



Allosteric modulators could be especially valuable in controlling receptors for which the design of orthosteric agonists or antagonists has been elusive.

Keynote review: Allosterism in membrane receptors

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Allosteric modulation of membrane receptors has been intensively studied in the past three decades and is now considered to be an important indirect mechanism for the control of receptor function. The allosteric site on the GABA_A receptor is the target for the most widely prescribed sleep medicines, the benzodiazepines. Cinacalcet, an allosteric enhancer of the calcium-sensing receptor, is used to treat secondary hyperparathyroidism. Allosteric ligands might be especially valuable to control receptors for which the design of selective orthosteric agonists or antagonists has been elusive, such as muscarinic acetylcholine receptors.

Modulation of membrane receptor function is crucial for controlling cellular processes. An important mechanism of controlling receptor function is allosteric modulation, in which modulators bind to a regulatory site on a receptor distinct from the orthosteric site, which is the site of binding of the native ligand. Allosteric modulators induce conformational changes that profoundly influence the behavior of a membrane receptor in response to its native ligand [1–3]. There are positive, negative and neutral allosteric modulators of membrane receptors.

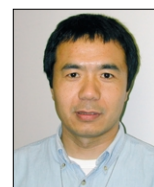
Christian Bohr [4] made the initial observation of the phenomenon of allostery. He carefully measured the oxygenation of hemoglobin and found that the binding curve was sigmoidal instead of hyperbolic, indicating cooperativity of binding of oxygen molecules to hemoglobin; he also found that carbon dioxide lowers oxygen affinity for hemoglobin. These properties make hemoglobin an efficient transporter of oxygen from the lungs to the tissues and of carbon dioxide from the tissues to the lungs.

Before the 1960s, hemoglobin was one of the few known examples of proteins that displayed allosteric properties. It was later demonstrated that many enzymes in bacteria and in higher organisms are subject to allosteric modulation. Researchers later discovered that in most allosteric proteins, indirect interactions between distinct specific binding sites account for the performance of their modulatory function [1]. An extension of the allosteric theory to membrane receptors was proposed in the late 1960s [3,5].

Although the history of allostery spanned over a century, it is still relevant to current research on protein function and signal transduction mechanisms [3]. Significant progress has been made in the investigation of allosteric modulation of protein action. The presence of allosteric sites

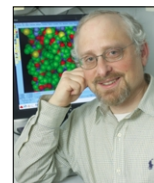
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on membrane receptors offers a novel pharmacological means of modulating receptor function. Allosteric sites have been found on ligand-gated ion channels (LGICs). An allosteric site on the γ -aminobutyric acid (GABA)_A receptor provides the basis for the therapeutic effects of benzodiazepines [6]. Another germane example is the anticholinesterase inhibitor galantamine. In addition to its anticholinesterase activity, galantamine is also a positive allosteric modulator of $\alpha 4\beta 2$ and $\alpha 7$ nicotinic receptors. Its successful use in the treatment of Alzheimer's disease might be an impetus for further development of more-potent and selective allosteric modulators [7]. Allosteric sites have also been demonstrated in G-protein-coupled receptors (GPCRs) [2]. Cinacalcet, a positive allosteric modulator of the calcium-sensing receptor (CaR), has been successfully used for the treatment of secondary hyperparathyroidism [8,9].

Therapy using receptor agonists is often prone to side effects owing to the widespread distribution of the target receptor in the body. An advantage of a positive allosteric modulator of a membrane receptor over its native, orthosteric activator is that, in principle, greater selectivity can be achieved. The positive allosteric modulator would enhance the action of the native agonist but might have no effect of its own on the unoccupied receptor. Thus, the agonist effect, which might be insufficient in a particular disease state, might be magnified through allosteric modulation. The higher subtype selectivity commonly exerted by allosteric modulators, and the fact that the allosteric action is ideally coupled to the simultaneous presence of the endogenous ligand, both help to prevent over-dosage compared with the administration of a conventional nonselective orthosteric agonist.

GPCRs

GPCRs, which constitute the largest family of cell-surface receptors, are major targets of drugs currently in clinical use. The GPCRs display the characteristic motif of seven transmembrane helices (TMs) and thus are also referred to as 7TM receptors. The 7TM proteins account for ~4% of the human genome. Mammalian GPCRs can be divided into three major subfamilies: class A, rhodopsin-like receptors; class B, secretin-like receptors; and class C, metabotropic glutamate-like and pheromone receptors. The rhodopsin-like receptors represent the predominant class of GPCRs. Members of a particular subfamily feature a substantial degree of amino acid homology, whereas different subfamilies demonstrate very low amino acid homology. Among the class A GPCRs, only the crystal structure of bovine rhodopsin has been determined [10].

The orthosteric ligand-binding site of a GPCR would be located either among TM helices or within the extracellular domain [3,10,11]. The third intracellular loop and carboxy-terminal end could interact with a G protein. Originally, a single receptor unit was thought to mediate the allosteric interactions between a modulator and the receptor. However, recent experiments suggest that the active forms of GPCRs can occur as GPCR dimers [12]. A dimerized nature of GPCRs is possibly associated with a diversification in their interactions with different G proteins, as well as in their desensitization and sequestration properties. It can also be argued that the functional unit of a GPCR is monomeric rather than dimeric or oligomeric [13]. Indeed, dimerization does not seem to be required for functional coupling of some GPCRs to G proteins but rather for its intracellular trafficking [14,15]. Thus, it is

possible that, at least in some cases, the monomer is sufficient as a functional unit for some of the class A GPCRs but this might not be the case for other classes of GPCRs. For example, a heterodimer is essential for the GABA_B receptor to function properly [16]. Taste receptors also exhibit this property [16]. Evidence suggests that the T1R1 and T1R3 taste receptors, which respond to sweet taste, combine as one functional unit [17].

An increasing percentage of GPCRs has been found to be modulated allosterically by various compounds. These receptors include all five subtypes of muscarinic receptors [18], at least three of the four subtypes of adenosine receptors [19], dopamine receptors [20], several class B GPCRs [21] and class C GPCRs [11]. The flexible nature of the interactions between the receptors and various allosteric modulators, combined with the potential for subtype selectivity, makes allosteric sites attractive for therapeutic intervention [2,22]. An allosteric modulator of CaR, cinacalcet, has been successfully administered for the treatment of secondary hyperparathyroidism [8].

A detailed mathematical analysis of the action of allosteric modulators of GPCRs has been described by Christopoulos [3], and Birdsall and Lazareno [18]. The allosteric ternary complex model (ATCM) features a term for the degree of cooperativity (α). The cooperativity factor $\alpha > 1$ denotes a positive cooperativity; $\alpha < 1$ results in negative cooperativity; neutral cooperativity occurs in cases where $\alpha = 1$. It should be noted that ATCM details the action of all allosteric modulators (not just enhancers) on the binding of orthosteric ligands to GPCRs but not on the signaling. The ATCM has been validated by direct binding measurements at an allosteric site on the muscarinic M2 receptor using an allosteric radioligand [23]. The model has recently been extended by Hall [24], Price *et al.* [25] and Ehlert [26] to accommodate the effects of allosteric modulators on efficacy. The effect of an allosteric modulator on agonist affinity and efficacy has been described in detail. A given allosteric modulator might have different effects on each of these parameters.

Class A GPCRs: rhodopsin-like receptors

Adenosine receptors

The A₁ adenosine receptor (AR) was the first subtype in the AR family for which selective orthosteric and allosteric ligands were developed. The aminobenzoylthiophene derivative 2-amino-4,5-dimethyl-3-thienyl-[3-trifluoromethylphenyl]methanone (PD81723; Figure 1) was the first allosteric enhancer in the GPCR field, as originally observed by Bruns and Fergus [27]. They found that PD81723 enhances the binding of agonist radioligand [³H]cyclohexyladenosine to A₁ ARs and decreases the rate of dissociation of [³H]cyclohexyladenosine from these receptors [27], suggesting that these compounds act at an allosteric site distinct from the orthosteric adenosine binding site to stabilize agonist–receptor–G-protein complexes. The aminobenzoylthiophenes were found to be highly selective for A₁ ARs, with little or no effect on the binding of agonists to other ARs or other GPCRs [27].

The behavior of A₁ AR enhancers has traditionally been complicated by both enhancement at low concentrations and inhibition at high concentrations [27]. Recently, several new series of 2-amino-3-benzoylthiophenes have been synthesized [28,29]. Several compounds proved to be superior to PD81723: they are more potent than PD81723 in enhancing the binding of A₁ AR agonist N⁶-cyclopentyladenosine to the receptor and their antagonistic

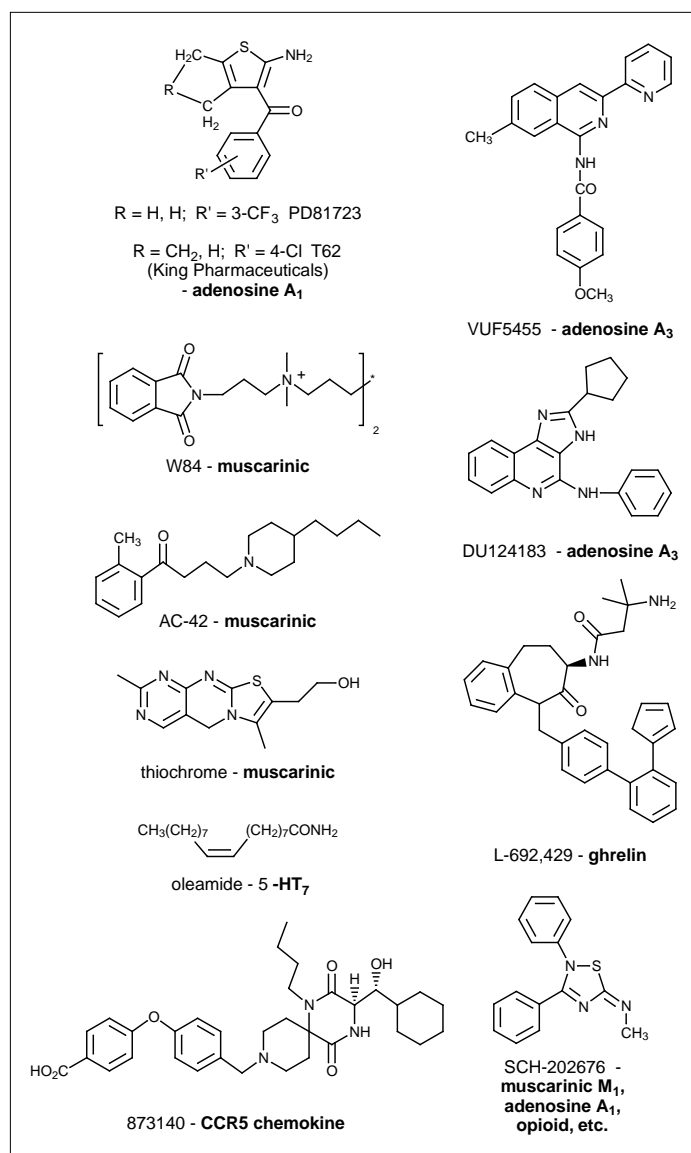


FIGURE 1

Structures of representative allosteric modulators for class A GPCRs that are in clinical trials or used as research tools.

activity on the A_1 ARs was diminished. A 4-chloro analog, in which the methyl groups of PD81723 have been cyclized, T62 (King Pharmaceuticals) (Figure 1), has been in preclinical development for the treatment of chronic pain [30,31] and is now in Phase I clinical trials. The benzoylthiophene allosteric enhancers also appear to act as coactivators of the A_1 AR – that is, it is capable of activating the receptor through binding to an allosteric site, even in the absence of an orthosteric agonist [32]. The thiadiazole SCH-202676 (Figure 1) and its analogs are also modulators for A_1 ARs and several other GPCRs [33,34]. It was recently demonstrated that the thiadiazoles are sulfhydryl-modifying agents rather than allosteric modulators because they appear reversibly to modify the sulfhydryl groups of cysteine residues in cell membrane preparations [35].

The allosteric modulation of A_{2A} and A_3 ARs has also been studied recently [36–39]. Although an allosteric enhancer for the A_{2A} ARs has not yet been developed, amiloride and analogs were demonstrated to be allosteric inhibitors for the A_{2A} AR. There are

at least three classes of allosteric modulators for the A_3 AR, including DU124183 (Figure 1), VUF5455 (Figure 1) and amiloride analogs [19]. All of these modulators influence the dissociation kinetics of the radioligand from the A_3 AR. They also influence the A_3 AR function, as demonstrated by *in vitro* cyclic AMP functional assays in intact cells [19]. It should be noted that the functional enhancement of these allosteric modulators can only be observed in the presence of a high concentration of competitive antagonist, thus overwhelming their antagonistic activity. An exception is DU124183 and some of its analogs, which enhance agonist efficacy even in the absence of a competitive antagonist. DU124183 achieved a 30% potentiation of the maximal efficacy of a potent A_3 receptor agonist. Thus, functional enhancement by DU124183 and its analogs could be further pursued for eventual therapeutic applications. A_3 allosteric enhancers might be predicted to be useful against ischemic conditions.

A_3 AR mutagenesis studies have implicated F182^{5,43} and N274^{7,45} in the action of the enhancers DU124183 and VUF5455 [39]. The mutagenesis data were interpreted using a rhodopsin-based A_3 AR molecular model, suggesting multiple binding modes of the enhancers either at the orthosteric site or at a distinct putative allosteric site.

Muscarinic receptors

Central muscarinic cholinergic neurotransmission has been implicated in psychosis and cognitive disorders [40]. Both acetylcholine esterase inhibitors and weakly selective muscarinic agonists improved neuropsychiatric symptoms and cognitive function in Alzheimer's disease patients. However, the lack of truly selective drugs for the five muscarinic receptors has prevented an unambiguous determination of the role of each receptor subtype in human behavior. Thus, the muscarinic receptors are prime candidates for the development of allosteric modulators to fill an unmet medical need [18].

An allosteric site on a GPCR was first discovered in muscarinic receptors through studies with isolated organs [41,42]. Subsequent receptor-binding studies confirmed the nature of the allosteric modulation of muscarinic receptors by gallamine [43]. Allosteric modulation of GPCRs has been most extensively studied in muscarinic receptors [18]. The existence of a secondary allosteric site on the muscarinic receptor suggests that it might be possible to develop novel allosteric enhancers to potentiate the effects of endogenous acetylcholine in the same way that benzodiazepines potentiate the action of GABA at the GABA_A receptor complex [6].

None of the orthosteric muscarinic receptor agonists or antagonists is highly selective for a given subtype, whereas highly subtype-selective allosteric modulators have been identified. Thus, the allosteric site could be a promising target in drug development. Therapeutically, allosteric enhancement of the effect of endogenous acetylcholine on a given subtype could be an effective way to relieve several disorders. For example, an M_1 receptor enhancer could be applied for treatment of Alzheimer's disease by compensating for the local acetylcholine deficit.

A wide range of molecules have been reported to be allosteric modulators of muscarinic receptors, including strychnine, neuro-muscular blockers, staurosporine and analogs, and other amine derivatives of alkaloids [18]. One of the allosteric modulators, dimethyl-W84 (Figure 1), has been radiolabeled to study the allosteric

site on the muscarinic receptor [44]. A recently synthesized positive allosteric modulator for the M2 muscarinic receptor was shown to be ~100-fold more potent than W84 [45]. Potential allosteric agonists of this receptor include AC-42 (Figure 1) [46,47] and *N*-desmethylozapine [48]. Some pirenzepine analogs were reported to interact with both binding domains for acetylcholine and the allosteric modulator brucine [49]. Brucine and its analogs act as allosteric modulators of various subtypes of muscarinic receptors in a complex manner. For example, *N*-chloromethylbrucine allosterically enhances and inhibits the binding and function of acetylcholine at M3 and M2 muscarinic receptors, respectively [50]. Analogs of the steroid derivative WIN 62,577 define a second allosteric site on the M3 muscarinic receptor that is nonoverlapping and pharmacologically distinct from the strychnine site [51]. Thiochrome (Figure 1), a metabolite of thiamine, enhances acetylcholine affinity selectively at the M4 receptor subtype via positive cooperativity but shows neutral cooperativity at M1, M2 and M3 receptors [52]. Felder *et al.* [53] have identified a novel selective positive allosteric modulator of the M4 receptor, which displayed an α of >20 in binding experiments and also enhanced functional responses. Trankle *et al.* [54] and Wess [55] recently described evidence suggesting that two allosteric agents and one orthosteric ligand might be able to bind to the M2 muscarinic receptor simultaneously. It has been suggested that the complex behavior might be rationalized by allosteric interactions transmitted within a receptor dimer [54,55].

Dopamine receptors

Dopamine receptors are intimately involved in several diseases, including Parkinson's disease and schizophrenia. Evidence suggests that dopamine receptors are subject to allosteric modulation [20]. Allosteric modulators of dopamine receptors include sodium ions, zinc ions, amiloride and its analogs and the tripeptide *L*-proline-*L*-leucine-*L*-glycine. All three types of allosteric modulation (positive, negative and neutral cooperativity), previously demonstrated in muscarinic receptors, have also been shown to occur in dopamine receptors. The ability of an allosteric modulator to fine tune the pharmacological response has attracted much interest in discovering new clinical applications. In the case of Parkinson's disease, an allosteric modulator that enhances the effect of the residual amount of endogenous dopamine could be extremely helpful. One of the promising allosteric modulators, *L*-proline-*L*-leucine-*L*-glycine, has been extensively modified for the potential treatment of Parkinson's disease [56].

Growth hormone secretagogue receptors

Growth hormone secretagogue receptors (GHSR) have been suggested as an important drug target for several diseases, including obesity and diabetes. Ghrelin is the endogenous agonist for the GHSR. Recently, Holst *et al.* [57] reported that a nonpeptide agonist, MK-0677, behaved essentially like ghrelin in activating the GHSR in all systems tested. However, it was found that another nonpeptide agonist, L-692,429 (Figure 1), and a peptide agonist, GH-releasing peptide 6, behave as positive and negative allosteric modulators of GHSR, respectively, in addition to their allosteric agonist activity. It is suggested that an optimal drug candidate could be an agonist which is also a positive modulator of ghrelin receptor signaling [57].

Chemokine receptors

Chemokines are a family of structurally related low molecular weight proteins acting on at least 18 GPCR members [58]. Their function is predominantly related to leukocyte trafficking [59]. Both allosteric agonists and allosteric antagonists have recently been found, and are of potential clinical application. Two peptide allosteric agonists have been pinpointed for the CXCR4 chemokine receptor, a co-receptor for T-tropic strains of HIV-1 [60]. Studies demonstrated that noncompetitive allosteric inhibitors of CXCR1 and CXCR2 chemokine receptors, including repertaxin, were protective against reperfusion-induced organ injury [61]. Targeting the allosteric site on the CXCR1 receptor has been suggested as a general strategy to modulate the activity of chemoattractant receptors. The allosteric noncompetitive HIV entry inhibitor 873140 (Figure 1) exerts its effect by interacting with an allosteric site on the CCR5 chemokine receptor [62].

Allosteric modulation of other members of class A GPCRs has also been described. These members include α_1 -adrenoceptor [63,64], serotonin 5-HT_{2C} [65], CB1 cannabinoid [25], purinergic P2Y₁ [66,67] and oxytocin receptors [68]. Interestingly, oleamide (Figure 1), which is an allosteric modulator of the 5-HT receptor, was recently found also to be an endogenous agonist for the CB1 cannabinoid receptor [69].

Class B GPCRs: secretin-like receptors

Class B GPCRs (also known as the secretin-like receptor family) are a family of peptide-binding receptors comprising 15 members which share little sequence homology with class A (rhodopsin-like) or class C (mGlu) GPCRs. Several class B GPCRs, including corticotropin-releasing factor (CRF), growth hormone-releasing hormone, glucagon, secretin, calcitonin and parathyroid hormone (PTH) receptors, are crucially involved in controlling numerous important physiological processes [21].

Unlike some of the class A GPCRs in which the binding of small-molecule ligands occurs within the TM region, peptides bind to the class B GPCRs in a putative two-domain model. The carboxy-terminus of the peptide ligand binds to the extracellular amino-terminal domain (~100–160 residues) of the receptor. Binding causes the interaction of the amino-terminus of ligands with the juxtamembrane domain (J-domain) of the receptor. Nonpeptide ligands can bind either the amino-terminus or the J-domain and allosterically modulate the peptide binding [21]. Thus, although it is unknown if all peptides bind orthosterically, allosteric modulation using small molecules seems to be a classical means of controlling the class B receptor function.

Hoare *et al.* [70] quantitatively evaluated the allosteric inhibitory mechanism of the nonpeptide modulators for the CRF₁ receptor using an allosteric ternary complex model. Mutagenesis studies of the CRF₁ receptor indicated that nonpeptide antagonists predominantly bind to the J-domain of the receptor. Two mutations (H199V and M276I) in the J-domain affected the nonpeptide but not peptide binding [71]. It should be noted that the experimental evidence from mutagenesis is not unequivocal. Hoare *et al.* [70] argued that there should be caution in the interpretation of the interaction as allosteric because of a lack of conclusive evidence. The nonpeptide glucagon receptor antagonist L-168,049 (Figure 2) also binds to the J-domain of the receptor and allosterically modulates glucagon binding [72]. Nonpeptide agonists have also been found

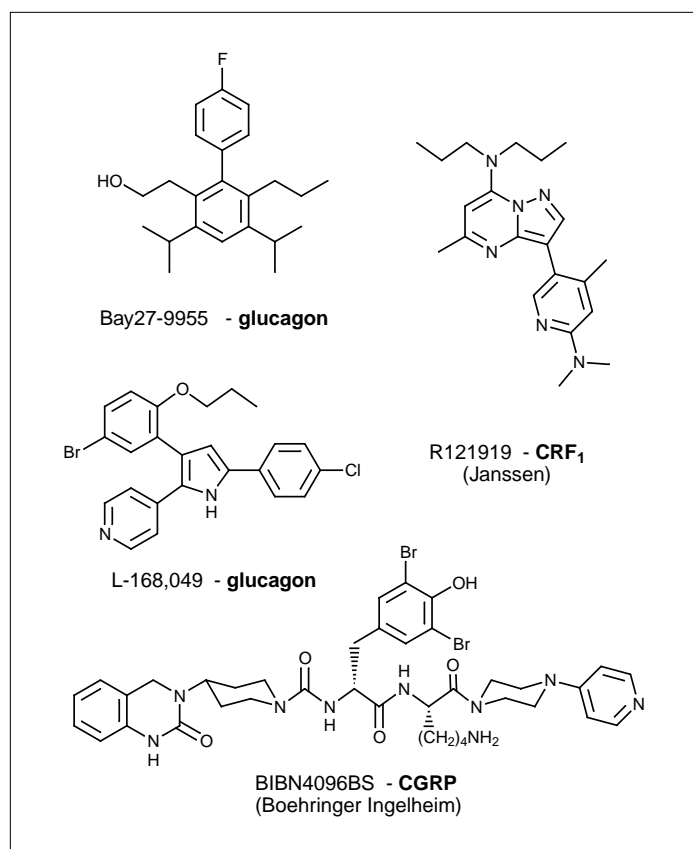


FIGURE 2

Structures of representative allosteric modulators for class B GPCRs that are in clinical trials or used as research tools.

for the calcitonin receptor [73] and the glucagon-like protein-1 receptor [74].

The first-generation nonpeptide CRF₁ antagonist R121919 (Figure 2) is being developed by Janssen Pharmaceuticals NV and Neurocrine Biosciences. It is in Phase II clinical trials for treating depression and anxiety [75]. Neurocrine Biosciences and GlaxoSmithKline (GSK) jointly initiated Phase I clinical trials of GSK 876008 (structure not disclosed) in late 2004. This compound will be entering Phase II clinical trials in the first half of 2006. The calcitonin gene-related peptide receptor antagonist BIBN4096BS (Boehringer Ingelheim Pharma, Germany and University of Copenhagen, Denmark) (Figure 2) is in Phase II clinical trials for migraine relief [76]. A glucagon antagonist (Bay27-9955; Figure 2) has been proposed for the treatment of type 2 diabetes [77].

Class C GPCRs: metabotropic glutamate-like receptors

Glutamate is the major excitatory transmitter in the brain and binds to both LGICs and a family of class C GPCRs known as metabotropic glutamate receptors (mGluRs). Unlike most class A and class B GPCRs, class C GPCRs contain three domains: the extracellular Venus flytrap domain (VFD), which contains the agonist-binding site, the cysteine-rich domain (CRD) and the heptahelical domain (HD) involved in G protein activation [11]. In addition, the carboxy-terminal tail can be unusually long – for example, 376 residues in the mGluR5b. The fact that in class C receptors the native agonists bind in an entirely separate domain of the receptor, with respect to class A GPCRs, provides an opportunity

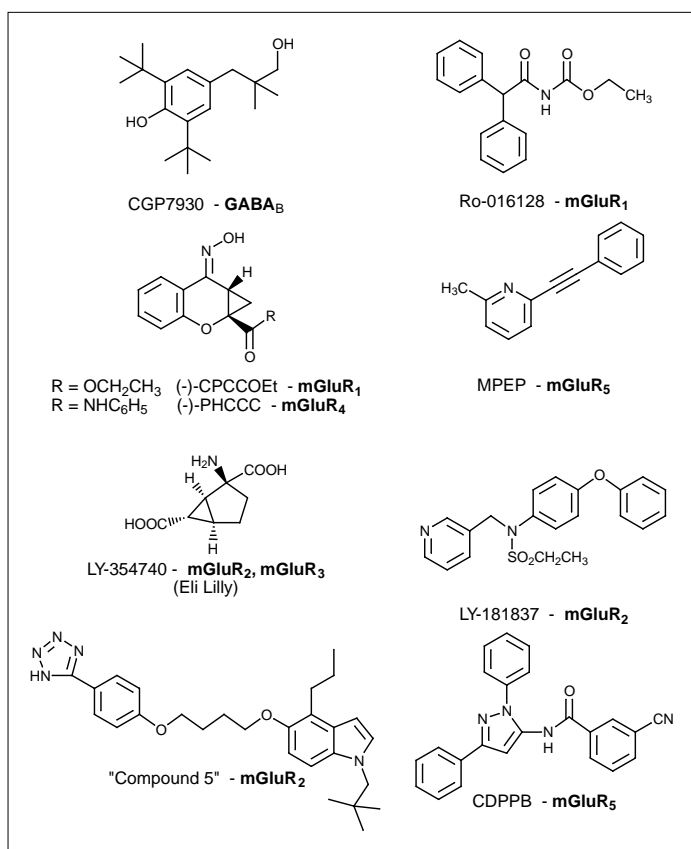


FIGURE 3

Structures of representative allosteric modulators for class C GPCRs (GABA_B and mGlu) that are in clinical trials or used as research tools.

to modulate receptor action through interactions of small molecules within the HD region.

Class C GPCRs are constitutive homo- or heterodimers. mGluRs exist in homodimers but GABA_B receptors form heterodimers (GABA_{B1} and GABA_{B2}). Full activation of the homodimers requires two agonists, whereas one agonist is sufficient to activate the heterodimeric receptors. In the case of GABA_B receptors, the GABA_{B1} VFD binds to an agonist but cannot activate the G protein, whereas GABA_{B2} is responsible for G protein activation. It was recently demonstrated that, although GABA binds to the VFD of GABA_{B1} through allosteric transition to activate the receptor, the GABA_{B2} HD can be activated directly by an allosteric modulator, CGP7930 [78] (Figure 3).

Experiments have indicated that some class C receptors exhibit agonist-independent constitutive activity. Spontaneous closure of the VFD was observed for the GABA_B receptors but competitive antagonists behave as inverse agonists to block the spontaneous closure of the VFD. However, in the case of mGluR1 and mGluR5, the spontaneous closure of the VFD was not blocked by competitive antagonists. Noncompetitive antagonists were found to bind to the HD and showed inverse agonist properties. In addition, the positive allosteric modulators of the class C GPCRs can also bind to HD, potentiating efficacy and potency of agonists, although they themselves did not show any agonist activity [79]. Interestingly, when the extracellular domain was removed from the receptors, the positive allosteric modulators acted as full agonists [80], suggesting that the HD of class C GPCRs might behave independently,

similarly to the class A receptors. Thus, Pin *et al.* [11] concluded that the HD of mGluRs can be either inhibited or activated by synthetic small molecules. Two noncompetitive antagonists are required per receptor dimer to block receptor activity, whereas a single positive allosteric modulator is sufficient for activation. This analysis supports the view that in GPCR dimers, a single monomeric HD is turned on at a given time.

Although the role of the CRD in receptor activation is less clear, it was recently found that the CRD of T1R3 receptors determines responses to intensely sweet proteins [81]. Also, it appears to be necessary for the activation of mGluRs and the CaR [82]. Thus, all three domains of class C GPCRs are amenable to orthosteric and allosteric modulation.

As a result of the emerging roles of class C GPCRs in numerous physiological and pathophysiological conditions, allosteric modulators have emerged as a classical means of control for several the class C GPCRs, and are the subject of intensive research owing to their therapeutic potential [83].

mGluRs

The mGluRs comprise eight members in three classes. Group I includes mGluR1 and mGluR5, which are predominantly coupled to Gq proteins, the activation of which results in an increase in intracellular calcium levels. Group I mGluRs are mainly located postsynaptically, generally enhancing postsynaptic signaling. Both group II (mGluR2 and mGluR3) and group III (mGluR4, -6, -7 and -8) are coupled to Gi/o proteins. Groups II and III are located presynaptically and have an inhibitory effect on neurotransmitter release.

Allosteric modulation has been well studied in the mGluRs. Glutamate and other known agonists and competitive antagonists bind to the amino-terminal region. All known positive and negative allosteric modulators bind to the 7TM domain. Development of subtype-selective allosteric modulators and their potential therapeutic applications have recently been reviewed [11,84–88].

The first allosteric modulators characterized for the mGluRs, 7-hydroxyiminocyclopropan[b]chromen-1a-carboxylic acid ethyl ester [(–)-CPCCOEt] and 2-methyl-6-(phenylethynyl)-pyridine (MPEP), are negative allosteric modulators for mGluR1 and mGluR5, respectively [89,90]. Selective positive allosteric modulators have recently been reported for mGluR1, mGluR2, mGluR4 and mGluR5 [91]. Ligands acting at orthosteric sites have not been demonstrated to be highly selective, suggesting that the allosteric sites might be able to be readily targeted by ligand optimization, a strategy that has been less successful for orthosteric glutamate binding sites. This finding supports the observation that putative allosteric sites generally have less sequence conservation between subtypes relative to orthosteric agonist-binding sites.

Positive allosteric modulators for mGluR1 include Ro-016128 (Figure 3), Ro-677476 and Ro-674853. These compounds are selective for mGluR1, with the exception of Ro-674853, which also showed some effect on mGluR5. Species differences between humans and rats are typical for these compounds, which are generally more potent at rat mGluR5 than at the human homolog. LY-181837 (Figure 3) was the first selective mGluR2 positive enhancer [92]. Subsequently, more-potent and selective enhancers, such as LY-487379, were developed. Other series of compounds acting as positive allosteric modulators for mGluR2 were also recently developed [85,93]. (–)-CPCCOEt (Figure 3) and MPEP (Figure 3) were initially

found to be negative allosteric modulators for mGluR1 and mGluR5, respectively, and were later found to be positive for mGluR4. An analog of (–)-CPCCOEt, (–)-N-phenyl-7-(hydroxylimino)cyclopropa[b]chromen-1a-carboxamide [(–)-PHCCC] (Figure 3), was found to be potentially useful for the treatment of Parkinson's disease [94]. 3,3'-Difluorobenzaldazine was the first positive allosteric modulator to be identified for mGluR5 [95]. Interestingly, two close analogs, 3,3'-dimethoxybenzaldazine and 3,3'-dichlorobenzaldazine, showed a negative allosteric effect and neutral cooperativity, respectively. Other novel classes of positive modulators for mGluR5, including N-[4-chloro-2-[(1,3-dioxo-1,3-dihydro-2H-isoin-dol-2-yl)methyl]phenyl]-2-hydroxybenzamide (CPPHA) and 3-cyano-N-(1,3-diphenyl-1H-pyrazol-5-yl)benzamide (CDPPB; Figure 3), were later identified [96,97]. Benzazoles, such as compound 5 (Figure 3), display efficacy as allosteric potentiators of mGlu2 in animal model for schizophrenia [98]. CDPPB exhibited a significant antipsychotic effect. These modulators have the potential to treat a range of central nervous system diseases, including anxiety, Parkinson's disease, schizophrenia and cognition disorders.

LY-354740 (Eli Lilly) (Figure 3), an allosteric enhancer of mGlu2 and mGlu3 receptors, is in Phase II clinical trials for the treatment of anxiety-related disorders [99,100].

GABA_B receptors

GABA_B receptors are emerging drug targets. Agonists can be used in the treatment of pain, drug-dependence and anxiety, whereas antagonists can prove beneficial in cognitive disorders and depression. To date, drug development has been impeded by the difficulty in achieving receptor subtype selectivity. Allosteric modulators of GABA_A channels, including diazepam, have been administered successfully, whereas directly acting GABA_A agonists have not been applied clinically, owing to the severe side effects [6]. Thus, the same approach might be applied to the GABA_B receptor. Indeed, recently synthesized compounds acting via an allosteric mechanism have been identified and are providing hope that future drugs might be developed to target this receptor [16]. CGP7930 and CGS13501 are the first positive allosteric modulators of the GABA_B receptor to be identified. These compounds do not activate the receptor on their own; rather, they potentiate the agonist efficacy and affinity, as observed with diazepam on GABA_A receptors.

CaRs

Secondary hyperparathyroidism (HPTH) is a common, life-threatening complication of chronic kidney disease, and can lead to cardiovascular calcification. The CaR is a class C GPCR that regulates the secretion of PTH and is most highly expressed in parathyroid, thyroid parafollicular cells and renal tubular cells. It is downregulated in chronic renal failure. Medicinal chemical studies of allosteric modulators of the CaR have led to the novel treatment of HPTH with calcimimetic agents.

Certain phenylalkylamine compounds, typified by the first-generation compounds NPS467 and NPS568, are among the positive allosteric modulators identified for the CaR [101]. At nanomolar concentrations, they were found to increase the concentration of cytoplasmic calcium in bovine parathyroid cells and to inhibit PTH secretion. Acidic residues in the extracellular loops of the CaR have been shown to be crucial for the response to both Ca²⁺ and NPS568

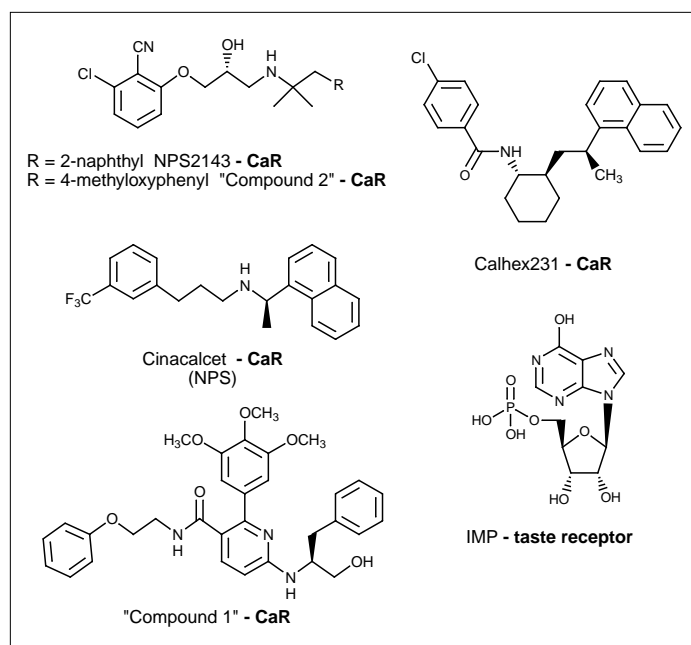


FIGURE 4

Structures of representative allosteric modulators for class C GPCRs (CaR) that are used in the clinic or as research tools.

[102]. Both positive and negative CaR allosteric modulators in this chemical class interact with the HD [103]. The second-generation calcimimetic cinacalcet (Figure 4) (NPS Pharmaceuticals) was the first allosteric modulator for a GPCR to be marketed for the treatment of HPTH [8,9]. Certain L-amino acids were also shown to be allosteric activators of the CaR [104]. The VFD of CaR has been shown to be the site of action of L-amino acid modulators [105].

Calcilytic agents, such as NPS2143 and CalHex231 (Figure 4), have also been designed [106,107]. Mutagenesis of the CaR has identified a mutation (P823A) at the TM6–TM7 junction that mimics the action of the negative allosteric modulator NPS2143, blocking the activation of the CaR occurring in autosomal dominant hypocalcemia. The finding that NPS2143 corrects a molecular defect associated with autosomal dominant hypocalcemia suggests that such negative allosteric modulators could offer an effective form of treatment for this disease [108].

Arey *et al.* [109] have designed a novel class of orally active calcilytic agents that increase serum PTH levels *in vivo*. Compound 1 (Figure 4) possesses potent antagonist activity at the human CaR, with an IC_{50} value of 64 nM in inhibiting intracellular Ca^{2+} flux. Such compounds might offer an improved treatment for osteoporosis.

Taste receptors

The perception of taste depends, to a large extent, upon an individual's sensitivity to taste, including sweet, bitter, sour, salty and unami. It has been proposed that sodium chloride stimulates taste receptors in an allosteric manner [110]. Activation of the unami receptor by disodium 5'-guanylate (GMP), but not by monosodium glutamate (MSG), was blocked by amiloride in a competitive manner [111]. MSG has been shown to be an allosteric modulator of the GMP-stimulated taste response [112]. The family of taste receptors has recently been cloned and functionally characterized [113].

It was suggested that T1R1 and T1R3 receptors, which respond to sweet taste, combine into one functional unit [17]. Certain nucleotides, such as inosine 5'-monophosphate (IMP; Figure 4), do not activate the functional unit but potentiate the ability of L-(+)-2-amino-4-phosphonobutanoic acid (L-AP4), MSG and other amino acids to activate the receptor [17].

LGICs

LGICs are membrane receptors responsible for rapid synaptic transmission and modulation of cellular activity. These channels are proteins spanning the cell membrane and forming both the binding site for the natural ligand and the ion-conducting pore, which can be opened or closed upon ligand binding. Upon activation, ion channels open to enable ion flux across the cell membrane. The flux can cause depolarization or hyperpolarization, depending on the charge and concentration of the ions. Allosteric modulators of LGICs are important therapeutic agents [114,115].

In mammals, LGICs comprise three subfamilies, based on the number of TMs. The first family is the P2X receptors, activated by ATP, which are cationic channels containing three subunits. Each subunit contains one large extracellular loop and two TMs with both amino- and carboxy-termini in the cytosol. The second family is glutamate-activated cationic channels, including N-methyl-D-aspartate (NMDA), alpha-amino-3-hydroxy-5-methylisoxazole-propionate (AMPA) and kainate receptors, all comprising four subunits. The amino-terminus is located extracellularly and the carboxy-terminus is located intracellularly. The third family is the largest family, and includes nicotinic, glycine, 5-HT₃, GABA_A, GABA_C, zinc-activated ion channel receptors and several others. Members of the third family are made up of five subunits, each containing an amino-terminus and four TMs. Both the carboxy- and amino-termini are located extracellularly. The ligand-binding site lies on the interface between adjacent subunits. Almost all members of the LGIC family are subject to allosteric modulation.

P2X receptors

The allosteric sites on the P2X receptors have been well defined [116]. In the rat dorsal root ganglion neuron, calcium ions selectively modulate the P2X₃ receptor, which is inhibited by magnesium [117]. Cibacron blue, which is an antagonist of P2X₁ and P2X₂ receptors, enhanced the P2X₃-mediated nociceptive responses induced by 3'-O-(4-benzoylbenzoyl)-ATP and attenuated by the effects of the antagonist trinitrophenyl-ATP [118]. Tetramethylpyrazine used in Chinese medicine as an analgesic negatively modulates ATP-activated currents in rat dorsal root ganglion neurons [119]. Ethanol has also been found to act as a negative modulator of P2X receptors by right-shifting the activation curve [120]. Ivermectin is a positive allosteric modulator of P2X₄ receptors [121]. Zinc has been shown to act as allosteric modulator of P2X₂ receptors, potentiating channel opening [122]. Although these molecules have not translated to therapeutic application, they could represent the optimizable leads for eventual clinical use [123].

Glutamate receptors

Glutamate is the major excitatory neurotransmitter in the brain [124]. Glutamate receptors are the only LGICs for which multiple high-resolution crystal structures have been solved. The agonist-binding site is between two regions of the extracellular domain.

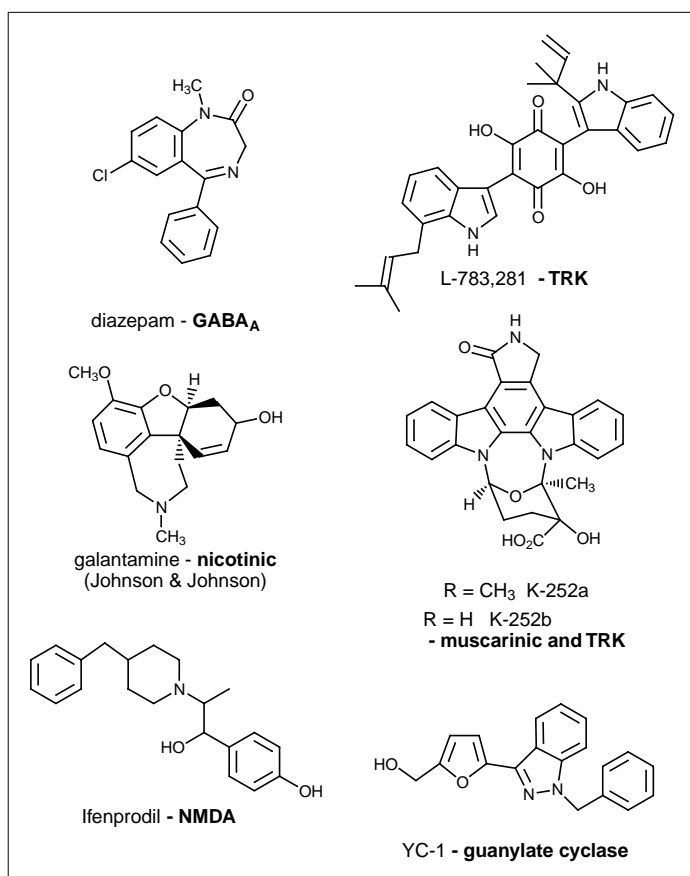


FIGURE 5
Structures of representative allosteric modulators for LGICs, Trk and guanylate cyclase receptors that are used in the clinic or as research tools.

NMDA receptors are cation-permeable channels and can be modulated by the positive allosteric modulator glycine and by the negative allosteric modulators zinc and ifenprodil (Figure 5) [125]. ATP was recently shown to act as both a competitive antagonist and a positive allosteric modulator at the NMDA receptor [126]. Positive and negative allosteric modulators have also been identified for the AMPA receptors [124].

GABA_A receptors

GABA, acting on LGICs, GABA_A and GABA_C, and G-protein-coupled GABA_B receptors, is the principal inhibitory neurotransmitter in the brain [6,127]. GABA_A receptors are important drug targets for anxiety, epilepsy and sleep disorders. The GABA_A receptors are the target for the most widely prescribed sleep medications, the benzodiazepines.

Activation of GABA_A receptors leads to opening of channels that are permeable to HCO₃⁻ and Cl⁻. Multiple allosteric sites have been found in GABA_A receptors [127,128]. Directly acting GABA_A agonists have not found clinical applications, owing to various side effects. An alternative approach was to design ligands that could potentiate the effect of the endogenous agonist, and thereby gain selectivity. Benzodiazepines, including diazepam (Figure 5), were among the first generation of allosteric modulators for a membrane receptor to be used clinically. Benzodiazepines bind to a site on the receptor distinct from the site where GABA binds, potentiating the functional response upon receptor activation by GABA. Drugs

acting at the allosteric sites have shown important advantages in comparison with conventional agonists or antagonists.

It was found recently that benzodiazepines show binding selectivity for a subset of GABA_A receptors [128,129], which is encouraging from the perspective of drug discovery. Efforts have been made to develop more selective ligands for a given binding site on the receptor [129,130].

Nicotinic receptors

Nicotinic receptors were among the first membrane receptors in which allosterism was studied [3,114]. Various molecules were shown to be allosteric modulators of the nicotinic receptors. Positive modulators included divalent cations, steroid hormones, ivermectin, cocaine methiodide and 5-hydroxyindole [131]. Galantamine (Johnson and Johnson) (Figure 5) is a positive allosteric modulator of $\alpha 4\beta 2$ and $\alpha 7$ nicotinic receptors [132,133], in addition to its anticholinesterase activity. Galantamine has been successfully used in the clinical treatment of Alzheimer's disease [7].

Other membrane receptors

Tyrosine kinase receptors

Tyrosine kinases are classified as transmembrane tyrosine kinase receptors (or receptor tyrosine kinases) and intracellular nonreceptor tyrosine kinases. Tyrosine kinase receptors are transmembrane proteins with a ligand-binding extracellular domain and a catalytic intracellular kinase domain. Emerging targets for cancer therapy [134], tyrosine kinase receptors have a central role in cellular growth, differentiation and oncogenesis. The activation of tyrosine kinase receptors by growth factors leads to dimerization and activation of an intrinsic tyrosine-specific kinase activity by an allosteric mechanism [135]. The insulin receptor, which belongs to the tyrosine kinase receptor family, functions as an allosteric protein in which the binding of the hormone to the α -subunit leads to a series of conformational changes resulting in the activation of the β -subunit tyrosine kinase [136].

Neurotrophins activate two types of cell-surface receptor, the Trk receptor tyrosine kinase and the shared p75 neurotrophin receptor [137,138]. p75 binds along the homodimeric interface of nerve growth factor, which disables the symmetry-related second p75 binding site of nerve growth factor through an allosteric conformational change [137]. Moreover, p75 affects Trk subdomain utilization in ligand-dependent activation, possibly by conformational or allosteric control [139]. L-783,281 (Figure 5) is a prototype for a small-molecule neurotrophin mimetic that activates Trk by interacting at a site different from the neurotrophin-binding site and induces conformational change [140].

Nerve growth factors as Trk receptor activators have not met with clinical success owing to their poor pharmacokinetic behavior, whereas small-molecule mimetics have shown promise for future treatment of neurodegenerative diseases [141]. These compounds include allosteric modulators and other indirectly acting agents. Given the difficulty of designing directly acting agonists, an alternative approach is to identify allosteric modulators that might potentiate the endogenous agonists, neurotrophins, which are physiologically secreted at low levels. Ganglioside GM1 and related compounds were among the first to be identified to potentiate the action of neurotrophins following Trk receptor activation [142]. Two staurosporine-like compounds, K-252a and K-252b

(Figure 5), which are also allosteric modulators for muscarinic receptors [143], were found either to inhibit or to enhance the action of the Trk receptor, depending on the concentrations used [141]. It was found that the staurosporine-like compound L-753,000 potentiates the neurotrophic effects of neurotrophin 3 by acting selectively at the TrkA receptor [144]. Additionally, Trk receptors in PC12 cells have been demonstrated to be activated indirectly by an adenosine A_{2A} receptor agonist, CGS21680, and this effect was blocked by a selective A_{2A} antagonist, ZM241385. The effect could also be blocked by the Trk inhibitor K252a. Thus, indirect activation by small molecules could open a new avenue for manipulating Trk receptors.

Guanylate cyclase receptors

Guanylate cyclases (GCs) are membrane receptors which, upon activation, catalyze the conversion of GTP to cGMP, and which can be classified into two subgroups: soluble and particulate GCs. The soluble GC is a ubiquitous receptor for nitric oxide (NO), which is involved in neurotransmission and vasodilation. The atrial natriuretic peptide receptor (particulate GC) is one of the best-studied membrane GCs and is a target for congestive heart failure [145].

3-(5'-hydroxymethyl-2'-furyl)-1-benzyl indazole (YC-1) (Figure 5) is an allosteric activator of soluble GC that increases the GC catalytic rate and sensitizes GC towards its gaseous activators, NO and carbon monoxide [146]. Bay58-2667 activates soluble GC in a NO-independent manner and its binding site is distinct from the binding site of the analog BAY41-2272, which activates soluble GC [147,148]. Thus, drug development for membrane GCs could target a distinct allosteric site to increase the prospect of selectivity.

Concluding remarks

Increasing evidence suggests that GPCR dimerization is common to all three classes of GPCRs. Original models describing the activation of a GPCR, especially the class A GPCRs, is generally related to the conformational change within a monomer. In the case of a

dimer, it can be speculated that an orthosteric agonist for one dimerized monomer could be a positive or negative allosteric modulator for another monomer. From this point of view, most, if not all, membrane receptors could be naturally allosterically modulated. Thus, allosteric modulation is a classical controlling mechanism for receptor behavior and cellular processes.

Allosteric sites are an increasingly attractive target for drug development; thus, future screening assay systems should be re-engineered to accommodate the requirement of allosteric sites. It is necessary to note the use of dissociation kinetics to detect the allosteric phenomenon. It has been demonstrated that some molecules, such as SCH202626 and its analogs, which do not act through an allosteric mechanism, might also increase or decrease the dissociation rate. Therefore, multiple binding and functional assays should be utilized for the study and validation of a potential allosteric modulator.

Allosteric modulators could be particularly useful to control receptors for which the design of selective orthosteric agonists or antagonists has been elusive, such as muscarinic receptors. Allosteric modulators for chemokine receptors are poised to join the next generation of anti-HIV drugs. Neurotrophins have not proven successful as therapeutic agents owing to their poor pharmacokinetics and lack of selectivity. Thus, identification of small-molecule allosteric modulators to produce a conformation change in the receptor tyrosine kinase structure to attenuate or potentiate its interaction with a large endogenous ligand is likely to be an intensive future effort. Owing to the fact that increasing effort has been invested in the study of the allosteric sites, and that increasing numbers of allosteric modulators are in clinical trials, it can be speculated that more drugs targeting allosteric sites will be used therapeutically in the near future.

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