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Allosteric Modulation of Seven Transmembrane Spanning Receptors: Theory, Practice, and Opportunities for Central Nervous System Drug Discovery

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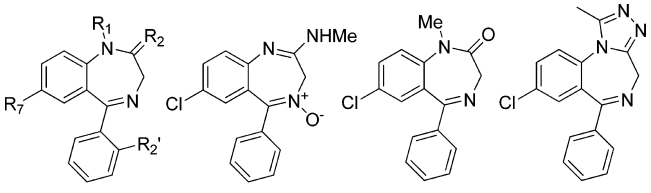
1. INTRODUCTION

1.1. Allosteric Modulation: A Historical Perspective. Early ideas regarding allosterism emerged over 50 years ago but gained little traction in the receptor field because of limitations in molecular pharmacology and screening technology.[1,2] Allosterism is a critical biochemical mechanism, as it enables proteins to sense changes in their environment and to respond to them; Fenton has recently referred to this as "second secret of life", preceded only by the genome.[1−3] The term "allostery" comes from the Greek *allos* (ἄλλος), "other", and *stereos* (στερεος), "solid (object)", meaning that an allosteric site of a regulatory protein is physically distinct from the classic, active, site.[1−8] In terms of receptor-based small molecule drug discovery, the binding site for the endogenous ligand is referred to as the orthosteric site.[1,4−8] In this setting, an allosteric modulator is a small molecule that binds at a topographically distinct allosteric site and either potentiates or inhibits the binding and/or signaling of an orthosteric ligand.[1,4−8] Fueled by the clinical success of the first allosteric modulator drugs **1**−**4** (benzodiazepines, referred to as "benzo" or BZD), which potentiate the effect of the neurotransmitter γ-aminobutyric acid (GABA) at the ionotropic $GABA_A$ receptor, the concept of allosteric modulation for a wide range of molecular targets has gained momentum in modern drug discovery (Figure 1).[4,9] Benzodiazepines, for example, possess a number of modes of pharmacology and include positive allosteric modulators (PAMs), which potentiate $GABA_A$ receptor response, and negative allosteric modulators (NAMs), which decrease channel activity and modulate the ability of these GABAergic receptors to elicit sedative, hypnotic, and anxiolytic effects. In addition to PAMs and NAMs, silent allosteric modulators (SAMs, or neutral allosteric ligands) bind at allosteric sites and can block the activity of PAMs and NAMs but importantly have no effect on orthosteric ligand responses. In contrast to the potentially deadly effects of direct acting $GABA_A$ agonists, allosteric modulation of $GABA_A$ by the benzodiazepine class has proven to be clinically safe and effective.[4,9] With advances in molecular pharmacology and screening technology, allosteric modulators have now been developed for other ion channels, kinases, phospholipases and seven transmembrane spanning receptors (7TMRs, also known as G-protein-coupled receptors (GPCRs)).[1,4−8,10−15]



1 | **2**, Chlordiazepoxide (Librium™) | **3**, Diazepam (Valium™) | **4**, Alprazolam (Xanax™)

Figure 1. Benzodiazepines, the first allosteric modulators with clinical success and marketed as $GABA_A$ allosteric modualtors. A generic benzodiazepine scaffold **1** highlighting the classical substitution patterns is shown. **2** (Librium) was the first benzodiazepine launched by Hoffmann-La Roche in 1960, and many other congeners followed such as **3** (Valium) and the tricylic analogue **4** (Xanax).

1.2. 7TMR Structures and Ligands. 7TMRs are the largest class of cell surface receptors, accounting for over 30% of currently marketed drugs and over 50% of all known drugs.[4−7] 7TMRs are plasma membrane proteins that receive stimuli (in the form of hormones, neurotransmitters, light, ions, or odorants) on the extracellular surface to alter receptor conformation, which in turn activates signaling cascades and effector systems located within the intracellular cytosol via coupling to G proteins and other accessory proteins.[4−7] Much of our understanding of the basic structure and function of 7TMRs is based on biochemical, genetic, imaging, and molecular pharmacological research, as crystal structures of 7TMRs (rhodopsin, opsin, β2 and β1 (agonist and antagonist bound), dopamine D_3, adenosine 2A (agonist and antagonist bound), chemokine $CXCR_4$, histamine H_1) have only recently been solved definitively.[4−7,16−32] However, these crystal structures have powered the development of homology models for multiple 7TMRs and afforded avenues for ligand design efforts. Structurally, all 7TMRs possess seven transmembrane helices, three extracellular and three intracellular loops, with an extracellular N-terminal tail and an intracellular C-terminal tail (Figure 2).[4−7,16−32] The heptahelical transmembrane domain is largely hydrophobic, whereas the extracellular (e1−e3) and intracellular (i1−i3) segments, or loops, are

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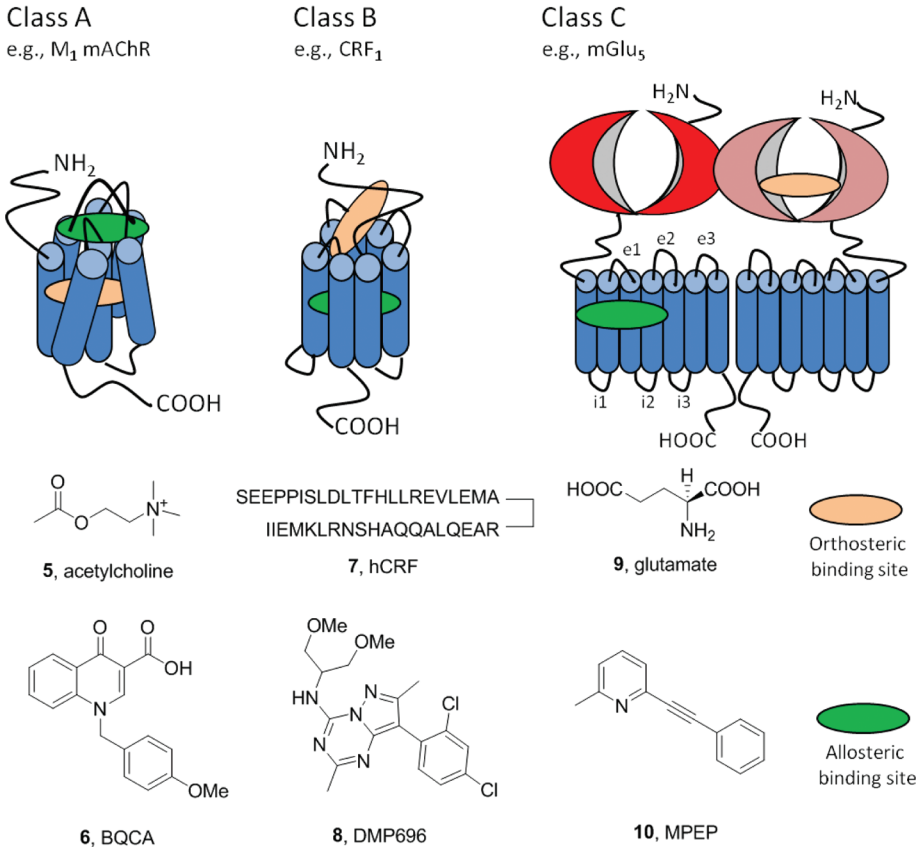

Figure 2. Structural topology of typical orthosteric and allosteric sites of families A, B, and C 7TMRs, highlighting representative orthosteric and allosteric ligands for each family.

generally hydrophilic, as would be anticipated for amino acids exposed to the phospholipid-rich membrane and the water-rich environments, respectively. The seven transmembrane helices are each approximately two-dozen amino acids long, while the C- and N-terminal tails as well as the loops can vary widely in length with up to hundreds of amino acids.[4−7,16−32] On the basis of sequence homology and functional roles, 7TMRs commonly are divided into three main families (or classes): A (e.g., M_1 mAChR), B (e.g., CRF_1), and C (e.g., $mGlu_5$) (Figure 2). The families are readily distinguished by comparing their amino acid sequences. Family B is distinguished from family A by the presence of a larger extracellular loop, and family C has a large, bi-lobed N-terminal Venus fly trap (VFT) domain. A second major difference between the families concerns the location of the orthosteric binding site and the nature of the orthosteric ligand. As shown in Figure 2, the orthosteric binding site of many family A 7TMRs is located with the 7TM domain whereas the orthosteric binding site is located in the large extracellular loop within family B and within the VFT domain in family C. The orthosteric ligands for families A and C are neurotransmitters, for example, **5** (acetylcholine, for the mAChRs) and **9** (glutamate, for the mGluRs), respectively.[4−7] The orthosteric ligands for family B 7TMRs are large peptide ligands with usually >30 amino acids, such as the 41 amino acid peptide **7** (hCRF) for corticotrophin releasing factor 1 (CRF_1). In contrast, allosteric ligands are structurally distinct from orthosteric ligands and bind at distinct sights, often, but not always, topologically distant from the orthosteric site.[4−7] For example, the family A M_1 mAChR PAM **6** (BQCA)[33] is believed to bind in a region above the TMs among the extracellular loops, whereas the family B PAM, **8** (DMP696),[34] and the family C NAM, **10** (MPEP),[35,36] bind within the TM domains.

Are there naturally occurring allosteric modulators? This question is invariably posed during any discussion of allosteric modulators, and one must understand the complexity of identifying such ligands within the chemical diversity of ligands within the human body.[1,2,37] However, a few natural allosteric modulators have been described, including the unnatural amino acid D-serine (an allosteric modulator of the NMDA receptor),[38] L-phenylalanine, and L-tryptophan (allosteric modulators of the calcium receptor)[39] and the tetrepeptide Leu-Ser-Ala-Leu, also known as 5-HT moduline (an allosteric modulator of the 5-HT_{1B} receptor).[40,41]

1.3. Orthosteric and Allosteric 7TMR Pharmacology. Historically, almost all of the FDA-approved drugs that act at 7TMRs bind at the orthosteric site and regulate receptor function by classical agonism (directly stimulating a receptor response), inverse agonism (blocking constitutive receptor activity), or competitive antagonism (blocking the binding of the native agonist).[4−8] This is somewhat expected, as the many of these ligands were discovered by employing assays that biased targeting of the orthosteric binding site. Despite this success, synthetic ligands exist for only a fraction of the known 7TMRs, and many efforts have failed to produce highly selective compounds suitable as drug leads because of the highly conserved orthosteric binding site across a family of 7TMRs and/or because of unfavorable physicochemical and drug metabolism/pharmacokinetic (DMPK) properties of synthetic orthosteric ligands. In many cases, direct acting agonists are toxic or lead to

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Table 1. Reported Allosteric Modulators of Family A G-Protein-Coupled Receptors[a]

receptor	modulator example
adenosine A_1	(PD 81723, PD 117975, PD 78416, PD 71605, LUF 5484, T-62);[45] (VCP 520, VCP 333)[46]
adenosine A_2	amiliorides
adenosine A_3	VU5455Z, VU8504Z, DU124183, [LUF6000 (compound **3** in paper)],[47] (AM 251, 2-arachidonylglycerol or 2-AG)[48,49]
adrenoceptor α_1	amilorides, benzodiazepines, conopeptide, ρ-TIA
adrenoceptor α_{2A}, α_{2B}	amilorides, sodium ions[50]
adrenoceptor β_2	zinc
cannabinoid CB_1	Org27569,[51] Org27759,[52] PSNCBAM-1,[53] (JHW 007, RTI-371)[54]
chemokine CXCR1	reparixin,[55] SCH527123 (compounds **2**−**27** inhibit CXCL8 which activates CXCR1, overall inhibition of CXCR1),[56,57] SCH-479833[58]
chemokine CXCR2	reparixin,[55] SCH527123,[56] SB656933,[59] DF2162,[59] SCH-479833[58]
chemokine CXCR3	IP-10, I-TAC
chemokine CXCR4	RSVM,[60] ASLW,[60] trichosanthin,[61] plerixafor[62]
chemokine CCR1	BX-471,[63] CP-481-715,[64] UCB35625[65]
chemokine CCR3	UCB35625[65]
chemokine CCR5	trichosanthin,[61] TAK779,[66] aplaviroc, AK602, 873140,[66,67] AK530,[68] TAKK 220,[69] SCH351125, ancriviroc,[70] vicriviroc,[71] maraviroc[72]
dopamine D_1	zinc[73]
dopamine D_2	amiloride,[74] zinc
endothelin ET_A	aspirin, sodium salicylate[75]
gonadotropin-releasing hormone receptor (GnRH)	furan derivitive -1 (bitopic), TAK-013[76]
GH secretagogue	L-629,429, GHRP-6, MK-677[77,78]
luteinizing hormone	Org 41841, [^{3}H]Org 43553[79]
mAChR M1	brucine, BQCA, TBPB, AC-42, 77-LH-28-1, N-DMC,[3,43,80,81] VU0119498,[82] staurosporine, ML169,[83] ML137,[84] ML071[85]
mAChR M2	McN-A-343, BR384, gallamine,[86] W84,[87] AC-42, 77-LH-28-1[88]
mAChR M3	VU0119498,[82] amiodarone, *N*-ethylamiodarone
mAChR M4	LY2033298,[89,90] VU0010010,[91] VU0152099,[92] VU0152100[92,93] ML108,[92] thiochrome,[3,43,80,81] WIN 62577, alcuronium, ML173[93]
mAChR M5	ML129,[94] VU0119498,[82] VU0365114,[95] VU0400265,[96] ML172[95]
neurokinin NK_1	heparin
opioid μ, δ	cannabidiol[97]
purine $P2_{Y1}$	2,2-*o*-pyridylisatogen tosylate
serotonin $5HT_{1B/1D}$	5HT-modulin
serotonin $5HT_{2A}$, $5HT_2$	oleamine[98]
serotonin $5HT_{2C}$	oleamine,[98] PNU-69176E[99]

[a]GH, growth hormone; mAChR, muscarininc acetylcholine receptor.

receptor desensitization, internalization, or down-regulation due to being "turned on" for prolonged periods.[4−8]

In recent years, extraordinary progress has been made in the discovery, chemical optimization, pharmacological understanding, and in some cases, clinical development of allosteric modulators for multiple 7TMRs to treat a wide range of peripheral and CNS pathologies (Tables 1−3).[4−8,10,11,42−180] This is due in large part to the development of functional assays that allow discovery of ligands that modulate a receptor without regard to the binding site and in effect identify ligands that do not bind at the orthosteric site. These new allosteric ligands include PAMs, NAMs, SAMs, as well as allosteric agonists (allosteric compounds that activate the receptor in the absence of the orthosteric ligand), partial antagonists (ligands that fully occupy the NAM site but only partially block receptor signaling), and ago-PAMs (PAMs that have inherent allosteric agonist activity).[4−8,10,11,42−44] However, many reported "allosteric" agonists may actually be "bitopic" ligands, that is, hybrid orthosteric/allosteric ligands that bind to both the orthosteric and allosteric sites within a given 7TMR.[181−183] Allosteric ligands offer numerous advantages: (1) Allosteric binding sites

Table 2. Reported Allosteric Modulators of Family B G-Protein-Coupled Receptors[a]

receptor	modulator example
CRF1 receptor	NBI 35965,[100] NBI 27914,[101] antalarmin,[102] DMP696,[34] SSR125543A,[103,104] DMP904,[34] NBI 30775/R121919[105,106]
CGRP receptor	compounds **1**, **3**, and **4**[107]
glucagon	L-168049,[108] DAB, and CP-91149[109]
GLP-1 receptor	T-0632, NovoNordisk compounds **1**−**6**,[110] compound **2**[111]

[a]CGRP, calcitonin gene related peptide; CRF1, corticotrophin releasing factor 1; GLP-1, glucagon-like peptide 1.

may be under less evolutionary pressure for their conservation, thus enabling high subtype selectivity to be achieved. (2) The effects of an allosteric modulator are saturable; once allosteric sites are occupied, no additional effects are observed, i.e., a "ceiling effect" (this is in contrast to "ceiling" of a partial agonist that will vary with receptor density and stimulus response coupling; this is thus far more variable than a ceiling level driven by cooperativity (at the level of binding; i.e., an α of

Table 3. Reported Allosteric Modulators of Family C G-Protein-Coupled Receptors[a]

receptor	modulator example
calcium sensing receptor	NPS 467, NPS 568, L-amino acids,[112−114] cinacalcet,[115] NPS 2143,[116] calhex 231,[117] SB-423557,[118] SB-423562,[119] calindol, ronacalceret[37]
$GABA_B$	BHF177,[120] rac-BHFF, BHFI,[121] CGP7930, GS39783, CGP13501[122−125]
$mGluR_1$	(−)-CPCCOEt,[126] BAY36-7620,[127] R214127,[128] EM-TBPC,[129] JNJ16259685,[130] YM-298198,[131] A841720,[132] FTIDC,[133] YM-230888,[134] CFMMC,[135] VU-71,[136] Ro 01-6128, Ro 67-4853, Ro 67-7476,[137] Ro 07-11401[138,139]
$mGluR_2$	LY181837, LY487379, 3-MPPTS, cyPPTS, 2,2,2-TEMPS, CBiPES,[140−143] BINA,[144] GSK1331258,[145] MNI-136, MNI-137[146−148]
$mGluR_3$	MNI-136, MNI-137[146,147]
$mGluR_4$	(−)-PHCCC,[149,150] ML128,[151] VU0001171, VU0080241, VU0092145, VU0155041,[152−154] VU0359516,[155] SIB-1893[156,157]
$mGluR_5$	MPEP,[35] MTEP,[158] fenobam,[159] VU0285683, VU0360172,[160] DMeOB, DFB, DCB,[161] VU0365396, VU0357121,[162] CPPHA,[163,164] CDPPB,[165,166] VU-29,[167] ADX-47273[168−171]
$mGluR_7$	AMN082,[172] MDIP, MMPIP[173]
T1R1	S807, IMP[37,174−176]
T1R2	S819, SE-2, SE-3[37,177,178]
T1R3	cyclamate, lactisole[37,179,180]

[a]mGluR, metabotropic glutamate receptor; T1R, taste receptors (sweet and umami).

10 sets a limit of 10, whereas a partial agonist can scale up to a full agonist or down to an antagonist depending on the tissue and the disease)). (3) A modulator that lacks agonistic activity will only exert its effects when the endogenous agonist is present, resulting in temporal and spatial activity (also referred to as state dependence) of the endogenous ligand and (4) improved chemical tractability.[4−8] While the majority of these advantages over orthosteric ligands have been realized, allosteric modulation is far from a panacea for drug discovery, and there are many caveats to consider. First, the lack of evolutionary pressure on allosteric sites can, and has, lead to significant species differences, which complicates preclinical pharmacodynamic and safety studies in mice/rats/dogs if a primary assay employs recombinant human receptor or vice versa.[4−8,10,11] Second, the state dependence of allosteric modulators could be a liability in degenerative pathologies because of the progressive loss of endogenous orthosteric tone. For example, Alzheimer's disease is characterized by a decrease in cholinergic tone with disease progression, potentially rendering an mAChR PAM ineffective over time, as there is no acetycholine to potentiate. In these situations, an allosteric agonist might prove to be more optimal for disorders in which the orthosteric ligand is lost as the disease progresses.[4−8,10,11,42−44,181−183]

By their very nature, allosteric ligands promote distinct conformations of 7TMRs such that the interactive properties of the receptor toward orthosteric ligands, as well as intracellular cytosolic proteins, can be modified in a ligand- and signaling protein-specific manner. This phenomenon has been termed "probe dependence"[184] and has substantial implications for the functional characterization and classification of allosteric modulators, as well as challenges associated with assigning quantitative parameters to facilitate allosteric ligand SAR. For example, the allosteric modulator **11** (LY2033298) positively modulates the binding affinity of the orthosteric agonist, ACh at the M_4 muscarinic receptor, but is neutral when tested against the orthosteric antagonist **12** ([^{3}H]-QNB).[90] Use of the endogenous agonist in a compound screen would thus reveal the allosteric activity of a ligand such as **11**, whereas a radioligand-based screen using **12** as the probe would fail to identify **11** (Figure 3). This highlights the requirement for careful consideration in the choice of orthosteric ligands to assess the effects of an allosteric modulator. Although the endogenous agonist for a given 7TMR should be the orthosteric probe of choice, this may not always be possible because of issues such as compound stability or in

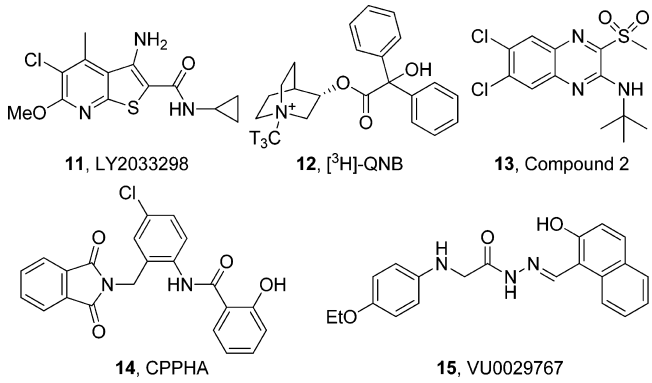

Figure 3. Structures of GPCR allosteric ligands **11**, **13**, **14**, and **15** that demonstrate the concept of "probe dependence", with **12**, an mAChR orthosteric radioligand discussed in the text.

situations such as screening for ligands for orphan 7TMRs where the endogenous agonist is not known. In these cases, the use of a surrogate orthosteric probe is common, but the ensuing pharmacology may prove to be misleading because of the potential for differential probe dependence between the modulator and the surrogate agonist relative to the therapeutically relevant endogenous agonist. These considerations also extend to the potential for off-target activities of allosteric modulators. Although the aforementioned mAChR allosteric ligand **11** is a selective PAM for the M_4 mAChR *when tested against ACh*, it displays remarkable positive *and* negative allosteric effects at the M_2 mAChR when tested against *other* orthosteric agonists, such as oxotremorine and xanomeline. If the latter agents were used as surrogates to characterize mAChR activity in modulator screens, then the resultant pharmacology would reflect activity at an undesired target (e.g., M_2 mAChR) in addition to the desired target (e.g., M_4 mAChR).[90] Finally, there are many 7TMRs that have more than one endogenous orthosteric agonist but that may not all respond the same way to allosteric ligands. A striking example of this phenomenon was recently observed at the glucagon-like peptide 1 (GLP1) receptor, where the small molecule allosteric agonist **13** (Novo Nordisk's compound **2**) had no effect on the signaling of the endogenous orthosteric peptide agonist GLP1(7−36) but significantly potentiated the signaling of another endogenous GLP1 receptor peptide, oxyntomodulin (Figure 3).[110]

As a final point, when an allosteric ligand binds to a 7TMR, the receptor adopts a unique, novel conformation (vide infra), enabling it to activate any number of downstream signaling cascades to the exclusion of other possible receptor states.[4−8,10,11] Here, probe dependence manifested at the level of the cytosolic interacting protein (e.g., G protein; β-arrestin, etc.) would result in signal pathway-dependent allosteric modulation. This has been coined "stimulus bias", "stimulus trafficking", "differential receptor trafficking", or "functional selectivity".[185−187] In short, an allosteric ligand may activate all downstream signaling cascades or a "surgical" selection of cascades with clear ramifications in pharmacodynamic models; therefore, it is critical to possess the requisite assays to understand how an allosteric ligand will modulate multiple signaling events. For example, the $mGlu_5$ PAM **14** (CPPHA, family C 7TMR) was shown to have differential effects on DHPG-mediated calcium signaling and ERK1/2 phosphorylation in a native astrocyte system.[188] In addition, an allosteric modulator of the M_1 muscarinic receptor **15** (VU0029767) potentiates ACh-mediated intracellular calcium mobilization but not phospholipase D activation; therefore, depending on the pathway/assay assessed, **15** would alternatively be classed as a PAM or a SAM, respectively (Figure 3).[82]

2. MODE OF ACTION OF ALLOSTERIC MODUALTORS

7TMRs are highly flexible proteins capable of assuming multiple conformations, of which some are active, some are inactive (pharmacologically silent), and some are partially active. In fact, 7TMRs should be thought of as ensembles of tertiary conformations randomly sampled by the receptor, and very subtle changes (as small as 1 Å) can engender profound effects on receptor activity.[1,4−8,10,11] Thus, when an allosteric modulator binds to a site topographically distinct from the orthosteric site, a change in receptor conformation occurs that can modify receptor activity in a positive, negative, or neutral direction. As mentioned before, the allosterically bound receptor is a "new" receptor type, with novel behavior and activity potential. Oligomerization of GPCRs, as either hetero- or homodimers, adds additional opportunities for modulation and probe dependence.[189] Operationally, 7TMR allosteric modulators exhibit affinity modulation, efficacy modulation, or varying degrees of both modes of modulation (Figure 4A).

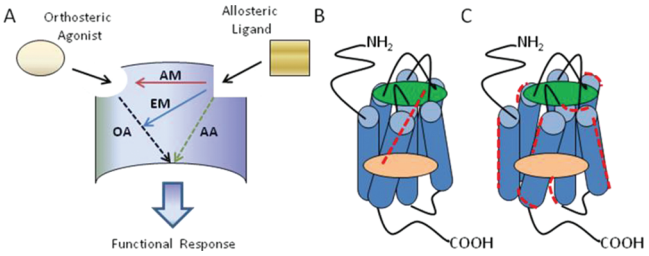

Figure 4. Mode of action of 7TMR allosteric modulators. (A) Allosteric ligands bind to a site topographically distinct from the orthosteric site on the 7TMR to modulate either the affinity (AM, affinity modulation) or efficacy (EM, efficacy modulation). This is in contrast to direct orthosteric agonism (OA) by the native ligand or allosteric agonism (AA) by the allosteric ligand alone. (B) "Hot wire" mode of allostery, suggesting a direct energy link between the allosteric binding site (green) and the orthosteric binding site (peach). (C) "Global allosteric modulation" mode, suggesting that changes at the orthosteric site are derived from global conformational variants within an ensemble of conformations.

With affinity modulation, the conformational change in the 7TMR upon allosteric ligand binding can affect either the association or dissociation rate (or both) of the orthosteric ligand.[1,4−8,10,11] For example, a PAM that displays affinity modulation will result in a more potent orthosteric ligand (agonist). For efficacy modulation, the conformational change in the 7TMR upon allosteric ligand binding leads to a change in signaling capacity (also termed intrinsic efficacy) and thereby either facilitates or inhibits receptor coupling to downstream effectors. There are two models proposed to account for the interactions between the allosteric and orthosteric ligand (Figure 4B and Figure 4C).[1,4,7] In one model, termed the "allosteric hot wire", the allosteric modulation site is directly linked to the orthosteric site through specific pathways.[1,190,191] In a more recent model, termed "global allosteric modulation", allosteric communication to the orthosteric site relies on long-range interaction through order/disorder transitions from multiple receptor conformations (i.e., population dynamics).[192,193] A number of mass-action schemes, based on variants of the ternary complex model, have been presented to describe the molecular effects of allosteric ligands on orthosteric pharmacology in terms of one or more "cooperativity factors", which indicate the magnitude and direction of an allosteric modulator-mediated stabilization of different 7TMR states.[4] From these models, it can be appreciated that the functional potency of an allosteric modulator will depend not only on its affinity for the allosteric site but also on the degree of cooperativity with the orthosteric ligand. Thus, a PAM (e.g., **11**) may bind to the receptor with weak affinity but possess potent functional activity due to a high cooperativity factor.[4,10,90] In contrast, another PAM (e.g, benzodiazepines) may bind with very high affinity but low positive cooperativity, thus also displaying potent functional activity. The low affinity observed with some PAMs can preclude them from serving as radioligands and PET tracers, and the affinity/functional activity balance must be carefully assessed when considering receptor occupancy, for example, as a potential biomarker strategy.

Unfortunately, most mass-action-based molecular models of allosteric modulation contain too many parameters to be fitted to real experimental data and thus cannot be used to rationalize structure−activity studies in a manner that can inform drug candidate selection matrices. A useful means for placing these issues on a more practical quantitative level is through the use of an "operational" model of allosterism and agonism, which has been developed to describe allosteric effects in terms of a minimum number of experimentally accessible parameters.[194] This model is illustrated conceptually in (Figure 5), and the equation describing the signaling of an orthosteric agonist in the presence of an allosteric modulator according to the model is as follows:

$$E = \{E_m(\tau_A[A](K_B + \alpha\beta[B]) + \tau_B[B]K_A)^n\}/$$
$$\{([A]K_B + K_AK_B + [B]K_A + \alpha[A][B])^n$$
$$+ (\tau_A[A](K_B + \alpha\beta[B]) + \tau_B[B]K_A)^n\}$$

where E is the effect, [A] and [B] are the concentrations, K_A and K_B are the equilibrium dissociation constants of the orthosteric and allosteric ligand, respectively, α is the cooperativity factor describing the allosteric effect of each ligand on the other's binding affinity, β is a scaling factor (from zero to infinity) that quantifies the magnitude by which the allosteric modulator modifies the efficacy of the orthosteric

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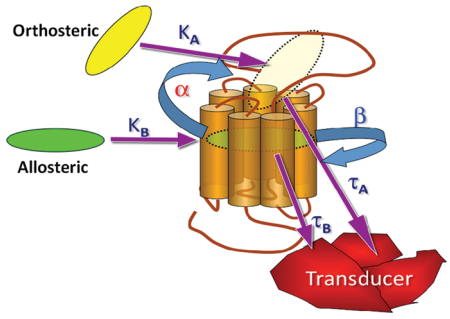
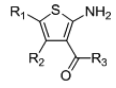


Figure 5. Schematic representation of the parameters underlying the operational model of allosterism and agonism. Parameters are defined in the main text.

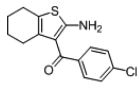
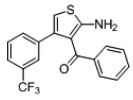
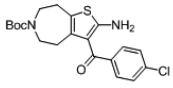
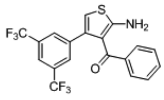
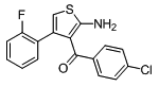
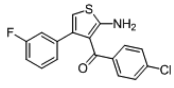
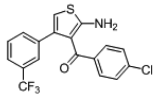
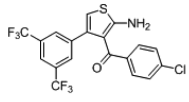
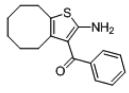
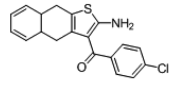
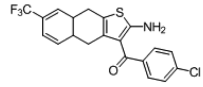
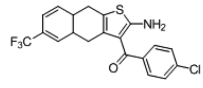
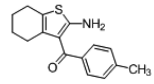
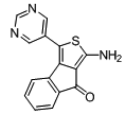
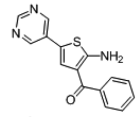
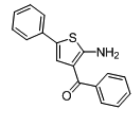
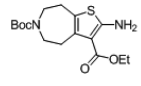
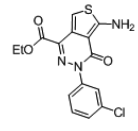
agonist at a given signal pathway, and the parameters τ_A and τ_B relate to the ability of the orthosteric and allosteric ligands, respectively, to promote receptor activation (direct agonism). These latter parameters incorporate the intrinsic efficacy of each ligand, the total density of receptors, and the efficiency of stimulus-response coupling. The parameters E_m and n denote the maximal possible system response and the slope factor of the transducer function that links occupancy to response, respectively.[4,10,194]

Importantly, the operational model can be fitted to experimentally derived data to provide estimates of some, or all, of its parameters.[47,52,90,195−197] At a minimum, there are three key parameters that can be routinely derived from application of this model to most functional screening data, as long as full concentration response and curve-shift relationships are determined. These three parameters are the allosteric modulator K_B, which provides information on the interaction of the allosteric ligand with the allosteric binding pocket on the free receptor, the composite cooperativity parameter $\alpha\beta$, which provides information on the overall allosteric effect on the orthosteric agonist in the chosen functional assay, and the modulator efficacy parameter τ_B, which provides information on the ability of the allosteric ligand to promote agonism in its own right in the absence of orthosteric ligand. Table 4 illustrates an example of such allosteric modulator SAR determined through analysis of the functional effects of a series of 2-amino-3-benzoylthiophenes (2A3BT) on A_1 adenosine receptor-mediated

Table 4. Allosteric Operational Model Parameters Describing the Functional Effect of Various 2-Amino-3-benzoylthiophenes on ERK1/2 Phosphorylation mediated by the Orthosteric Agonist *R*-PIA at Adenosine A_1 Receptors



16

Compound	Structure	pK_B	$\log\alpha\beta$ ($\alpha\beta$)	$\log\tau_B$ (τ_B)
16a (T62)[a]		5.49 ± 0.09	0.58 ± 0.08 (3.8)	-0.32 ± 0.05 (0.5)
16b 9a[a]		6.37 ± 0.16	0.38 ± 0.07 (2.4)	-0.33 ± 0.04 (0.5)
16c (VCP333)[a]		5.23 ± 0.25	0.64 ± 0.12 (4.4)	-1.34 ± 0.75 (0.05)
16d (9o)[a]		5.22 ± 0.07	1.06 ± 0.08 (11)	0.62 ± 0.24 (4.1)
16e (13c)[a]		5.10 ± 0.18	1.04 ± 0.20 (11)	0.43 ± 0.11 (2.7)
16f (13d)[a]		5.88 ± 0.16	0.67 ± 0.10 (4.7)	-0.30 ± 0.08 (0.5)
16g (13a)[a]		5.74 ± 0.12	0.55 ± 0.06 (3.5)	-0.48 ± 0.08 (0.3)
16h (13o)[a]		5.46 ± 0.32	1.31 ± 0.40 (20)	0.50 ± 0.20 (3.2)
16i (12j)[a]		5.08 ± 0.17	0.46 ± 0.07 (2.9)	-0.41 ± 0.09 (0.4)
16j (25b)[a]		6.01 ± 0.16	0.44 ± 0.07 (2.7)	-0.48 ± 0.08 (0.3)
16k (25f)[a]		5.11 ± 0.41	0.58 ± 0.17 (3.8)	-1000 (~0)
16l (25d)[a]		5.12 ± 0.32	0.82 ± 0.16 (6.6)	-1000 (~0)
16m (12c)[a]		5.10 ± 0.14	0.98 ± 0.14 (10)	0.31 ± 0.08 (2.0)
16n (17d)[a]		6.67 ± 0.13	-1000 (~0)	-1000 (~0)
16o (22d)[a]		5.82 ± 0.10	-1000 (~0)	-1000 (~0)
16p (22a)[a]		6.36 ± 0.13	-1000 (~0)	-1000 (~0)
16q (2c)[a]		6.07 ± 0.05	-1000 (~0)	-1000 (~0)
16r (13b)[a]		8.30 ± 0.24	-1000 (~0)	-1000 (~0)

[a]Compound nomenclature refers to compound identifier in the manuscript in which it originally appeared.

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ERK1/2 phosphorylation.[46] From this analysis, it can be seen that the increase in trifluoromethylphenyl substitutions to the R_2 group of the 2A3BT scaffold can increase positive cooperativity while having a detrimental effect on modulator affinity (compare **16b** to **16d**, and **16g** to **16h**), whereas conformational constraint in the R_2/R_3 regions tends to convert positive modulators into highly negative modulators (e.g., **16n**, **16r**). It is also noted that the reference compound, **16a**, progressed into phase IIB clinical trials for the treatment of neuropathic pain (King Pharmaceuticals) prior to failing due to lack of efficacy; it is possible that this failure may be attributed to the rather low degree of positive cooperativity, as revealed by the application of the operational model to the in vitro data. Although speculative, this finding highlights some of the advantages of operational modeling when applied to allosteric SAR, namely, the ability to link the chemistry to the key, measured biological parameters and the ability to facilitate hypothesis generation to understand biological mechanisms and their relevance to the desired therapeutic profile. For instance, one can ask questions such as the following: How much cooperativity ($\alpha\beta$) or allosteric agonism (τ_B) is required to achieve in vivo efficacy? How do structural modifications affect compound affinity (K_B) versus cooperativity ($\alpha\beta$)? The latter is important because these properties are not correlated, and thus, different structural manipulations can change them in different directions. Probe dependence will manifest as different $\alpha\beta$ values depending on the orthosteric agonist used and/or the signal pathway being assessed as a readout of receptor activation. In terms of drug discovery programs, these insights can be used to more rationally inform the design of candidate selection matrices for drugs acting allosterically.[46,47,52,90,195−197]

3. IN VITRO PHARMACOLOGY OF ALLOSTERIC MODULATORS

The development of high-throughput functional (kinetic) assays have enabled scientists to perform screens of large compound collections and to identify small molecules capable of modulating the activity of a receptor through novel, allosteric mechanisms.[4,160,198−201] While there are multiple approaches and technologies to accomplish this for 7TMRs, one of the most common approaches measures receptor-induced mobilization of intracellular calcium using an imaging-based plate reader that makes simultaneous measurements of calcium levels in each well of a multiwell microplate containing cells transfected with the receptor of interest and loaded with calcium-sensitive fluorescent dye. In the early stages of drug discovery for allosteric modulators of 7TMRs, HTS campaigns targeted the identification of either PAMs or NAMs, running single-point screens with either an EC_{20} or EC_{80} concentration of the orthosteric agonist, respectively. More recently, "triple add" protocols have supplanted "single add" screens to allow for, in a single assay, the identification of PAMs, NAMs, agonists, and antagonists (Figure 6A).[4,160,198−201] Because of

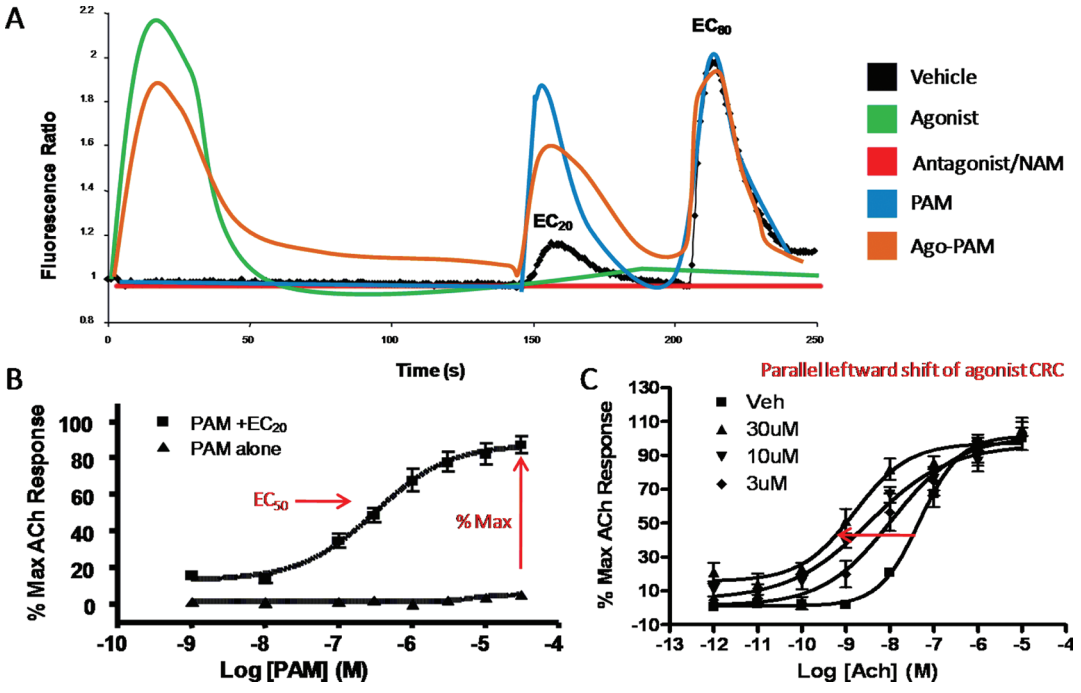

Figure 6. Functional assays, measuring calcium fluorescence as a surrogate for 7TMR receptor activation, employed to identify and profile 7TMR allosteric modulators. (A) "Triple add" paradigm for both HTS campaigns and primary assay for lead optimization: vehicle (black) trace where an EC_{20} of orthosteric agonist is added 150 s into the kinetic run, followed by an EC_{80} of orthosteric agonist. Compounds are added at $T = 0$, and an agonist (green) elicits calcium fluorescence immediately upon addition. Secondary assays with orthosteric radioligands and/or mutant receptors will determine if the compound is an orthosteric or allosteric agonist. An antagonist (red) will block both the EC_{20} and the EC_{80}; once again, secondary assays will distinguish competitive from noncompetitive (NAM) antagonists. A pure PAM (blue) will not elicit receptor activation alone but will potentiate the EC_{20} to varying degrees of efficacy, while an ago-PAM (orange) will activate the receptor alone, plus potentiate the EC_{20}. Hence, a single assay protocol will identify agonists, allosteric agonists, PAMs, ago-PAMs, antagonists, and NAMs. (B) In vitro pharmacology of an mAChR PAM, once again with a calcium fluorescence readout. The PAM has no effect alone on receptor activation, but in the presence of an EC_{20} (or subthreshold concentration of orthosteric agonist, ACh in this case), a classical concentration−response curve (CRC) results, from which an EC_{50} for potentiation can be calculated. Also, the %Max, the degree of potentiation above the EC_{20}, can be measured, and both the EC_{50} and %Max must be optimized. (C) "Fold shift" assay. Here, the concentration of the orthosteric agonist (ACh) is held constant, and increasing concentrations of the PAM cause a parallel leftward shift of the ACh CRC, in effect making ACh a more potent agonist.

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the emerging concept of "molecular switches" (see below),[202] this screening paradigm is also optimal for use as a program's primary assay to identify subtle structural changes leading to opposing modes of pharmacology.

As shown in Figure 6B, a prototypical PAM has no effect on the transfected cells in the absence of the orthosteric agonist, but in the presence of a subthreshold concentration of the orthosteric agonist, increasing PAM concentrations provide a classical concentration–response curve (CRC) from which an EC_{50} for potentiation can be quantified as an empirical measure of modulator potency under a defined set of assay conditions.[4,5] This also affords a %Max value, the maximum increase in activity of the orthosteric agonist above the EC_{20}, i.e., from an EC_{20} to an EC_{80}. In this type of experimental paradigm, the observed EC_{50} reflects both the affinity of the modulator for the allosteric site and the degree of cooperativity that it exerts on the orthosteric ligand. In contrast, the %Max only reflects the cooperativity of the interaction. Each of these parameters must be optimized, as it is possible to have allosteric modulators with high potency and low %Max (reflecting high affinity and low cooperativity), weak potency and high %Max (low affinity and high cooperativity), and various combinations thereof. If the assay is run in an alternative mode, whereby the complete orthosteric agonist CRC is determined in the absence or presence of a fixed concentration of allosteric modulator, then another property that can be evaluated and optimized is the degree of the agonist curve "fold shift", either to the left (potentiation) or right/down (antagonism) of an agonist CRC (Figure 6C).[4,5] This is typically reported as a fold shift at a single concentration, such as a 30-fold shift at 10 μM. Optimally, if multiple modulator concentrations are utilized in this latter type of experiment, then the data can be fitted to the operational model (above) and direct estimates of affinity and cooperativity can be obtained. Irrespective, each of the properties described in the preceding section (%Max, potency, fold shift or operational model affinity, cooperativity, and efficacy) must be simultaneously optimized for optimal in vivo efficacy.[4,5] A common question posed in small molecule allosteric modulators programs is the follwing: Which of these parameters is most important for in vivo efficacy? Unfortunately, the answer is not clear and may vary by 7TMR, allosteric binding site, allosteric ligand chemotype, and therapeutic indication. For now, a general empirical guideline followed by most researchers in the field is to optimize for potency (EC_{50}) and %Max and aim for at least a 5-fold shift of the agonist CRC; however, estimates of fold shift for some family A and family C 7TMR allosteric ligands may approach >70-fold, depending on the assay.[4,5] Other properties of a CNS compound, for example, the ability of a compound to penetrate the blood–brain barrier, the clearance of a compound from the brain and systemic compartments, the amount of compound that is non-protein bound and able to interact with the target, and the in vivo situation being assessed (for example, how long does a receptor need to be occupied with drug to maintain efficacy), certainly must be balanced with in vitro pharmacology profiles when assessing in vivo efficacy and potency of allosteric modulators.

4. STRUCTURE–ACTIVITY RELATIONSHIPS (SARS) WITHIN mGluRs AND mAChRs

Another common observation from numerous structure–activity relationship (SAR) studies performed to date on 7TMR allosteric modulators is the finding of "flat" or "shallow" SAR for different classes, often making optimization of micromolar potency ligands either difficult or impossible.[1,4−12] The literature in this arena is filled with tales of heroic optimization campaigns wherein hundreds or thousands of compounds were synthesized and evaluated, affording only 5−10% active molecules. However, there are also cases wherein SAR is robust and tractable, though rare. Thus, for the chemical optimization of allosteric ligands,[4,197,202] focused, iterative library synthesis provides a distinct advantage over traditional singleton approaches; however, multiple dimensions of a scaffold must be surveyed to identify regions tolerant of modification. As SAR is often extremely "shallow", the concept of walking fluorine atoms around an allosteric ligand, i.e., "the fluorine walk", has achieved some success in identifying positions tolerant of change. For example, **6** displayed "shallow" SAR, and multiple attempts at optimization, adding groups larger than fluorine (methyl, OR, Cl, Br, alkyl, etc.) to multiple positions (i.e., R_1 and/or R_2) on the scaffold of **6**, led to primarily inactive compounds **17**.[33,203] The strategic installation of fluorine atoms to both the core R_1 and the benzyl side chain R_2 of **6** led to compounds with significant improvements in M_1 PAM activity (Figure 7) but only in the 5- (**18**), 8- (**19**), or 5,8-positions (**20**); introduction of fluorine atoms in any other position led to inactive compounds. Once identified, these new fluorinated cores opened up new avenues for diverse functionalization on the benzyl core that were not tolerated on the parent core, leading to potent M_1 PAMs such as **21** (M_1 EC_{50} = 41 nM).[203] A very similar "fluorine walk" strategy has proven successful in developing submicromolar M_1 PAMs in other chemotypes.[83,84] It is important to note that in these cases and many other published studies, only fluorine substitutions were tolerated, making this approach a potential first tier strategy in allosteric ligand optimization.

In addition to the "flat" or "shallow" allosteric structure–activity relationships (SAR), the emerging concept of "molecular switches", i.e., subtle structural changes that can change the mode of pharmacology (from PAM to NAM and/or SAM) and/or subtype selectivity within a family of receptors, threatens to diminish the application of rational drug design approaches to allosteric modulators.[202] Moreover, unexpected occurrences of "molecular switches" require alterations to routine screening paradigms for optimization efforts. Here, the "triple add" approach is ideal,[4,160,198−201] identifying allosteric agonists, PAMs, and NAMs in a single screen. Although examples of "molecular switches" developed for mGluRs and mAChRs are presented herein, this subtle effect has been reported for allosteric kinase and phospholipase ligands as well.[202]

In the field of mGluRs, the first allosteric modulators disclosed were (−)-ethyl (7*E*)-7-hydroxyimino-1,7α-dihydrocyclopropa[*b*]chromene-1α-carboxylate (CPCCOEt, $mGlu_1$ selective) and **10** ($mGlu_5$ selective), both NAMs, followed by the earliest $mGlu_5$ PAMs, 3,3′-difluorobenzaldazine (DFB), **14**, and 3-cyano-*N*-(1,3-diphenyl-1*H*-pyrazol-5-yl)benzamide (CDPPB).[4,44] While various molecular switches on the difluorobenzaldazine scaffold had the ability to engender the full range of PAM, NAM, and SAM pharmacology (structures not shown),[161] an even more surprising example of pharmacological mode switching can be seen in Figure 8. A functional HTS with the $mGlu_5$ receptor identified the "partial antagonist" **22**, which maximally inhibited 71% of the glutamate response with $mGlu_5$ IC_{50} = 486 nM.[202,204−206] This simple and very low molecular weight hit was rapidly explored using a parallel iterative library approach to reveal numerous molecular switches. Introduction of a 3′-methyl group transformed the

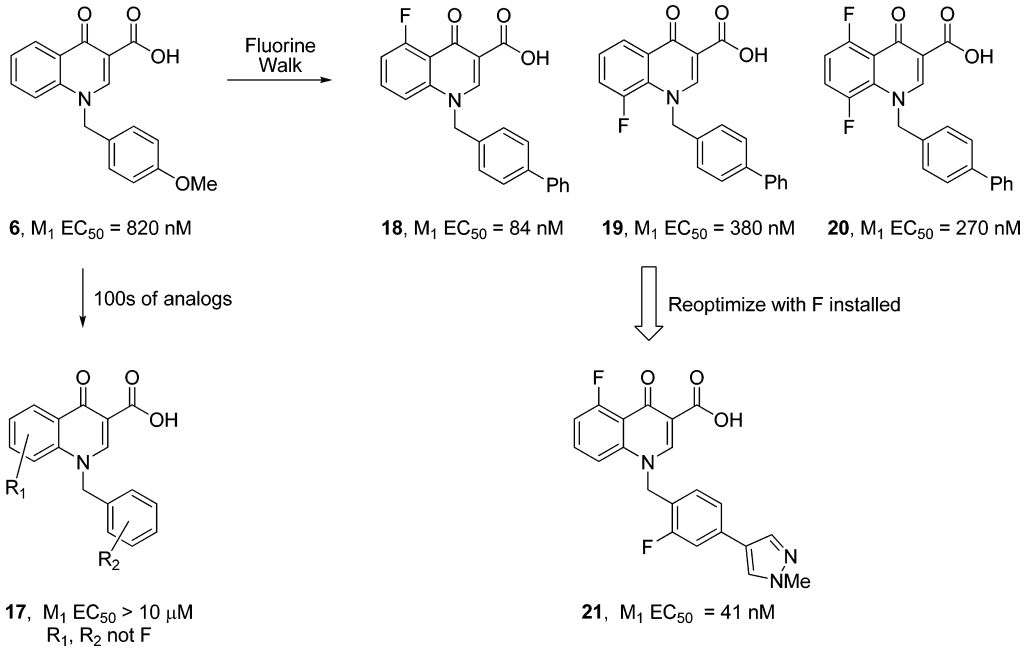

Figure 7. SAR within the M_1 PAM **6** chemotype. Groups R_1 and R_2 other than F, as in **17**, were not tolerated and afforded inactive compounds. Walking fluorine atoms around the core identified three positions, leading to cores **18**–**20** that engendered M1 PAM activity. Reoptimization with the fluorinated cores led to potent M_1 PAMs such as **21**.

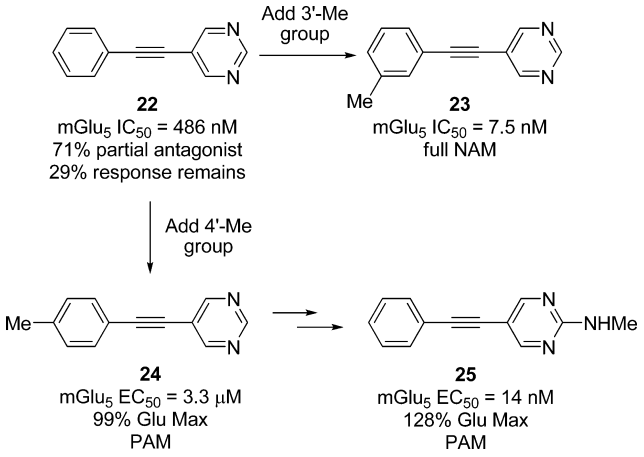

Figure 8. Subtle "molecular switches" within a series of $mGlu_5$ allosteric ligands giving rise to partial antagonists **22**, full NAMs **23**, and PAMs **24** and **25**.

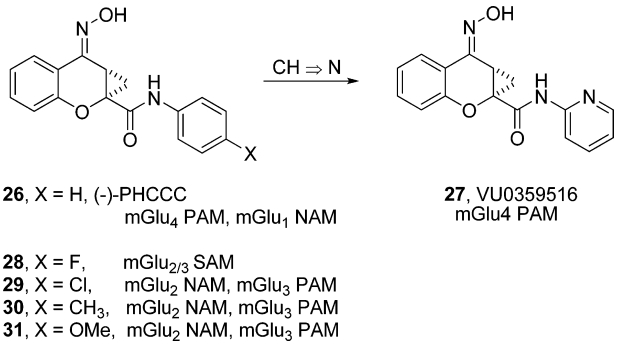

Figure 9. Subtle "molecular switches" within a series of $mGlu_4$ allosteric ligands providing a selective $mGlu_4$ PAM **27** or allosteric ligands that cross over into the group II family of mGlus and displaying $mGlu_2$ and $mGlu_3$ PAM, NAM, and SAM activities.

partial antagonist into a very potent full NAM **23** ($mGlu_5$ IC_{50} = 7.5 nM). This methyl group exquisitely illustrates the idea of a "molecular switch" as it is moved just one position over to provide compound **24**, which is now a weak PAM ($mGlu_5$ EC_{50} = 3.3 μM) with the ability to potentiate a submaximal dose of glutamate up to a full glutamate response (99% Glu Max). Further optimization within this series of PAMs provided compound **25**, a very potent $mGlu_5$ PAM that possessed robust in vivo efficacy in a rodent amphetamine-induced hyperlocomotion assay.[202,204−206]

Also within the mGluR field, molecular switches have been reported that profoundly affect the receptor subtype selectivity of a given allosteric ligand and also the mode of pharmacology. Figure 9 shows **26** ((−)-PHCCC), which possess both $mGlu_4$ PAM and $mGlu_1$ NAM activity.[155] Introduction of a weakly basic nitrogen to arrive at **27** (VU0359516) effectively abolished all $mGlu_1$ NAM activity to provide a pure $mGlu_4$ PAM (EC_{50} > 30 μM versus $mGlu_{1-3,5-8}$) with good potency and efficacy ($mGlu_4$ EC_{50} = 380 nM, Glu Max = 121%, 20-fold shift).[155] Subsequent to this work, an $mGlu_2$ FRET-based binding assay identified the fluorinated analogue **28** which displayed $mGlu_2$ K_i = 6.6 μM while at the same time lacked all functional activity at $mGlu_2$ and $mGlu_3$.[207] Importantly, **28** could also block (silence) the activities of related $mGlu_2$ and $mGlu_3$ allosteric modulators **29**–**31**, the definition of an $mGlu_{2/3}$ SAM. As alluded to above, the position of the fluorine in **28** represented a productive location for the introduction of alternate molecular switches (Figure 9, X = Cl, Me, OMe) which could transform an $mGlu_1$ NAM/$mGlu_4$ PAM **26** or an $mGlu_{2/3}$ SAM **28** into a series of $mGlu_2$ NAM/$mGlu_3$ PAMs **29**–**31**.[207]

These types of molecular switches that govern receptor subtype selectivity have similarly been reported in the mAChR literature. Figure 10 shows **32**, which was an attractive, low-molecular weight PAM hit from a functional HTS assay with the M_1 mAChR.[82,84,94−96] Although originally identified as only an M_1 PAM, subsequent characterization revealed **32** to be a

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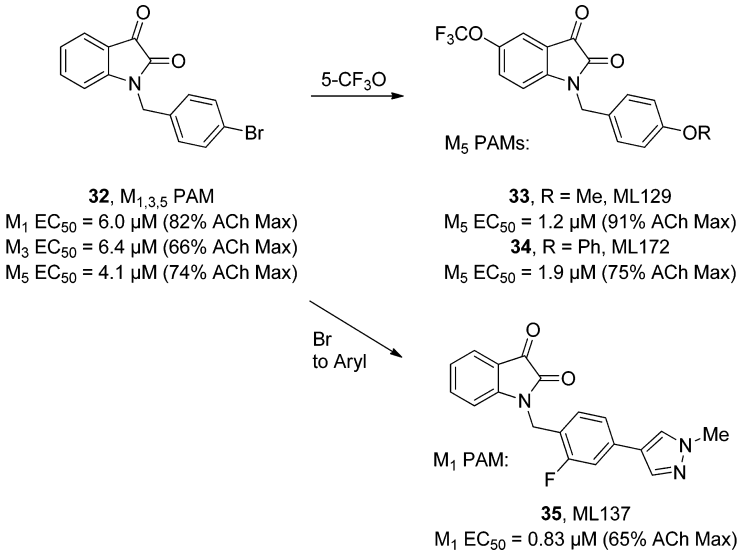

Figure 10. Subtle "molecular switches" within a series of mAChR allosteric ligands that afford highly selective M_5 PAMs **33** and **34** or a selective M_1 PAM **35**.

nonselective G_q-coupled mAChR PAM displaying activity at the $M_{1,3,5}$ receptor subtypes while being devoid of activity at the G_i-coupled M_2 and M_4 mAChRs.[82] This interesting selectivity for the different G-protein-coupled signaling pathways spurred medicinal chemistry efforts to determine if subtle molecular switches could be discovered that might provide selectivity for a single mAChR subtype. The first breakthrough came with the discovery that placing a 5-trifluoromethoxy substituent on the isatin core led to a profound preference for M_5 PAM activity. This molecular switch employed during an exploration of substituents about the benzyl group ultimately provided **33** (R = Me, ML129) and **34** (R = Ph, ML172), the first and most highly selective M_5 PAMs, respectively, reported to date.[94−96] Further structural modifications around the nonselective lead **32** revealed that replacing the bromine with various aryl groups identified the *N*-methylpyrazole as a powerful molecular switch for establishing high levels of M_1 selectivity. Fine-tuning this M_1 PAM activity through the strategic introduction of a fluorine atom, i.e., the "fluorine walk", provided the highly selective M_1 PAM **35** (ML137).[84] Ongoing studies exploring the utility of these and other allosteric ligands promise to reveal numerous additional molecular switches, and as these compounds progress into more animal models and detailed DMPK evaluations, it will only be a matter of time before metabolism-induced molecular switches are reported.[201] Such molecular switches may not be observed within all allosteric ligand scaffolds or at all allosteric binding sites; in fact, some allosteric ligands and binding sites are considered "molecular locks" with robust tractable SAR.

5. OPPORTUNITIES FOR CNS DISORDERS AND THERAPEUTICS

Despite these challenges, allosteric ligands have enabled researchers to develop small molecule tools (Tables 1−3)[4−8,10,11,42−180] with exquisite selectivity for a particular target, not possible with orthosteric ligands, and to achieve proof of concept in preclinical models of various CNS disorders. For example, $mGlu_5$ NAMs have provided preclinical target validation in models of anxiety, fragile X syndrome, chronic pain, migraine, and GERD.[170] In fact, $mGlu_5$ NAMs are one of the most advanced with multiple compounds in clinical development and displaying efficacy in phase II (Table 5).[208−230] Diverse chemotypes of both $mGlu_5$[171] and $mGlu_2$[148] PAMs have shown robust activity in preclinical models of schizophrenia and cognition, while selective $mGlu_4$ PAMs[157] have validated the target for both pain and Parkinson's disease.[4]

The biogenic amine receptors, the prototypes for promiscuous pharmacology, have benefited greatly from allosteric approaches.[4,42−180] For example, the mAChRs have been targets of interest for multiple CNS disorders since the 1950s, but the highly conserved orthosteric (acethycholine) binding site led to unselective small molecules. Because of the therapeutic relevance of the mAChRs, multiple companies advanced orthosteric pan-mAChR agonists into the clinic and noted efficacy in phase II and phase III trials in Alzheimer's disease and schizophrenia patients; however, the adverse events from activation of peripheral M_2 and M_3 prevented further development. Recently, allosteric ligands, both allosteric/bitopic agonists and PAMs, have been developed for M_1, M_4, and M_5.[4,43] These tools are now dissecting the individual contributions of these three mACh receptor subtypes in the clinical efficacy of pan-mAChR agonists. Importantly, this is but one example against the backdrop of the allosteric ligands in Tables1−3,[4,42−180] that are validating discrete targets within large families of receptors and shedding light on the therapeutic potential of GPCRs, long obscured by the lack of selective tool compounds.

Finally, it is very important to point out that allosteric ligands have been advanced into marketed therapeutics, suggesting that the approach of targeting allosteric mechanisms is sound and tractable (Figure 11). **36** is a PAM of the calcium sensing receptor (a family C GPCR) and is used to treat hyperparathyroidism.[115] **37** is a NAM of chemokine receptor 5 (a family A GPCR) that inhibits HIV entry into cells and is used to treat HIV infections.[72] Thus, both PAMs and NAMs have advanced to the market, with many other allosteric ligands in clinical trials and late preclinical development.[4]

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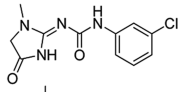
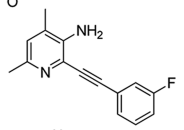
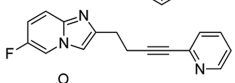
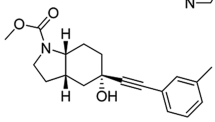
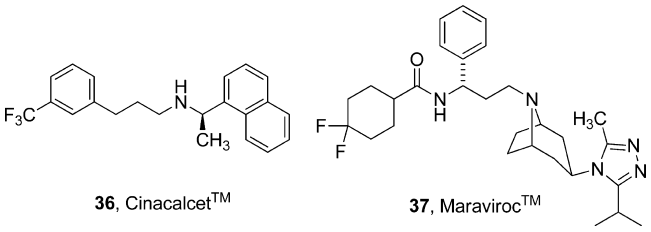
Table 5. $mGlu_5$ NAM Clinical Compounds

Drug Name	Structure	Organization	Clinical Trials
fenobam™ (NPL-2009)[208-210]		Neuropharm	anxiety, fragile X syndrome
raseglurant™ (ADX10059)[211-214]		Addex	anxiety, GERD, migraine
dipraglurant™ (ADX48621)[211-216]		Addex	PD-LID
AFQ056[217-222]		Novartis	fragile X syndrome, PD-LID, GERD, nicotine addiction
	Exact Structure Unknown		
AZD2066[223,224]	Structure Unknown	AstraZeneca	GERD, chronic neuropathic pain, major depressive disorder, diabetic neuropathy
AZD2516[223,225]	Structure Unknown	AstraZeneca	GERD, chronic neuropathic pain, major depressive disorder
RG7090[226]	Structure Unknown	Roche	treatment resistant depression
RO4917523[227]	Structure Unknown	Roche	fragile X syndrome, treatment resistant depression
STX107[228,229]	Structure Unknown	Seaside Therapeutics	fragile X syndrome



36, Cinacalcet™ **37**, Maraviroc™

Figure 11. Structures of the two marketed GPCR allosteric modulators: **36**, a PAM of the calcium sensing receptor, and **37**, a NAM of CCR_5.

6. CONCLUSION

The broad uptake of the concept of allosteric modulation has led to a renaissance in pharmacological approaches toward 7TMR pharmacology, promising new and exciting avenues to the pursuit of receptor-subtype and pathway-selective small molecules. Although the promises of this approach are apparent, there remain significant challenges with regard to optimal means for detecting, validating, and quantifying allosteric ligand effects in a manner that routinely informs SAR and candidate selection matrices and is also ultimately predictive of therapeutic efficacy. To meet these challenges, further convergence is required between the disciplines of pharmacology, which can shed insight into the nature and mechanisms underlying phenomena such as probe dependence and pathway-biased modulation, medicinal/synthetic chemistry, which can overcome the issue of "flat" allosteric SAR and explore the full potential of molecular switches in creating new allosteric behaviors, and structural biology, which can identity the underlying structural basis of allosteric ligand binding and the associated receptor conformations that mediate allosteric effects. Fortunately, recent work in the field is illustrating how truly translational approaches toward 7TMR pharmacology promise to overcome many of these challenges and to realize the therapeutic potential of allosteric modulators as novel medicines.

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Biographies

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Corey R. Hopkins is Associate Director of Medicinal Chemistry for the Vanderbilt Center for Neuroscience Drug Discovery (VCNDD) at Vanderbilt University, TN. Corey completed his doctorate in 2002 with Professor Peter Wipf at the University of Pittsburgh, PA, on the total synthesis of tetrazomine and naphthyridinomycin/bioxalomycin class of compounds. He also developed novel ring expansion methodology to make pharmacophore analogues of Dnacin. Corey moved to Sanofi-Aventis Pharmaceuticals in 2001 and later became Senior Research Investigator in CNS medicinal chemistry. He worked on therapeutic targets for several diseases including multiple sclerosis, rheumatoid arthritis, osteoporosis, respiratory diseases, and auto-immune disorders. In 2008, Corey accepted a Research Assistant Professor position in the Department of Pharmacology and serves as Co-Director of the Vanderbilt Specialized Chemistry Center for Accelerated Probe Development.

Michael R. Wood is Associate Director of Medicinal Chemistry for the Vanderbilt Center for Neuroscience Drug Discovery (VCNDD) at

Vanderbilt University, TN. Mike completed his doctorate in 1995 with Professor Bruce H. Lipshutz at the University of California, Santa Barbara, where he developed catalytic copper conditions for the 1,4-addition of functionalized organometallic reagents. In 1996, he accepted a postdoctoral position with Professor David A. Evans at Harvard University, MA, and concluded the first total synthesis of the vancomycin aglycon. In 1998, he moved to Merck and Co., Inc. to develop antagonists of bradykinin B1 and CGRP receptors. In 2009, Mike accepted a Research Assistant Professor position in the Department of Pharmacology and serves as the Co-Director of the Vanderbilt Specialized Chemistry Center for Accelerated Probe Development.

Kyle A. Emmitte is Associate Director of Medicinal Chemistry for the Vanderbilt Center for Neuroscience Drug Discovery (VCNDD) at Vanderbilt University, TN. Kyle completed his doctorate in 2001 with Professor Michael T. Crimmins at the University of North Carolina at Chapel Hill on the development of asymmetric alkylation ring-closing metathesis strategies for the synthesis of medium-ring ether natural products. Kyle joined GlaxoSmithKline in Research Triangle Park, NC, and made significant contributions to the discovery of GSK461364, an inhibitor of polo-like kinase 1. Kyle also co-led the team that discovered GSK1904529, an inhibitor of insulin-like growth factor-1 receptor. In 2008, Kyle accepted a Research Assistant Professor position in the Department of Pharmacology and serves as Co-Director of the Vanderbilt Specialized Chemistry Center for Accelerated Probe Development.

Colleen M. Niswender is the Director of Molecular Pharmacology for the Vanderbilt Center for Neuroscience Drug Discovery (VCNDD) at Vanderbilt University, TN. Colleen obtained her doctorate in pharmacology in 1996 under the direction of Professor Ronald Emeson at Vanderbilt University, studying regulation of RNA editing in the mammalian central nervous system and characterizing molecular determinants regulating RNA editing events within the AMPA subtype glutamate receptor, $GluR_2$, and the G-protein-coupled 5-HT_{2C} serotonin receptor. She pursued postdoctoral studies with Professor G. Stanley McKnight at the University of Washington. She accepted an Associate Professor position in Pharmacology in 2004, serving as Director of Molecular Pharmacology. She has extensive experience with GPCRs, cell signaling, assay development, HTS and lead optimization, and concepts of allosteric modulation.

Arthur Christopolous is a Professor and National Health and Medical Research Council (NHMRC) Senior Research Fellow at Monash University (Melbourne, Australia). Arthur received his doctorate in Pharmacology from the Victorian College of Pharmacy at Monash University in 1997 and then pursued postdoctoral studies at the University of Minnesota. In 1999, Arthur returned to the University of Melbourne as a Senior Research Fellow. He currently serves as the Co-Director of the Drug Discovery Biology Laboratory at the Monash Institute of Pharmaceutical Sciences and Department of Pharmacology. His research interests include understanding novel modes of regulation of GPCRs to identify novel targets or approaches for drug discovery, investigating virtually all levels of GPCR structure/function, including analysis of the functional significance of single nucleotide polymorphisms and RNA-editing.

P. Jeffrey Conn is the Director of the Vanderbilt Center for Neuroscience Drug Discovery (VCNDD) at Vanderbilt University, TN. He received his doctorate in Pharmacology from Vanderbilt in 1986 and pursued postdoctoral studies at Yale University, CT. He accepted a faculty position in Pharmacology at Emory University, GA, in 1988, establishing himself as a leader in studies of neurotransmitter receptor function. In 2000, he moved to Merck and Co., Inc., as Director of the Department of Neuroscience. In 2003, he moved to Vanderbilt University and founded the VCNDD. He serves as Editor-in-Chief of *Molecular Pharmacology*. Dr. Conn is the Lee E. Limbard Professor of Pharmacology at Vanderbilt University. Dr. Conn's current research is focused on development of novel treatment strategies for schizophrenia, Parkinson's disease, and other disorders.

Craig W. Lindsley is Director of Medicinal Chemistry for the Vanderbilt Center for Neuroscience Drug Discovery (VCNDD) at Vanderbilt University, TN. Craig received his doctorate in 1996 from the University of California, Santa Barbara, and pursued postdoctoral studies at Harvard University, MA. In 2001, Craig moved to Merck & Co., Inc., and developed a streamlined approach for lead optimization, resulting in delivery of six preclinical candidates. He also provided preclinical proof-of-concept for the first isoenzyme selective, allosteric AKT kinase inhibitors, the first mGluR5 and M_1 PAMs. In 2006, Craig accepted Associate Professor appointments in Pharmacology and Chemistry at Vanderbilt University. He serves as Editor-in-Chief of *ACS Chemical Neuroscience*. Now Full Professor, Craig serves as Principal Investigator of the Vanderbilt Specialized Chemistry Center for Accelerated Probe Development.

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■ ABBREVIATIONS USED

CNS, central nervous system; GPCR, G-protein-coupled receptor; 7TMR, seven transmembrane receptor; TM, transmembrane; PAM, positive allosteric modulator; NAM, negative allosteric modulator; SAM, silent allosteric modulator; BZD, benzodiazepine; $GABA_A$, γ-aminobutyric acid; VFT, Venus fly trap; mAChR, muscarinic acetylcholine receptor; ACh, acetylcholine; mGluR, metabotropic glutamate receptor; NMDA, *N*-methyl-D-aspartate; 5-HT_{1B}, 5-hydroxytryptamine receptor 1B; DMPK, drug metabolism/pharmacokinetic; SAR, structure−activity relationship; GLP1, glucagon-like peptide 1; DHPG, dihydroxyphenylglycine; PET, positron emission tomography; CRC, concentration−response curve; FRET, fluorescence resonance energy transfer; HTS, high-throughput screening; GERD, gastroesophageal reflux disease; CPCCOEt, (−)-ethyl (7*E*)-7-hydroxyimino-1,7α-dihydrocyclopropa[*b*]-chromene-1α-carboxylate; DFB, 3,3′-difluorobenzaldazine; CDPPB, 3-cyano-*N*-(1,3-diphenyl-1*H*-pyrazol-5-yl)benzamide; MPEP, 2-methyl-6-(phenylethynyl)pyridine; [^{3}H]-QNB, [^{3}H]-quinuclidinyl benzylate

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