthosteric iperoxo binding site. The allosteric binding site of LY2119620 is formed by residues from the extracellular side of helices II, VI, and VII and the ECL2 and ECL3. (B) In the allosteric LY2119620 binding site, the piperidine group is engaged in a charge−charge interaction with $Glu172^{ECL2}$; the amide oxygen and nitrogen make hydrogen bonds with the side chains of $Tyr80^{2.61}$ and $Asn419^{ECL3}$, respectively; the bridging oxygen accepts a hydrogen bond from the side chain of $Asn410^{6.58}$. In addition to polar contacts, the hydrophobic interactions between M_2R and LY2119620 are mainly from the aromatic residues, including $Tyr80^{2.61}$, $Tyr83^{2.64}$, $Trp422^{7.35}$, $Tyr426^{7.39}$, and $Tyr177^{ECL2}$ (PDB code 4MQT). *Credit: Adapted with permission from J. Med. Chem. 2019, https://doi.org/10.1021/acs.jmedchem.7b01844. Copyright (2019) American Chemical Society.*

neurotransmission (Saleh et al., 2018). To date, CB_1R 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 (Di Marzo, 2008). Alternatively, the development of both NAMs and PAMs of the CB_1R has been of high interest in recent years.

CB_1R PAMs. The structural diversity of the small molecules reported as CB_1R PAMs remains relatively small, mostly containing 2-phenyl-1*H*-indole scaffold. Interestingly, GAT211 (Fig. 11.7**)** acts as an ago-PAM as its *R*-enantiomer, while the *S*-enantiomer lacks intrinsic agonist activity (Laprairie et al., 2017). Structurally related ZCZ011 (Fig. 11.7) exhibited broad allosteric activity across signaling pathways and multiple agonists, and reduced neuropathic pain in the mouse with no psychoactive effects (Ignatowska-Jankowska et al., 2015).

CB_1R NAMs. The past decade has seen the identification of multiple promising compounds as NAMs targeting the CB_1R, mainly comprising two scaffolds that have been extensively characterized: indolecarboxamides and the diarylurea analogs. The discovery of Org27569, Org29647, and Org27759 (Fig. 11.7) displaying a reduction of CB_1R agonists efficacy demonstrated, for the first time, the existence of an allosteric binding site at the receptor that can be recognized by synthetic small-molecule ligands (Price et al., 2005). Subsequent SAR studies have revealed both the 2-carboxamide function and the 5-halogen substituent in the indole ring are required to maintain allosteric activity (Khurana et al., 2014; Mahmoud et al., 2013; Nguyen et al., 2015; Piscitelli et al., 2012).

PSNCBAM-1 (Fig. 11.7), discovered by HTS, was the first CB_1R NAM bearing a diarylurea scaffold (Horswill et al., 2007). Numerous studies have now reported thorough SAR around diarylurea analogs (Baillie et al., 2013; Khajehali et al., 2015). In particular, RTICBM-74 (Fig. 11.7) has shown a good PK profile and in vivo efficacy in a rat model of cocaine withdrawal (Nguyen et al., 2017). Nevertheless, in vivo studies on promising CB_1R NAMs are still limited and will need to progress toward proof-of-concept studies to show therapeutic utility.

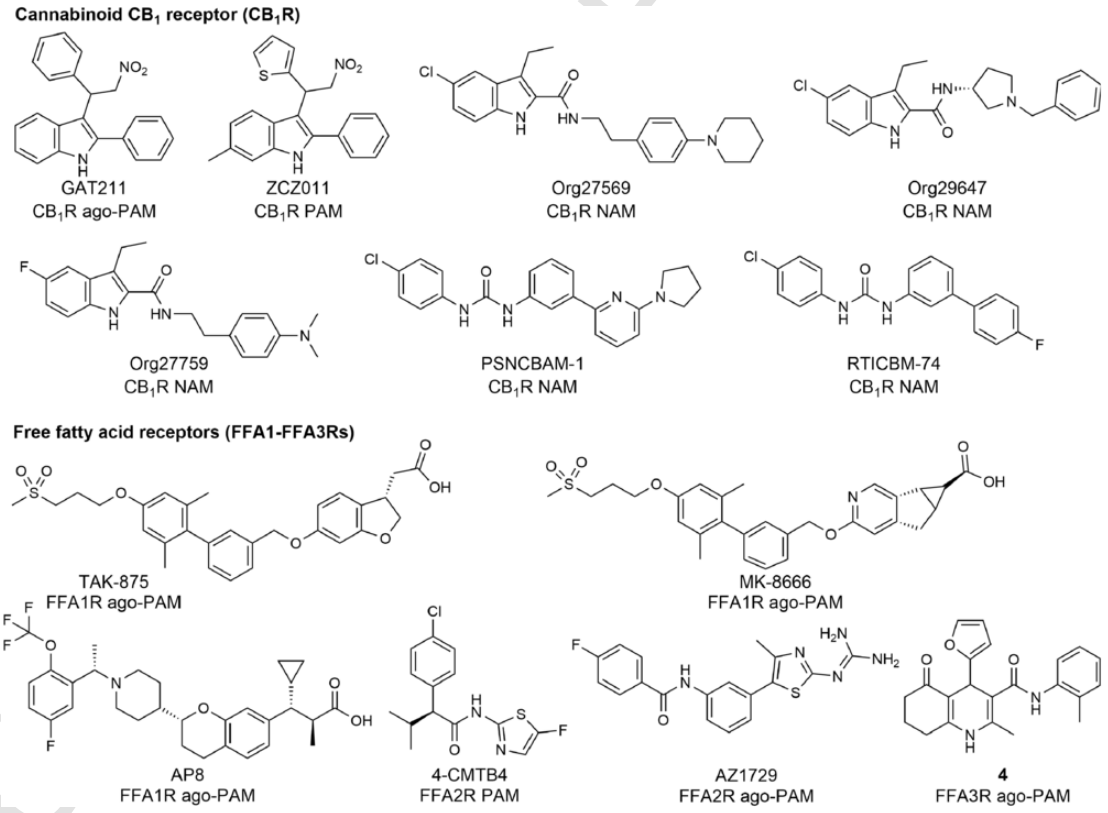

FIGURE 11.7 Representative allosteric ligands targeting lipidic cannabinoid and free fatty acid GPCRs of class A.

11.2.6 Free fatty acid receptors (FFA1−FFA3Rs)

Free fatty acid receptors (FFARs) are a recently "deorphanized" family of receptors that are activated by nonesterified or "free" fatty acids (FFAs), the members of which are now receiving substantial interest as novel targets for the treatment of inflammatory and metabolic diseases (Milligan et al., 2017).

FFA1R (GPR40). The FFA1R, which was designated GPR40 prior to the discovery of endogenous signaling ligand, 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. In recent years, numerous pharmacologically diverse allosteric ligands of FFA1R have been discovered and structural work has described their binding to distinct sites. The cocrystal complexes of the structurally related ago-PAMs TAK-875 and MK-8666 (Fig. 11.7) with FFA1R reveal that they both bind to a far uncommon site formed by helices III−V and the ECL2 and that is adjacent to the exterior lipid membrane surface (Figs. 11.8 and 11.9) (Lu et al., 2017; Srivastava et al., 2014). In contrast, the ternary complex structure of FFA1R-MK-8666-AP8 (Figs 11.7 and 11.9) reveals that the latter, a novel and more potent FFA1R ago-PAM, binds to a lipid-facing pocket formed by helices II−V and ICL2 that is a site completely distinct from the allosteric binding site of MK-8666 (Lu et al., 2017).

Importantly, TAK-875 (fasiglifam) was progressed into phase III clinical trials for the treatment of type 2 diabetes mellitus, but the trial underwent early termination due to toxicity. Likewise, MK-8666 has recently been approved to advance into a phase I clinical trial for the treatment of the same disease.

FFA2R (GPR43). FFA2 and FFA3 receptors have been found expressed in various cancer cells, including breast, colon, and liver (Milligan et al., 2017). Phenylacetamide derivatives such as 4-CMTB4 (Fig. 11.7) were identified via HTS and represent the first series of synthetic small molecules that display PAM-like effects at the FFA2R (Lee et al., 2008). However, their poor PK properties have limited further development as preclinical candidates (Lee et al., 2008). Recently, structurally related AZ1729 (Fig. 11.7) has been characterized as a FFA2R ago-PAM displaying an interesting pharmacological biased profile (Bolognini et al., 2016).

FFA3R (GPR41). Hexahydroquinolone-3-carboxamide derivatives discovered by Arena Pharmaceuticals are the predominantly reported allosteric FFA3R ligands (Leonard et al., 2006). Representative compound **4** (Fig. 11.7) is an ago-PAM with modest potency and selectivity over FFA2R (Bolognini et al., 2016). However, this series displays a complex pharmacology and considerable care must be taken to use their activity for defining specific roles of FFA3R in either in vitro or in vivo settings.

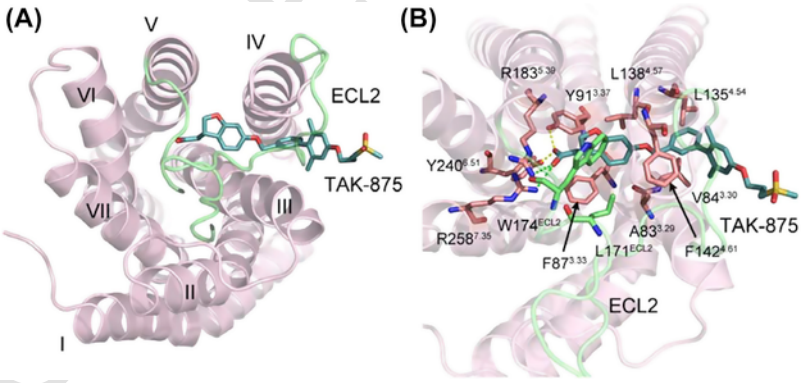

FIGURE 11.8 Cocrystal structure of GPR40 in complex with ago-PAM TAK-875 (see Fig. 11.7). (A) TAK-875 is positioned outside the 7TM, in a site formed by helices III−V and the ECL2; this site is adjacent to the exterior membrane surface. (B) In the allosteric binding site, the carboxylate group of TAK-875 is engaged in salt bridge interactions with the side chains of Arg183$^{5.39}$ and Arg258$^{7.35}$ and hydrogen binding interactions with the side chains of Tyr91$^{3.37}$ and Tyr240$^{6.51}$. The dihydrobenzofuran group is encapsulated by residues Ala83$^{3.29}$, Phe87$^{3.33}$, Leu138$^{4.57}$, Phe142$^{4.61}$, Leu171^{ECL2}, and Trp174^{ECL2} defining a hydrophobic pocket. The benzyl group is situated between helices III and IV and forms hydrophobic interactions with residues Val84$^{3.30}$, Leu135$^{4.54}$, and Phe142$^{4.61}$ (PDB code 4PHU). *Credit: Reprinted with permission from J. Med. Chem. 2019, https://doi.org/10.1021/acs.jmedchem.7b01844. Copyright (2019) American Chemical Society.*

11.2.7 Adenosine receptors (A_1, A_{2A}, A_{2B}, and A_3Rs)

Adenosine receptors (ARs) comprise a family of GPCRs that mediate the physiological actions of adenosine. The four identified AR subtypes —A_1, A_{2A}, A_{2B}, and A_3Rs— have distinct localization and signal transduction pathways (Sheth et al., 2014). Historically, both agonists and antagonists have been used to indiscriminately modulate adenosine receptor subtypes and have been involved in the treatment of diseases that span cardiovascular disease, CNS disorders, inflammatory and allergic disorders, and cancer. Allosteric modulation may provide multiple benefits for targeting subtype selective ligands that are pursued to avoid side effects therapeutically.

A_1R. Bruns et al. first developed a novel series of allosteric enhancers, represented by PD 81,723 (Fig. 11.10), selectively enhancing the binding of agonist N_6-cyclopentyladenosine (CPA) to the A_1R (Bruns and Fergus, 1990; Bruns et al., 1990). Following this pioneering work, multiple PAM derivatives were designed around 2-amino-3-benzoylthiophene scaffold, and detailed SAR studies on the 4- and 5-positions of the thiophene ring have been conducted (Van Der Klein et al., 1999). Within these series, analog T-62 (Fig. 11.10) was developed by King Pharmaceuticals and advanced into clinical trials for the potential treatment of neuropathic pain associated with hyperalgesia and allodynia (Baraldi et al., 2007). However, the program was terminated after failure to meet the end point for efficacy in phase IIb (Childers et al., 2005; Kimatrai-Salvador et al., 2013).

To avoid observed intrinsic antagonist activity at high concentrations and moderate efficacy (Ferguson et al., 2008), further chemical modifications and optimization generated a new series of 2-amino-3-benzoylthiophene derivatives bearing an arylpiperazine ring linked to a methylene at the 4-position of the thiophene. Substantially higher efficacy than PD 81,723 without significant antagonist activity at the A_1R, A_2R, or A_3R was achieved in compound **5** (Fig. 11.10), a representative analog of the series (Romagnoli et al., 2012).

As mentioned previously, some AMs 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. While no structure of an adenosine receptor bound to its AM has been disclosed to date, the understanding of the complex A_1R pharmacology has

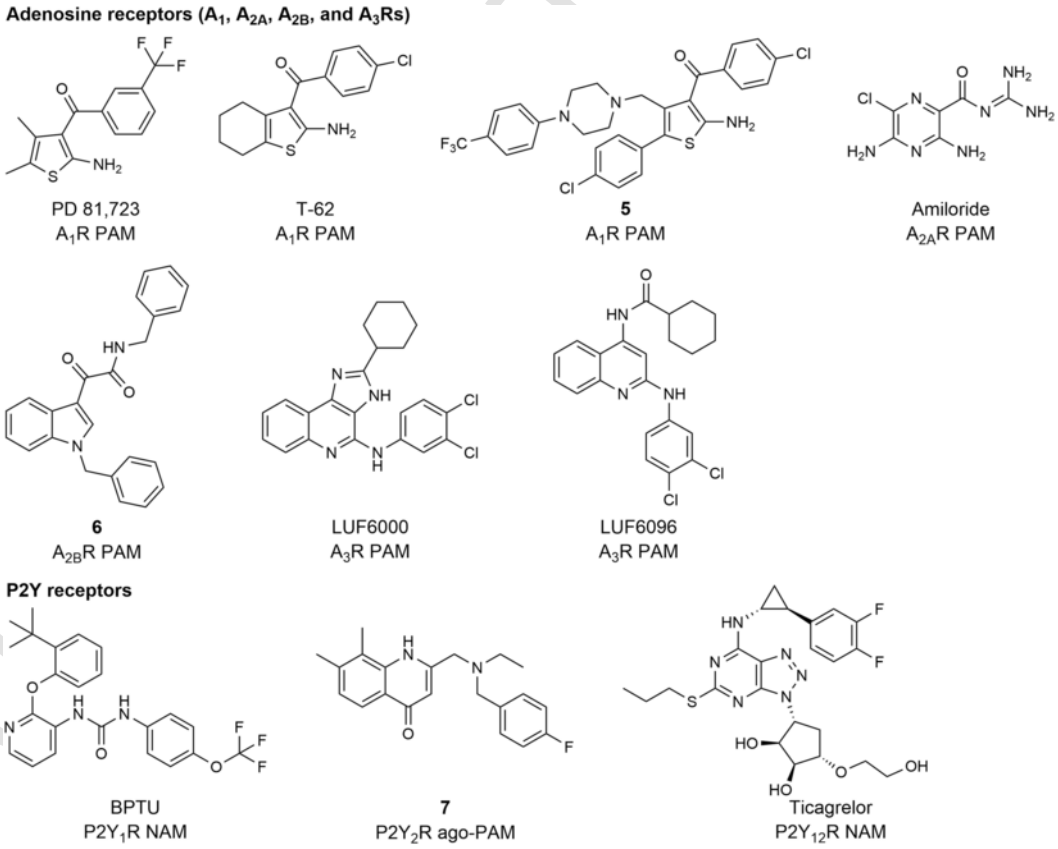

FIGURE 11.10 Representative allosteric ligands targeting nucleotide adenosine and P2Y GPCRs of class A.

greatly benefited from the recent reports of solved structures of both the active and inactive state of A_1R and cryo-electron microscopy of active state of A_1R (Draper-Joyce et al., 2018a; Glukhova et al., 2017). Together with previous mutagenesis studies, this structural information provides additional insight for AM binding, and docking studies have identified ECL2 as a critical domain for PAM activity (Nguyen et al., 2016).

A_{2A}R and A_{2B}Rs. Amiloride (Fig. 11.10) and derivatives thereof have been reported as adenosine $A_{2A}R$ PAMs, and docking studies based on the high resolution crystal structure of agonist-bound $A_{2A}R$ describe their interactions in the sodium ion pocket of the receptor (Gao and Ijzerman, 2000; Massink et al., 2016).

Compounds with a 1-benzyl-3-ketoindole scaffold, for example, compound **6** (Fig. 11.10), are the only reported AMs for $A_{2B}R$ (Taliani et al., 2013; Trincavelli et al., 2014). These PAMs and NAMs may provide useful chemical probes to elucidate the therapeutic potential of the least characterized adenosine subtype.

A_3R. 1*H*-imidazo[4,5-*c*]quinolin-4-amine —for example, LUF6000— and 2,4-disubstituted quinoline —for example, LUF6096— are the core scaffolds of the modest number of selective A_3R AMs reported to date (Goblyos et al., 2006; Heitman et al., 2009) (Fig. 11.10). Interestingly, LUF6000 shows significant potentiation of agonist efficacy and no enhancing effect on agonist potency, whereas open-ring LUF6096 displays positive allosteric effects on both agonist efficacy and potency.

11.2.8 P2Y receptors ($P2Y_1$, $P2Y_2$, and $P2Y_{12}$Rs)

P2Y receptors are a family of purinergic GPCRs, stimulated by nucleotides such as ATP, ADP, UTP, UDP, and UDP-glucose. To date, eight P2Y receptors have been cloned in humans —$P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$, $P2Y_{12}$, $P2Y_{13}$, and $P2Y_{14}$— and the biological effects of their activation depend on how they couple to downstream signaling pathways, either via G_i, $G_{q/11}$, or G_s protein (Von Kugelgen and Hoffmann, 2016).

Nonnucleotide allosteric antagonists of the $P2Y_1R$ such as BPTU (Fig. 11.10), discovered by Bristol-Myers Squibb, and other diarylurea derivatives are also being considered as antithrombotic agents with improved safety profiles (Chao et al., 2013; Qiao et al., 2013; Yang et al., 2014). In the recent cocrystal complex, BPTU is the first $P2Y_1R$ antagonist shown to bind to an allosteric site entirely outside of the 7TM, not only outside of the orthosteric site. The allosteric binding site of BPTU is situated adjacent to the lipid membrane and engages the ligand by mostly hydrophobic and aromatic residues located in helices I−III as well as minor involvement of ECL1 (Fig. 11.11).

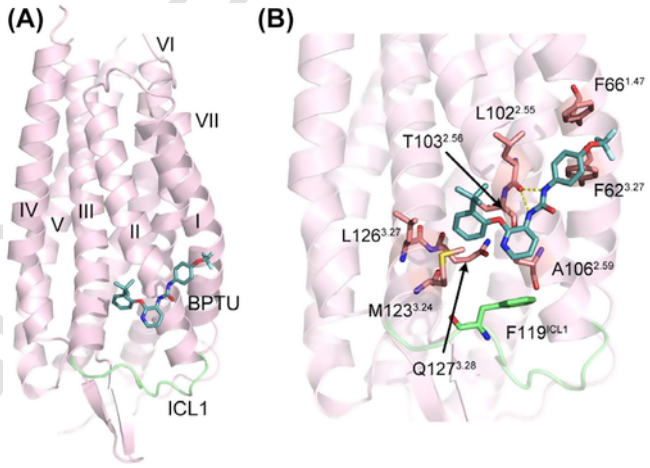

FIGURE 11.11 Cocrystal structure of $P2Y_1R$ in complex with allosteric antagonist BPTU (see Fig. 11.10). (A) BPTU binds to $P2Y_1R$ on the lipidic interface of the transmembrane domain; this BPTU binding site is distinct from the endogenous ADP binding site and is formed by residues from helices I−III and ICL1. (B) The allosteric binding pocket, formed by aromatic and hydrophobic residues, accommodates BPTU predominantly through hydrophobic interactions. The pyridyl group of BPTU comes in contact with residues Ala106$^{2.59}$ and F119^{ICL1}. The benzene ring within the phenoxy group of BPTU is situated in a hydrophobic subpocket formed by residues from helices II and III, including Thr103$^{2.56}$, Met123$^{3.24}$, Leu126$^{3.27}$, and Gln127$^{3.28}$; by contrast, the *tert*-butyl substituent on the phenoxy group engages in a hydrophobic interaction with Leu102$^{2.55}$. At the opposite side of the ligand, the ureido phenyl ring is involved in aromatic−aromatic interactions with Phe62$^{3.27}$ and Phe66$^{1.47}$. The only polar interactions observed are two hydrogen bonds between the NHs of the BPTU's urea group and the backbone carboxyl of Leu102$^{2.55}$ (PDB code 4XNV). *Credit: Reprinted with permission from J. Med. Chem. 2019, https://doi.org/10.1021/acs.jmedchem.7b01844. Copyright (2019) American Chemical Society.*

Compound **7** (Fig. 11.10), with a novel 4(1*H*)-quinolinone scaffold, is among the first nonnucleotide selective $P2Y_2R$ AMs, characterized as an ago-PAM, and preclinical studies support their therapeutic potential for the treatment of cardiovascular disorders (Sakuma et al., 2017).

The $P2Y_{12}R$ is activated by ADP to trigger glutamate release, facilitating thrombus formation, and has been validated as a drug target for the treatment of thromboembolisms and other clotting disorders (Dorsam and Kunapuli, 2004). The FDA approval of ticagrelor (AZD6140, Fig. 11.10), a $P2Y_{12}R$ allosteric antagonist discovered by AstraZeneca, has paved the way for antithrombotic drugs in this class with safer bleeding profiles to emerge as therapeutics (Springthorpe et al., 2007).

11.2.9 Chemokine receptors (CCR2, CCR4, CCR5, CCR9, CXCR1, CXCR2, CXCR3, and CXCR4)

At least 18 chemokine GPCRs have been identified in humans that are classified in four subfamilies –CCR, CXCR, CX3CR, and XCR— based on the relative positioning of conserved cysteine residues in the *N*-terminal domain of their endogenous ligands (Bachelerie et al., 2014). Chemokine receptors are activated by more than 50 chemokine ligands in a concerted manner in response to various immunologic or inflammatory events (Allegretti et al., 2016). Thus, probe dependence may be a primary advantage for AMs of chemokine receptors. The development of AMs for chemokine receptors represents a profound advance for AMs of class A GPCRs with marketed drugs (maraviroc, NAM of CCR5; plerixafor, NAM of CXCR4, see Fig. 11.12) and structural studies of AMs binding to the receptors at high resolution (for CCR2, CCR5, and CCR9) (Lu and Zhang, 2018). These structures have revealed allosteric modulation via binding intracellular allosteric sites, which is still uncommon for class A GPCRs but may have important therapeutic implications, especially for chemokine receptors.

CCR subfamily. CCR2 subtype is implicated in numerous inflammatory and neurodegenerative diseases (O'connor et al., 2015). CCR2-RA-[R] (Fig. 11.12) is a selective NAM of CCR2 with an excellent PK profile. The cocrystal structure of CCR2 in a ternary complex with CCR2-RA-[R] and orthosteric antagonist BMS-681 demonstrates that the former occupies an intracellular allosteric binding site, as seen in other chemokine receptors (Fig. 11.13) (Zheng et al., 2016).

CCR4 subtype is crucial for recruiting T cells during the inflammatory response upon exposure to allergens, and has thus been a target for the discovery of small-molecule therapeutics due to its central role in pathogenesis such as asthma,

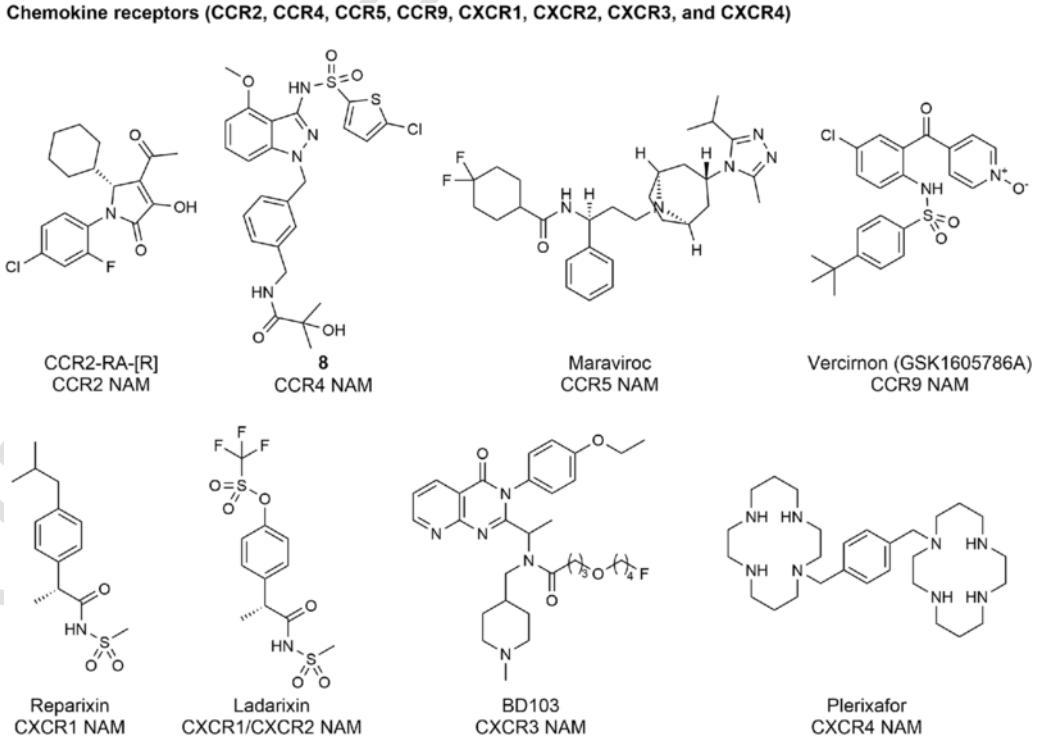

FIGURE 11.12 Representative allosteric ligands targeting protein chemokine GPCRs of class A.

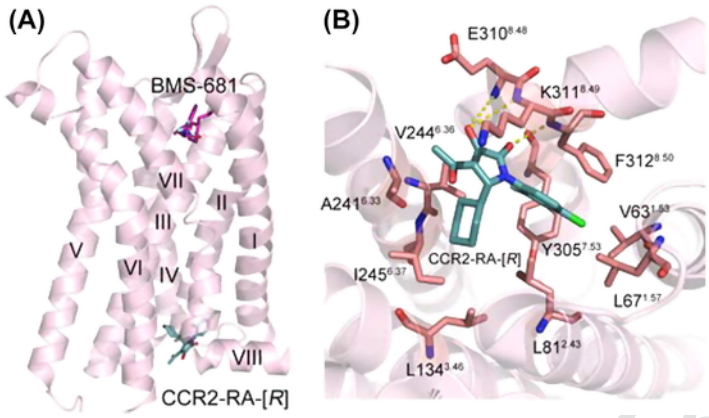

FIGURE 11.13 Cocrystal structure of CCR2 in a ternary complex with orthosteric antagonist BMS-681 and NAM CCR2-RA-[R] (see Fig. 11.12). (A) CCR2-RA-[R] occupies an allosteric site on the intracellular side, which is more than 30 Å away from the orthosteric site on the extracellular side where BMS-681 is bound. The allosteric site of CCR2-RA-[R] is formed by residues from the intracellular ends of helices I−III and VI−VIII. (B) In the allosteric site, the hydroxyl group of CCR2-RA-[R] forms a bifurcated hydrogen bond with the backbone amines of Glu310$^{8.48}$ and Lys311$^{8.49}$, and the pyrrolone carbonyl group is hydrogen-bonded to the backbone amide of Phe312$^{8.50}$. For the hydrophobic interactions, the phenyl group contacts with Val63$^{1.53}$, Leu67$^{1.57}$, Leu81$^{2.43}$, Tyr305$^{7.53}$, and Phe312$^{8.50}$, and the cyclohexane ring interacts with Leu81$^{2.43}$, Leu134$^{3.46}$, Ala241$^{6.33}$, Val244$^{6.36}$, and Ile245$^{6.37}$ (PDB code 5T1A). *Credit: Adapted with permission from J. Med. Chem. 2019, https://doi.org/10.1021/acs.jmedchem.7b01844. Copyright (2019) American Chemical Society.*

atopic dermatitis, cancer, and mosquito-borne tropical diseases. Representative indazole sulfonamide **8** (Fig. 11.12) is a potent antagonist with a good PK profile that binds to the intracellular allosteric binding site of the CCR4 and has been selected for further development (Procopiou et al., 2013). Subsequent studies suggest there are two additional allosteric sites to which small molecules bind.

CCR5 subtype is widely implicated in the process of HIV type 1 infection (Maeda et al., 2012) and has been validated as a drug target with the FDA approval of maraviroc (Fig. 11.12) as an anti-HIV agent. The cocrystal complex of CCR5 and maraviroc demonstrates that the allosteric ligand binds to the receptor occupying an extracellular site of the 7TM (Fig. 11.14) (Tan et al., 2013b). Recently, the first probe dependent CCR5 PAMs have been discovered based on 2-benzazepine, bipyridine, and terpyridine scaffolds (Thum et al., 2017). The identification of probe dependent CCR5 PAM and NAM ligands will broaden the biological knowledge of chemokine signaling and the therapeutic relevance.

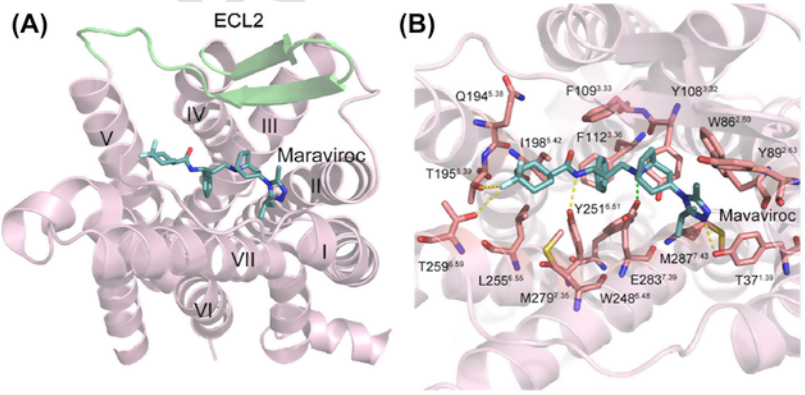

FIGURE 11.14 Cocrystal structure of CCR5 in complex with NAM maraviroc (see Fig. 11.12). (A) Maraviroc occupies an extracellular cavity on top of the 7TM from the helices I−III and V−VII and does not interact with the ECL2, which is among the major binding determinants for CCR5 chemokine agonists. (B) In the binding site, Glu283$^{7.39}$ forms a salt bridge with the protonated nitrogen of the tropane group of maraviroc. Tyr251$^{6.51}$ forms a hydrogen bond with the carboxamide nitrogen of the ligand. Tyr37$^{1.39}$ forms a hydrogen bond with the amine of the triazole group of the ligand. Thr195$^{5.39}$ and Thr259$^{6.59}$ are involved in a hydrogen bond with one of the fluorines in the cyclohexane ring of the ligand. For the hydrophobic interactions, the cyclohexane ring of the ligand is located inside the hydrophobic pocket formed by Gln194$^{5.38}$, Ile198$^{5.42}$, Leu255$^{6.55}$, and Met279$^{7.35}$; the phenyl group protrudes into a deep hydrophobic cleft formed by a cluster of aromatic residues, Tyr108$^{3.32}$, Phe109$^{3.33}$, Phe112$^{3.36}$, Trp248$^{6.48}$, and Tyr251$^{6.51}$; and the triazole group is engaged in hydrophobic interactions with Trp86$^{2.60}$, Tyr89$^{2.63}$, and Met287$^{7.43}$ (PDB code 4MBS). *Credit: Reprinted with permission from J. Med. Chem. 2019, https://doi.org/10.1021/acs.jmedchem.7b01844. Copyright (2019) American Chemical Society.*

CCR9 subtype is a member of the CC chemokine receptor subfamily implicated in inflammatory bowel disease. Vercirnon (GSK1605786A, Fig. 11.12) is a selective allosteric antagonist of CCR9 that entered phase III clinical trials for the treatment of Crohn disease, but the study was terminated due to the lack of efficacy (Wendt and Keshav, 2015). The cocrystal structure of CCR9 with vercirnon shows that the allosteric ligand binds to the intracellular side of the receptor, which is similar to that of CCR2-RA-[R] bound to CCR2 (Fig. 11.15) (Oswald et al., 2016).

CXCR subfamily. CXCR1 subtype is largely expressed on T lymphocytes and natural killer cells, playing a key role in acute and chronic inflammatory conditions (Allegretti et al., 2016). Reparixin (Fig. 11.12) is a noncompetitive NAM for CXCR1 that binds the receptor at an allosteric site between helices I, III, and VI and has been advanced into a phase III clinical trial for pancreatic islet autotransplantation (Bertini et al., 2004). Also, a phase I study is ongoing to evaluate the safety of orally administered reparixin in combination with paclitaxel in HER 2-negative metastatic breast cancer patients. Ladarixin (Fig. 11.12) is a highly potent allosteric inhibitor of CXCR1/CXCR2 that has been advanced into clinical trials for type 1 diabetes (Allegretti et al., 2016).

CCXR3 subtype is viewed as a promising drug target for a myriad of inflammatory diseases, such as rheumatoid arthritis, multiple sclerosis, cancer, atherosclerosis, and allograft rejection. Azaquinazolinone derivatives such as representative compound BD103 (Fig. 11.12) have been characterized as promising AMs of the CXCR3 endowed with signaling bias and probe dependence (Bernat et al., 2015; Brox et al., 2018).

CXCR4 subtype is a chemokine receptor essential for hematopoietic stem cell colonization of fetal bone marrow during development (Allegretti et al., 2016). Interestingly, plerixafor (Fig. 11.12), an NAM of CXCR4 with tetraazacyclotetradecane scaffold, 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 lymphoma or multiple myeloma (Miller et al., 2018).

11.2.10 Opioid receptors (μ-OR, δ-OR, and κ-OR)

Opioid GPCRs are widely distributed in the brain, the spinal cord, peripheral neurons, and the digestive tract. Opioid receptors (ORs), especially the μ-OR subtype, have been targeted for the treatment of pain and related disorders for thousands of years, and remain the most widely used analgesics in the clinic (Al-Hasani and Bruchas, 2011). Although μ-OR activation produces profound analgesia, tolerance develops for opioid drugs and addiction can be severely problematic (Morgan and Christie, 2011). Additionally, side effects such as respiratory depression, nausea, constipation, and others have highlighted the urgent need for novel OR therapeutics that have a safer profile without the loss of

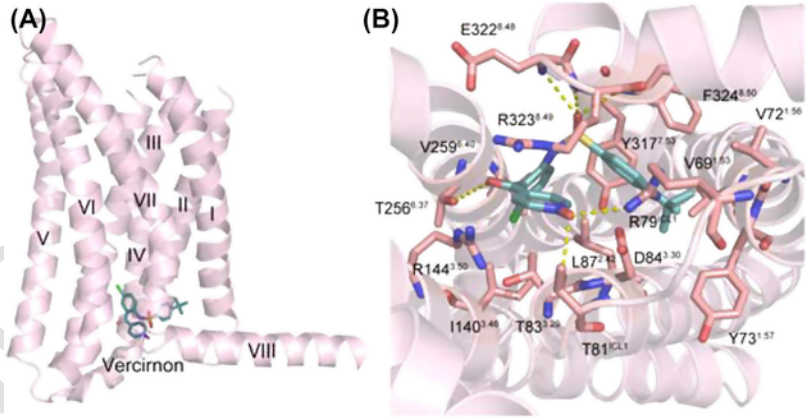

FIGURE 11.15 Cocrystal structure of CCR9 in complex with allosteric antagonist vercirnon (see Fig. 11.12). (A) Vercirnon binds to the intracellular side of the CCR9, which is approximately 33 Å away from the orthosteric site. The allosteric site of vercirnon is formed by residues from the intracellular ends of helices I–III and VI–VIII. (B) In the allosteric binding site, the sulfone group of vercirnon is engaged with hydrogen bonding interactions with the backbone amine of Glu322$^{8.48}$, Arg323$^{8.49}$, and Phe324$^{8.50}$. The pyridine-*N*-oxide group protrudes toward the intracellular face of the CCR9 and forms a bifurcated hydrogen bond with the side chains of Arg78^{ICL1} and Thr81^{ICL1}. The ketone group is hydrogen bonded to the side chain of Thr256$^{6.37}$. For the hydrophobic interactions, the *tert*-butylphenyl group makes numerous contacts with the hydrophobic cleft formed by Val69$^{1.53}$, Val72$^{1.56}$, Tyr731.57, Leu87$^{2.42}$, Tyr317$^{7.53}$, and Phe324$^{8.50}$. The chlorophenyl group is located in a narrow, hydrophobic cleft surrounded by residues Leu87$^{2.43}$, Ile140$^{3.46}$, Val259$^{6.40}$, and Tyr317$^{7.53}$, whereas the pyridine-*N*-oxide group is located in a polar cavity surrounded by residues Thr83$^{2.39}$, Asp84$^{2.40}$, Arg144$^{3.50}$, Arg323$^{8.49}$, and Thr81^{ICL1} (PDB code 5LWE). *Credit: Adapted with permission from J. Med. Chem. 2019, https://doi.org/10.1021/acs.jmedchem.7b01844. Copyright (2019) American Chemical Society.*

efficacious analgesia. AMs may be suitable in this scenario, as they have a generally safer profile, owing to the ceiling effect (Remesic et al., 2017). Both NAMs and PAMs have been explored for ORs, and while most work on allosteric modulation has been directed toward the μ-OR, a few ligands have been reported as hits for the δ-OR (Remesic et al., 2017).

Cannabidiol and salvinorin-A are among the earliest identified NAMs for the μ-OR and δ-OR (Kathmann et al., 2006; Remesic et al., 2017). Recently, BMS-986121 and BMS-986122 (Fig. 11.16) were identified via an HTS as the first selective PAMs for the μ-OR (Burford et al., 2013, 2015). Subsequent chemical SAR study around the latter led to the discovery of BMS-986124 (Fig. 11.16) as an SAM (Burford et al., 2013, 2015). Additionally, diterpene alkaloid ignavine (Fig. 11.16) has shown positive modulatory activity for μ-OR and an analgesic effect in vivo (Ohbuchi et al., 2016).

BMS-986187 (Fig. 11.16), with a chemically novel core compared to previous BMS series, was discovered as an effective PAM at the δ-OR and at the κ-OR rather than the μ-OR (Livingston et al., 2018).

Allosteric modulation of ORs holds promise of delivering safer analgesics and other therapeutics to the clinic; however significant optimization and development are still needed.

11.2.11 Proteinase-activated PAR2 receptor

PAR2 belongs to protease-activated receptors (PARs), a unique family of GPCRs that are not activated by endogenous ligands but rather activated by the cleavage of their extracellular amino terminus at a specific site by proteases (Adams et al., 2011). The newly formed *N*-terminus, also called a tethered ligand, subsequently binds to the receptor to initiate receptor activation.

PAR2 has recently been recognized as an important modulator of coagulation and inflammatory responses (Ramachandran et al., 2012). AZ3451 (Fig. 11.16) is a small-molecule allosteric antagonist of PAR2. The cocrystal structure of PAR2 bound to AZ3451 shows that the ligand binds to a remote allosteric site outside the helical bundle (Fig. 11.17), which is distinct from the orthosteric site of antagonist AZ8838 located near the extracellular surface (Cheng et al., 2017).

11.3. ALLOSTERIC MODULATORS TARGETING CLASS B GPCRS

Class B GPCRs, also known as the secretin family, are a subfamily of receptors that bind paracrine or endocrine peptide hormones involved in the physiology and pathophysiology of major functions, and represent targets of particular interest for the treatment of diseases such as type 2 diabetes mellitus, osteoporosis, hypercalcaemia, Paget disease, migraine, depression, anxiety, Crohn disease, and cancer. Historically, the discovery and development of small-molecule orthosteric ligands for class B receptors have proven to be challenging (Bortolato et al., 2014). More recently, the search for AMs has become an intense field of research, as it represents an attractive strategy for the development of novel agents as an alternative to peptides in clinical use or as first-generation therapeutics. The calcitonin (CT), corticotropin-releasing factor (CRF), vasoactive intestinal peptide (VIP), pituitary adenylate cyclase-activating polypeptide (PACAP), and glucagon (GCG) receptor families have been allosterically targeted, and their most relevant AMs will be reviewed in this section.

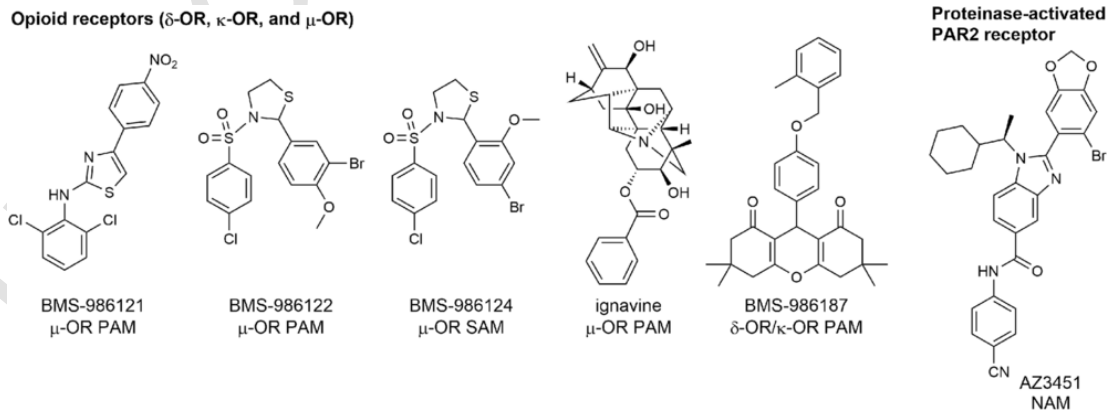

FIGURE 11.16 Representative allosteric ligands targeting protein opioid and proteinase-activated GPCRs of class A.

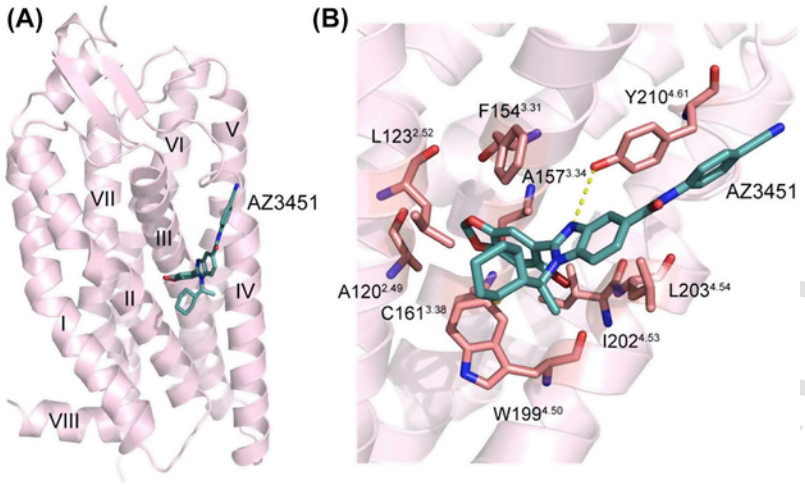

FIGURE 11.17 Cocrystal structure of PAR2 in complex with allosteric antagonist AZ3451 (see Fig. 11.16). (A) The allosteric AZ3451 binding site of PAR2 is located in the extracellular side, outside the 7TM, and lined by residues from helices II−IV. (B) The only hydrogen bond in the allosteric binding site is formed between the benzimidazole nitrogen of AZ3451 and the side chain of Tyr210$^{4.61}$. For the hydrophobic interactions, the central benzimidazole moiety of AZ3451 engages in a hydrophobic interaction with Leu203$^{4.54}$. The 1,3-benzodioxole ring is located inside a hydrophobic pocket, which is defined by residues Ala120$^{2.49}$, Leu123$^{2.52}$, Phe154$^{3.31}$, Ala157$^{3.34}$, Cys161$^{3.38}$, Trp199$^{4.50}$, and Ile202$^{4.53}$. The cyclohexyl ring protrudes into the binding site and is positioned between the interface of the lipid layer and the receptor, which contacts with Leu123$^{2.52}$. The benzonitrile group with the cyclohexyl ring also sits at the interface of the lipid layer and the receptor and forms an aromatic−aromatic interaction with Tyr210$^{4.61}$ (PDB code 5NDZ). *Credit: Reprinted with permission from J. Med. Chem. 2019, https://doi.org/10.1021/acs.jmedchem.7b01844. Copyright (2019) American Chemical Society.*

11.3.1 Calcitonin receptors (CT and CGRPRs)

The CT family of peptides includes CT, amylin, CT gene-related peptide (CGRP), and adrenomedullin, and their receptors have therapeutic relevance for many pathologies, such as cardiovascular disease, migraine, osteoporosis, diabetes, obesity, and lymphatic insufficiency (Poyner et al., 2002).

CTR. Agonists of the CT receptor (CTR) are of interest as biological tools and therapeutic agents in the treatment of bone-related disorders such as osteoporosis and hypercalcaemia of malignancy, since CT peptide is involved in maintenance of calcium homeostasis, mainly with respect to bone formation and metabolism. Few nonpeptide ligands have been reported for CTR and among them, only a series of pyrazolopyridine derivatives has been deeply studied to define the molecular mechanism of action (Boros et al., 2005; Dong et al., 2009). These ligands, represented by compound **9** (Fig. 11.18), weakly potentiated the cAMP production of the endogenous ligand and exhibited incomplete inhibition of [^{125}I] labeled CT, results that contributed to propose an allosteric mode of action. Importantly, mutagenesis and chimeric studies supported that these molecules bind in TM1 and the extracellular domain of the receptor, a distinct site of action relative to that of CT. These results represented the first identification of the juxtamembranous region of the amino-terminal domain of the CTR as a critical site for allosteric drug action at class B GPCRs.

CGRPR. The CGRP is one of the most potent endogenous vasodilators and has been implicated in the pathogenesis of migraine headache, which is directly related to dilatation of cranial vessels and activation of the trigemino-vascular system (Brain et al., 2002). Therefore, blocking CGRP receptors (CGRPRs) has been suggested as a potential antimigraine strategy. CGRPRs are heterodimeric complexes composed by the CT receptor-like receptor (CLR) and the receptor activity modifying protein 1 (RAMP1) (Mclatchie et al., 1998). Although the complex structural nature of the CGRPR has hampered the research, efforts within the pharmaceutical industry have led to the identification of small-molecule inhibitors, which have turned out to act as AMs. Olcegepant (BIBN4096BS, Fig. 11.18) was the first potent and selective CGRPR antagonist reported. It was developed from a series of dipeptide-like compounds identified by HTS (Doods et al., 2000). Olcegepant inhibited induced neurogenic vasodilation in human brain vessels (Moreno et al., 2002) and its efficacy in the acute treatment of migraine was confirmed (Olesen et al., 2004). Telcagepant (MK-0974, Fig. 11.18) (Salvatore et al., 2008) is another highly selective nonpeptide antagonist that displayed good oral bioavailability. This molecule resulted from a lead optimization process of a benzodiazepine CGRPR antagonist identified by HTS. In

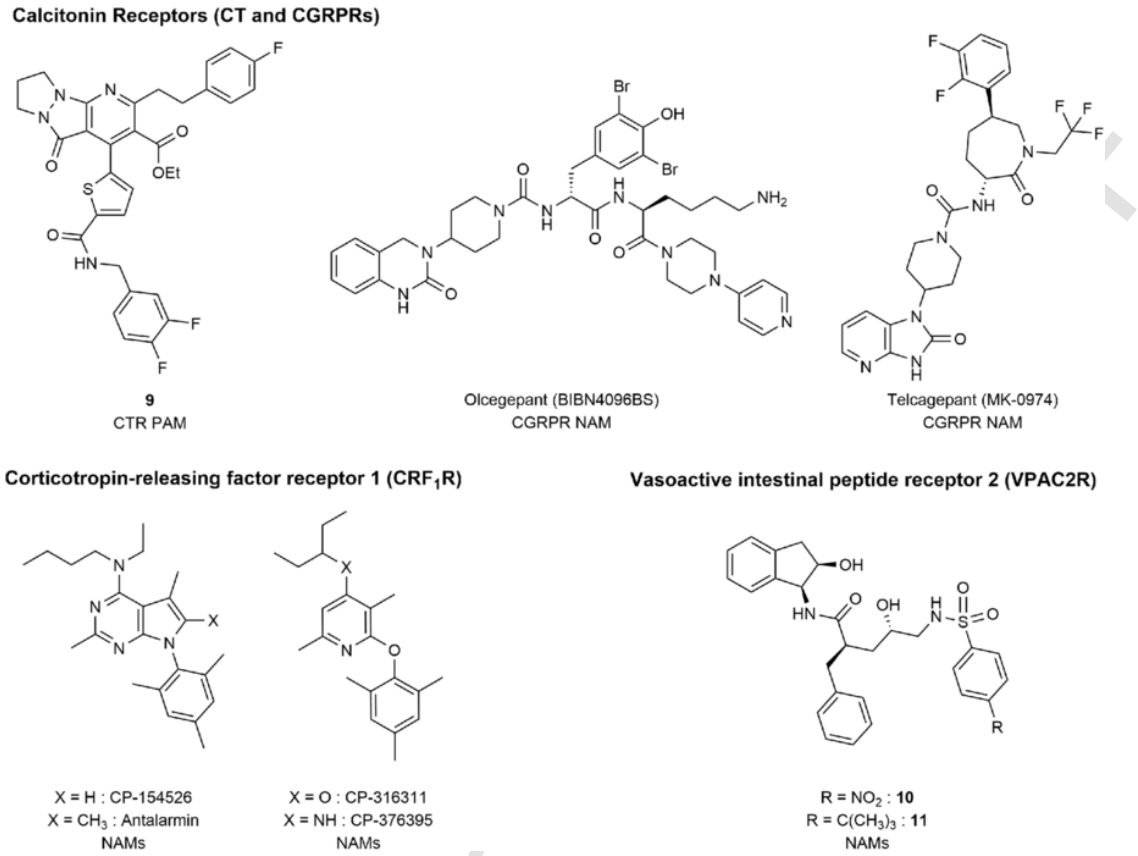

FIGURE 11.18 Representative allosteric ligands targeting calcitonin, corticotropin-releasing factor, and vasoactive intestinal peptide GPCRs of class B.

vivo evaluation of telcagepant showed a concentration-dependent inhibition of dermal vasodilation, revealing its value in the acute treatment of migraine (Ho et al., 2008).

In-depth research with olcegepant and telcagepant suggested an allosteric mechanism of antagonism as their plausible mode of action (Hay et al., 2006; Miller et al., 2010). Both the CLR and RAMP1 components of the CGRPR have extracellular domains, which interact with each other and together form part of the peptide-binding site. The small-molecule binding site was suggested to be located on these extracellular domains. Mutagenic screen with CLR and RAMP1 indicated that olcegepant and telcagepant share a similar binding site and occupy a region close to the interface of the *N*-terminal domains of CLR and RAMP1.

11.3.2 Corticotropin-releasing factor receptor 1 (CRF_1R)

The corticotropin-releasing factor receptors (CRF_1 and CRF_2R) are activated by the endogenous CRF peptide and the related urocortin peptides, which are known to play important biological roles in the regulation of central and peripheral stress responses.

It is well established that CRF_1R antagonism represents an interesting mechanism for treating anxiety, depression, and other stress-related disorders (Holsboer, 2001; Smagin et al., 2001), and allosteric modulation of this receptor has proven to be a valuable approach for therapeutic intervention (Hoare, 2007). To date, many small-molecule, selective, and high-affinity CRF_1R ligands have been characterized as allosteric antagonists. CP-154526 (Schulz et al., 1996) and antalarmin (Fig. 11.18) (Webster et al., 1996) were the first modulators reported as effective blockers of the neuroendocrine effects and behavioral changes induced by CRF. Since then, as a result of HTS campaigns and subsequent medicinal chemistry optimization programs, several compounds have been disclosed as selective CRF_1R AMs and have reached different stages of clinical evaluation, aimed at testing the hypothesis that CRF_1 inhibitors could be used clinically as antidepressant drugs (Kehne and Cain, 2010).

Among a series of 2-aryloxy-4-alkoxypyridines derived from CP-154526 and developed by Pfizer, CP-316311 (Fig. 11.18) stood out as a potent and selective allosteric antagonist with oral efficacy in the CNS, which advanced to phase II depression trials. Further optimization of these pyridine-based AMs addressing physicochemical properties led to CP-376395 (Fig. 11.18) (Chen et al., 2008), which displayed improved potency over CP-316311 in relevant in vivo models. CP-376395 was selected as backup candidate to CP-316311, entering also into clinical evaluation.

All these CRF_1R agents have been described as NAMs rather than as competitive CRF_1R antagonists. They keep the receptor in an inactive conformation, and peptide and small-molecule ligands that bind to different conformational states of the receptor do not compete for the same binding region (Chen et al., 2008; Grigoriadis et al., 2009; Hauger et al., 2006). This binding mode has been confirmed by the crystal structure of CRF_1R in complex with CP-376395 (Figs 11.18 and 11.19) (Hollenstein et al., 2013). The structure shows that the antagonist binds to an allosteric site very deep within the 7TM toward the intracellular face of the receptor, inhibiting the binding and signaling of peptide-agonist ligands. Importantly, this cocrystal structure of CRF_1R has provided a model for class B GPCRs, revealing an unanticipated antagonist-binding site and enabling future structure-based small-molecule drug discovery approaches.

11.3.3 Vasoactive intestinal peptide receptor 2 ($VPAC_2R$)

The $VPAC_1$, $VPAC_2$, and PAC_1 receptors ($VPAC_1$, $VPAC_2$, and PAC_1Rs), mediated by neuropeptides VIP and PACAP, have emerged as potentially useful therapeutic targets for inflammatory, metabolic, or circadian functions (Dickson and Finlayson, 2009; Sherwood et al., 2000). They represent a clear example where the development of nonpeptide ligands is of high interest since the endogenous agonists are not practical as therapeutics due to their short half-lives and lack of oral bioavailability and brain penetration. However, small-molecule drug discovery for these class B GPCRs has been certainly difficult. Compound **10** (Fig. 11.18) was the first specific antagonist reported for human $VPAC_2R$ (Chu et al., 2010), and it was discovered via HTS of a 1.67 million-compound collection. This ligand is a specific NAM that noncompetitively antagonizes $VPAC_2R$-mediated cAMP accumulation and β-arrestin2 binding, with no activity on $VPAC_1R$ or PAC_1R. A structural similarity search starting from **10** yielded structurally related $VPAC_2R$ NAM **11** (Fig. 11.18), which also displayed some activity for $VPAC_1R$ and PAC_1R. β–arrestin assays using chimera receptors indicated that both compounds bind to an allosteric site in the 7TM of the receptor as opposed to the *N*-terminal extracellular domain, where the endogenous ligand binds. The fact that compounds **10** and **11** are the only $VPAC_2R$ NAMs known to date makes them a valuable tool for further study of $VPAC_2R$ and related receptors.

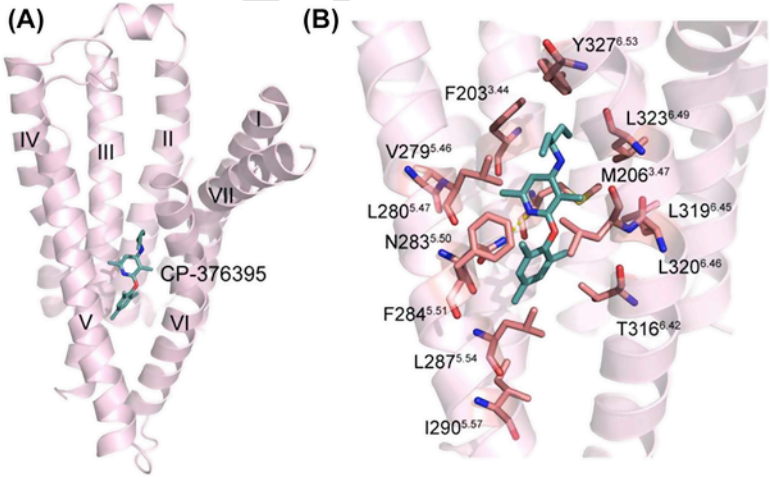

FIGURE 11.19 Cocrystal structure of CRF_1R in complex with allosteric antagonist CP-376395 (see Fig. 11.18). (A) The allosteric CP-376395 binding site of CRF_1R is located in the intracellular half of the receptor, which is approximately 18 Å away from the orthosteric peptide agonist-binding site located at the center of the large cavity presented to the extracellular side of the receptor. (B) CP-376395 binds in a deep site created by residues from helices III, V, and VI. The pyridine nitrogen of CP-376395 in the allosteric binding site is involved in a hydrogen bond with the side chain of $Asn283^{5.50}$. For the hydrophobic interactions, the aryloxy moiety is located inside a hydrophobic pocket created by residues $Phe284^{5.51}$, $Leu287^{5.54}$, $Ile290^{5.57}$, $Thr316^{6.42}$, $Leu319^{6.45}$, and $Leu320^{6.46}$. The central pyridine ring forms hydrophobic interactions with $Met206^{3.47}$ and $Val279^{5.46}$. The exocyclic alkylamino group binds in a hydrophobic pocket defined by residues $Phe203^{3.44}$, $Leu280^{5.47}$, $Leu323^{6.49}$, and $Tyr327^{6.53}$ (PDB code 4K5Y). *Credit: Reprinted with permission from J. Med. Chem. 2019, https://doi.org/10.1021/acs.jmedchem.7b01844. Copyright (2019) American Chemical Society.*

11.3.4 Glucagon receptors (GCG and GLPRs)

GCGR. The GCGR is considered an important drug target in the treatment of diabetes due to the key role of its endogenous ligand in glucose homeostasis (Ahren, 2015). Despite many small-molecule GCGR antagonists from multiple chemical classes being discovered, detailed studies about the nature of their inhibition mechanism are very scarce. Among early disclosed antagonists, compound L-168049 (Fig. 11.20) was characterized as a noncompetitive antagonist. This pyrrole derivative was identified from novel structural classes of nonpeptidyl ligands based on triarylimidazole and triarylpyrrole scaffolds that showed significant binding affinity for the human GCGR (Cascieri et al., 1999). L-168049 behaves as a high affinity and potent NAM that affects both affinity and activity of GCG. Site-directed mutagenesis experiments indicated that the binding site of L-168049 is located within the 7TM, while the endogenous peptide binds to the amino-terminal domain. Unfortunately, L-168049 displayed poor affinity for the different species orthologs of GCGR, hampering its testing in preclinical animal models of disease.

NNC 25-2504 (Fig. 11.20) is another highly potent noncompetitive antagonist. This alkylidene hydrazide was found to inhibit GCG-stimulated glucose production in isolated primary rat hepatocytes and to suppress exogenous GCG-induced glucose increase in blood. NNC 25-2504 was orally bioavailable in dogs (Madsen et al., 2002).

MK-0893 (Fig. 11.20) (Xiong et al., 2012) was the first AM of GCGR entering clinical trials, and advanced to phase IIa in diabetic patients. This NAM displayed good potency and selectivity profile, and was developed from an optimization effort around a series of pyrazole-based antagonists, in which the *N*-heterocycle was found to be a good replacement of a urea core present in a previously identified GCGR competitive antagonist (Lau et al., 2007). The cocrystal structure of the complex GCGR-MK-0893 has been recently published (Fig 11.21) (Jazayeri et al., 2016). The structure shows an unexpected binding site for the AM, located outside the 7TM, in a position between TM6 and TM7 and extending into the lipid bilayer, in contrast with the TM-inner site described for the CRF_1R AM CP-376395 (Fig. 11.19). The role of the key residues of the allosteric site identified in the X-ray structure was confirmed by mutagenesis.

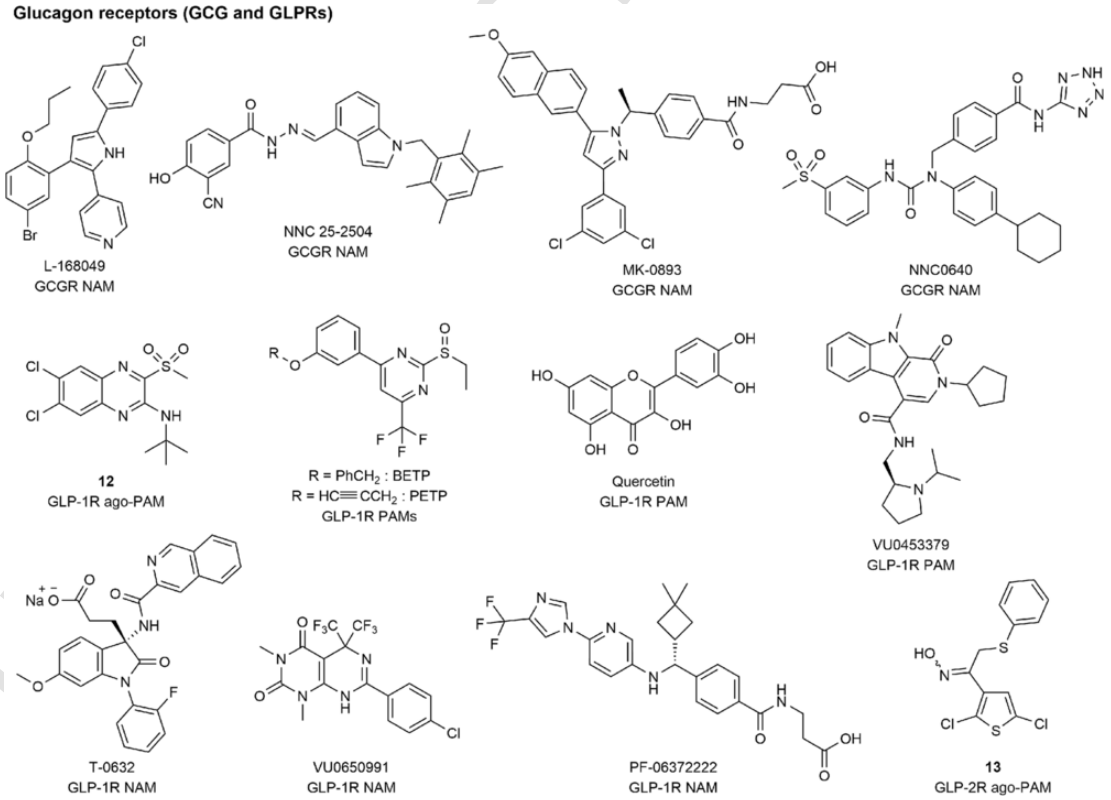

FIGURE 11.20 Representative allosteric ligands targeting glucagon GPCRs of class B.

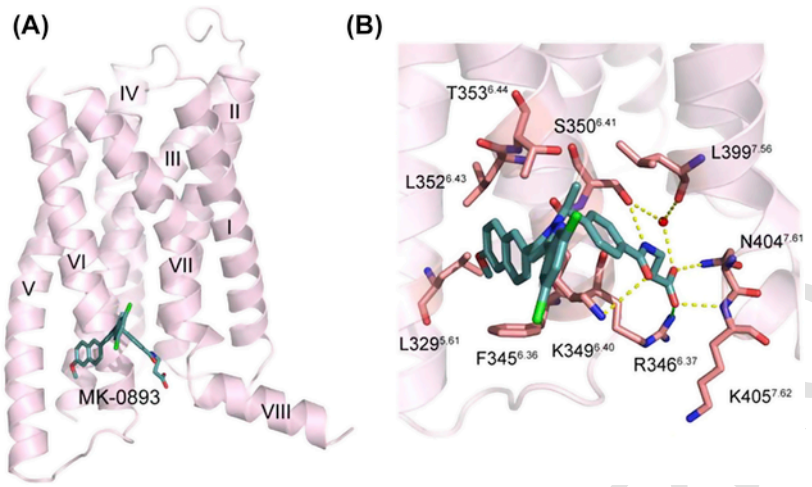

FIGURE 11.21 Cocrystal structure of GCGR in complex with allosteric antagonist MK-0893 (see Fig. 11.20). (A) MK-0893 binds to an allosteric site outside the 7TM between helices VI and VII, extending into the lipid bilayer. (B) For polar interactions in the allosteric binding site, the terminal anionic carboxylic acid group of MK-0893 forms a salt bridge with Arg346$^{6.37}$, hydrogen bonds with the side chain of Asn404$^{7.61}$ and the backbone amine of Lys405$^{7.62}$, and a water-mediated hydrogen bond with the side chain of Ser350$^{6.44}$ and the backbone carbonyl of Leu399$^{7.56}$. In addition, the amide group of the ligand engages in hydrogen bonding interactions with the side chains of Lys349$^{6.40}$ and Ser350$^{6.41}$. For hydrophobic interactions, the methoxynaphthalene moiety forms numerous hydrophobic interactions with residues Leu329$^{5.61}$, Phe345$^{6.36}$, Leu352$^{6.43}$, Thr353$^{6.44}$, and the alkyl chain of Lys349$^{6.40}$. The benzylpyrazole core forms hydrophobic interactions with residues Thr353$^{6.44}$ and Leu399$^{7.56}$ and a π−cation interaction with Lys349$^{6.40}$ (PDB code 5EE7). *Credit: Reprinted with permission from J. Med. Chem. 2019, https://doi.org/10.1021/acs.jmedchem.7b01844. Copyright (2019) American Chemical Society.*

The exact antagonist mechanism of MK-0893 remains unclear, but it has been suggested that the activation of the receptor is hampered by restriction of the outward helical movement of TM6 required for G-protein coupling activation.

Competition binding assays with [^{3}H]MK-0893 have been used to characterize the binding of previously reported GCGR antagonists, chemically related or totally distinct to MK-0893. In this context, NAM NNC0640 (Fig. 11.20) (Lau et al., 2007) was fully displaced by [^{3}H]MK-0893, indicating a common allosteric binding site (Jazayeri et al., 2016). Interestingly, the cocrystal structure of the full-length human GCGR in an inactive conformation in complex with NNC0640 and an antigen-binding fragment of an inhibitory antibody has been recently published (Zhang et al., 2017). This new structure has shown that NAM NNC0640 occupies a region on the external surface of the 7TM of GCGR and binds in a site similar to that reported for MK-0893, which confirms previous radioligand binding assay results.

GLP-1R. Class B GLP-1R is activated by several endogenous GLP-1 peptide hormones and represents a key target for type 2 diabetes mellitus, where it has a critical role in the potentiation of insulin secretion and suppression of GCG secretion (Graaf et al., 2016). To date, several peptide GLP-1R agonists have demonstrated their utility for the treatment of obesity and type 2 diabetes mellitus and have successfully reached the market (Tomlinson et al., 2016). However, the discovery of nonpeptide small-molecule drugs remains highly desirable, since they may constitute a significant advance due to their enhanced bioavailability, particularly oral absorption. In this sense, a number of nonpeptide GLP-1R agonists have been reported and, interestingly, most of them behave as AMs that do not compete with GLP-1 in the orthosteric site. Starting from a quinoxaline scaffold, a hit identified from a functional screening, Novo Nordisk developed the first GLP-1R PAMs (Knudsen et al., 2007). Among them, compound **12** (Fig. 11.20) was the most potent agonist found and has been extensively characterized. It acts as an ago-PAM, potentiating GLP-1-induced receptor activation in the presence of GLP-1, and it is selective over other class B GPCRs. Interestingly, this PAM displays probe dependence for the effects on peptide agonists of GLP-1R, such as exendin or extended GLP-1 peptides (Willard et al., 2012; Wootten et al., 2013b). Compound **12** was capable of releasing glucose-dependent insulin from wild-type mouse islets, but not from GLP-1R knockout mice. However, its poor PK properties precluded clinical development.

BETP (Fig. 11.20) is another remarkable GLP-1R PAM (Sloop et al., 2010). This compound was obtained by structural modifications of a pyrimidine hit, which was identified from a small library generated from a three-dimensional pharmacophore model. Despite key structural differences, BETP possesses a very similar biological profile in terms of activation of receptor signaling and probe-dependence to that of compound **12**.

An interesting aspect of both compound **12** and BEPT is their high electrophilic character, which allows them to establish covalent interactions with free cysteine residues of the receptor (Eng et al., 2013; Nolte et al., 2014). This

covalent mechanism of action was studied using a clickable analog of BETP named PETP (Fig. 11.20). Thus, Western blot analysis of GLP-1R protein purified from CHO-K1 cells treated with PETP and subjected to click chemistry using biotin-azide demonstrated the incorporation of the probe into GLP-1R, confirming the covalent modification of the receptor. Additionally, mass-spectrometry-based proteomics of GLP-1R from cells treated with BEPT and subsequent mutagenesis studies indicated that cysteine residue Cys347 was the specific residue covalently modified by the PAM. In fact, adduct formed with Cys347 is critical for both the intrinsic efficacy and the cooperative allosteric effect. However, this covalent modification is not enough to explain the differences in intrinsic efficacy and the cooperative effect of compound **12** and BEPT.

Nonelectrophilic small-molecule ligands have been also reported to act as GLP-1R modulators. Among them, a series of flavonoids represented by quercetin (Fig. 11.20) has been characterized as AMs that positively modulated affinity and efficacy of GLP-1R peptide ligands, lacking intrinsic activity (Wootten et al., 2011). Quercetin displayed functional selectivity and enhanced calcium signaling of endogenous truncated GLP-1 peptides and exendin, but not cAMP production. However, polypharmacology and a flat SAR precluded further development of this family of compounds (Perry et al., 2002).

VU0453379 (Fig. 11.20) is a CNS penetrant GLP-1R modulator (Morris et al., 2014) that represents an opportunity for the assessment of the therapeutic potential of GLP-1 potentiation in the CNS, where the receptor has been linked to neuroprotection, learning, neurogenesis, and memory (During et al., 2003; Perry et al., 2002). VU0453379 lacks probe dependence, potentiating endogenous GLP-1 as well as synthetic peptide agonists exenatide and liraglutide. Importantly, the compound exhibited efficacy in a haloperidol-induced catalepsy model, a preclinical model of Parkinson disease, and current efforts are focused on improving both allosteric potency and PK profile to confirm Parkinson disease as an exciting indication for GLP-1R PAMs.

Vivia Biotech has also contributed to the field of GLP-1R modulators with the identification of a series of oxadiazole derivatives able to act as PAMs, enhancing the efficacy of GLP-1R stimulation dose-dependently and potentiating insulin secretion (Rodriguez De Fonseca et al., 2012).

Although the main therapeutic strategy targeting GLP-1R has been the development of agonists for the treatment of hyperglycemia, recent studies have revealed that antagonism of GLP-1R also plays an important role in the treatment of acute and chronic stress as well as anxiety. The number of small-molecule GLP-1R antagonists identified to date is scarce. T-0362 (Fig. 11.20), one of the first nonpeptide GLP-1R ligands disclosed, was described as an NAM, which binds to GLP-1R, blocking GLP-1-induced cAMP production (Tibaduiza et al., 2001).

More recently, the first CNS penetrant and orally bioavailable NAM VU0650991 (Fig. 11.20) has been reported (Nance et al., 2017). Molecular pharmacology assays showed that VU0650991 did not display probe dependence, affording similar inhibitory activity for the endogenous agonist GLP-1 (7−36) amide and the synthetic agonist exendin-4. VU0650991 reduced blood insulin levels while increasing blood glucose levels in rats upon oral dosing, validating the role of GLP-1R antagonism in vivo.

Crystal structures of the human GLP-1R were obtained in complex with the two nonselective NAMs PF-06372222 (Figs. 11.20 and 11.22) and NNC0640 (Figs. 11.20 and 11.23) (Song et al., 2017). These compounds had previously been optimized as GCGR antagonists and, in fact, NNC0640 was reported as a GCGR NAM and its crystal structure in complex with the GCGR was resolved (Zhang et al., 2017). The crystal structures have shown that both NAMs bind to the same pocket located outside helices V–VII proximal to the intracellular half of the GLP-1R, similar to the site occupied by MK-0893 in the crystal structure of the GCGR (Fig. 11.21).

Regarding the GLP-2R, this receptor subtype is an interesting therapeutic target for intestinal damage. In fact, a peptide GLP-2R agonist, teduglutide, is under clinical development for short bowel syndrome and Crohn disease. However, unlike the case of GLP-1R, there is only one report on compounds having allosteric activity toward human GLP-2R. In that work, a series of thiophene derivatives represented by compound **13** (Fig. 11.20) was characterized as ago-PAMs of different GLP-2 peptides in recombinant cells expressing the GLP-2R (Yamazaki et al., 2012). These modulators present a sulfanyl group in their structures; however, it is not clear whether this electrophilic group establishes a covalent interaction with the receptor, as described for GLP-1R PAMs BEPT and compound **12**. Overall, although these results are preliminary they may be useful for future development of small-molecule AMs of the GLP-2R.

11.4. ALLOSTERIC MODULATORS TARGETING CLASS C GPCRS

Class C GPCRs are characterized by two unique features: the large extracellular and ligand-binding *N*-terminal domain in addition to the generic 7TM, and the formation of homo- and heterodimers where orthosteric and allosteric ligands can

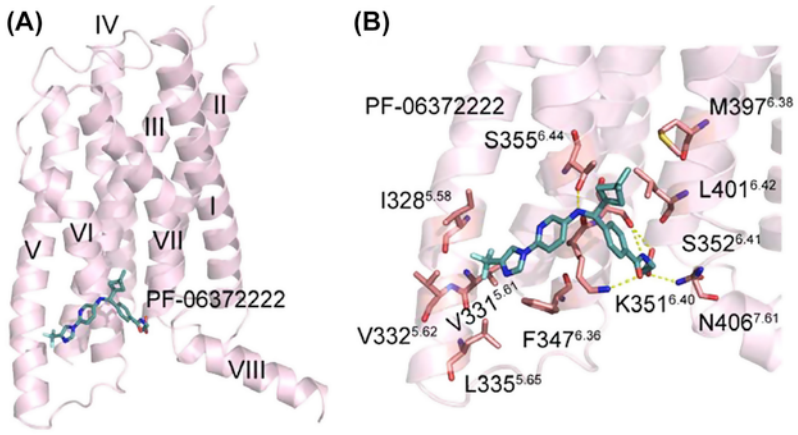

FIGURE 11.22 Cocrystal structure of GLP-1R in complex with allosteric antagonist PF-06372222 (see Fig. 11.20). (A) PF-06372222 binds to an allosteric site located outside helices V−VII proximal to the intracellular half of the GLP-1R. (B) The terminal anionic carboxylic acid group of PF-06372222 in the allosteric binding site is involved in hydrogen bonding interactions with the side chains of residues Ser352$^{6.41}$ and Asn406$^{7.61}$. The amide group of the ligand forms hydrogen bonds with the side chains of Ser352$^{6.41}$ and Lys351$^{6.40}$, and the aminopyridine moiety engages a hydrogen bond with the side chain of Ser355$^{6.44}$. For hydrophobic interactions, the trifluoromethylpyrazole moiety of the ligand is located in a hydrophobic pocket created by residues Ile328$^{5.58}$, Val331$^{5.61}$, Val332$^{5.62}$, Leu335$^{5.65}$, and Phe347$^{6.36}$. The pyridine ring creates hydrophobic contacts with the alkyl chain of Lys351$^{6.40}$, and the phenyl ring establishes hydrophobic interactions with the alkyl chain of Lys351$^{6.40}$ and Leu401$^{7.56}$. Finally, the dimethylcyclobutane group forms hydrophobic interactions with Ser355$^{6.44}$, Met397$^{6.38}$, and Leu401$^{6.42}$ and protrudes into the lipid bilayer (PDB code 5VEW). *Credit: Adapted with permission from J. Med. Chem. 2019, https://doi.org/10.1021/acs.jmedchem.7b01844. Copyright (2019) American Chemical Society.*

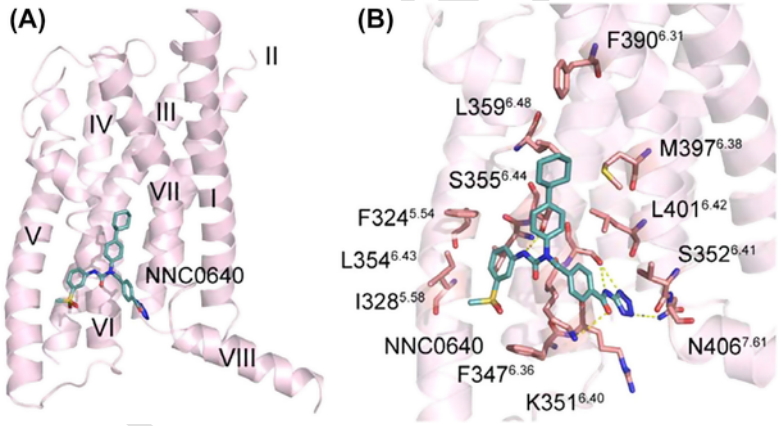

FIGURE 11.23 Cocrystal structure of GLP-1R in complex with allosteric antagonist NNC0640 (see Fig. 11.20). (A) NNC0640 binds to the same allosteric site than PF-06372222, located outside helices V−VII proximal to the intracellular half of the GLP-1R. (B) The tetrazole group of NNC0640 in the allosteric binding site forms hydrogen bonds with the side chains of Ser352$^{6.41}$ and Asn406$^{7.61}$. The amide group of the ligand forms hydrogen bonds with the side chains of Ser352$^{6.41}$ and Lys351$^{6.40}$, and the urea amine moiety of the ligand engages in a hydrogen bond with the side chain of Ser355$^{6.44}$. For hydrophobic interactions, the methylsulfonephenyl group of the ligand is located in a hydrophobic pocket defined by residues Phe324$^{5.54}$, Ile328$^{5.58}$, Phe347$^{6.36}$, Leu354$^{6.43}$ and the alkyl chain of Lys351$^{6.40}$. The tetrazolylbenzamide group creates hydrophobic contacts with the alkyl chains of Arg348$^{6.37}$ and Lys351$^{6.40}$, Leu401$^{7.56}$, and Val405$^{7.60}$. The phenylcyclohexyl group forms hydrophobic interactions with Ser355$^{6.44}$, Leu359$^{6.48}$, Phe390$^{6.31}$, Met397$^{6.38}$, and Leu401$^{6.42}$ and protrudes into the lipid bilayer (PDB code 5VEX). *Credit: Adapted with permission from J. Med. Chem. 2019, https://doi.org/10.1021/acs.jmedchem.7b01844. Copyright (2019) American Chemical Society.*

occupy the same receptor or a canonical transmode. Another important characteristic of this class of receptors is that the orthosteric binding site is not located within the 7TM.

Given the important (patho)physiological roles class C GPCRs play and their therapeutic potential, extensive drug discovery efforts have been undertaken toward the identification of small-molecule ligands (Brauner-Osborne et al., 2007). In particular, class C AM drug discovery has been fruitful, with an increasing number of selective PAMs and NAMs discovered in the last two decades for the calcium-sensing receptor (CaSR), the GPCR class C group 6 subtype A

receptor (GPRC6AR), the γ-aminobutyric acid type B receptor ($GABA_BR$), and the metabotropic glutamate receptors ($mGlu_{1\text{-}8}Rs$) (Table 11.1) (Engers and Lindsley, 2013; Leach and Gregory, 2017; Urwyler, 2011).

11.4.1 Calcium-sensing receptor (CaSR)

The CaSR is responsible for the maintenance of a stable blood Ca^{2+} concentration (Brown et al., 1993; Hofer and Brown, 2003). It controls Ca^{2+} homeostasis by modulating the secretion of parathyroid hormone in parathyroid glands, and regulating the reabsorption of Ca^{2+} in kidney and bone (Brown, 2013). The CaSR is able to activate many different signaling pathways in a ligand- and tissue-specific manner, which allows it to play crucial roles in Ca^{2+} homeostasis and in biological processes unrelated to calcium balance. Thus, the CaSR has become an important target for hyperparathyroidism, hypocalcaemia, and hypercalcaemia of malignancy but also for diseases such as cancer, Alzheimer disease, and diabetes mellitus (Ward et al., 2012).

Extracellular Ca^{2+} is the principal endogenous agonist of the CaSR and therefore mimicking it with selective drug-like molecules is not obvious. However, the activation effect of Ca^{2+} and other direct agonists can be modulated and the use of AMs, either agonists (calcimimetics) or antagonists (calcilytics), has been proposed as treatment for a variety of Ca^{2+} related diseases.

CaSR PAMs. Alkylbenzylamine NPS R568 and its dechloro-derivative NPS R467 (Fig. 11.24) were the first CaSR PAMs reported and were obtained in a derivatization program starting from calcium channel blocker fendiline (Hammerland et al., 1998; Nemeth et al., 1998). Both compounds increased the concentration of cytoplasmic Ca^{2+} and inhibited parathyroid hormone secretion in cells. In a small clinical study, oral administration of NPS R568 lowered parathyroid hormone and Ca^{2+} plasma levels in postmenopausal women with mild primary hyperparathyroidism (Silverberg et al., 1997). However, both NPS R568 and NPS R467 caused hypocalcaemia due to a calcitonin-mediated reduction in Ca^{2+} efflux from bone and activation of renal CaSRs.

Optimization of NPS R568 and NPS R467 resulted in the identification of cinacalcet (Fig. 11.24) (Nemeth et al., 2004), with an improved metabolic profile, making this compound the first GPCR AM approved by FDA. Cinacalcet is prescribed for the treatment of secondary hyperparathyroidism and hypercalcaemia in patients with parathyroid

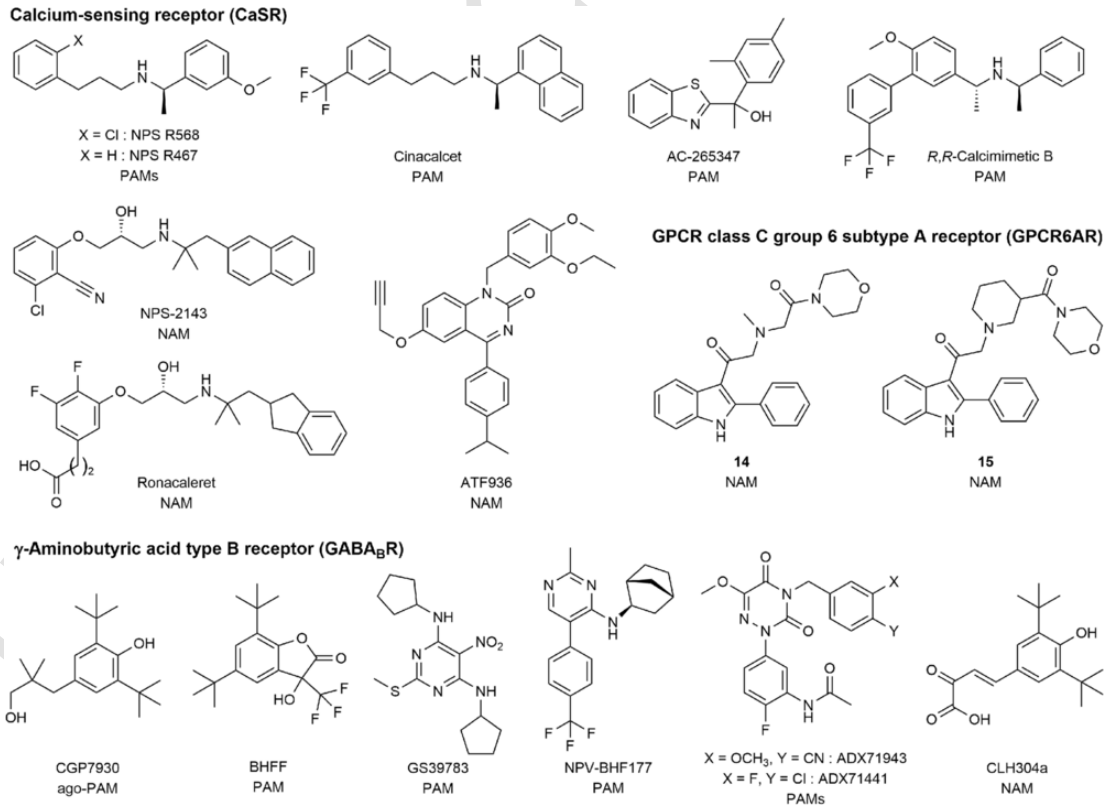

FIGURE 11.24 Representative allosteric ligands targeting calcium-sensing, group 6 subtype A, and γ-aminobutyric acid type B GPCRs of class C.

carcinoma. However, this drug also presents hypocalcaemia side effects and its use is restricted to severely affected patients.

After the launch of cinacalcet, and with the aim of improving its side effect profile, the research focused on the selective stimulation of the CaSR signaling in the parathyroid gland without affecting the CaSR in other tissues. These efforts led to the identification of two novel PAMs, AC-265347 (Ma et al., 2011) and the dibenzylamine (*R*,*R*)-calcimimetic B (Fig. 11.24) (Henley et al., 2011). Both compounds decreased parathyroid hormone release in vivo at doses that did not promote CT secretion, which resulted in nonsignificant reductions in serum Ca^{2+} levels. This activity profile has been explained by the ability of these new PAMs to bias signaling toward inositol phosphate accumulation and ERK1/2 phosphorylation, whereas alkylbenzylamine calcimimetics were biased toward allosteric modulation of Ca^{2+} immobilization and inositol phosphate accumulation (Cook et al., 2015). Therefore, these results demonstrated that it was possible to normalize serum parathyroid hormone and calcium levels by using CaSR PAMs without causing uncontrolled hypocalcaemia. Despite these PAMs displaying good PK profiles, none of them have advanced to clinical trials.

CaSR NAMs. Although constant and high levels of parathyroid hormone stimulate bone resorption, short and intermittent increase of serum parathyroid hormone can stimulate new bone formation and therefore improve bone mineral density in osteoporosis patients. In this context, inhibition of CaSR via orally available NAMs could represent an interesting alternative to current injected treatment with recombinant parathyroid hormone to achieve a burst of parathyroid release. The first NAM reported was NPS-2143 (Fig. 11.24) (Gowen et al., 2000), which is the result of an optimization process of a hit identified in a functional screening from the former SmithKline Beecham compound collection (Marquis et al., 2009). NPS-2143 is a potent antagonist of the CaSR and promotes parathyroid hormone secretion in cells. When tested in a rodent model of osteoporosis, the compound produced sustained elevations of plasma parathyroid hormone after oral administration, which led to increased bone turnover. These results confirmed for the first time that in vivo treatment with an NAM of the parathyroid cell CaSR may have a net bone forming effect and it represents a potential new treatment for osteoporosis. However, NPS-2143 treatment did not produce any change in bone mass and density. This effect was initially attributed to the long-lasting increase (>4 h) in parathyroid hormone levels, and subsequent research was focused on the development of short-lived NAMs that would produce bursts of parathyroid hormone release and stimulate bone formation.

Ronacaleret (Balan et al., 2009) and ATF936 (Fig. 11.24) (John et al., 2011) are NAMs that are rapidly metabolized and display adequate PK and pharmacodynamic profiles. In fact, both compounds were able to trigger transient elevations of endogenous parathyroid, but neither produced a significant improvement in bone mineral density. Further research demonstrated that the caveat of these NAMs might be insufficient maximal parathyroid levels, and current work is focused on the design of ligands that display the required pharmacological properties.

11.4.2 GPCR class C group 6 subtype a receptor (GPCR6AR)

The GPCR6AR, closely related to CaSR, is widely expressed in humans, with high expression levels in brain, skeletal muscle, testis, leukocytes, liver, and kidney. This receptor is activated by L-amino acids, with a preference for L-Arg, L-Orn, and L-Lys (Kuang et al., 2005; Wellendorph et al., 2005). These amino acids were found to directly activate or positively modulate the receptor depending on its signaling pathway. Testosterone and osteocalcin, a hormone secreted by osteoblasts, have been also reported to stimulate GPCR6AR (Pi et al., 2011). Early studies suggested that GPCR6AR activation via L-amino acids or osteocalcin promote insulin release from β-islet cells, linking the receptor to metabolic and endocrinological disorders (Oury et al., 2011). However, subsequent research has resulted in partly contradictory (Rueda et al., 2016) and development of new specific GPCR6AR ligands, including AMs, which is highly desirable for the elucidation of the physiological roles of the receptor.

The first GPRC6AR allosteric antagonists have been recently discovered by chemogenomic analysis using known class A GPCR privileged structures. Thus, 2-arylindole derivatives were identified as AMs, which bind in a hydrophobic pocket in the 7TM of the mouse GPRC6AR with inhibitory activity (Gloriam et al., 2011). Of these compounds, only indole compound **14** (Fig. 11.24) was selective for the GPCR6AR, and it was selected as lead scaffold for a medicinal chemistry program. Systematic modifications in each of the four regions of the pharmacophore afforded several compounds displaying good activity and selectivity profiles (Johansson et al., 2015). Among them, compound **15** (Fig. 11.24) was characterized as the most potent GPRC6AR NAM, with improved selectivity over related class A and class C GPCRs. Although further optimization of compound **15** is necessary for future pharmacological studies, this allosteric antagonist constitutes an important tool to elucidate the physiological and therapeutic relevance of the GPRC6AR.

11.4.3 γ-Aminobutyric acid type B receptor ($GABA_BR$)

The $GABA_BR$ exists as a heterodimer of two subunits, $GABA_{B1}$ and $GABA_{B2}$. Activation of the receptor occurs via agonist binding to the B1 subunit, which transactivates the B2 subunit, subsequently stimulating the Gi/o proteins (White et al., 1998). $GABA_BR$ is involved in the pathophysiology of many diseases such as muscle spasticity, pain, anxiety, depression, epilepsy, drug addiction, schizophrenia, and neurodegenerative diseases (Froestl, 2010). Currently, there is only one FDA-approved drug targeting the $GABA_BR$, baclofen, a selective agonist prescribed as a muscle relaxant (Brogden et al., 1974). However, typical activation of the $GABA_BR$ orthosteric site is associated to different adverse effects, and baclofen causes dizziness, sedation, and mental confusion. In addition, baclofen also displays poor brain penetration, short duration of action, rapid tolerance, and a narrow therapeutic margin (Bowery, 2006). Therefore, positive allosteric modulation of the $GABA_BR$ constitutes an attractive strategy to retain the benefits of receptor activation while eliminating the adverse effects and limitations of the classic orthosteric approach (Filip et al., 2015).

The first characterized $GABA_BR$ PAM was CGP7930 (Fig. 11.24) (Urwyler et al., 2001), which potentiated GABA-induced signals. CGP7930 increased both potency and efficacy in cultured cells and rat brain membranes. Later research showed that the compound is an ago-PAM of the $GABA_BR$ that binds to an allosteric site located on the heptahelical domain of the $GABA_{B2}$ subunit, according to experiments with chimeric receptors (Binet et al., 2004). CGP7930 showed antidepressant and anxiolytic properties in several rodent models, and was also able to reduce drug-seeking behavior with substances such as alcohol, nicotine, and cocaine.

Optimization of CGP7930 structure by Hoffmann-La Roche led to BHFF (Fig. 11.24) (Malherbe et al., 2008), a 3-hydroxybenzofuran-2-one derivative that increased the potency and the efficacy with which GABA stimulated binding of $[^{35}S]GTP\gamma S$. Furthermore, this PAM exhibited anxiolytic-like activity in a mouse model of stress-induced hyperthermia.

New chemotype GS39783 (Fig. 11.24) and structurally related compounds were also reported as $GABA_BR$ PAMs. These ligands, as previously described PAMs, acted via a dual mechanism, enhancing both the affinity and the maximal efficacy of GABA (Urwyler et al., 2003). GS39783 was a more potent PAM than CGP7930, and showed anxiolyticlike effects in rodents (Cryan et al., 2004).

NVP-BHF177 (Fig. 11.24) is an optimized derivative of GS39783 where the nitro group has been removed. Interestingly NVP-BHF177 is devoid of the genotoxicity side effects observed with GS39783. NVP-BHF177 exhibited antinicotine and antialcohol effects as well as anxiolytic properties in mice (Paterson et al., 2008).

ADX71943 (Fig. 11.24) is a potent and selective $GABA_BR$ PAM that exhibited consistent and target-related efficacy in acute and chronic pain tests, while having no effect in studies associated with centrally mediated anxietylike reactivity (Kalinichev et al., 2014). Further optimization of this compound led to ADX71441 (Fig. 11.24), which had outstanding preclinical efficacy and tolerability in different rodent models of pain, anxiety, and addiction (Hwa et al., 2014). ADX71441 is the first $GABA_BR$ PAM entering clinical development, with phase I studies in addiction expected to start in 2018 (Addextherapeutics).

Overall, since the discovery of first $GABA_BR$ PAM CGP7930, several new and potent scaffolds acting at the $GABA_BR$ allosteric site have been identified with interesting pharmacological activities. However, clinical translation of such effects is now necessary to confirm their potential as therapeutic agents.

The first reported NAM of the $GABA_BR$, CLH304a (Fig. 11.24), was identified by an optimization process starting from PAM CGP7930 (Chen et al., 2014). This compound decreased GABA-induced inositol phosphate production and was selective over other GPCR class C members, such as Group I mGluRs. CLH304a negatively modulated $GABA_BR$ activity through the heptahelical domain of the $GABA_{B2}$ subunit (Sun et al., 2016). However, CLH304a and its derivatives presented several limitations in terms of their PK properties due to presence of an aromatic hydroxyl group and an electrophilic α,β-unsaturated ketone, which may be responsible for toxicological liabilities.

11.4.4 Metabotropic glutamate receptors ($mGlu_{1\text{-}5}$ and $mGlu_7Rs$)

mGluRs are activated by glutamate, the main neurotransmitter in vertebrates, and modulate synaptic transmission. This family of receptors includes eight members that are classified in three subgroups based on their sequence homology and signaling pathway, namely, Group I ($mGlu_1$ and $mGlu_5Rs$), Group II ($mGlu_{2\text{-}3}Rs$), and Group III ($mGlu_4$ and $mGlu_{6\text{-}8}Rs$) (Niswender and Conn, 2010). mGluRs have a very rich pharmacology with broad therapeutic potential, mostly in CNS-related pathologies such as pain, anxiety, depression, Parkinson disease, Alzheimer disease, cognition, addition, and schizophrenia.

The traditional strategy to develop subtype selective ligands by targeting the glutamate binding site, the orthosteric approach, has been highly challenging due to high conservation of this binding site across the eight mGlu subtypes. Moreover, most orthosteric ligands are of peptidic nature and therefore display limited CNS exposure and poor oral bioavailability. Therefore, allosteric modulation has emerged as an attractive approach for selectively targeting individual mGluR subtypes (Engers and Lindsley, 2013; Gregory and Conn, 2015; Lindsley et al., 2016; Lutjens and Rocher, 2017). In the next sections, we summarize key data and therapeutic potential for the most representative mGluR AMs.

mGlu$_1$R. The mGlu$_1$R has been described to play an important role in addiction, anxiety, epilepsy, pain, and psychotic disorders. In this context, the positive allosteric modulation of the mGlu$_1$R has been scarcely explored. Early PAMs displayed high potency but suffered from poor CNS penetration and PK properties, limiting their utility in vivo (Knoflach et al., 2001). Among them, only Ro-07-11401 (Fig. 11.25) presented acceptable CNS exposure and has been used as tool compound for in vivo preclinical validation of mGlu$_1$R activation in CNS disorders (D'amore et al., 2014). Interestingly, Ro-07-11401 was found to potentiate the response to glutamate in schizophrenic mGlu$_1$ mutant receptors, suggesting that mGlu$_1$R PAMs could represent a potential treatment for schizophrenic patients harboring these mutations (Cho et al., 2014b).

In the search for in vivo tool compounds with improved properties, new PAMs were obtained by molecular switches starting from an mGlu$_4$R PAM (Garcia-Barrantes et al., 2015a). Among them, VU0486321 (Fig. 11.25) was characterized as a highly potent, selective, and CNS-penetrant mGlu$_1$R PAM. Moreover, VU0486321 does not induce epileptiform activity and seizures in rats at drug concentrations far above its EC_{50}, suggesting that the adverse effect liability of Group I mGluR nonselective agonists is solely mediated by agonism at mGlu$_5$R (Garcia-Barrantes et al., 2015b).

The development of mGlu$_1$R NAMs has been an active area of research and structurally diverse ligands have been described. CPCCOEt (Fig. 11.25) was the first mGlu$_1$R NAM reported. The compound is a noncompetitive inhibitor with moderate potency at the mGlu$_1$R and good selectivity profile over the other mGluR subtypes, being a useful tool for in vitro studies (Annoura et al., 1996; Hermans et al., 1998). More potent NAMs were subsequently reported (Owen, 2011; Urwyler, 2011). Among them, JNJ-16259685, FTIDC, and A-841720 (Fig. 11.25) displayed potencies in the low nanomolar range. These molecules have been used to evaluate the role of mGlu$_1$R in several diseases with mixed results. Thus, although JNJ-16259685 and FTIDC showed anxiolytic-like activity in the lick suppression test, they were found to

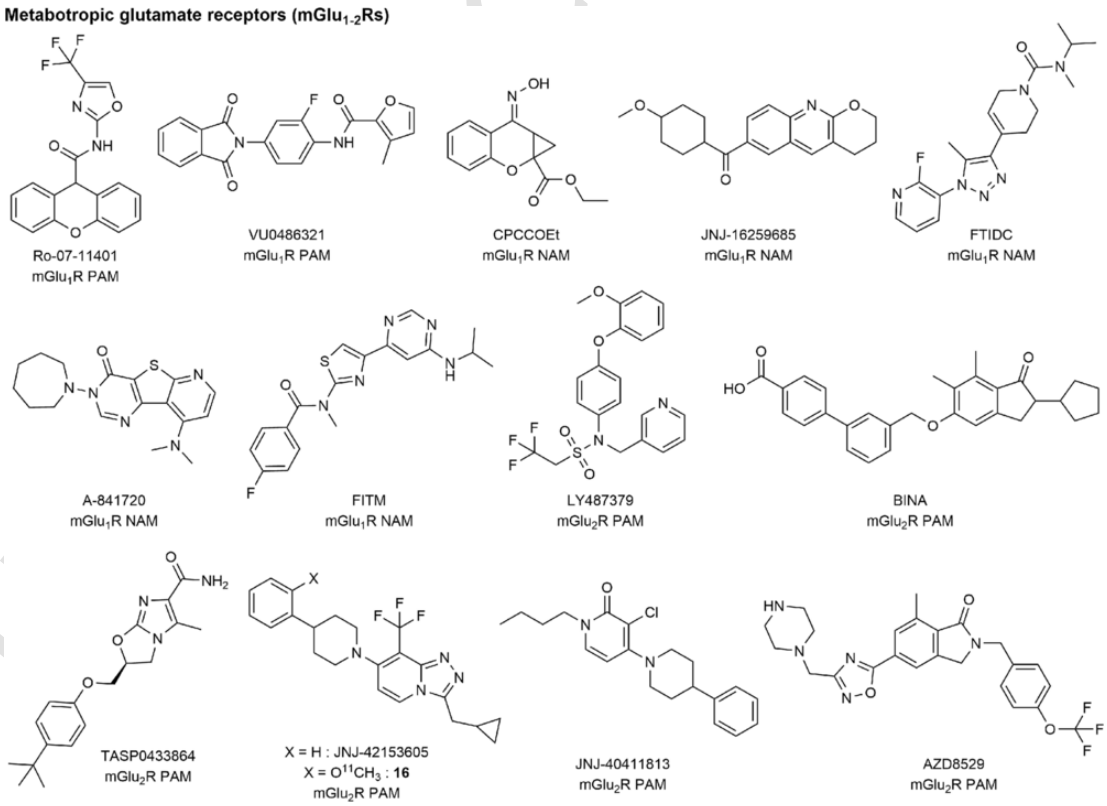

FIGURE 11.25 Representative allosteric ligands targeting metabotropic glutamate receptors 1–2 GPCRs of class C.

be inactive in the elevated plus maze (Satow et al., 2008; Steckler et al., 2005). Results in models of chronic pain were more coherent; however, different side effects were found. Hence, A-841720 was active against Freund's adjuvant induced inflammatory pain and reduced mechanical allodynia in sciatic nerve constriction and spinal nerve ligation models of neuropathic pain, but significant motor side effects occurred at analgesic doses (El-Kouhen et al., 2006).

FITM (Fig. 11.25) is an $mGlu_1R$ NAM with excellent selectivity against the $mGlu_5R$ that has been cocrystallized bound to the 7TM of the $mGlu_1R$ (Fig. 11.26) (Wu et al., 2014). FITM binds within the 7TM adjacent to the extracellular side. Interestingly, study of the ligand-receptor interactions by docking of FITM analogs into the crystal structure predicted the involvement of amino acid Leu757 in binding interactions, a residue that has been reported as critical for NAM activity (Cho et al., 2014a).

$mGlu_{2-3}Rs$. Most of the research on potentiation of Group II receptors has been focused on the development of selective $mGlu_2R$ PAMs, since it was established that selective activation of $mGlu_2R$ is directly implicated in driving the antipsychotic efficacy of orthosteric $mGlu_{2/3}R$ agonists. LY487379 (Johnson et al., 2003) and BINA (Galici et al., 2006) (Fig. 11.25) are the prototypical $mGlu_2R$ PAMs. They have been used as preclinical tools and have recapitulated much of the preclinical pharmacology of $mGlu_{2/3}R$ agonists, sparking the interest for selective PAMs (Ellaithy et al., 2015). Therefore, in recent years, a number of $mGlu_2R$ PAMs has been reported. TASP0433864 (Fig. 11.25) is a selective $mGlu_2R$ PAM, which has shown antipsychotic effects. It was able to modulate the *N*-methyl-D-aspartate (NMDA) signaling pathway involved in pathophysiology of schizophrenia and to inhibit induced hyperlocomotion in rodents (Hiyoshi et al., 2014). Janssen has contributed with different chemotypes to the $mGlu_2R$ PAM arena. JNJ-42153605 (Fig. 11.25) (Cid et al., 2012) is a highly optimized PAM with central activity in vivo by suppressing REM sleep stage in the rat sleep-wake electroencephalogram paradigm, a phenomenon previously demonstrated to be mediated by $mGlu_2R$. JNJ-42153605 represented the first example of efficacy of an $mGlu_2R$ PAM in the conditioned avoidance response test, a well-established antipsychotic model, inhibiting avoidance without impairing the escape response (Megens et al., 2014). Interestingly, its ^{11}C-labeled derivative compound **16** (Fig. 11.25) was used in PET studies, which confirmed its specific and reversible binding to $mGlu_2R$ in vivo (Andrés et al., 2012).

JNJ-40411813 (ADX71149) (Fig. 11.25) is a pyridone-based CNS penetrant $mGlu_2R$ PAM with an attractive PK profile, which was active in multiple antipsychotic animal models. JNJ-40411813 entered in an exploratory phase IIa clinical study in patients with schizophrenia (Cid et al., 2014). It met the primary objectives of safety and tolerability and demonstrated positive effect as adjunctive treatment to patients with residual negative symptoms. JNJ-40411813 was also

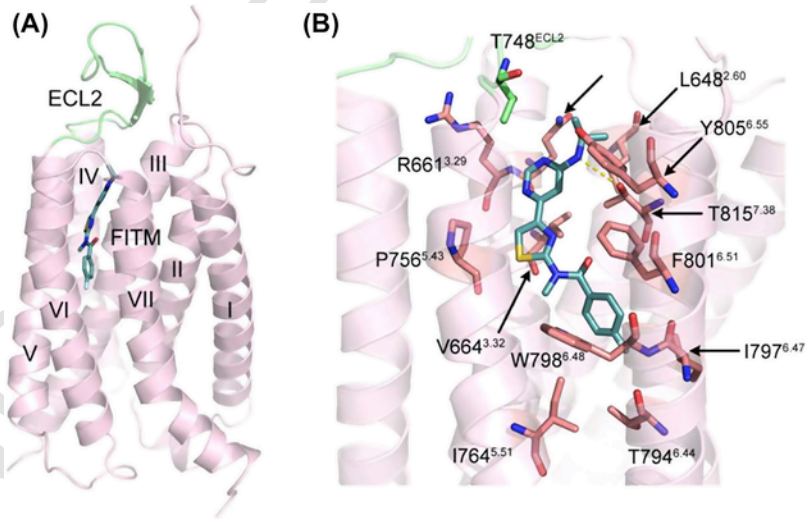

FIGURE 11.26 Cocrystal structure of $mGlu_1R$ in complex with NAM FITM (see Fig. 11.25). (A) FITM binds inside the 7TM adjacent to the extracellular side. The allosteric FITM binding site of the $mGlu_1R$ partially overlaps with the orthosteric binding site for the class A GPCRs, but it is distinct from its own orthosteric glutamate binding site at the *N*-terminal extracellular domain. The allosteric FITM binding site is formed by residues from the ECL2, helices II, III, and V−VII. (B) The pyrimidineamine group of the FITM in the allosteric FITM binding site forms a hydrogen bond with the side chain of Thr815$^{7.38}$. For hydrophobic interactions, the *p*-fluorophenyl group of the ligand is situated deep into a pocket defined by residues Ile764$^{5.51}$, Thr794$^{6.44}$, Ile797$^{6.47}$, Trp798$^{6.48}$, and Phe801$^{6.51}$. The central thiazole linker of the ligand creates hydrophobic interactions with residues Val664$^{3.32}$, Pro756$^{5.43}$, and Phe801$^{6.51}$. The 5-*N*-isopropylpyrimidine group of the ligand establishes hydrophobic interactions with residues Leu648$^{2.60}$, Gln660$^{3.28}$, Val664$^{3.32}$, Thr748^{ECL2}, Tyr805$^{6.55}$, Thr815$^{7.38}$ and the alkyl chain of Arg661$^{3.29}$ (PDB code 4OR2). *Credit: Reprinted with permission from J. Med. Chem. 2019, https://doi.org/10.1021/acs.jmedchem.7b01844. Copyright (2019) American Chemical Society.*

evaluated in a phase II proof-of-concept study in patients with major depressive disorder with significant anxiety symptoms. Although efficacy signals were met on some measures, the primary outcome measure was not significant, and the development of the compound in anxious depression was discontinued.

A different $mGlu_2R$ PAM chemotype was developed by AstraZeneca. AZD8529 (Fig. 11.25) is an isoindolinone derivative that advanced into a phase IIa proof-of-concept study in schizophrenic patients. However, although it had demonstrated activity in several preclinical antipsychotic and anxiolytic models, the compound was discontinued due to lack of efficacy.

Inhibition of Group II receptors has been linked to depression and cognition. Research in this field yielded a number of mixed $mGlu_{2/3}R$ NAMs, which have proven to be valuable pharmacological tools. Thus, benzodiazepine derivatives RO4491533 and RO4432717 (Fig. 11.27) have shown efficacy in rodent models of depression (Goeldner et al., 2013; Woltering et al., 2010). Decoglurant (RO4995819, Fig. 11.27), a nonselective NAM, advanced to clinical phase II trial in patients with major depressive disorder.

A new pyrazolo[1,5-*a*]quinazolin-5-one chemotype was recently proposed for the development of $mGlu_{2/3}R$ NAMs (Mayer et al., 2014). The initial hit was identified from an FRET assay and it was optimized to compound **17** (Fig. 11.27), a potent NAM with oral bioavailability and CNS exposure that improved spatial working memory in a dose-dependent manner in mice challenged with scopolamine (Schann et al., 2013).

The search for selective $mGlu_2R$ NAMs has been elusive, although they are highly necessary to understand the physiological roles of the individual subtypes of Group II. MRK-8-29 (Fig. 11.27) is a potent and selective $mGlu_2R$ NAM discovered by Merck and its use contributed to establish the specific role of $mGlu_2R$ in long-term depression as a major regulator of prefrontal cortex function and cognition (Walker et al., 2015). Scaffold-hopping around MRK-8-29 considering its similarity with a reported M_1R PAM resulted in a new series of 4-oxo-1,4-dihydroquinoline-3-carboxamides represented by VU6001192 (Fig. 11.27), a potent and highly selective $mGlu_2R$ NAM with an interesting PK profile (Felts et al., 2015).

Selective $mGlu_3R$ NAMs were developed at Vanderbilt University starting from an $mGlu_5R$ PAM, which displayed weak $mGlu_3R$ NAM activity and selectivity over $mGlu_2R$. Optimization of the initial hit afforded VU0469942 (ML337, Fig. 11.27), selective against $mGlu_2$ and $mGlu_5Rs$, but displaying suboptimal CNS penetration and high protein binding

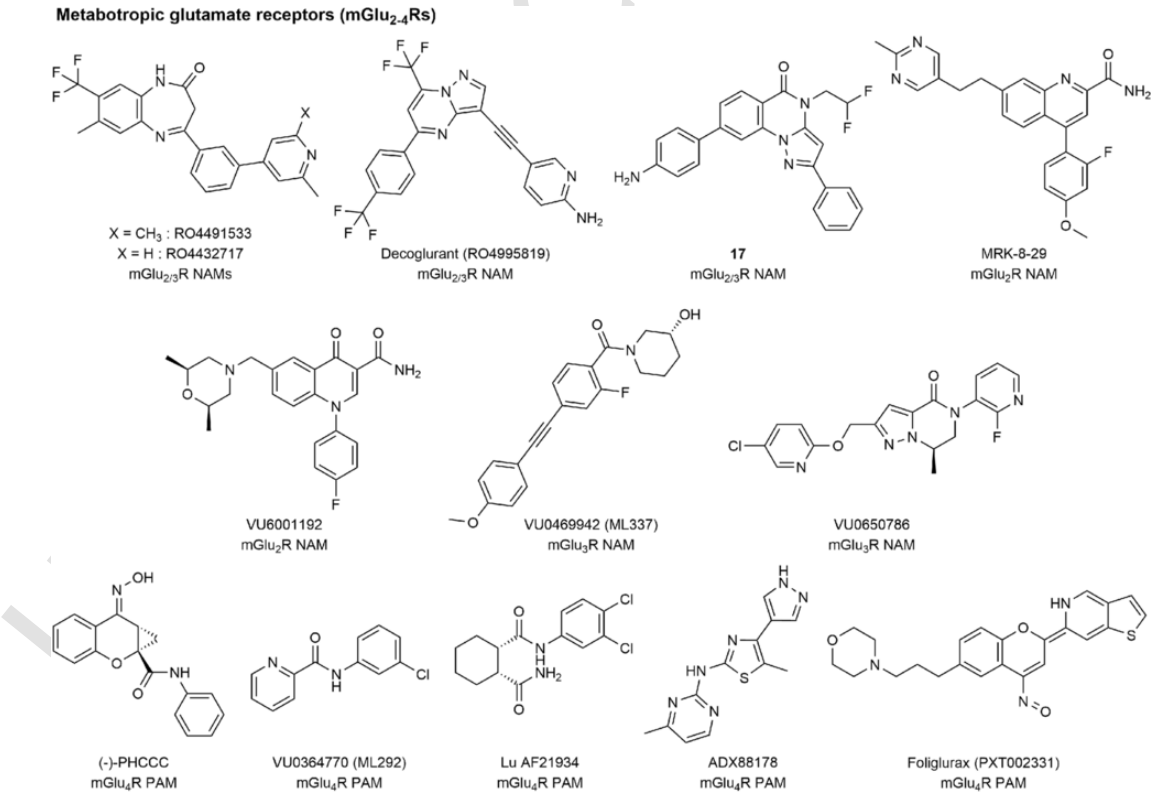

FIGURE 11.27 Representative allosteric ligands targeting metabotropic glutamate receptors 2–4 GPCRs of class C.

in rats. However, VU0469942 could be used as an in vivo tool in mice to demonstrate the utility of a selective $mGlu_3R$ NAM in a long-term depression model (Walker et al., 2015). Removal of the 1,2-diarylethyne fragment present in VU0469942 and an extensive SAR exploration led to VU0650786 (Fig. 11.27). In this case, the introduction of a chlorine atom in the molecule was key for good PK properties and VU0650786 displayed efficacy in anxiolytic and antidepressant models.

$mGlu_4R$. Among Group III mGluRs, $mGlu_4R$ research is the most advanced due to the implication of this receptor subtype in Parkinson disease, both for symptomatic motor disturbances and for neuroprotection of dopaminergic neurons (Bennouar et al., 2013; Valenti et al., 2005). In this context, research has been focused on the development of $mGlu_4R$ PAMs due to the difficulty in identifying selective orthosteric ligands. (-)-PHCCC (Fig. 11.27) was the first $mGlu_4R$ PAM disclosed. Although it is a relatively weak and nonselective PAM with a poor PK profile, (-)-PHCCC was able to relieve motor symptoms in an animal Parkinson disease model after intracerebroventricular injection (Maj et al., 2003). Since then, other $mGlu_4R$ PAMs with improved drug-like properties have been reported and contributed to validate the potential of this approach for the symptomatic treatment of Parkinson disease. VU0364770 (ML292, Fig. 11.27) was active in several Parkinson disease models when administered alone or in combination with inactive doses of L-DOPA (Jones et al., 2012). $mGlu_4R$ PAMs Lu AF211934 (Bennouar et al., 2013) and ADX88178 (Fig. 11.27) (Le Poul et al., 2012) showed activity in the 6-OHDA model in combination with L-DOPA, offering additional cotreatment options for Parkinson disease and reducing the dosage of L-DOPA. Foliglurax (PXT002331, Fig. 11.27) is the first $mGlu_4R$ PAM that successfully completed phase I trials, initiating a phase II study with Parkinson disease patients in 2017.

$mGlu_5R$. Allosteric modulation of $mGlu_5R$ affords opportunities for the treatment of diverse CNS disorders and has been extensively investigated, yielding a wealth of pharmacological tools as well as compounds that have entered clinical trials. Below we cover key examples of $mGlu_5R$ AMs and recent advances in the field.

Multiple $mGlu_5R$ PAMs have been reported; however, activation of $mGlu_5R$ via PAMs has been limited due to severe side effects found preclinically (Rook et al., 2013). These side effects have been associated to the level of intrinsic agonist activity shown by the ligand, and therefore, compounds with ago-PAM activity should be avoided. Particularly, the adverse effect liabilities of $mGlu_5R$ PAMs have been linked to NMDA receptor activation, and development of signal-biased PAMs could be an alternative to circumvent these side effects. In this context, VU0409551 (Fig. 11.28) is a potent,

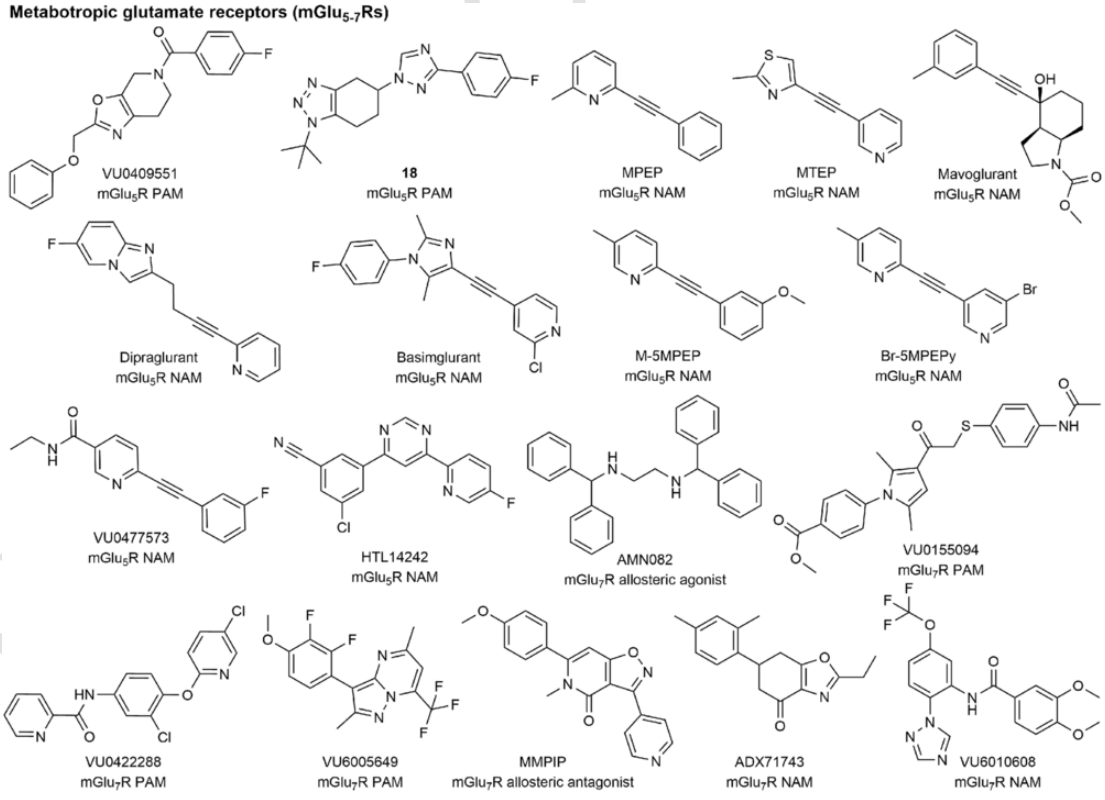

FIGURE 11.28 Representative allosteric ligands targeting metabotropic glutamate receptors 5–7 GPCRs of class C.

selective, and orally bioavailable $mGlu_5R$ PAM that displayed robust antipsychotic and cognition enhancing efficacy, without modulating NMDA receptor. Based on these results, VU0409551 was selected for further development, and represents the first $mGlu_5R$ PAM preclinical candidate for schizophrenia, suggesting that modulation of NMDA receptor activity may not be critical for in vivo efficacy (Conde-Ceide et al., 2015; Rook et al., 2015).

Compound **18** (Fig. 11.28) is a novel and safe PAM with activity in a preclinical model of cognition, wherein adverse effect liability has been avoided by reducing maximal glutamate fold-shift, that is, cooperativity (Ellard et al., 2015). Therefore, these examples demonstrated that target-related side effects can be overcome by different strategies.

Alkyne derivatives MPEP (Gasparini et al., 1999) and MTEP (Fig. 11.28) (Cosford et al., 2003) are prototypical $mGlu_5R$ NAMs. These compounds exhibited high potency, selectivity, and brain penetrance and have helped to validate preclinically therapeutic indications such as Parkinson disease (Gasparini et al., 2013). Since the discovery of MPEP and MTEP, diverse NAM chemotypes have been developed and have shown preclinical efficacy for the treatment of disorders associated with glutamatergic neurotransmission hyperactivity and/or dysfunction, with several of them entering clinical trials (Li et al., 2013). Thus, mavoglurant (AFQ056) (Berg et al., 2011) and dipraglurant (Fig. 11.28) (Tison et al., 2016) are $mGlu_5R$ NAMs that have shown efficacy in reducing levodopa-induced dyskinesia in Parkinson disease and have progressed to phase II trials. Additionally, mavoglurant (Fig. 11.28) is currently in phase II studies for fragile X syndrome, cocaine-related disorder, and alcohol drinking. More recently, basimglurant has reached clinical development for major depressive disorder (Fig. 11.28) (Lindemann et al., 2015).

However, some $mGlu_5R$ NAMs have demonstrated adverse effects, including psychotomimetic-like effects in animals and psychosis in humans, revealing a narrow therapeutic window (Emmitte, 2011). In this context, the identification of $mGlu_5R$ partial NAMs represented a new mode of pharmacology that could limit potential adverse events associated to complete blockade of receptor signaling with full NAMs. These new ligands fully occupy the allosteric site but only partially block signaling, allowing to vary degrees of antagonist activity (Rodriguez et al., 2005). M-5MPEP, Br-5MPEPy, and VU0477573 (Fig. 11.28) are partial $mGlu_5R$ NAMs with activity in selected preclinical models. Thus, M-5MPEP and Br-5MPEPy exhibited antidepressan-tlike and anxiolytic-like activity, corresponding to in vivo $mGlu_5R$ occupancy, and without enhancing hyperlocomotion, as is the case with MTEP (Gould et al., 2016). VU0477573, with an excellent PK profile, displayed efficacy in rodent models of anxiolytic activity (Nickols et al., 2016). Therefore, the efficacy of partial $mGlu_5R$ NAMs is similar to that observed with full NAMs but with a broader therapeutic window.

Regarding the binding site of these NAMs to $mGlu_5R$, two crystal structures have been published. The cocrystal structure of binary $mGlu_5R$-mavoglurant complex has been recently reported showing that mavoglurant binds deep within the 7TM (Figs. 11.28 and 11.29) (Dore et al., 2014). When compared to the crystal structure of $mGlu_1R$ in complex with

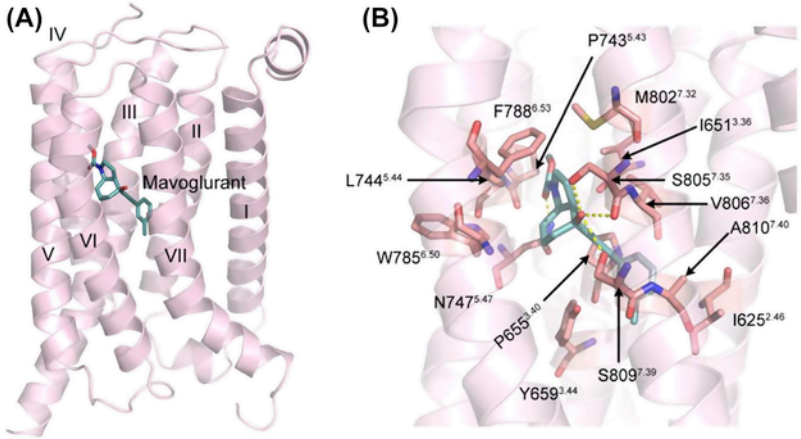

FIGURE 11.29 Cocrystal structure of $mGlu_5R$ in complex with NAM mavoglurant (see Fig. 11.28). (A) Mavoglurant is located in the extracellular side, deep within the 7TM that is wrapped by residues from helices II, III, and V−VII. (B) The carbamate carbonyl of the mavoglurant in the allosteric binding site forms a hydrogen bond with the side chain of Asn747$^{5.47}$, and the hydroxyl group of the ligand forms hydrogen bonds with the side chains of Ser805$^{7.35}$ and Ser809$^{7.39}$ as well as the backbone carbonyl of Ser805$^{7.35}$. For hydrophobic interactions, the bicyclic ring of the ligand is located in the main hydrophobic pocket created by residues Ile651$^{3.36}$, Pro655$^{3.40}$, Leu744$^{5.44}$, Trp785$^{6.50}$, Phe788$^{6.53}$, Met802$^{7.32}$, and Val806$^{7.36}$. The 3-methylphenyl ring fills a hydrophobic pocket created by residues Ile625$^{2.46}$, Pro655$^{3.40}$, Tyr659$^{3.44}$, and Ala810$^{7.40}$. The alkyne linker establishes contacts with residues Pro655$^{3.40}$, Tyr659$^{3.44}$, Ser809$^{7.39}$, and Val806$^{7.36}$. The methoxy terminal portion of the carbamate creates hydrophobic contacts with residues Ile651$^{3.36}$, Pro743$^{5.43}$, and Leu744$^{5.44}$ (PDB code 4OO9). *Credit: Reprinted with permission from J. Med. Chem. 201-, https://doi.org/10.1021/acs.jmedchem.7b01844. Copyright (201-) American Chemical Society.*

FITM, the two structures are in agreement with a number of molecular features conserved across subtypes. Interestingly, FITM is found higher in the $mGlu_1R$ allosteric site, highlighting the potential for different allosteric binding modes across the same class of receptors. HTL14242 has also been cocrystalized with the $mGlu_5R$ (Figs. 11.28 and 11.30) (Christopher et al., 2015). Despite lacking the alkyne function, the binding mode of HTL14242 is very similar to that one identified for mavoglurant.

$mGlu_7R$. Group III $mGlu_7R$ is widely distributed in the CNS, where it is thought to play a critical role in modulating normal neuronal function and synaptic transmission (Dalezios et al., 2002). Thus, $mGlu_7R$ has been proposed as therapeutic target for various CNS disorders, including Parkinson disease, autism, depression, bipolar disorder, and schizophrenia (O'connor et al., 2010; Peterlik et al., 2016). The lack of small-molecule tool compounds with appropriate selectivity and drug-like properties has hampered the progression of drug discovery across $mGlu_7R$. In this context, AMs have emerged as valuable tools with good potency, selectivity and physicochemical properties to study the receptor therapeutic potential.

AMN082 was the first selective ligand reported for $mGlu_7R$ (Fig. 11.28) (Mitsukawa et al., 2005). This compound acted as an allosteric agonist, which directly activated receptor signaling by binding to an allosteric site in the transmembrane domain without affecting the affinity of orthosteric binders. AMN082 is orally active and brain penetrant, and has been a valuable in vivo tool to unveil the role of the $mGlu_7R$, mainly in stress-related CNS disorders. However, the compound presented a rapid metabolism in rat liver microsomes toward a metabolite that displayed significant binding affinity for the dopamine, serotonin, and norepinephrine transporters. Therefore, the in vivo activity of AMN082 may not be purely mediated by $mGlu_7R$, and other mechanisms cannot be discarded (Sukoff Rizzo et al., 2011).

Although different $mGlu_7R$ allosteric agonists have been described since the discovery of AMN082, the number of pure PAMs has been scarce. VU0155094 and VU0422288 (Fig. 11.28) were disclosed as pan-Group III PAMs and, despite their lack of selectivity, have represented useful tool compounds for the validation of the $mGlu_7R$ in different pathological processes (Jalan-Sakrikar et al., 2014). VU0155094 was discovered in an HTS while looking for $mGlu_8R$ PAMs, and potentiated mGluR subtypes 4, 6, 7, and 8, but not the other mGluRs. VU0422288, identified within an $mGlu_4R$ PAM lead optimization program, is the most potent pan-Group III AM reported to date. VU0422288 and VU0155094 have been studied by electrophysiological experiments in the hippocampus of adult rats, and data proved that both compounds produced relevant and robust effects modulating synaptic transmission via a presynaptic mechanism, which confirmed that $mGlu_7R$ can be modulated by a PAM (Jalan-Sakrikar et al., 2014).

A novel chemotype based on a pyrazolo[1,5-*a*]pyrimidine scaffold has yielded a new family of $mGlu_7R$ PAMs, wherein VU6005649 (Fig. 11.28) stood out as an $mGlu_7R$-preferring PAM with no activity at $mGlu_{1\text{-}6}Rs$ and four-fold selectivity versus $mGlu_8R$ (Abe et al., 2017). VU6005649 was found to be CNS penetrant and showed modest but significant efficacy in a mouse contextual fear conditioning model, exhibiting procognitive effects on associative

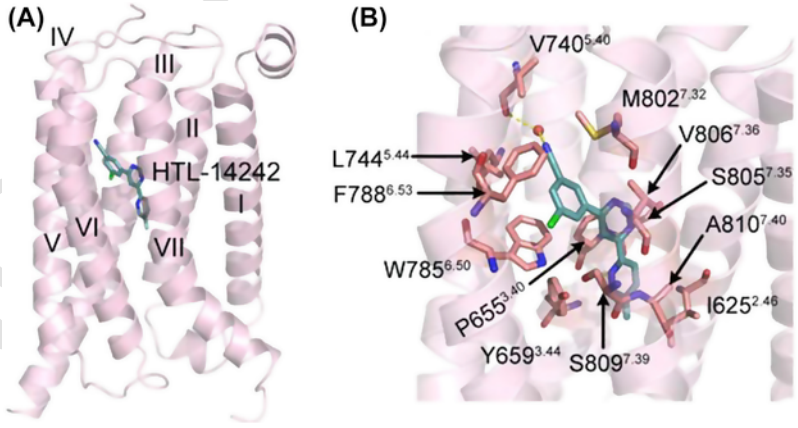

FIGURE 11.30 Cocrystal structure of $mGlu_5R$ in complex with NAM HTL14242 (see Fig. 11.28). (A) HTL14242 occupies an extracellular allosteric pocket similar to that of mavoglurant in the $mGlu_5R$ receptor, lined by residues from helices II, III, and V−VII. (B) The pyridine nitrogen of HTL-14242 in the allosteric HTL-14242 binding site engages a hydrogen bond with the side chain of $Ser809^{7.39}$, and the 5-cyano of the ligand is involved in a water-mediated hydrogen bond with the backbone carbonyl oxygen of $Val740^{5.40}$. For hydrophobic interactions, the pyridine ring of the ligand fits into a hydrophobic pocket defined by residues $Ile625^{2.46}$, $Tyr659^{3.44}$, $Ser809^{7.39}$, and $Ala810^{7.40}$. The pyrimidine linker is situated in a hydrophobic pocket created by residues $Ser654^{3.39}$, $Pro655^{3.40}$, and $Val806^{7.36}$. The phenyl ring creates hydrophobic contacts with residues $Trp785^{6.50}$ and $Phe788^{6.53}$. The cyano substituent establishes contacts with residues $Leu744^{5.44}$, $Phe788^{6.53}$, and $Met802^{7.32}$. (PDB code 5CGD). *Credit: Adapted with permission from J. Med. Chem. 201-, https://doi.org/10.1021/acs.jmedchem.7b01844. Copyright (201-) American Chemical Society.*

learning. Although the compound also induced some level of sedation, which could contribute in diminishing mice capacity for associative learning, this PAM constituted the first example of $mGlu_{7/8}R$-mediated efficacy in this cognition model.

Regarding $mGlu_7R$ antagonism, several allosteric antagonists and NAMs have been reported. MMPIP (Fig. 11.28) is an allosteric antagonist selective for $mGlu_7R$, orally bioavailable and CNS penetrant that has been used to study the pharmacology of $mGlu_7R$ in rodents (Suzuki et al., 2007). The first potent and selective $mGlu_7R$ NAM, ADX71743 (Fig. 11.28), was reported by Addex Therapeutics (Kalinichev et al., 2013). It was developed from an HTS hit and its NAM activity was confirmed in Schild plot experiments, where ADX71743 induced a concentration-dependent rightward shift of reference agonist L-AP4 concentration response curve together with a decrease of its efficacy. This compound showed good plasma exposures in rodents and was evaluated in several rodent behavioral models of psychosis, anxiety, and depression. Specifically, ADX71743 was active in the marble burying and elevated plus maze tests in mice, suggesting an anxiolytic-like effect mediated by $mGlu_7R$ (Klar et al., 2015). Last research published on $mGlu_7R$ NAMs corresponds to the identification of VU6010608 (Fig. 11.28). This compound is the result of a challenging optimization program around a hit identified from an HTS campaign. VU6010608 increased $mGlu_7R$ affinity and good selectivity over the other mGluR subtypes, but it suffered from a poor PK profile. Nonetheless, this NAM represents a good tool compound for in vitro studies thanks to its robust antagonist effect (Reed et al., 2017).

The growing pool of $mGlu_7R$ AMs available to date will spur further research around $mGlu_7R$ that should afford more potent and selective ligands suitable for in vivo preclinical validation of the receptor.

11.5. ALLOSTERIC MODULATORS TARGETING CLASS F GPCRS

Recent research has shown that class F GPCRs, traditionally considered undruggable receptors, also possess allosteric binding sites for exogenous ligands. This class includes the frizzled (FZD) and smoothened (SMO) receptors.

11.5.1 Frizzled receptors (FZD_4R)

Human FZDRs comprise 10 members ($FZD_{1\text{-}10}Rs$) that respond to the extracellular ligands of the Wingless/Integrated (Wnt) family of lipo-glycoproteins (Dijksterhuis et al., 2014), and are implicated in many aspects of embryonic development and in homeostasis of adult tissues (Clevers and Nusse, 2012). In this context, the FZD_4R is of particular interest since it regulates stemness during cellular development and in adult life, and misregulation of the receptor activity is involved in cancer stem cell genesis in many types of malignancies and tumor proliferation.

In 2015, the first small molecules able to target the FZD_4R and to inhibit Wnt signaling were reported (Generoso et al., 2015). These compounds had been designed to behave as pharmacological chaperones for a misfolded mutant of FZD_4R, but they were indeed Wnt–β-catenin inhibitors. Among them, FzM1 (Fig. 11.31) stood out as an NAM that binds to an allosteric site located near the ICL3 of the receptor and induces conformational changes that ultimately inhibit the Wnt-β-catenin cascade. In that manner, FzM1 was able to affect the growth and differentiation state of U87MG and

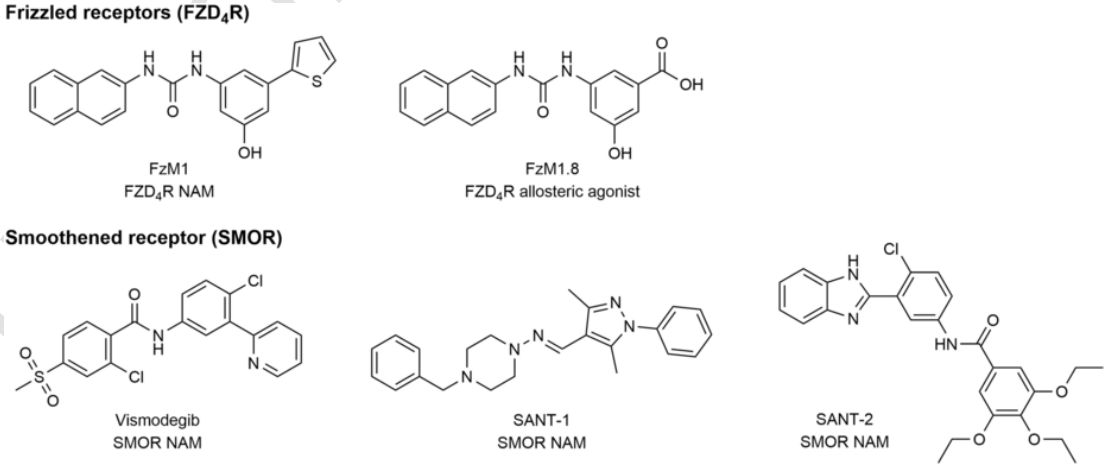

FIGURE 11.31 Representative allosteric ligands targeting GPCRs of class F.

Caco-2 cells, two cells lines known to rely on an active Wnt pathway to maintain their undifferentiated proliferative state. These results are of great importance, as they suggest the applicability of these compounds as antitumor drugs.

Further research conducted with this FZD_4R NAM led to the identification of the first allosteric agonist of the FZD_4R (Riccio et al., 2018). Thus, the replacement of the thiophene ring present in FzM1 by a carboxylic acid group induced a

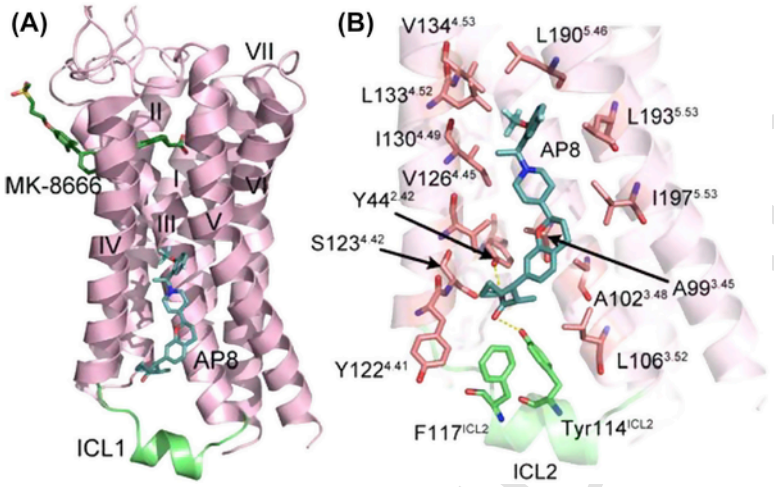

FIGURE 11.9 Cocrystal structure of GPR40 in a ternary complex with ago-PAMs MK-8666 and AP8 (see Fig. 11.7). (A) AP8 binds to a well-defined lipid-facing pocket formed by helices II−V and ICL2, which is outside the intracellular halves of the 7TM, and this site is completely distinct from that of MK-8666 located in the extracellular halves of the 7TM. (B) In the allosteric binding site, the carboxylate group of AP8 accepts three hydrogen bonds from the side chains of Tyr44$^{2.42}$, Ser123$^{4.42}$, and Tyr114^{ICL2}. The methyl and cyclopropyl groups engage in hydrophobic and aromatic interactions with residues Leu106$^{3.52}$, Tyr114^{ICL2}, Phe117^{ICL2}, and Tyr122$^{4.41}$. The piperidine-chroman group makes hydrophobic contacts with residues Ala99$^{3.45}$, Ala102$^{3.48}$, Val126$^{4.45}$, and Ile197$^{5.53}$. The terminal trifluoromethoxyphenyl group fits into a hydrophobic pocket formed by residues Ile130$^{4.49}$, Leu133$^{4.52}$, Val134$^{4.53}$, Leu190$^{5.46}$, and Leu193$^{5.47}$ (PDB code 5TZR). *Credit: Adapted with permission from J. Med. Chem. 2019, https://doi.org/10.1021/acs.jmedchem.7b01844. Copyright (2019) American Chemical Society.*

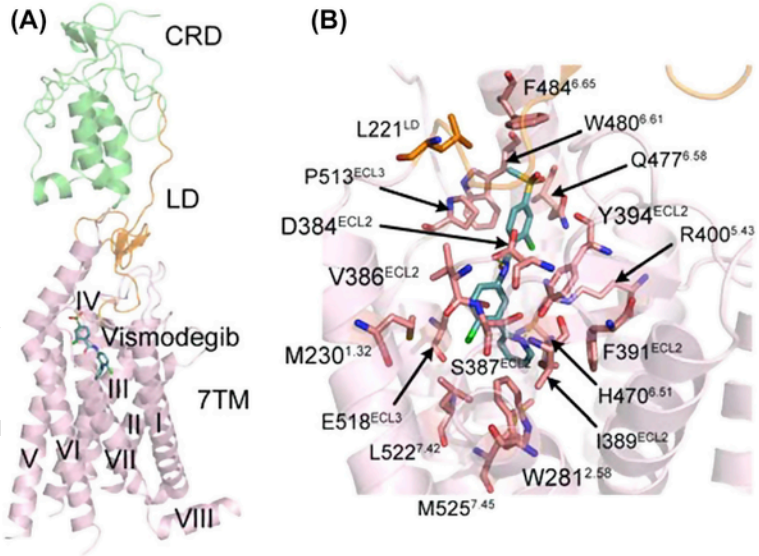

FIGURE 11.32 Cocrystal structure of SMOR in complex with NAM vismodegib (see Fig. 11.31). (A) Vismodegib is bound to the 7TM adjacent to the extracellular side. The vismodegib binding site is defined by residues from the linker domain, ECL2, ECL3, and helices I, II, and V−VII. (B) The pyridine nitrogen and the amide NH of vismodegib in the allosteric vismodegib binding site are involved in a hydrogen bond with the side chain of $Tyr394_{ECL2}$ and Asp384^{ECL2}, respectively. For hydrophobic interactions, the pyridinechlorophenyl moiety of the ligand is deeply buried, forming numerous hydrophobic interactions with residues Met230$^{1.32}$, Trp281$^{2.58}$, Val386^{ECL2}, Ser387^{ECL2}, Ile389^{ECL2}, Phe391^{ECL2}, Tyr394^{ECL2}, Arg400$^{5.43}$, His470$^{6.51}$, Glu518^{ECL3}, Leu522$^{7.42}$, and Met525$^{7.45}$. The methylsulfonephenyl moiety of the ligand creates hydrophobic contacts with residues Leu221 in the linker domain, Tyr394^{ECL2}, Gln477$^{6.58}$, Trp480$^{6.61}$, Phe484$^{6.65}$, and Pro513^{ECL3} (PDB code 5L7I). *Credit: Adapted with permission from J. Med. Chem. 2019, https://doi.org/10.1021/acs.jmedchem.7b01844. Copyright (2019) American Chemical Society.*

molecular switch that transformed the molecule into an activator of Wnt signaling, the allosteric agonist FzM1.8 (Fig. 11.31). This ligand potentiated the β-catenin pathway in the absence of any Wnt ligand. In particular, FzM1.8 promoted recruitment of heterotrimeric G proteins, and biased Wnt signaling toward a noncanonical route that involves phosphatidylinositol-3-kinase. The treatment of a colon cancer line with FzM1.8 left unaltered the expression of colon stem cell markers, indicating that FZD_4R/phosphatidylinositol-3-kinase pathway stimulates proliferation and preserving the stemness of the cells.

11.5.2 Smoothened receptor (SMOR)

The SMOR mediates signal transduction in the Hedgehog pathway, which is critical for development, differentiation, growth, and cell migration (Arensdorf et al., 2016). The Hedgehog pathway plays key roles in a wide type of cancers (Wu et al., 2017) and therefore, small molecules able to inhibit SMOR are under intensive development as antitumor agents. In fact, several lead compounds are currently in clinical trials, including the SMOR NAM vismodegib (GDC-0449) (Fig. 11.31) (Robarge et al., 2009), which has been the first agent targeting the Hedgehog signaling pathway approved by the FDA. Vismodegib was the result of an optimization program looking for improved potency, PK, and drug-like properties by explorations around the amide moiety. This NAM produced complete tumor regression in a medulloblastoma allograft mouse model that is wholly dependent on the Hedgehog pathway for growth. Vismodegib is prescribed for the treatment of basal cell carcinoma.

Recently, the cocrystal structure of vismodegib bound to SMOR has been solved, revealing two allosteric binding sites (Figs. 11.31 and 11.32) (Byrne et al., 2016). This structure clearly showed that vismodegib is bound to the 7TM adjacent to the extracellular side. The second allosteric site, located in the extracellular domain, was occupied by cholesterol, and appears to promote the binding of the natural agonist, the Hedgehog protein.

SANT-1 and SANT-2 (Fig. 11.31) are other SMOR antagonists whose mode of action has been characterized in terms of allosteric modulation (Rominger et al., 2009). Both compounds exhibited partial competition of the radioligand [^{3}H]SAG-1.3 binding, but fully inhibited the activation of the Hedgehog pathway induced by agonist SAG-1.5 in a β-lactamase reporter gene cellular assay. The crystal structure of the human SMOR in complex with SANT-1 has been solved and revealed that the compound is bound within the 7TM (Wang et al., 2014), providing also a structural explanation for the allosteric modulation exerted by SANT-1. Vismodegib and SANT-1 bind the same site of the SMOR, although SANT-1 binds more deeply in the 7TM.

11.6. CONCLUSIONS

Studies in the past decade highlight the 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. Therefore, utilizing allosteric modulation to precisely alter the function of the receptors may enable the therapeutic targeting of previously intractable GPCRs or provide safer therapeutics for currently targeted receptors. At present, GPCR allosteric modulation is a fundamental approach in drug discovery, 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.

A key challenge in the discovery of AMs targeting GPCRs includes the identification of nonconserved allosteric sites where AMs can bind, as a prerequisite for discovering potent and selective drugs. The recent great progress in GPCR structural biology using X-ray crystallography has solved distinct GPCRs in complex with their small-molecule AMs across all human GPCR classes: A, B, C, and F. Knowledge of the detailed receptor−modulator interactions at the allosteric sites pave the way for virtual screening to identify novel allosteric leads or structure-based drug design to improve the binding affinity of existing AMs. This breakthrough is expected to contribute to the discovery of GPCR allosteric drugs with an improved therapeutic action.

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