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Allosteric Modulation of Family C G-Protein-Coupled Receptors: from Molecular Insights to Therapeutic Perspectives

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***Abstract*——Allosteric receptor modulation is an attractive concept in drug targeting because it offers important potential advantages over conventional orthosteric agonism or antagonism. Allosteric ligands modulate receptor function by binding to a site distinct from the recognition site for the endogenous agonist. They often have no effect on their own and therefore act only in conjunction with physiological receptor activation. This article reviews the current status of allosteric modulation at family C G-protein coupled receptors in the light of their specific structural features on the one hand and current concepts in receptor theory on the other hand. Family C G-protein-coupled receptors are characterized by a large extracellular domain containing the orthosteric agonist binding site known as the "venus flytrap module" because of its bilobal structure and the dynamics of its activation mechanism. Mutational analysis and chimeric constructs have revealed that allosteric modulators of the calcium-sensing, metabotropic glutamate and $GABA_B$ receptors bind to the seven transmembrane domain, through which they modify signal transduction after receptor activation. This is in contrast to taste-enhancing molecules, which bind to different parts of sweet and umami receptors. The complexity of interactions between orthosteric and allosteric ligands is revealed by a number of adequate biochemical and electrophysiological assay systems. Many allosteric family C GPCR modulators show in vivo efficacy in behavioral models for a variety of clinical indications. The positive allosteric calcium sensing receptor modulator cinacalcet is the first drug of this type to enter the market and therefore provides proof of principle in humans.**

I. Introduction

A. Allosteric Modulation of Receptor Activation—an Appealing Therapeutic Principle

The concept of allosteric receptor modulation in drug targeting has attracted considerable interest in recent years. On the one hand, this is probably because of the clinical success of the sedative/anxiolytic benzodiazepines, which were introduced into clinical use decades ago. They remain the pioneer example of drugs acting as allosteric modulators, in this case enhancing the function of the ionotropic $GABA_A$ receptor (Möhler, 2006). On the other hand, drugs acting in a similar manner at other receptors, including G-protein-coupled receptors, are undoubtedly of great potential (Conn et al., 2009a; De Amici et al., 2010), and modern drug discovery techniques have already allowed the identification of many such compounds in recent years. "Allosteric" (from Greek "αλλο" meaning *other* and "στερεο" meaning *object, shape*) drugs are compounds that act at a site on a given receptor that is topographically distinct from the "orthosteric" ("ορτηο" meaning *correct*) recognition site for the natural or exogenous agonists or competitive antagonists. They act primarily by changing the three-dimensional receptor conformation and thereby the affinities and/or efficacies of orthosteric ligands. Because they bind to sites on a given receptor other than those to which orthosteric ligands bind, they are in general structurally diverse and not at all related to orthosteric ligands, particularly the endogenous natural agonists.

Allosteric receptor modulators promise to have numerous advantages over orthosteric drugs. First, whereas an orthosteric agonist will stimulate a given receptor independently of its physiological state, a positive allosteric modulator will frequently not stimulate the receptor by itself, but only enhance the function of receptors activated by endogenous agonist. Thus, a positive allosteric modulator acts much more in concert with the temporal and spatial organization of physiological receptor activation and is therefore expected to have a *much lower side effect potential than orthosteric agonists*. For example, the positive allosteric $GABA_B$ receptor modulators 2,6-di-tert-butyl-4-(3-hydroxy-2,2-dimethylpropyl)phenol (CGP7930[1]) and

[1]Abbreviations: 3-MPPTS, 2,2,2-trifluoro-*N*-[3-(2-methoxyphenoxy)phenyl]-*N*-(pyridin-3-ylmethyl)ethanesulfonamide; 5MPEP, 5-methyl-6-(phenylethynyl)-pyridine; 6-OHDA, 6-hydroxydopamine; 7TM, 7-transmembrane; ABP688, 3-(6-methyl-pyridin-2-ylethynyl)-cyclohex-2-enone-*O*-methyl-oxime; ACPT-II, (1*R*,3*R*,4*S*)-1-aminocyclopentane-1,3,4-tricarboxylic acid; ADX47273, *S*-(4-fluorophenyl)-{3-[3-(4-fluorophenyl)-[1,2,4]-oxadiazol-5-yl]-piperidin-1-yl}-methanone; AMN082, *N*,*N*′-dibenzhydrylethane-1,2-diamine dihydrochloride; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; BAY36-7620, (3a*S*,6a*S*)-hexahydro-5-methylene-6a-(2-naphthalenylmethyl)-1*H*-cyclopenta[*c*]furan-1-one; BHF177,*N*-[(1*R*,2*R*,4*S*)-bicyclo[2.2.1]hept-2-yl]-2-methyl-5-[4-(trifluoromethyl)phenyl]-4-pyrimidinamine; BHK, baby hamster kidney; BINA, biphenylindanone A; CA, cornu ammonis; CaSR, calcium-sensing receptor; CBiPES, *N*-(4′-cyano-[1,1′-biphenyl]-3-yl-*N*-(3-pyridinylmethyl)-ethanesulfonamide hydrochloride; CDPPB, 3-cyano-*N*-(1,3-diphenyl-1*H*-pyrazol-5-yl)benzamide; CGP13501, 3,5-*bis*(1,1-dimethylethyl)-4-hydroxy-α,α-dimethylbenzenepropana; CGP35348, (3-aminopropyl)(diethoxymethyl)phosphinic acid; CGP54626, [*S*-(*R**,*R**)]-[3-[[1-(3,4-dichlorophenyl)ethyl]amino]-2-hydroxypropyl](cyclohexylmethyl) phosphinic acid; CGP55845A, (2*S*)-3-[[(1*S*)-1-(3,4-dichlorophenyl)ethyl]amino-2-hydroxypropyl](phenylmethyl)phosphinic acid hydrochloride; CGP62349, 3-[(1*R*)-1-[[(2*S*)-2-hydroxy-3-[hydroxy-[(4-methoxyphenyl)methyl]phosphoryl]propyl]-methylamino]ethyl]benzoic acid; CGP7930, 2,6-di-tert-butyl-4-(3-hydroxy-2,2-dimethylpropyl)phenol; CHO, Chinese hamster ovary; CPCCOEt, 7-hydroxyiminocyclopropan[*b*]chromen-1α-carboxylic acid ethyl ester; CPPHA, *N*-{4-chloro-2-[(1,3-dioxo-1,3-

N,*N*′-dicyclopentyl-2-methylsulfanyl-5-nitro-pyrimidine-4,6-diamine (GS39783) (Urwyler et al., 2001, 2003) do not have the sedative, hypothermic, and muscle relaxant effects of the orthosteric agonist baclofen (Carai et al., 2004; Cryan et al., 2004). It should be noted, however, that the advantage of *state or activity dependence*, which is a fundamental difference between positive allosteric modulators and orthosteric agonists, in theory does not apply to antagonists. In fact, as long as competitive antagonists, like negative modulators, are truly "silent" (i.e., they are not partial or inverse agonists), they also have no effect in the absence of an endogenous agonist. The important difference between competitive antagonists and negative modulators lies in the fact that the inhibition produced by the former, but not that by the latter, can be overcome by increasing agonist concentrations. Whether this is an advantage or not depends on the pathophysiological mechanisms of the disease to be addressed. Second, because of their use-dependent mechanism of action, positive allosteric modulators are expected to have no or only *low propensity for receptor desensitization*, whereas persistent agonist treatment often leads to receptor down-regulation. Gjoni and Urwyler (2008) have shown that this is actually the case for the example of the G-protein-coupled $GABA_B$ receptor. The receptors for many hormones or neurotransmitters exist in families comprising a number of subtypes with differing characteristics and functions. Whereas the orthosteric binding sites of these receptor subtypes usually remained well conserved during evolution, because they have to accommodate their natural ligands, there was no selective pressure to maintain the apparently nonessential allosteric binding sites unchanged. Therefore, and third, many positive or negative allosteric modulators bear a *high selectivity for a given receptor subtype*. It should be noted that subtype selectivity can be achieved on the basis of affinity (selective binding to a subtype) or cooperativity (binding to more than one subtype, with positive or negative cooperativity at only one and neutral cooperativity at the others). A typical example is given by muscarinic receptors, for which "absolute subtype selectivity" has been achieved in different allosteric modulators such as brucine derivatives or thiochrome (Birdsall et al., 2004). In the course of this review, we encounter similar examples for subtypes of metabotropic glutamate (mGlu) receptors. Moreover, as a fourth point, for some receptors it is inherently difficult to design synthetic orthosteric ligands. For example, numerous attempts to find low-molecular-weight agonists for peptide receptors have failed because of the size and number of attachment sites of the recognition domain for these relatively large endogenous ligands. Receptor modulation via an allosteric site, allowing completely new molecular scaffolds, seems a much more promising approach in such a case. Likewise, the design of orthosteric ligands for mGlu receptors has proven to be difficult. Most agonists and antagonists for the glutamate binding sites of mGlu receptors are amino acid derivatives, often with heavy stereochemical constraints, which can make the synthesis of such molecules most demanding (Pellicciari et al., 1996; Marinozzi and Pellicciari, 2000). Because of the hydrophilic nature of these compounds, their oral bioavailability and brain permeability is rather

dihydro-2*H*-isoindol-2-yl)methyl]phenyl}-2-hydroxybenzamide; CRC, concen ration-response curve; CRD, cysteine-rich receptor domain; CREB, cAMP-responsive element-binding protein; cyPPTS, 2,2,2-trifluoro-*N*-[3-(cyclopentyloxy)phenyl]-*N*-(3-pyridinylmethyl)-ethanesulfonamide; DCG-IV, (2*S*,2′*R*,3′*R*)-2-(2′,3′-dicarboxycyclopropyl)glycine; DFB, *N*,*N*′-bis-(3-fluoro-benzylidene)-hydrazine; DHPG, (*R*,*S*)-3,5-dihydroxyphenylglycine; ECD, extracellular domain; EM-TBPC, 1-ethyl-2-methyl-6-oxo-4-(1,2,4,5-tetrahydro-benzo[*d*]azepin-3-yl)-1,6-dihydro-pyrimidine-5-carbonitrile; EPSP, excitatory postsynaptic potential; ERK, extracellular signal-regulated kinase; FLIPR, fluorescence imaging plate reader; FMRP, fragile X mental retardation protein; FRET, fluorescence resonance energy transfer; FTIDC, 4-[1-(2-fluoropyridin-3-yl)-5-methyl-1*H*-1,2,3-triazol-4-yl]-*N*-isopropyl-*N*-methyl-3,6-dihydropyridine-1(2*H*)-carboxamide; GERD, gastroesophageal reflux disease; GIRK, G-protein-regulated inwardly rectifying potassium channel; GPCR, G protein-coupled receptor; GS39783, *N*,*N*′-dicyclopentyl-2-methylsulfanyl-5-nitro-pyrimidine-4,6-diamine; GTPγ^{35}S, guanosine 5′-*O*-(3-[^{35}S]thio)triphosphate; HEK, human embryonic kidney; HTS, high-throughput screening; IPTE, 2-[(4-indan-2-ylamino)-5,6,7,8-tetrahydroquinazolin-2-ylsulphanyl]-ethanol; JNJ16259685, (3,4-dihydro-2*H*-pyrano[2,3-*b*]quinolin-7-yl)-(*cis*-4-methoxycyclohexyl)-methanone; L-AP4, L-(+)-2-amino-4-phosphonobutyric acid; LTP, long-term potentiation; LY181837, *N*-(4-phenoxyphenyl)-*N*-(3-pyridinylmethyl)ethanesulfonamide; LY341495, (2*S*)-2-amino-2-(1*S*,2*S*-2-carboxycyclopronan-1-yl-3-(xanth-9-yl)propanoic acid; LY354740, (+)-2-aminobicyclo[3.1.0] hexane-2,6-dicarboxylic acid; LY379268, (−)-2-thia-4-aminobicyclo [3.1.0]hexane-4,6-dicarboxylate; LY404039, (−)-(1*R*,4*S*,5*S*,6*S*)-4-amino-2-sulfonylbicyclo[3.1.0]-hexane-4,6-dicarboxylic acid; LY487379, 2,2,2-trifluoro-*N*-[4-(2-methoxyphenoxy)phenyl]-*N*-(3-pyridinylmethyl)-ethanesulfonamide; M-5MPEP, 2-(3-methoxyphenylethynyl)-5-methylpyridine; mGlu, metabotropic glutamate; MK-801, dizocilpine maleate; MMPIP, 6-(4-methoxyphenyl)-5-methyl-3-pyridin-4-ylisoxazolo[4,5-*c*]pyridin-4 (5*H*)-one; MNI-136, 7-bromo-4-(3-pyridin-3-yl-phenyl)-1,3-dihydro-benzo[*b*][1,4]diazepin-2-one; MNI-137, 4-(7-bromo-4-oxo-4,5-dihydro-3 *H*-benzo[*b*][1,4]diazepin-2-yl)-pyridine-2-carbonitrile; MPEP, 2-methyl-6-(phenylethynyl)-pyridine; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydro-pyridine; MS, multiple sclerosis; MTEP, 3-((2-methyl-4-thiazolyl) ethynyl)pyridine; NMDA, *N*-methyl-D-aspartate; NPS2143, 2-chloro-6-[3-[1,1-dimethyl-2-(2-naphthyl)ethylamino]-2(*R*)-hydroxypropoxy]benzonitrile; NPS2390, *N*-(1-adamantyl)quinoxaline-2-carboxamide; NPS467, *N*-(3-methoxy-α-phenylethyl)-3-phenyl-1-propylamine; NPS568, (*R*)-*N*-(3-methoxy-α-phenylethyl)-3-(2′-chlorphenyl)-1-(propylamine hydrochloride); NTD, N-terminal domain; PCP, 1-(1-phenylcyclohexyl)piperidine (phencyclidine); PET, positron emission tomography; PHCCC, *N*-phenyl-7-(hydroxyimino)cyclopropa[*b*]chromen-1*a*-carboxamide; PLC, phospholipase C; PPI, prepulse inhibition; PTH, parathyroid hormone; R214127 1-(3,4-dihydro-2*H*-pyrano[2,3-*b*]quinolin-7-yl)-2-phenyl-1-ethanone; rac-BHFF, 5,7-bis(1,1-dimethylethyl)-3-hydroxy-3(trifluoromethyl)-2(3*H*)-benzofuranone; Ro 01-6128, diphenylacetyl-carbamic acid ethyl ester; Ro 67-4853, butyl (9*H*-xanthene-9-carbonyl)carbamate; Ro 67-7476, (*S*)-2-(4-fluorophenyl)-1-(toluene-4-sulfonyl)pyrrolidine; S807, *N*-(heptan-4-yl)benzo[d][1,3]dioxole-5-carboxamide; S819, 1-((1*H*-pyrrol-2-yl) methyl)-3-(4-isopropoxyphenyl)thiourea; SAR, structure-activity relationship; SIB-1757, 6-methyl-2-(phenylazo)-3-pyridinol; SIB-1893, (*E*)-2-methyl-6-(2-phenylethenyl)pyridine; SIH, stress-induced hyperthermia; SKF97541, 3-aminopropyl(methyl)phosphinic acid; SP203, 3-fluoro-5-(2-(2-(fluoromethyl)thiazol-4-yl)ethynyl)benzonitrile; VFTM, venus flytrap module; VGCC, voltage-gated calcium channel; VU0155041, *cis*-2-[[(3,5-dichlorophenyl)amino]carbonyl]cyclohexanecarboxylic acid; VU-29, 4-nitro-*N*-(1,3-diphenyl-1*H*-pyrazol-5-yl)benzamide;YM-230888, 4-(cycloheptylamino)-*N*-[[(2*R*)-tetrahydro-2-furanyl]methyl]-thieno[2,3-*d*] pyrimidine-6-methanamine; YM-298198, 6-amino-*N*-cyclohexyl-*N*,3-dimethyl-thiazolo[3,2-*a*] benzimidazole-2-carboxamide hydrochloride.

poor in most cases. Because of the structural diversity and more lipophilic nature of allosteric ligands, such agents may show an elegant way out of these dilemmas. In fact, as is discussed in detail, numerous positive and negative modulators of mGlu receptors are known today, with good pharmacokinetic properties and subtype selectivities.

Potential disadvantages of allosteric GPCR modulators and pitfalls or caveats in their discovery and development should also be mentioned, however (Raddatz et al., 2007). First, the often-encountered low evolutionary conservation of allosteric sites, which allows for receptor subtype selectivity, may also result in significant *species differences*. We encounter a few examples in this review (e.g., Table 7). This aspect will, of course, become problematic when rodent receptors are used for drug screening, or when rodent models are used for the investigation of drug effects in vivo after the characterization of allosteric effects at recombinant human receptors in vitro. Second, the advantageous activity-dependence of positive allosteric modulators might turn into a handicap in neurodegenerative diseases in which the loss of neurons results in decreased availability of the endogenous agonist. In such a case, a positive allosteric modulator might progressively lose efficacy in parallel with the progression of the disease. Third, from a medicinal chemistry point of view, we will see in this review that allosteric GPCR modulators often have "*flat,*" *nontractable structure-activity relationships* (SAR), which makes it most difficult to improve on lead molecules with micromolar affinities. In addition, only slight structural modifications sometimes result in important changes in pharmacological responses, from positive to negative allosteric modulation over neutral cooperativity, making the hurdles for rational ligand design almost insurmountable. Last, at present it seems impossible to find the optimal profile of cooperative and/or agonistic activity of an allosteric drug, matching the complexity of the underlying biology of a disease for which a thorough understanding is most often missing. Examples of these and other challenges in the development of allosteric GPCR modulators are discussed in this review. Nevertheless, these hurdles and difficulties do certainly not diminish the great therapeutic potential that these novel drugs have and the enthusiasm that they have generated among the research community in recent years.

B. Family C G Protein-Coupled Receptors, a Promising Group of New Drug Targets

G-protein-coupled receptors (GPCRs) are most important drug targets; 40 to 50% of all marketed therapeutics are estimated to act through approximately 50 different GPCRs, and more than 300 of these receptors remain to be exploited (Lundstrom, 2005; Overington et al., 2006; Schlyer and Horuk, 2006; Lagerström and Schiöth, 2008). GPCRs form one of the largest and most diverse protein superfamilies; at least 1% of the mammalian genome encodes GPCRs (Takeda et al., 2002). Roughly half of the total of approximately 950 GPCRs are believed to have endogenous ligands, whereas the other half are sensory (taste or odorant) receptors. In fact, GPCRs are probably the most important type of cellular signal transducing proteins, mediating the messages of stimulants as diverse as ions, hormones and neurotransmitters, odorants, pheromones, gustative molecules, lipids, peptides, proteins, and even light (photons). This multiplicity of stimulants is matched by the broad diversity of existing GPCRs. Signal transduction through GPCRs is a proven "evolutionary success" (Bockaert and Pin, 1999), and genes coding for GPCRs have expanded from a common ancestor into a large number of species with little sequence homology between the branches of their evolutionary trees. Different nomenclatures have classified mammalian GPCRs into at least five families, and the phylogenetic relationships of more than 800 human GPCR sequences have been thoroughly established (Bockaert and Pin, 1999; Fredriksson et al., 2003). A common structural feature of all GPCRs is a central core domain made up of seven transmembrane-spanning α-helices (7TM or heptahelical domain) containing 25 to 35 amino acid residues each that are connected by three intracellular and three extracellular loops. The receptors are believed to convey their signals through interaction of their 2nd and 3rd intracellular loops with the α-subunits of heterotrimeric G-proteins, although this has not actually been proven for most of the members of the seven transmembrane (7TM) receptor superfamily. In the case of the rhodopsin-like receptors, the most ancient and largest GPCR family, the 7TM domain contains the ligand binding site, although in other families the N-terminal extracellular domains also contribute to various extents to ligand binding (Bockaert and Pin, 1999). This is most markedly the case for family C GPCRs, which are the subject of this review.

Family C GPCRs comprise various pheromone, vomeronasal, and taste receptors, the calcium-sensing receptor (CaSR), as well as "metabotropic" $GABA_B$ and glutamate receptors (for review, see Pin et al., 2003; Bräuner-Osborne et al., 2007; Rondard et al., 2011) (Fig. 1). The receptors belonging to this family have been identified only relatively recently, and therefore very few drugs targeting these receptors are in clinical use today. One of them is the antispastic agent baclofen (Lioresal), which was marketed long before its target, the $GABA_B$ receptor, was pharmacologically identified (Bowery et al., 1980). From there to the molecular cloning of this elusive receptor (Kaupmann et al., 1997) again almost 2 decades went by. Likewise, the first biochemical experiments describing the stimulation of the phospholipase C (PLC) pathway via "metabotropic" glutamate receptors (mGlu receptors) were described in the 1980s (Sladeczek et al., 1985; Nicoletti et al., 1986); the first of these, the mGlu1 receptor, was cloned in 1991 (Masu et al., 1991), followed by seven others (see section III).

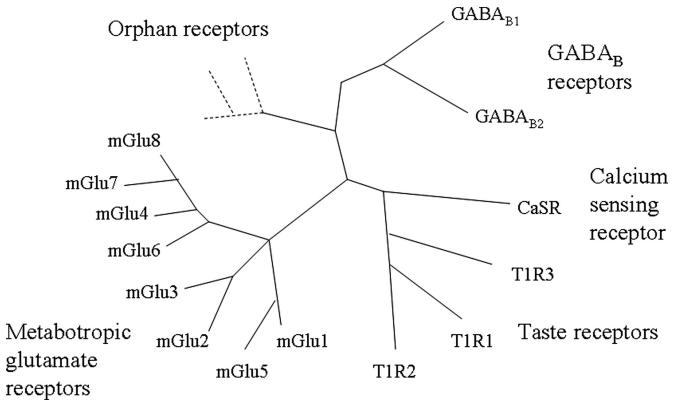


FIG. 1. Phylogenetic tree based on the 7TM sequences showing the human family C GPCRs. Orphan receptors are shown only summarily; vomeronasal (pheromone) receptors are not included because these are pseudogenes in the human genome. For a more detailed representation of the phylogenetic relationships of family C GPCRs, see Pin et al. (2003).

The structure and activation mechanism of family C GPCRs have recently been reviewed by Rondard et al. (2011). The molecular structure of family C GPCRs, like that of other GPCRs, contains an N-terminal extracellular domain (ECD), 7TM domains that are linked by three intracellular and three extracellular loops, and an intracellular C-terminal amino acid sequence (Fig. 2). The structure of family C GPCRs is characterized by an exceptionally large ECD, which is linked (except in the GABA$_B$ receptor) to the 7TM domain by an amino acid stretch rich in cysteine residues (Fig. 2). The ligand binding site is located in the ECD (Malitschek et al., 1999) and consists of a bilobal structure that closes upon agonist binding; this is in contrast to rhodopsin-like (family A) GPCRs, in which the ligand binding site is in the 7TM domain. The ECD of family C GPCRs has been found to be closely related to bacterial periplasmic amino acid binding proteins (O'Hara et al., 1993), which transport nutrients from the extracellular to the intracellular space in bacteria. On the other hand, the 7TM domain of family C GPCRs is phylogenetically related to rhodopsin-like (family A) GPCRs. When an evolutionary tree is reconstructed on the basis of the "venus flytrap module" (VFTM) sequences of family C GPCRs, the same distances are obtained as when the sequences of the 7TM domain are used (except for orphan receptors lacking the VFTM) (Pin et al., 2003). It appears, therefore, that this receptor family might have its evolutionary origin in a gene fusion between bacterial periplasmic binding proteins and rhodopsin-like receptors (Pin et al., 2003).

This modular structure of family C GPCRs raises questions about the role of their different building blocks in the signal transduction mechanisms. Parmentier et al. (2002) have proposed a model for the functioning of family C GPCRs that addresses this question; its essential assumptions are strongly supported by a large number of precedent and subsequent experimental findings. The model posits that both the extracellular and the 7TM domains of these receptors exist in active and inactive states. Because the 7TM domain interacts with the G-protein, receptor activation of the ECD by agonist binding must stabilize the active state of the 7TM domain. On the other hand, allosteric interactions being reciprocal, a stabilization of the 7TM domain (for example by the binding of positive allosteric modulators) will result in facilitation of agonist binding to and activation of the ECD. This coupling seems to be tight in the case of GABA$_B$ receptors but rather loose in the case of mGlu receptors, probably because of the presence of the cysteine-rich amino acid stretch separating the two domains in the latter. In fact, as in rhodopsin-like receptors, nonhydrolyzable GTP analogs such as GTPγS decrease agonist binding affinities at GABA$_B$ but not at mGlu receptors (Parmentier et al., 2002). This loose coupling also led Parmentier et al. (2002) to predict that receptor stimulation must be possible by direct activation of the 7TM domain even in the absence of agonist bound to the ECD. This concept is known as "allosteric agonism" in the terminology of theoretical receptor models (see section I.C).

The ligand binding domain in the ECD consists of a bilobal structure that closes upon agonist binding in a way reminiscent of the functioning of a venus flytrap plant (Bessis et al., 2000; Galvez et al., 1999, 2000a). The concept of ligand binding to the VFTM has been convincingly confirmed by X-ray analysis of the mGlu1 receptor ECD in its unliganded form or containing bound glutamate (closed form) (Kunishima et al., 2000) or an antagonist (open form) (Tsuchiya et al., 2002). The ligand-binding region was found to exist as a disulfide-linked homodimer in the crystal; many aspects of the role of homo- and heterodimeric assemblies in family C GPCR function will be touched upon in this review. More recently, the crystal structures of the entire ECD of mGlu3 receptor and the ligand-binding domain of mGlu7 receptor ECDs have also been reported (Muto et al., 2007). Molecular modeling studies suggest that the mGlu8 receptor antagonists (1*R*,3*R*,4*S*)-1-aminocyclo-

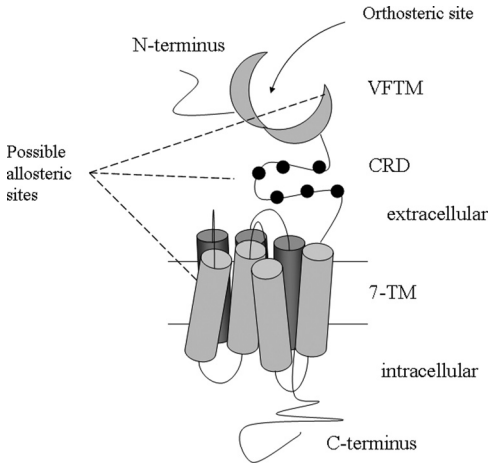


FIG. 2. Schematic representation of a family C GPCR protein. The GABA$_B$ receptor does not have the CRD domain, and orphan (retinoic acid-induced) receptors lack both the VFTM and the CRD.

pentane-1,3,4-tricarboxylic acid and M-AP4 prevent the closure of the VFTM because of ionic and sterical hindrance, respectively, at their receptor interaction sites. Site-directed mutagenesis of the amino acid residues responsible for these hindrances removed these constraints and allowed both compounds to fully activate the mGlu8 receptor (Bessis et al., 2002). On a similar line, Kniazeff et al. (2004) succeeded in locking the $GABA_B$ receptor in its active state by introducing two cysteine residues forming a disulfide bridge maintaining the binding domain in its closed form.

The ligand binding site for rhodopsin-like receptors is located inside the 7TM domain, and it is noteworthy that the binding sites for most allosteric modulators for family C GPCRs, which are the subject of this review, have also been located in this 7TM domain (references are given in the respective sections). This of course raises the question of whether this putative allosteric site corresponds to the agonist binding site for rhodopsin-like receptors. The interaction of allosteric ligands for family C GPCRs with the 7TM domain has been the subject of several reviews (Costantino, 2006; Noeske et al., 2006; Surgand et al., 2006).

The elucidation of the molecular structure of membrane bound proteins remains a major challenge; nevertheless, the high resolution X-ray structures of bovine rhodopsin and, more recently, β-adrenergic and adenosine A2 receptors are available (for review, see Weis and Kobilka, 2008; Mustafi and Palczewski, 2009). Although the sequence homology between the 7TM domain of family C GPCRs and that of rhodopsin-like (family A) receptors is low, the two share several common topological features (Costantino, 2006). On this basis, homology models of several family C GPCRs using bovine rhodopsin as a template have been used for the docking of positive and negative allosteric modulators into their putative binding pockets. On the basis of such models, amino acid residues involved in the binding of allosteric compounds have been identified using site directed mutagenesis. Although in this way allosteric binding sites have been found at different locations in the 7TM domain of family C GPCRs (see references in the respective sections), the fact that at least some allosteric family C GPCR modulators bind to a pocket in the 7TM domain, which is the equivalent of the retinal binding site in bovine rhodopsin strongly suggests, in line with the model of Parmentier et al. (2002), that such compounds should be able to activate these receptors in their own right. Indeed, Goudet et al. (2004) have elegantly shown that the 7TM domain of the mGlu5 receptor functionally behaves like rhodopsin-like receptors: they were able to demonstrate that the negative allosteric modulator 2-methyl-6-(phenylethynyl)-pyridine (MPEP) (Table 2) and the positive allosteric modulator *N*,*N*′-bis-(3-fluorobenzylidene)-hydrazine (DFB) (Table 5) act as an inverse agonist or full agonist, respectively, on mGlu5 receptor constructs lacking the extracellular domain. Direct receptor activation via the 7TM domain as observed with DFB is the basis for the phenomenon of ago-allosterism outlined in section I.C (Langmead and Christopoulos, 2006; Schwartz and Holst, 2006). In the course of this review, we will encounter several other examples of compounds acting as allosteric agonists or inverse agonists at family C GPCRs via the 7TM domain.

C. Allosteric Receptor Modulation in the Light of Theoretical Receptor Models

A number of classic and more recent theoretical models have attempted to formalize the mechanisms of interaction of receptors with their ligands. The "two-state model" (Leff, 1995) assigns an active (R*) and an inactive (R) conformation to the receptor, which are interconvertible and in thermodynamic equilibrium. The spontaneous formation of R* without agonist binding is the basis for constitutive activity. The binding of an agonist stabilizes the active receptor state R* (intrinsic efficacy $\alpha > 1$), whereas an inverse agonist ($\alpha < 1$) reduces constitutive activity by shifting the equilibrium to the resting state R. Increasing or decreasing values of α account for various degrees of low or higher partial to full agonism (or inverse agonism). "Silent" antagonists block the binding site, but are neutral in terms of receptor activation ($\alpha = 1$). This model, however, does not accommodate the effects of allosteric drugs. Changes in binding affinities of orthosteric ligands induced by allosteric modulators have been described by a "ternary complex model," which was originally designed for the modulation of agonist binding by receptor-G-protein coupling (De Lean et al., 1980) but can also be applied instead to the binding cooperativity between two small molecules interacting with distinct sites on the same receptor protein. Because the thermodynamic stability of the ternary complex is independent of the order of ligand binding, the allosteric interaction between the orthosteric and allosteric ligands is always reciprocal. Its strength depends on the chemical nature of both molecules [i.e., is ligand- (or "probe") dependent]. The more recent allosteric two state model introduced by Hall (2000) (Fig. 3) combines these two previous models, but at the same time accounts for additional aspects of receptor-ligand interactions, which were not covered by the ternary complex model and the two-state model. In particular, it introduces the allosteric constant δ, by which the efficacy α of an orthosteric ligand (in addition to its affinity) can be modified. Moreover, it also accounts for allosteric agonism ($\beta > 1$) or inverse agonism ($\beta < 1$) (i.e., the stabilization of the active or inactive receptor states, respectively, by ligands binding to a site distinct from the orthosteric site). This model treats the allosteric interactions in purely numerical terms; i.e., the constants γ and δ are independent of the qualitative nature of the orthosteric ligand. Thus, this model predicts that partial or full agonists or inverse agonists, as

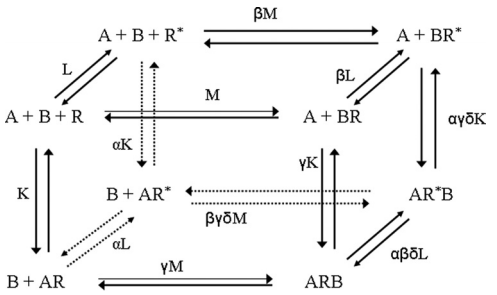


FIG. 3. The allosteric two-state model. R, inactive state of the receptor; R*, active state of the receptor; A, orthosteric ligand; B, allosteric ligand; K, binding constant of A; L, receptor isomerization constant; M, binding constant of B; α, intrinsic efficacy of A; β, intrinsic efficacy of B; γ, binding cooperativity between A and B; δ, activation cooperativity between A and B. Slightly modified after Hall (2000).

well as competitive antagonists, should be equally amenable to modulation by allosteric drugs (for examples and further discussion, see Urwyler et al., 2005). The model of Hall was extended in a further step to a "quaternary complex model" by Christopoulos and Kenakin (2002), to include allosteric modulation of the orthosteric site not only by low molecular weight compounds, but at the same time also by receptor-G-protein interactions.

These theoretical considerations, together with the structural and functional aspects discussed above, show that the allosteric binding site is, just as the orthosteric site, a ligand binding site in its full own right (Fig. 4). In addition to cooperative effects modulating affinities and/or efficacies of orthosteric ligands, agonistic or inverse agonistic effects of compounds acting at the allosteric site (often in the 7TM domain) are possible, just as for those binding to its orthosteric counterpart (here the VFTM) (Langmead and Christopoulos, 2006; Schwartz and Holst, 2006). There are mixed forms of modulating and agonistic effects, and for both there is a continuum from negative to positive efficacies to different degrees (Fig. 4). This continuum also comprises neutral allosteric ligands (i.e., compounds without any positive or negative cooperativity or agonistic or inverse agonistic efficacy). In analogy to "silent" orthosteric antagonists, such compounds will act as antagonists at the allosteric site, competitively blocking the effects of positive or negative allosteric modulators, allosteric agonists or inverse agonists, or mixed forms thereof. Examples for such drugs are the benzodiazepine receptor antagonist flumazenil, or the neutral allosteric site ligand at the mGlu5 receptor 5-methyl-6-(phenylethynyl)-pyridine (5MPEP) (Fig. 7), which blocks the actions of both positive and negative mGlu5 receptor modulators (Rodriguez et al., 2005). This diversity of possibilities of compound effects through allosteric sites shows that these are active sites on a receptor (although they seem to be "serendipitous" sites for which no natural ligands exist), which makes them malleable and suitable targets for pharmacological intervention.

D. Strategies for Discovering Allosteric Modulators

GPCRs leave open an enormous potential for drug development by their sheer number. For many of them, the natural ligands are not yet identified; i.e., they still have the status of "orphan receptors." Others, such as family C GPCRs, which are the subject of this review, have been identified by molecular cloning and pharmacologically characterized only relatively recently, and only a rather small number of drugs acting through them exist to date. Most pharmaceuticals developed so far act at GPCRs as orthosteric ligands (that is, as agonists or antagonists at the binding sites for their natural agonists) and have typically been identified in ligand binding assays (Hopkins and Groom, 2002; Maudsley et al., 2005). Modern assay techniques, in conjunction with

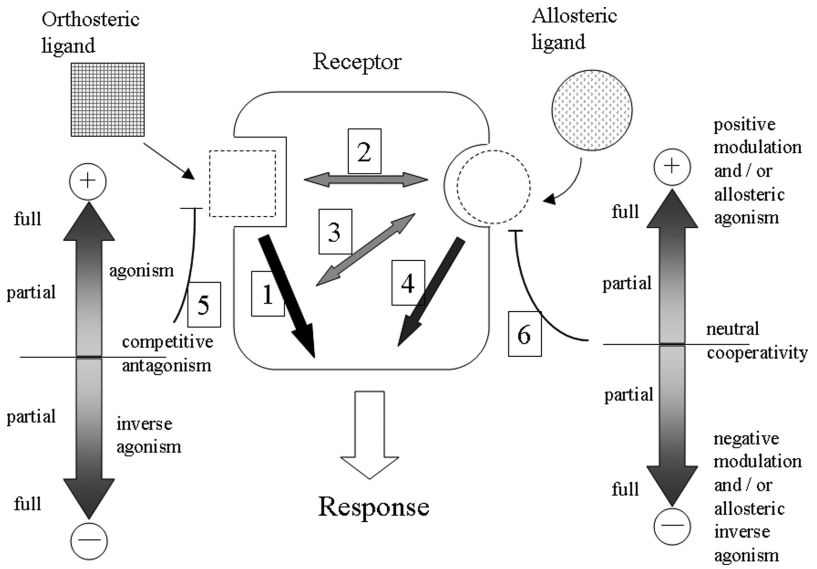


FIG. 4. Mechanisms of ligand-receptor interactions. Orthosteric and allosteric ligands bind to topographically distinct sites on a receptor. Both can positively or negatively influence signal transduction, either on their own or by modulating each other's affinity and/or efficacy. 1, orthosteric full or partial agonism or inverse agonism; 2, affinity modulation (positive or negative); 3, efficacy modulation (positive or negative); 4, allosteric agonism or inverse agonism; 5, neutral binding to the orthosteric site/competitive antagonism; 6, neutral binding to the allosteric site.

the stable expression of a drug target of interest in recombinant cell lines, now make it possible to screen large compound libraries containing hundreds of thousands of chemical entities for new pharmacological actions (McLoughlin et al., 2007). Moreover, computer-assisted ("in silico") searching methods such as ligand- or structure-based homology modeling, are gaining importance as a valuable add-on to experimental high-throughput screening (HTS) (Triballeau et al., 2005; Costantino, 2006; Schlyer and Horuk, 2006). Such modern drug discovery technologies have made it possible to find molecules acting at GPCRs through other sites and other mechanisms than orthosteric ligands (Rees et al., 2002; Brink et al., 2004).

The plurality and complexity of compound effects at GPCRs call for powerful assay techniques to adequately describe the nature of orthosteric and allosteric receptor ligands. For example, inverse agonism can only be detected in the presence of a measurable degree of constitutive activity, and the measurement of low-efficacy partial agonism depends on the availability of an assay system sufficiently sensitive to detect small responses (Binet et al., 2004; Urwyler et al., 2005; Langmead and Christopoulos, 2006). In this context, it should be noted that in sensitive, highly expressing recombinant systems with a high degree of receptor reserve, the apparent efficacy of partial agonists will increase and can even reach seemingly full agonism. Resulting apparent ligand effects are thus a combination of intrinsic and conditional factors, making the choice of the appropriate assay system crucial. Whereas orthosteric ligands can easily be detected in radioligand binding assays, such experiments are most often not suitable for HTS campaigns and will not always detect compounds acting through novel allosteric sites. Typically, whole cell or, in some cases, membrane-based functional assays will be performed in the presence of a submaximal agonist concentration to reveal positive or negative allosteric modulation by test compounds from a screening library. Because allosteric interactions are ligand-dependent, it is probably best to use the endogenous agonist for these experiments. A careful secondary characterization will then be needed to confirm that the compound of interest is indeed acting through an allosteric mechanism. This will include radioligand displacement assays to exclude interaction with the orthosteric binding site as well as kinetic assays to show indirect effects on the binding properties of an orthosteric probe. Moreover, detailed radioligand binding as well as a panel of functional assays are needed to determine whether an allosteric modulator primarily affects the affinity or the apparent efficacy of an orthosteric agonist, or both. Functional assays should also be conducted in the absence of an orthosteric agonist, to detect any putative intrinsic agonistic or inverse agonist activity of the allosteric compound. Last but not least, compound effects should be confirmed in native assay systems, especially because receptor interactions with effector systems may be different in recombinant and native environments. The natural agonist may not be the best to work with in native preparations, as it usually will be a substrate for enzymes and transporters. Therefore, in the light of the ligand dependence mentioned in section I.C. it should be ascertained beforehand in recombinant assays that a chosen synthetic agonist undergoes the same allosteric influences by the test compound as the natural one. Once compounds interacting with a novel allosteric site have been identified in this way, it might become possible to obtain radioligands directly labeling that site, provided they have high (nanomolar) binding affinity. As we will see in the course of this review, this is the case for only relatively few examples so far.

Using such approaches, a considerable number of molecules modulating family C and other GPCRs have been discovered and characterized in recent years. This novel therapeutic concept has opened exciting new avenues in modern pharmacology.

II. Allosteric Ligands for Taste Receptors: Not Only the Proof of Concept

A. Taste—More than Just a Pleasure

Only five different basic qualities make up the repertoire of taste sensation by humans and other organisms: sweet, bitter, salty, sour, and "umami" (the savory taste of meat, derived from Japanese "umai," delicious). At the entry door for food, taste perception exerts the role of a nutritional quality control (Lindemann, 2001): it monitors the content of energy-rich carbohydrates (sweet), amino acids (umami), it checks whether the salt content is within the tolerance of the organism's electrolyte balance, and it also detects the presence of potentially harmful or toxic ingredients, which often have a bitter or sour taste. This small number of only five taste qualities, which has proven during evolution to be sufficient for fulfilling this quality control task, corresponds to a low diversity of taste receptors. This implies, however, that the relatively few taste receptors are rather promiscuous (Wellendorph et al., 2009) in the sense that, for example, the sweet receptor recognizes different types not only of sugars but also of other sweetener molecules, and the umami receptor binds different L-amino acids, whereas, by comparison, mGlu receptors are highly specific for the canonical agonist L-glutamate. Frauli et al. (2006) have demonstrated that among the 20 natural L-amino acids, only glutamate is able to activate mGlu receptors.

Whereas beforehand the characterization of tastant molecules relied essentially on behavioral measures (Palmer, 2007), the relatively recent discovery and characterization of taste receptors (see section II.B) made the area of taste research amenable to the concepts and techniques of molecular pharmacology. In fact, in the search for taste additives, companies that specialize in

the development of such chemicals proceed in much the same way that pharmaceutical companies do when pursuing a new target [for example by expressing the receptors of interest in recombinant cell lines (Ozeck et al., 2004) and using these for HTS of large compound libraries (Palmer, 2007; Zhang et al., 2008a)]. The potencies of such molecules obtained in these in vitro assays can be expected to reliably predict their in vivo activity, because no biological barriers oppose them and no pharmacokinetic considerations apply.

The mechanisms of sensing by taste receptors have been the subject of several reviews (Mombaerts, 2004; Scott, 2004; Chandrashekar et al., 2006; Palmer, 2007; Temussi, 2009) and can be treated only superficially here. It has long been the prevailing view that taste cells are selective for only one of the five taste modalities (i.e., they express only one class of taste receptors) and that from them a neuronal signal is sent to the brain along "labeled lines" (Zhang et al., 2003; Scott, 2004; Chandrashekar et al., 2006). However, more recent adaptations of the model allow for some cross-talk between different taste signals (Tomchik et al., 2007; Temussi, 2009). In fact, although "type II" taste-specific cells express only one type of taste receptor, signals from different taste cells may converge on type III cells, thus adding some complexity to taste perception. Taste receptors fall into different categories in terms of their signal transduction mechanisms. Not surprisingly, receptors sensing sour and salty tastes are ion channels. On the other hand, bitter, sweet, and umami receptors are GPCRs and couple to gustducin, a G-protein specifically involved in taste processing (Chandrashekar et al., 2000), but also to other G-proteins (Kusakabe et al., 2000; Ozeck et al., 2004). Taste receptors for bitter, sweet, and umami are localized in type II cells, which are part of the taste buds on the surface of the tongue. The signal transduction machinery of type II cells, however, does not enable them to make synaptic contacts with sensory neurons. It is believed that indirect intercellular communication via synapse-capable "type III" cells relays the taste receptor input to sensory neuronal output. These mechanisms have been nicely reviewed by Palmer (2007). Approximately 30 genes encode a group of bitter receptors (T2R GPCRs) to accommodate for a large variety of potentially harmful food ingredients. These are class A (rhodopsin-like) receptors and are therefore beyond the scope of this review.

B. Molecular Mechanisms of Sweet and Umami Taste Sensing

Whereas the need for sensing a large number of potentially harmful food ingredients is met by at least 30 different bitter taste receptors, evolution has adopted a different strategy for the recognition of sweet- or umami-tasting molecules. Only three T1R receptor proteins encoded by separate genes (T1R1, T1R2, and T1R3) have been identified so far in mice and humans (Kitagawa et al., 2001; Max et al., 2001; Montmayeur et al., 2001; Nelson et al., 2001; Sainz et al., 2001). T1R3 is coexpressed in taste cells either with T1R1 or with T1R2. Studies in knockout animals lacking an individual subtype of T1R receptors led to the conclusion that T1R1+T1R3 are required for umami sensing, whereas T1R2+T1R3 are needed for sweet perception (Zhao et al., 2003). Members of the Felidae family, from the domestic cat to the tiger, have a deletion mutation in their *T1R2* gene that makes them indifferent to sweets and is likely to have played a role in the evolution of their carnivorous behavior (Li et al., 2005). Nelson et al. (2001) and Li et al. (2002) expressed T1R2 together with T1R3 in recombinant cell lines and were indeed able to demonstrate intracellular Ca^{2+} signals in response to a variety of sweet compounds. Cells lacking either subunit did not produce responses. Likewise, T1R1 and T1R3 produced a calcium signal in response to L-glutamate or other amino acids when expressed together, but not individually, in HEK cells (Li et al., 2002; Nelson et al., 2002). On the basis of these findings, it is now well established that, much like the $GABA_B$ receptor identified earlier (Fig. 12; section IV), sweet and umami receptors consist of heterodimers (Fig. 5), either between T1R2 and T1R3 for the former, or

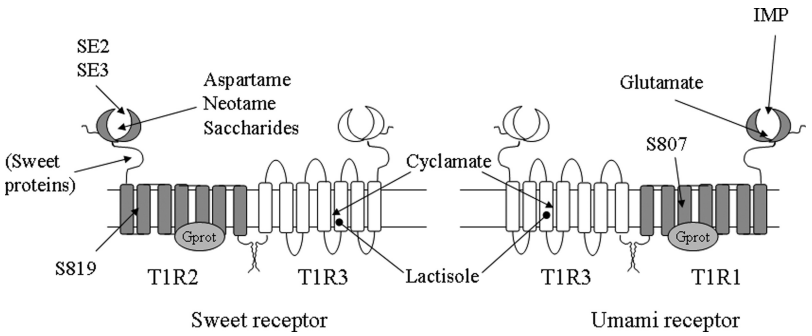


FIG. 5. Structure and ligand binding sites of sweet and umami taste receptors. The two receptors are heterodimeric complexes, each subunit protein belonging to family C GPCRs. The T1R3 subunit is common to both the sweet and umami receptors; in the former, it coassembles with T1R2 and in the latter, with T1R1. The orthosteric agonists shown bind to the hinge region of the VFTM; SE2/SE3 and IMP act allosterically at sweet and umami receptors, respectively, by binding to the opening end of the VFTM, thereby stabilizing its closed conformation (see also Fig. 6). Sweet proteins have been proposed to activate T1R2 via its CRD. The other allosteric ligands shown bind to the 7TM region. See Table 1 for structures of compounds and more details.

between T1R1 and T1R3 for the latter. Accordingly, taste cells express T1R1 or T1R2 but not both, as otherwise they would send an ambiguous message to the brain. Because the sweet taste receptor and the umami taste receptor have the subunit T1R3 in common, the unique subunits T1R1 (for umami) and T1R2 (for sweet) are expected to account for ligand binding (Zhang et al., 2008a). However, deviations from this expectation are in fact observed.

A multiplicity of different ligand binding sites is found on the sweet receptor T1R2/T1R3 (Fig. 5) (Cui et al., 2006). By analogy to other family C GPCRs, it seems reasonable to consider the binding pocket within the cleft that separates both lobes of the VFTM to be the "orthosteric" site, because the natural ligands such as sucrose or other sugars bind to this site. It is noteworthy that although family C GPCRs mostly sense amino acids, the canonical ligands for the sweet receptor are saccharides. The VFTM on T1R2 of the sweet receptor also binds the majority of artificial sweeteners, such as aspartame or neotame (Fig. 5). Xu et al. (2004) have exploited the fact that human but not rat T1R2/T1R3 are activated by several synthetic sweeteners to map the binding sites for aspartame and neotame. In agreement with behavioral observations, human but not rat receptors responded to aspartame, neotame, and cyclamate when expressed in HEK cells and assayed for intracellular calcium increases. Functional expression of rat/human chimeric constructs led to the conclusion that neotame and aspartame interact with the VFTM in T1R2. In fact, when the N-terminal domain of the human T1R2 was replaced with the rat sequence and coexpressed with human T1R3, the responsivity to neotame and aspartame was lost. Conversely, replacement of the T1R2 N-terminal domain in the rat receptor with its human counterpart resulted in the gain of ability to be activated by these two sweeteners.

Nie et al. (2005) monitored the interaction of mouse T1R2 and T1R3 N-terminal domains (expressed in *Escherichia coli* and purified) with sugars and synthetic sweeteners by circular dichroism spectroscopy and by measuring changes in intrinsic tryptophan fluorescence. They found that glucose and sucrose, but not cyclamate (Table 1), bound to both VFTMs, albeit with different affinities. It is noteworthy that the T1R3 NTD bound sucrose with higher affinity than the T1R2 NTD, whereas this was reversed for glucose. Both humans and mice perceive sucrose as sweeter than glucose, suggesting a key role of the T1R3 NTD in receptor activation. However, both T1R2 and T1R3 subunits, and their intersubunit coupling, are essential to form a functional saccharide receptor. This becomes clear from the fact that the umami receptor, which also contains the T1R3 subunit, is not activated by sugars (although it is modulated by cyclamate; see below). Obviously, intersubunit coupling between the heterodimer subunits is crucial; the sweet receptor, like other family C GPCRs, functions as an "allosteric machine." Moreover, the studies of Xu et al. (2004) indicate that the T1R2 subunit is responsible for the intracellular G-protein interaction.

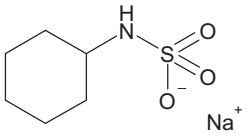
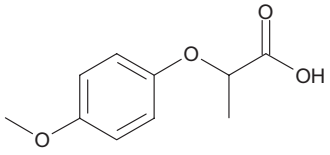
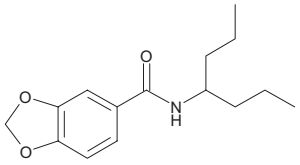
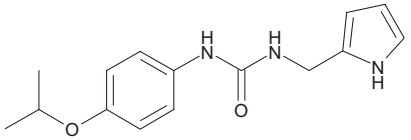
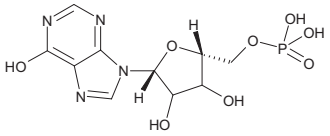
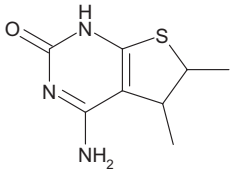
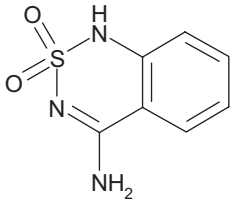
Somewhat controversial results have been reported concerning the binding sites and binding mode of sweet proteins. Temussi (2002) and Morini et al. (2005) have performed in silico docking experiments using homology models based on the ECD of the mGlu1 receptor. From this, they suggested that sweet proteins such as brazzein, monellin, and thaumatin can bind to a secondary site on the open end of the VFTM without entering the deep cleft ("wedge model"). Subsequently, Koizumi et al. (2007), using human/mouse chimeric constructs expressed in HEK cells and intracellular calcium mobilization assays, have demonstrated that the protein neoculin, which tastes sweet to man but not mice, is indeed received by the N-terminal domain of T1R3. However, the wedge model has now been found not to be consistent with the results of mutational analysis, at least in the case of brazzein (Assadi-Porter et al., 2010). On the other hand, Jiang et al. (2004), also by using human/mouse chimeras and by performing site-directed mutagenesis experiments, came to the conclusion that the cysteine-rich region of human T1R3 determines the responses to intensely sweet proteins such as brazzein or monellin and might be part of their binding site (Fig. 5). In this case, these proteins would act in a unique way as allosteric agonists through the cysteine-rich receptor domain (CRD). In this study it was also observed that an A537P point mutation in the CRD of hT1R3 resulted in a loss of responsiveness to all sweeteners, also those binding to T1R2, suggesting that the T1R3 CRD plays an important role in receptor coupling and again highlighting the importance of subunit interactions in the heterodimer.

Cyclamate (Table 1) also tastes sweet to humans, but not to rodents. Experiments similar to those outlined above revealed that the binding site for cyclamate is located in the 7TM domain of human T1R3 (Xu et al., 2004; Jiang et al., 2005). From the fact that cyclamate activates the receptor in the absence of any other ligand, it follows that this sweetener is an *allosteric agonist*. On the other hand, the Senomyx (San Diego, CA) compound 1-((1*H*-pyrrol-2-yl)methyl)-3-(4-isopropoxyphenyl)thiourea (S819; Table 1) is a sweet compound that interacts in a similar way with the T1R2 transmembrane domain (Zhang et al., 2008a). Like cyclamate, lactisole (Table 1), a sweet taste inhibitor in humans but not rodents, also requires the human T1R3 transmembrane domain to exert its effects (i.e., to inhibit the receptor's response to different agonists such as sugars, sweet proteins, and synthetic sweeteners) (Jiang et al., 2005). Lactisole thus behaves as a negative allosteric modulator; it is competitive against cyclamate. In fact, in molecular modeling studies, docking of cyclamate and lactisole in the T1R3 transmembrane domain revealed a considerable overlap in the binding pockets for the two ligands (Jiang et al., 2005). Site-directed mutagenesis studies have identified amino acid residues interacting with lactisole in the

TABLE 1

Allosteric ligands for sweet and umami taste receptors

Taste enhancers stabilize either the active form of the VFTM or that of the 7-TM region by binding to allosteric sites. Lactisole is an allosteric antagonist at both taste receptors. See section II.B for further details.

Compound	Structure	Potencies	Comments	References
Cyclamate		EC_{50} = 3 mM	Allosteric agonist (sweet) or modulator (umami) binding to the 7TM domain of T1R3	Xu et al., 2004; Jiang et al., 2005b
Lactisole		IC_{50} = 41 μM	Negative modulator binding to the 7TM domain of T1R3 (sweet/umami)	Xu et al., 2004; Jiang et al., 2005a
S807		EC_{50} ≈ 0.5 μM	Allosteric agonist binding to the 7TM domain of T1R1 (umami)	Zhang et al., 2008a
S819			Allosteric agonist binding to the 7TM domain of T1R2 (sweet)	Zhang et al., 2008a
IMP		EC_{50} ≈ 3 mM	Naturally occurring umami taste enhancer stabilizing the closed conformation of the T1R1-VFTM (Fig. 6)	Li et al., 2002; Nelson et al., 2002; Zhang et al., 2008a
SE-2		EC_{50} = 22 μM		
SE-3			Positive modulators of the sweet receptor, stabilizing the closed form of the T1R2-VFTM	Servant et al., 2010; Zhang et al., 2010a

pocket (Jiang et al., 2005) or have shown that only two amino acids in transmembrane region 5 are responsible for the insensitivity of rodent receptors to lactisole (Winnig et al., 2005).

The canonical, orthosteric agonists for the T1R1/T1R3 umami receptor are L-amino acids, which bind to the T1R1 VFTM receptor domain (Fig. 5) (Zhang et al., 2008a). Most mammalian species respond strongly to the taste of a broad range of L-amino acids, and cellular assays with recombinantly expressed mouse T1R1/T1R3 revealed that this combination functions as a broadly tuned amino acid-sensing receptor, with highest sensitivities for cysteine, alanine, glutamine, and serine (Nelson et al., 2002). In humans, on the other hand, the unique savory, meaty "umami" taste sensation is elicited mainly by monosodium L-glutamate. Accordingly, in calcium imaging assays in cells expressing the human T1R1/T1R3, the potency of L-glutamate (EC_{50} between 1 and 10 mM) is at least 1 order of magnitude higher than that of other amino acids (Li et al., 2002; Nelson et al., 2002). Thus, the relatively high selectivity of the human umami receptor for glutamate is reminiscent of that of its relatives, the mGlu receptors. It is noteworthy that the group III mGlu receptor subtype 4 (see section III.C)

agonist L-AP4 also elicits umami taste in humans, and it activates T1R1/T1R3 with a potency similar to that of glutamate.

The T1R3 subunit is shared by the sweet and the umami receptors; thus, it is not surprising that cyclamate and lactisole are also active at the human umami receptor (Fig. 5). Lactisole reduces umami taste perception and antagonizes recombinant human T1R1/T1R3 in an allosteric fashion; its IC_{50} value (0.2 mM) is not dependent on the glutamate concentration. On the other hand, unlike its effect on the sweet receptor, cyclamate does not activate the umami receptor by itself but enhances its response to glutamate, thus acting as a positive allosteric modulator (Xu et al., 2004). Cyclamate shifts the concentration-response curve of glutamate to lower concentrations by approximately 2-fold. As one might expect, cyclamate has comparable EC_{50} values (in the low millimolar range) for its effects on both the sweet and the umami taste receptors (Xu et al., 2004). This interesting change from allosteric agonism to allosteric modulation by cyclamate occurring in function of the partnering subunit can be easily detected in recombinant cell lines, but the enhancing effect on umami taste cannot be perceived in taste tests in humans because of the intense intrinsic sweet taste of cyclamate.

A hallmark of umami taste is its strong potentiation by purine 5′-ribonucleotides such as IMP (Table 1) or GMP, which do not elicit the umami taste by themselves. This potentiation was nicely reproduced in cellular calcium mobilization assays with human T1R1/T1R3 (Li et al., 2002; Nelson et al., 2002). The responses to glutamate were greatly enhanced by IMP and GMP, and in the presence of 0.2 mM IMP, the EC_{50} for glutamate was lowered by approximately 30-fold. CMP, which does not enhance umami taste, had no effect, and IMP did not enhance the responses to sweeteners at T1R2/T1R3. Most importantly, IMP and GMP did not activate the T1R1/T1R3 proteins on their own, indicating that they act as positive allosteric modulators at the human umami taste receptor. These compounds are rare examples of naturally occurring allosteric GPCR modulators.

Zhang et al. (2008a) undertook the task to elucidate the molecular mechanism for umami taste synergism by purine nucleotides and other allosteric modulators. Using chimeric T1R receptors, site-directed mutagenesis, and molecular modeling, they came to propose a cooperative ligand-binding model in which glutamate binds close to the hinge region of the T1R1 VFTM, whereas 5′-ribonucleotides bind to a site adjacent to the opening region of the flytrap, thereby further stabilizing the closed conformation apparently by coordinating positively charged amino acid residues via their phosphate groups (Fig. 6). This mechanism easily explains why these ribonucleotides have no activity on their own: binding to one lobe at the open end of the VFTM will not be sufficient to close the flytrap, because the other lobe remains too distant in the absence of an agonist such as

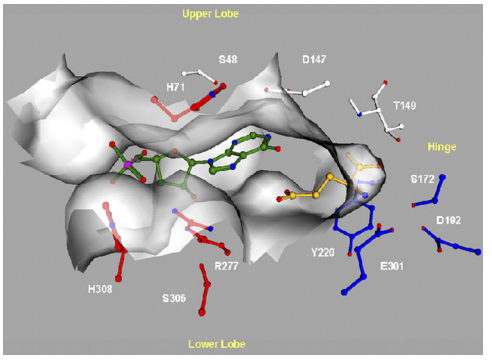


FIG. 6. Mechanism of stabilization of the closed conformation of the umami receptor VFTM by IMP (green). Whereas glutamate (golden) closes the two lobes by binding deep inside the VFTM near its hinge region (right), IMP further stabilizes the closed conformation by binding to the two lobes near the flytrap opening to the left. Because the upper (top) and lower (bottom) lobes are too far apart in the open conformation, IMP cannot activate the receptor by binding to one lobe in the absence of glutamate. Key residues for glutamate binding are blue, for IMP binding red. The two sweet taste enhancers SE2 and SE3 (Table 1) have been shown to act in a similar way at the sweet taste receptor. [Reproduced from Zhang F, Klebansky B, Fine RM, Xu H, Pronin A, Liu H, Tachdjian C, and Li X (2008a) Molecular mechanism for the umami taste synergism. *Proc Natl Acad Sci USA* **105:**20930–20934. Copyright © 2008 National Academy of Sciences U.S.A. Used with permission.]

glutamate, which closes the module by binding to the hinge region. Very recently, the two compounds SE-2 and SE-3 (Table 1) have been shown to act in the same way at the sweet receptor (Servant et al., 2010; Zhang et al., 2010a). This is a unique mechanism of allosteric modulation at family C GPCRs; most other modulating molecules, such as lactisole or cyclamate, exert their action via the 7TM region of a receptor subunit. *N*-(heptan-4-yl) benzo[*d*][1,3]dioxole-5-carboxamide (S807; Senomyx; Table 1) is indeed a molecule that stimulates recombinant umami receptors directly by interacting with the 7TM domain of T1R1 (Zhang et al., 2008a). As a result of the efforts in the food industry to identify novel taste enhancers, many other umami-tasting molecules obtained and derived from different sources and leads and with different binding sites and mechanisms have been described previously (Winkel et al., 2008).

C. Possible Benefits of Allosteric Modulators for Taste Receptors

There are multiple reasons for the interest in the discovery of taste enhancers from a health policy point of view. Pathologic conditions such as hypertension, obesity, diabetes, and many others are related to or have an impact on nutritional habits. Although artificial sweeteners already represent a large market in the food sector, there still is a need to expand this principle into other taste qualities. For example, an enhancer of salty taste would be of help for people on a low sodium diet. Elderly people often have a decline of taste function and appetite that may result in nutritional deficits. Taste enhancers might help them to enjoy their food and prevent such deficits. It is noteworthy that taste receptors are also located in the gastrointestinal tract and possibly

involved in the sensing of food intake. They respond to organic nutrients or their immediate breakdown products (e.g., sugars or amino acids). These receptors might also represent potential drug targets to treat, for example, type II diabetes by mimicking food intake by potent agonists or positive allosteric modulators (Wellendorph et al., 2009).

From a theoretical point of view, at first sight it might not matter whether taste enhancers act as orthosteric or allosteric agonists or as positive modulators. As outlined in the introduction, the potential advantage of positive allosteric modulators as pharmaceuticals lies in the fact that they act in a physiological context [i.e., only when and where receptors are activated by their endogenous ligands (hormones or neurotransmitters)]. Taste enhancers, however, are administered with food and therefore concomitantly with the natural ligands for taste receptors anyway. Thus, this consideration does not seem to apply here. However, none of the artificial sweeteners available at present can really reproduce the taste of sugar. Some of them have a metallic or bitter second taste at high concentrations that is apparently due to interactions with bitter and/or capsaicin receptors (Kuhn et al., 2004; Riera et al., 2008). An advantage of positive allosteric modulators would be to enhance the natural taste of sugar, thereby allowing reduction of intake and caloric supply. In addition, the problem with synthetic agonists often is that they are required in molar quantities similar to those of the natural ligands, making them expensive. On the other hand, because of their high affinities, agonists active at low concentrations would dissociate from their receptors slowly, leaving behind a not necessarily desirable aftertaste. Although this might also be an issue with positive allosteric modulators increasing the affinity of the natural agonist, a modulator predominantly acting through efficacy cooperativity would have the advantage of enhancing the taste of food only as long as we enjoy its presence in the palate.

III. Allosteric Modulators Allow for Subtype Selectivity among Multiple Metabotropic Glutamate Receptors

Metabotropic glutamate receptors serve mainly to modulate excitatory neurotransmission in the brain, whereas their ionotropic counterparts (AMPA, NMDA, and kainate receptors) mediate fast excitatory synaptic signaling. Eight different mGlu receptor subtypes have been identified by molecular cloning and are classified into three groups based on sequence homology (Fig. 1), pharmacological properties, and distinct coupling to intracellular signaling cascades. Group I (activating phospholipase C) comprises mGlu receptors 1 and 5, group II (coupled to G_i-mediated inhibition of adenylyl cyclase) includes mGlu2 and mGlu3 receptors, and group III (also inhibiting cAMP formation) consists of mGlu receptor subtypes 4, 6, 7, and 8. Various isoforms of these subtypes originating from alternative splicing of their messenger RNAs have also been found.

The design of agonist and antagonist ligands binding to the orthosteric site of glutamate receptors is a most demanding endeavor in medicinal chemistry and is hampered by hurdles of different kinds. First, the binding pocket for glutamate almost exclusively requires amino acid-like structures for binding. Such polar, charged molecules pose problems regarding bioavailability and blood-brain barrier permeability. Second, although agonists or antagonists that are relatively selective between mGlu receptor subgroups have been obtained, almost no orthosteric ligands that are truly selective for a given subtype within a subgroup are known today, because the glutamate binding site has been highly conserved during evolution. Allosteric ligands potentially offer a way out of these dilemmas. Indeed, their binding sites being located in the 7TM domain, they usually are neutral, lipophilic structures lacking the pharmacokinetic constraints of glutamate analogs. Moreover, because these binding sites are under less evolutionary selection pressure than the glutamate recognition site and are therefore less conserved, selectivity for subtypes within a given group can be achieved. Many allosteric mGlu receptor ligands with interesting pharmacological properties are known today, and some of them seem to have a considerable therapeutic potential (for review, see Niswender and Conn, 2010; O'Neill et al., 2010; Gregory et al., 2011).

A. Allosteric Ligands for Group I Metabotropic Glutamate Receptors: the First Success Stories

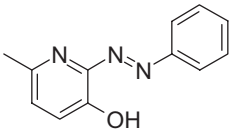
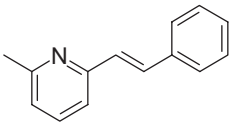
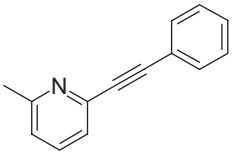
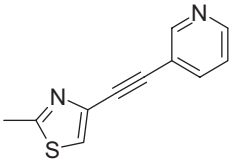
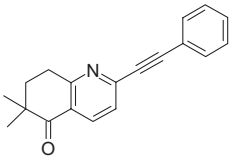
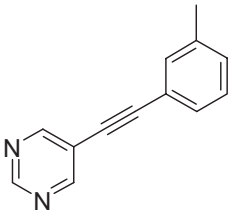
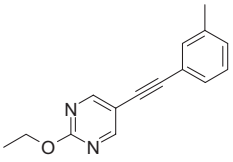
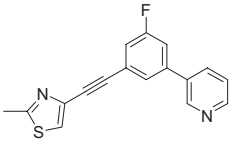
Allosteric ligands for group I mGlu receptors were among the first to be discovered for family C GPCRs. CPCCOEt (Table 6) was described by Annoura et al. (1996) as the first non–amino acid-like mGlu1 receptor antagonist. Its mode of action was subsequently characterized by Hermans et al. (1998) and Litschig et al. (1999). The discovery of a negative allosteric mGlu1 receptor modulator was soon followed by that of compounds acting in the same way at its mGlu5 receptor counterpart [6-methyl-2-(phenylazo)-3-pyridinol (SIB-1757), (*E*)-2-methyl-6-(2-phenylethenyl)pyridine (SIB-1893), 2-methyl-6-(phenylethynyl)-pyridine (MPEP); Table 2] (Gasparini et al., 1999; Varney et al., 1999). Since then, a great number of both positive and negative allosteric modulators have been described for both group I mGlu receptors, and their development has been brought forward to the stage of clinical trials for some of them (Gasparini et al., 2008; Jaeschke et al., 2008; Lindsley and Emmitte, 2009; Niswender and Conn, 2010).

1. Noncompetitive mGlu5 Receptor Antagonists Turned out to Be Prototype Allosteric mGlu Receptor Modulators. MPEP (Table 2) was the fruit of a collaboration between Novartis (Basel, Switzerland) and SIBIA Neurosciences Inc. (now part of Merck) that yielded SIB-1757 and SIB-1893 (Table 2) as hits from an mGlu5 receptor high-

TABLE 2

*Some acetylenic negative allosteric mGlu5 receptor modulators (***III–IX***) originally evolving from SIB-1757 (***I***) and SIB-1893 (***II***)*

Besides MPEP and MTEP, some recently published structures are shown. Earlier compounds have been comprehensively reviewed by Jaeschke et al. (2008) and by Lindsley and Emmitte (2009). The IC_{50} values shown are from a representative functional or radioligand binding assay. Because of the different assay conditions, they should be compared with caution. See Fig. 7 and Table 4 for more structures of acetylenic allosteric mGlu5 receptor ligands.

Compound	Structure	Potency	Comments	References
I: SIB-1757		IC_{50} = 3.7 μM	First negative mGlu5 receptor modulators resulting from an HTS program	Varney et al., 1999
II: SIB-1893		IC_{50} = 3.5 μM		Varney et al., 1999
III: MPEP		IC_{50} = 36 nM	Prototypical negative mGlu5 modulator, resulting from an optimization program based on compounds **I** and **II**; active in animal models for a number of indications (see section III.A.1)	Gasparini et al., 1999
IV: MTEP		IC_{50} = 16 nM	Somewhat better brain penetration and in vivo efficacy in some models compared to MPEP	Cosford et al., 2003a
V		IC_{50} = 23 nM		Vanejevs et al., 2008
VI		IC_{50} = 7.5 nM		Sharma et al., 2009
VII		IC_{50} = 21 nM		Sharma et al., 2009
VIII		IC_{50} = 4.8 nM		Kulkarni et al., 2009

throughput screening program. These structurally novel compounds inhibited mGlu5 receptor activity in a phosphoinositol turnover assay in a recombinant cell line expressing human mGlu5 receptors with IC_{50} values of 3.7 and 3.5 μM, respectively (Varney et al., 1999). The much more potent antagonist MPEP was then obtained as a result of a structural derivatization program starting from SIB-1757 and SIB-1893 (Gasparini et al., 1999). In a recombinant cell line expressing the human mGlu5a receptor, MPEP completely inhibited quisqualate-induced phosphoinositol hydrolysis with an IC_{50} of 36 nM, corresponding to a 100-fold increase in potency compared with

the two SIB compounds. MPEP was somewhat later found to inhibit constitutive activity in a cell line transiently overexpressing the rat mGlu5 receptor, suggesting inverse agonist activity of the compound (Pagano et al., 2000). In the context of an extensive characterization in vitro, Gasparini et al. (1999) tested MPEP in functional assays for all cloned mGlu receptor subtypes expressed in recombinant cell lines. It displayed no agonistic, inhibitory, or modulatory effects at any mGlu receptors other than mGlu5. The only noteworthy exception was observed with the mGlu4 receptor: a slight enhancement of the inhibition of forskolin-stimulated cAMP formation produced by L-AP4 at a high concentration (100 μM) of MPEP. This inconspicuous finding went unnoticed at the time, but it was later reported that MPEP is indeed a positive allosteric modulator of mGlu4 receptors, although at much higher concentrations than those needed for inhibition of mGlu5 receptors (see section III.C). Electrophysiological recordings in *Xenopus laevis* oocytes revealed no effect of MPEP (100 μM) on ionotropic (AMPA, NMDA, and kainate) glutamate receptors. When applied microiontophoretically to the CA1 area of the hippocampus, a brain region prominently expressing mGlu5 but only weakly mGlu1 receptors, in anesthetized rats, MPEP inhibited the increase in neuronal firing induced by the group I mGlu receptor agonist (*R*,*S*)-3,5-dihydroxyphenylglycine (DHPG). When MPEP was administered intravenously (1–20 mg/kg), it also inhibited excitatory responses evoked by DHPG in a dose-dependent manner, proving that the compound crosses the blood-brain barrier. Responses produced by AMPA were not affected.

Later, Cosford et al. (2003a) described MTEP (Table 2), another high-affinity mGlu5 receptor negative modulator with improved water solubility, somewhat better brain penetration, and greater in vivo potency compared with MPEP. In fact, although both compounds were approximately equipotent in mGlu5 receptor assays in vitro, and although both of them readily enter the brain, the ED_{50} in an in vivo receptor occupancy assay was lower for MTEP (1 mg/kg i.p. versus 2.1 mg/kg for MPEP after 1 h), and MTEP was 5-fold more potent in the fear-potentiated startle model of anxiety in rats (ED_{50} = 1 mg/kg i.p. versus 5 mg/kg). It was also reported that MTEP had fewer off-target effects than MPEP; however, this might not be too relevant, because the concentrations of MPEP needed to inhibit NMDA receptors or monoamine oxidase A or to positively modulate the mGlu4 receptor (see section III.C) were approximately a 1000-fold higher than those at which it inhibits mGlu5 receptors.

MPEP and MTEP being potent and selective mGlu5 receptor antagonists with central activity in vivo upon peripheral administration, it is not surprising that they have become most important research tool compounds. So far, several hundred published studies have used MPEP or MTEP to elucidate the role of mGlu5 receptors in biochemical, electrophysiological, or behavioral model systems in vitro and in vivo. However, these compounds do not seem suitable for therapeutic development because of the metabolic and toxicologic liability constituted by the potentially reactive acetylene linker. For example, MTEP inhibits cytochrome P450 1A2, and MPEP has been found to form adducts with glutathione (Milbank et al., 2007). For these reasons, big chemical efforts have been undertaken to find metabolically stable and safe acetylenic mGlu5 receptor antagonists, to remove the acetylene group by designing analogs maintain the relative geometry of the two aryl rings in MPEP and MTEP (examples shown in Table 3), or to identify completely new scaffolds by high-throughput screening campaigns. A special case in this context is the compound fenobam (Table 3), which was tested in clinical trials as an anxiolytic agent in the late 1970s and much later turned out to be a potent, negative mGlu5 receptor modulator binding to the same allosteric site as MPEP and MTEP (Porter et al., 2005; Malherbe et al., 2006). Fenobam has also become an important starting point for chemistry programs. Table 2 provides some recent examples of acetylenic, and Table 3 gives some nonacetylenic negative allosteric mGlu5 receptor modulators. It is not possible to review here the wealth of chemical structures acting in this way; excellent comprehensive reviews of negative mGlu5 receptor modulator chemistry have been provided by Jaeschke et al. (2008), Rodriguez and Williams (2007), and Lindsley and Emmitte (2009).

The fact that MPEP and MTEP have high potencies in the low nanomolar concentration range, which is rather unique for compounds binding to allosteric sites, makes this type of molecule suitable for use as radioligands. Several tritium-labeled derivatives of MPEP and MTEP have been characterized in binding studies in vitro (membrane binding, autoradiography) and in vivo (Anderson et al., 2002; Gasparini et al., 2002; Cosford et al., 2003b; Hintermann et al., 2007). Radioligand binding assays have routinely been used to determine whether novel molecules from synthetic chemistry programs bind to the same allosteric mGlu5 receptor site as MPEP and MTEP and to assess their affinity for that site.

The high affinity and good brain permeability of MPEP and MTEP derivatives should also make them suitable ligands for in vivo imaging by positron emission tomography (PET). However, despite the good in vitro properties of a great number of PET ligand candidates, the development of useful PET tracers has proven to be difficult (for review, see Yu, 2007). In fact, the utility of PET ligands strongly depends, besides selectivity and good brain penetration, on their metabolic stability and favorable pharmacokinetic properties. Moreover, many candidates show lack of specific binding in vivo or false "specific" binding in the cerebellum, a brain region with low mGlu5 receptor expression. Nevertheless, Hamill et al. (2005) have succeeded in obtaining three PET ligands (compounds **I–III** in Table 4) that specifically label

TABLE 3

Some examples of nonacetylenic negative allosteric mGlu5 receptor modulators and one recently discovered neutral allosteric ligand

Besides fenobam, some recently published structures are shown. Earlier compounds have been comprehensively reviewed by Jaeschke et al. (2008) and by Lindsley and Emmitte (2009). The potencies shown are IC_{50} values from functional or radioligand binding assays.

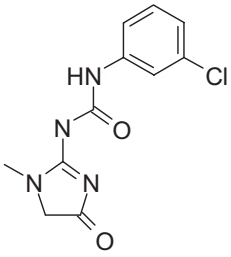
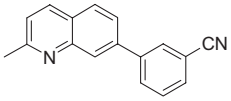
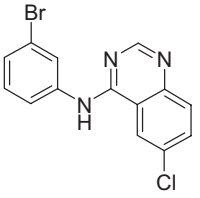
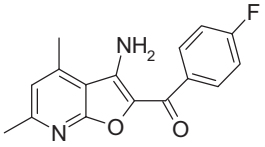
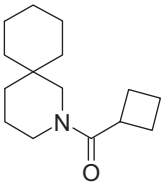
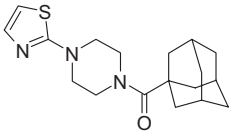
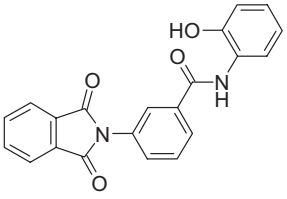
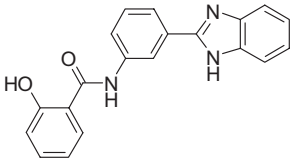
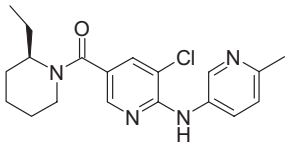
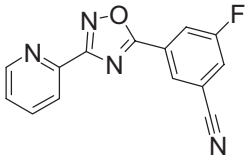
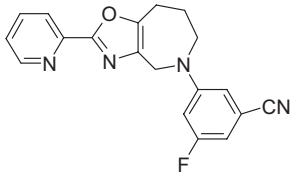
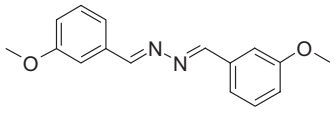
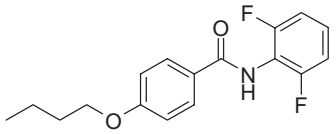
Compound	Structure	Potency (IC_{50})	Comments	References
I: Fenobam		58 nM	Anxiolytic in human clinical trials, later identified as negative mGlu5 receptor modulator	Porter et al., 2005
II		110 nM		Zhang et al., 2010b
III		124 nM		Felts et al., 2009
IV		150 nM		Rodriguez et al., 2009
V		820 nM		Rodriguez et al., 2009
VI		540 nM		Rodriguez et al., 2009
VII		420 nM	Structurally related to the positive modulator CPPHA (Table 5)	Zhou et al., 2009
VIII		3.5 μM		Zhou et al., 2009

TABLE 3—*Continued.*

Compound	Structure	Potency (IC_{50})	Comments	References
IX		32 nM	Anxiolytic activity in animal models: FPS, SIH, Vogel conflict test	Spanka et al., 2010
X: VU0285683		24 nM	Binds with high affinity to the MPEP site; anxiolytic-like activity in rodents	Rodriguez et al., 2010
XI		16 nM		Burdi et al., 2010
XII: DMeOB		3 μM	A close derivative of the positive mGlu5 modulator DFB (**I** in Table 5)	O'Brien et al., 2003
XIII: VU0365396			First non-MPEP site neutral allosteric mGlu5 receptor ligand	Hammond et al., 2010

DMeOB, 3,3′-dimethoxybenzaldazine; FPS, fear-potentiated startle.

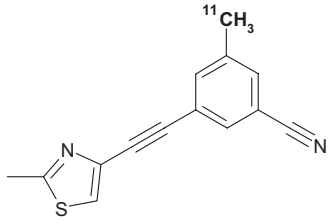
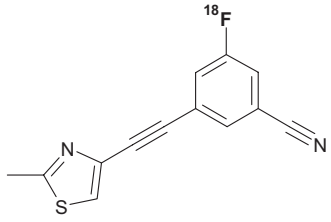
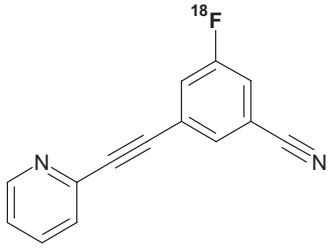
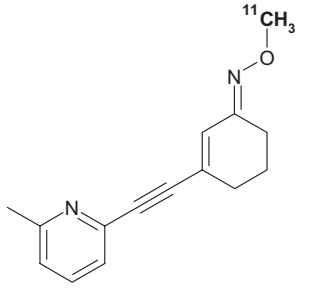
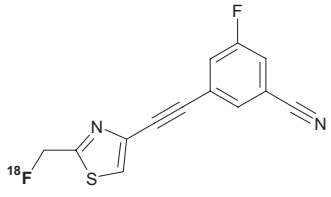
mGlu5 receptors in rhesus monkey brain in vivo with a regional distribution corresponding to that found in autoradiographic studies in vitro. 3-(6-Methyl-pyridin-2-ylethynyl)-cyclohex-2-enone-*O*-[^{11}C]methyl-oxime ([^{11}C]ABP688; **IV** in Table 4) also fulfills all the criteria for a suitable PET ligand (Ametamey et al., 2006; Hintermann et al., 2007). Ex vivo autoradiography and in vivo PET studies in rats and mice demonstrated preferential uptake of [^{11}C]ABP688 in brain regions known to be rich in mGlu5 receptors, whereas in mGlu5 receptor knockout mice, low binding levels with a uniform distribution were found. Blocking studies with an unlabeled MPEP analog further confirmed specific binding of [^{11}C]ABP688 to mGlu5 receptors in vivo. [^{11}C]ABP688 has subsequently been evaluated for in vivo PET imaging of mGlu5 receptors in humans (Ametamey et al., 2007); high radioactivity concentrations were observed in brain regions rich in mGlu5 receptors (anterior cingulate, medial temporal lobe amygdala, caudate, putamen), whereas labeling in the cerebellum was low. The tracer 3-fluoro-5-(2-(2-[^{18}F](fluoromethyl)thiazol-4-yl)ethynyl)benzonitrile ([^{18}F]SP203; compound **V** in Table 4) has also successfully been used for in vivo PET studies, first in monkeys (Siméon et al., 2007) and then in humans (Brown et al., 2008). This choice of good ligands should open up exciting possibilities for using PET studies to determine appropriate doses of candidate drugs that bind to mGlu5 receptors (by measuring their in vivo receptor occupancy), as well as for monitoring changes in mGlu5 receptor densities in the brains of patients suffering from neurological or psychiatric disorders.

There is robust evidence for a therapeutic potential of mGlu5 receptor antagonists in a number of clinical indications. The *anxiolytic* potential of negative allosteric mGlu5 receptor modulators began to emerge when MPEP was tested in conditioned and unconditioned anxiety models soon after its discovery (Spooren et al., 2000b; for review, see Swanson et al., 2005). The localization of mGlu5 receptors in the brain is mostly postsynaptic, often in conjunction with NMDA receptors to which they are linked by scaffolding proteins and the efficacy of which they enhance to regulate synaptic strength and plasticity. A brain region thought to be critically involved in anxiety is the amygdala, where mGlu5 receptors are localized on dendritic shafts and spines in the lateral nucleus postsynaptic to thalamic inputs (Rodrigues et al., 2002). MPEP has been reported to impair long-term potentiation (LTP) at these synapses in vitro, and intra-amygdalar administration of MPEP impaired the acquisition but not the consolidation or expression of conditioned fear (Rodrigues et al., 2002). Contextual fear conditioning also depends on the hippocampus, and it is of interest in this context that an

TABLE 4

Negative allosteric mGlu5 receptor modulators which have been used as PET ligands

The binding affinities for native or recombinant mGlu5 receptors have been obtained in radioligand binding experiments using the corresponding unlabeled compounds as displacers.

Compound	Structure	Binding Affinity	PET Studies	References
I		K_i = 0.23 nM (rat mGlu5)		
II		K_i = 0.08 nM	Used for PET imaging studies in Rhesus monkeys	Hamill et al., 2005
III		K_i = 0.2 nM		
IV: [^{11}C]ABP688		K_i = 3.5 nM (human mGlu5)	PET imaging studies in human subjects	Ametamey et al., 2006, 2007; Hintermann et al., 2007
V: [^{18}F]SP203		IC_{50} = 0.036 nM (rat mGlu5)	Used for PET imaging studies in Rhesus monkeys and humans	Siméon et al., 2007; Brown et al., 2008

increase in mGlu5 receptor expression has been found in CA3 one day after acquisition training, followed by a shift to CA1 10 days after training, supporting the role of these hippocampal regions in short- and long-term memory consolidation (Riedel et al., 2000). The interest in mGlu5 receptor antagonists as novel anxiolytic agents has been fostered further by the report that fenobam (Table 3), a nonbenzodiazepine anxiolytic with a hitherto unknown mechanism of action (Pecknold et al., 1982), is a potent negative allosteric mGlu5 receptor modulator interacting with the same site in the 7TM domain as MPEP (Porter et al., 2005; Malherbe et al., 2006). Clinical trials with fenobam were discontinued at the time because psychostimulant side effects were observed in some cases (Friedmann et al., 1980).

The mGlu5 receptor is also strongly expressed in the basal ganglia, suggesting a role in movement disorders. The effects of negative mGlu5 receptor modulators in animal models of Parkinson's disease are somewhat equivocal. Based on the reversal of the effects of haloperidol in rats (muscle rigidity, catalepsy), Ossowska et al. (2005) concluded that mGlu5 receptor antagonists

such as MPEP and MTEP may be more effective in inhibiting parkinsonian muscle rigidity than akinesia. In the hands of Breysse et al. (2002), MPEP reversed severe akinetic deficits in rats with bilateral 6-hydroxydopamine (6-OHDA)-induced nigrostriatal lesions after long-term but not after short-term administration. On the other hand, they saw no effect on haloperidol-induced catalepsy. However, several findings strongly suggest that mGlu5 receptor antagonists might be effective against tardive dyskinesias, an important complication of long-term treatment with L-DOPA in patients with Parkinson's disease. In 6-OHDA-lesioned rats, MTEP significantly attenuated the induction as well as the acute expression of abnormal involuntary movements after long-term treatment with L-DOPA. A biochemical correlate of abnormal involuntary movements was an increased GABA outflow in the substantia nigra (measured by in vivo microdialysis) that was counteracted by MPEP concomitantly with its behavioral effects (Mela et al., 2007). In autoradiographic studies with [^{3}H]MPEP, Samadi et al. (2008) observed a strong increase in mGlu5 receptor density in the posterior putamen (+41%) and in the pallidum (+56%) of MPTP-lesioned monkeys that had been treated with L-DOPA and developed dyskinesias, but not in tissue from monkeys in which dyskinesias were prevented by adjunct treatments. Ouattara et al. (2009) came to similar conclusions; moreover, they found that patients with Parkinson's disease who had dyskinesias had higher [^{3}H]ABP688-specific binding in putamen and globus pallidus compared with those without motor complications. The antidyskinetic effects of negative mGlu5 receptor modulators have been recently confirmed in a nonhuman primate model of Parkinson's disease at the behavioral level. In MPTP-lesioned monkeys, MPEP, MTEP, and fenobam significantly reduced the expression and, in the case of fenobam, also the development of dyskinesias associated with long-term L-DOPA treatment, with little or no reduction of the antiparkinsonian effect of L-DOPA (Johnston et al., 2010; Morin et al., 2010; Rylander et al., 2010). Promising clinical trials in this indication are ongoing with at least two compounds: AFQ056 (Novartis) (O'Neill et al., 2010) and ADX48621 [belonging to a series of butynyl pyridines, Addex Pharmaceuticals (Geneva, Switzerland); Bessis et al., 2005; see also Gasparini et al., 2008].

Another promising clinical indication for mGlu5 receptor antagonists is gastroesophageal reflux disease (GERD). mGlu5 receptors localized on terminal vagal afferents seem to trigger transient lower esophageal sphincter relaxations, resulting in gastric reflux, thus opening the possibility to block their increased frequency in GERD with mGlu5 receptor antagonists (Young et al., 2007). Transient lower esophageal sphincter relaxations and reflux episodes are potently and dose-dependently inhibited in ferrets by MPEP and MTEP (Frisby et al., 2005) and in dogs by MPEP (Jensen et al., 2005). Early clinical trials with the negative allosteric mGlu5 receptor modulator ADX10059 (a 3-aminopyridine derivative, Addex Pharmaceuticals; Bolea et al., 2004; see also Gasparini et al., 2008) have shown a significant reduction in the number and duration of reflux episodes in healthy subjects and patients with GERD (Keywood et al., 2009; Zerbib et al., 2010).

Fragile X syndrome, the most common hereditary form of mental retardation, encompasses hyperactivity, attention deficits, cognitive impairment, delayed neuronal development, increased incidence of epilepsy, and autistic-like behavior. It is caused by a loss-of-function mutation in the gene encoding the fragile X mental retardation protein (FMRP), which is an important regulator (repressor) of the transcription of specific mRNAs and is believed to be involved in synaptic plasticity by mechanisms involving the control of protein synthesis. Long-term depression in the hippocampus triggered by group I mGlu receptor activation, a process involving protein synthesis, is enhanced in FMRP knockout mice (Huber et al., 2002). This and other findings led to the "mGlu receptor theory of fragile X mental retardation" (Bear et al., 2004), which posits that FMRP and group I mGlu receptors normally oppose each other and that in the absence of the former, unchecked mGlu receptor functions dependent on protein synthesis are excessive. This hypothesis is buttressed by several observations in fragile X syndrome mouse models. Administration of MPEP corrects two phenotypes (enhanced sensitivity to audiogenic seizures and open field behavior) in FMRP knockout animals (Yan et al., 2005). Moreover, certain phenotypes relevant for the human disease (e.g., cortical development, hippocampus-dependent memory, seizure susceptibility) can be rescued by the generation of FMRP knockout animals with a concomitant 50% reduction in mGlu5 receptor expression (Dölen et al., 2007). Clinical trials with fenobam have been initiated, and first results of an open label safety and pharmacokinetics study in adult patients have been reported (Berry-Kravis et al., 2009).

Glutamate is a key neurotransmitter involved in pain perception pathways, and mGlu5 receptors are strategically located all along these pathways from the periphery (skin) to the central nervous system. The first investigations with MPEP found that the drug inhibits thermal hyperalgesia induced by the group I agonist DHPG and that peripheral but not central administration of MPEP reduces thermal hyperalgesia in inflammatory pain models in rodents (Bhave et al., 2001; Walker et al., 2001a,b). On the other hand, MPEP was found to be inactive in models of acute pain. In addition, systemic, local, and central application of MPEP was found to be efficacious in a model of postoperative pain in rats (Zhu et al., 2005), suggesting a role for both peripheral and central mGlu5 receptors in that condition. The early work on the role of mGlu5 receptors in chronic pain has been summarized by Slassi et al. (2005) and seems to indicate that negative allosteric mGlu5

receptor modulators are more effective in reversing thermal hyperalgesia compared with mechanical hyperalgesia or allodynia in both inflammatory and neuropathic pain models. Newer studies have extended these early findings and shed more light on the role of mGlu5 receptors in pain signaling. Transient receptor potential vanilloid 1 channels also play a critical role in the transduction of pain sensation. They have recently been shown to be functionally coupled to mGlu5 receptors on presynaptic terminals of sensory neurons in the dorsal horn of the spinal cord (Kim et al., 2009). Accordingly, transient receptor potential vanilloid 1 is involved in pain behaviors induced by spinal mGlu5 receptor activation by the agonist DHPG and blocked by MPEP. Li et al. (2010) have reported increased mGlu5 receptor expression on primary afferent neurons contributing to nociceptive transmission in diabetic neuropathic pain. Excitatory postsynaptic currents evoked from the dorsal root were increased in diabetic animals and inhibited by MPEP, but not by blocking mGlu1 receptors. In a visceral pain model, MTEP and MPEP have been found to dose-dependently reduce visceromotor and cardiovascular responses evoked by colorectal distension through an action at peripheral afferent endings (Lindström et al., 2008). The early results on the effects of MPEP in inflammatory pain have been confirmed by Montana et al. (2009) with fenobam, which reduced formalin-induced pain behaviors (licking or lifting the injected paw) and inflammation-induced thermal hypersensitivity in mice. These effects were not seen in mGlu5 receptor knockout mice. The beneficial effects of fenobam, however might, be limited by a lack of therapeutic window (Jacob et al., 2009). Apparently successful phase II clinical trials with the negative allosteric mGlu5 receptor modulator ADX10059 (Bolea et al., 2004) in the indication migraine have been summarized by Jaeschke et al. (2008) and by Gasparini et al. (2008). The development of ADX10059 has been discontinued, however, apparently as a result of the incidence of abnormalities in liver function tests (http://www.addexpharma.com).

Because the mGlu5 receptor is often closely associated with the NMDA receptor, with which it works in tandem, a potential concern is that blockade of mGlu5 receptors might result in the same side effects previously observed with NMDA receptor antagonists, namely cognitive deficits and psychotomimetic effects, which could seriously limit their clinical usefulness. Weak negative allosteric mGlu5 receptor modulators, which do not completely inhibit mGlu5 receptor-mediated responses even at saturating concentrations, might be a promising way to circumvent this concern. As discussed in the introduction, the intrinsic properties of allosteric ligands, just as those of orthosteric ones, are positioned along a continuum ranging from neutral (silent) binding to full or partial positive or negative effects. Rodriguez et al. (2005) have described MPEP-analogs that exert only weak negative functional cooperativity at mGlu5 receptors. In fact, M-5MPEP and 2-(2-(5-bromopyridin-3-yl) ethynyl)-5-methylpyridine at maximally active concentrations were found to only partially antagonize an mGlu5 receptor-mediated calcium signal in rat cortical astrocytes (Fig. 7). Moreover, as shown in Fig. 8 at the example of M-5MPEP, they reversed the full blockade produced by MPEP to the maximal level of inhibition that they were able to produce on their own (by completely displacing MPEP from its binding site). Like MPEP, both compounds inhibited the binding of [^{3}H]3-methoxy-5-(pyridine-2-ylethynyl)pyridine to membranes from mGlu5 receptor expressing cells in a competitive fashion (IC_{50} values 100–200 nM). A most interesting molecule described in the same study is 5MPEP, which also inhibited [^{3}H]3-methoxy-5-(pyridine-2-ylethynyl)pyridine binding (IC_{50} = 388 nM) but exerted no functional cooperativity; i.e., it is a neutral ligand at the mGlu5 receptor

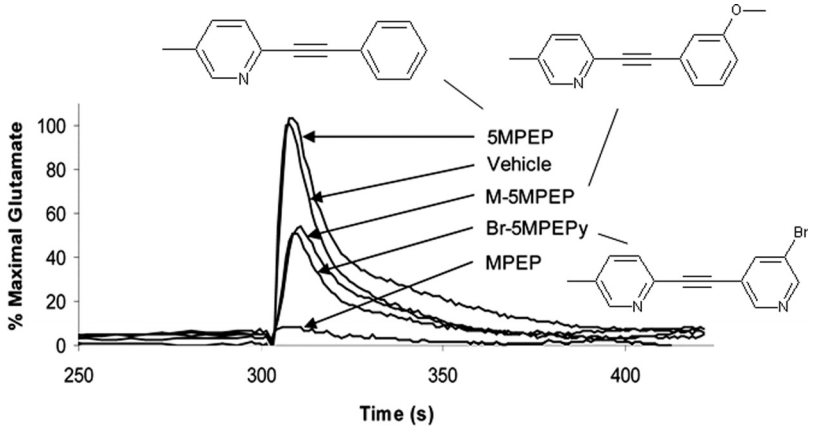


FIG. 7. mGlu5 receptor-mediated increase in intracellular calcium induced by glutamate in rat cortical astrocytes was measured by loading the cells with a calcium-sensitive dye. Cells were preincubated with test compounds at a maximally active concentration (10 μM). Whereas MPEP (Table 2) completely blocked the calcium signal in response to glutamate, M-5MPEP and 2-(2-(5-bromopyridin-3-yl)ethynyl)-5-methylpyridine (Br-5MPEPy) were able to only partially antagonize the mGlu5 receptor response, and 5MPEP did not inhibit the glutamate effect at all. [Modified from Rodriguez AL, Nong Y, Sekaran NK, Alagille D, Tamagnan GD, and Conn PJ (2005) A close structural analog of 2-methyl-6-(phenylethynyl)-pyridine acts as a neutral allosteric site ligand on metabotropic glutamate receptor subtype 5 and blocks the effects of multiple allosteric modulators. *Mol Pharmacol* **68:**1793–1802. Copyright © 2005 American Society for Pharmacology and Experimental Therapeutics. Used with permission.]

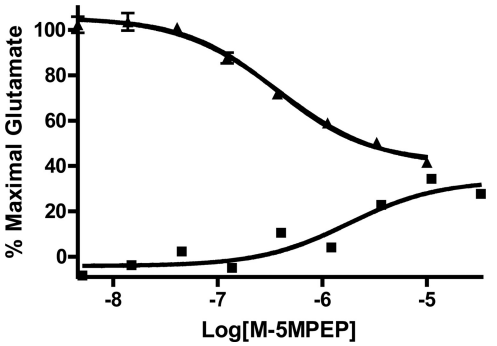


FIG. 8. Weak negative allosteric mGlu5 receptor modulation by M-5MPEP. The calcium response induced by glutamate in cells loaded with a calcium-sensitive dye (Fig. 7) was inhibited by M-5MPEP by maximally approximately 60% (▲). On the other hand, 50 nM MPEP completely antagonized the calcium response. This blockade by MPEP was partially reverted by the simultaneous addition of M-5MPEP (■) at increasing concentrations, maximally to the level of inhibition that M-5MPEP produces when added alone. [Reproduced from Rodriguez AL, Nong Y, Sekaran NK, Alagille D, Tamagnan GD, and Conn PJ (2005) A close structural analog of 2-methyl-6-(phenylethynyl)-pyridine acts as a neutral allosteric site ligand on metabotropic glutamate receptor subtype 5 and blocks the effects of multiple allosteric modulators. *Mol Pharmacol* **68:**1793–1802. Copyright © 2005 American Society for Pharmacology and Experimental Therapeutics. Used with permission.]

allosteric site (Fig. 4). Accordingly, it did not inhibit the glutamate-induced calcium signal in astrocytes on its own up to at least 10 μM (Fig. 7), but it antagonized the blockade of that signal produced by MPEP (Fig. 9). This inhibition of the negative allosteric effect of MPEP by 5MPEP was competitive; the Schild plot of the data shown in Fig. 9 revealed a pA_2 value of 6.25 (slope = 1), which is in excellent agreement with the binding affinity of 5MPEP. The four compounds shown in Fig. 7 are a beautiful representation of the continuum of pharmacological effects mediated via an allosteric site, as discussed in section I and illustrated in Fig. 4.

2. Positive Allosteric mGlu5 Receptor Modulators. Very small modifications in a molecule are sometimes sufficient to change the intrinsic properties of a given allosteric modulator template. Such molecular/pharmacological "switches" have been described recently in a series of compounds based on a weak negative modulator lead issued from an HTS campaign, resulting in negative as well as positive allosteric modulators (Sharma et al., 2008, 2009). In fact, compound **V** shown in Table 5 (Sharma et al., 2009) is one of the most potent mGlu5 receptor positive allosteric modulators known to date (EC_{50} = 14 nM), and it is based on an MPEP-like scaffold! Positive mGlu5 receptor modulators based on the MPEP-scaffold have also been described by other groups (Vanejevs et al., 2008; Ritzén et al., 2009) and, most recently, Rodriguez et al. (2010) (compound **VIII** in Table 5). The first positive mGlu5 receptor modulators, however, were reported by O'Brien et al. (2003). A family of benzaldazine analogs was found to exert a whole spectrum of effects, ranging from positive to negative allosteric modulation encompassing neutral antagonism at the allosteric site (O'Brien et al., 2003). DFB (compound **I** in Table 5) was identified as a selective positive modulator of human and rat mGlu5 receptors; in recombinant Chinese hamster ovary (CHO) cells expressing either of these receptors, it enhanced intracellular calcium mobilization elicited by glutamate, quisqualate, or DHPG with EC_{50} values in the low micromolar range. At a maximally active concentration (100 μM), DFB enhanced the potencies of these mGlu5 receptor agonists by approximately 2-fold, with no change in their maximal effects. The analog 2,2′-difluorobenzaldazine had a somewhat lower potency as a positive mGlu5 receptor modulator (O'Brien et al., 2003). On the other hand, other analogs were found to be negative modulators [such as 3,3′-dimethoxybenzaldazine (compound **XII** in Table 3)] or neutral allosteric ligands [such as 3,3′-dichlorobenzaldazine (compound **IX** in Table 5)]. 3,3′-Dichlorobenzaldazine had no modulatory effect on mGlu5 receptor activity, but it reversed the positive modulation produced by DFB (IC_{50} = 7.6 μM) and the negative modulation by 3,3′-dimethoxybenzaldazine (IC_{50} = 17 μM). None of these compounds had an effect on the binding of [^{3}H]quisqualate to the orthosteric agonist recognition site, but they all inhibited the binding of a tritiated MPEP analog to its allosteric site. Somewhat later, the same group presented, as a result of a screening and an iterative analog library approach (Zhao et al., 2007), another selective positive mGlu5 receptor modulator, CPPHA (Table 5, compound **II**), which had effects in calcium assays in recombinant cell lines similar to those of DFB, although with a somewhat higher potency and degree of cooperativity (greater enhancement of agonist potencies) (O'Brien et al., 2004). The CPPHA chemotype is a striking example of "flat" SAR around allosteric GPCR modulators: only 4.5% of 985 analogs had any activity (Zhao et al., 2007). In

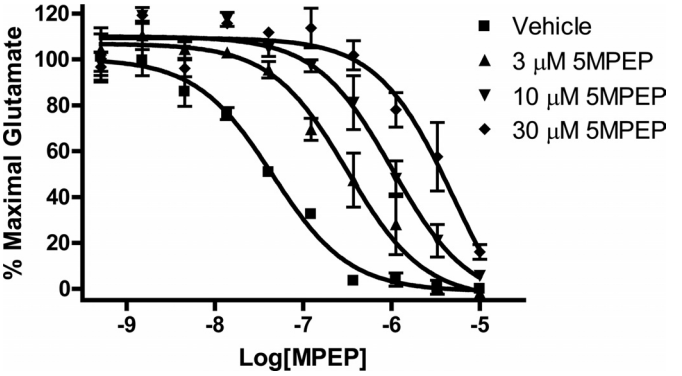


FIG. 9. 5MPEP reduces allosteric inhibition by MPEP in a competitive manner. An mGlu5 receptor-mediated calcium response was measured as shown in Fig. 7. The concentration-dependent inhibition of the glutamate effect by MPEP was measured in the absence (■) and in the presence of increasing concentrations of 5MPEP (▲, 3 μM; ▼, 10 μM; ♦, 30 μM). [Reproduced from Rodriguez AL, Nong Y, Sekaran NK, Alagille D, Tamagnan GD, and Conn PJ (2005) A close structural analog of 2-methyl-6-(phenylethynyl)-pyridine acts as a neutral allosteric site ligand on metabotropic glutamate receptor subtype 5 and blocks the effects of multiple allosteric modulators. *Mol Pharmacol* **68:**1793–1802. Copyright © 2005 American Society for Pharmacology and Experimental Therapeutics. Used with permission.]

TABLE 5

Positive allosteric mGlu5 receptor modulators and one ligand with neutral cooperativity

Information about compound potencies in vitro in one representative assay is given as an indication only. Different assay systems were used, so potencies should be compared with caution. Because of the complexity of allosteric interactions and the different possibilities of measuring modulation, information on cooperativity is not summarized here but can be found in section III.A.2.

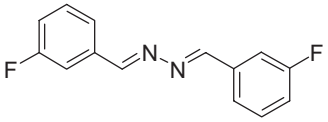
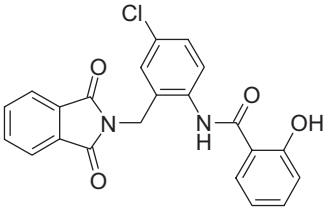
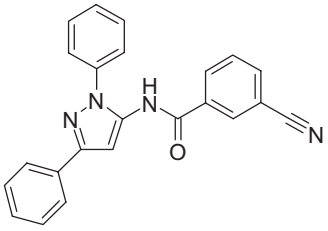
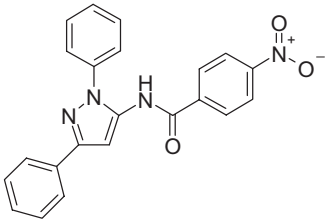
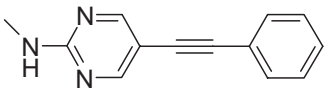
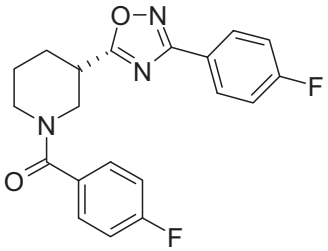
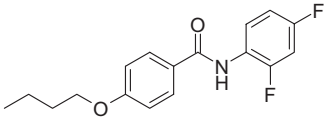
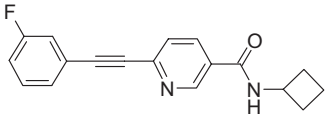
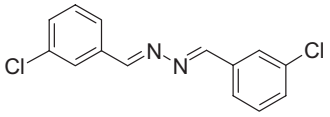
Compound	Structure	Potency	Comments	References
I: DFB		EC_{50} = 2.6 μM		O'Brien et al., 2003
II: CPPHA		EC_{50} = 250 nM	Acts through an allosteric site distinct from the MPEP binding site	Zhao et al., 2007
III: CDPPB		EC_{50} = 20 nM	Active in animal models of antipsychotic activity (e.g., PPI- or PCP-induced locomotion)	Lindsley et al., 2004; Kinney et al., 2005
IV: VU-29		EC_{50} = 11 nM		de Paulis et al., 2006
V		EC_{50} = 14 nM	Based on MPEP scaffold (pharmacological switch)!	Sharma et al., 2009
VI: ADX47273		EC_{50} = 170 nM	Active in animal models of antipsychotic activity	Liu et al., 2008
VII: VU0357121		EC_{50} = 33 nM	Interacts with a site distinct from the MPEP or CPPHA sites	Hammond et al., 2010
VIII: VU0360172		EC_{50} = 16 nM	Reverses amphetamine-induced hyperlocomotion	Rodriguez et al., 2010

TABLE 5—*Continued.*

Compound	Structure	Potency	Comments	References
IX: DCB		IC_{50} = 7.6 μM (to inhibit DFB)	A derivative of DFB (**I**) with neutral cooperativity at mGluR5	O'Brien et al., 2003

DCB, 3,3′-dichlorobenzaldazine;

contrast to DFB, CPPHA did not displace the radioligand from the MPEP site, indicating that the two positive modulators elicit similar allosteric effects through distinct binding sites. In electrophysiological experiments on brain slice preparations, CPPHA had no effects on its own but potentiated the enhancement of NMDA receptor currents by subthreshold levels of DHPG as well as DHPG-induced depolarizations of rat subthalamic nucleus neurons. Further important positive allosteric mGlu5 receptor modulators include CDPPB (Lindsley et al., 2004; Kinney et al., 2005) and *S*-(4-fluorophenyl)-{3-[3-(4-fluorophenyl)-[1,2,4]-oxadiazol-5-yl]-piperidin-1-yl}-methanone (ADX47273; Liu et al., 2008) (Table 5, compounds **III** and **VI**, respectively). These two compounds have essentially the same effects as DFB and CPPHA in fluorometric calcium assays in vitro (i.e., a shift of glutamate concentration-response curves to the left by approximately 10-fold, without a change of maximal stimulation by glutamate). However, the potencies of these new modulators are clearly higher than those of their predecessors (EC_{50} = 20 nM for CDPPB and 170 nM for ADX47273). Both compounds inhibit the binding of radioligands labeling the MPEP recognition site, but not [^{3}H]quisqualate binding. In line with this, the effects of CDPPB in calcium assays, like those of DFB, were inhibited by the neutral allosteric ligand 5MPEP described above. de Paulis et al. (2006) have undertaken an extensive derivatization program based on the CDPPB scaffold, yielding a few analogs with a slightly better activity [e.g., 4-nitro-*N*-(1,3-diphenyl-1*H*-pyrazol-5-yl)benzamide (VU-29); compound **IV** in Table 5]. Likewise, ADX47273 has served as a starting point for the synthesis of a large number of analogs (Engers et al., 2009b). As a result, compounds with improved physicochemical properties were obtained; furthermore, some of them also produced larger increases in glutamate potencies (up to 28-fold, the strongest effect found so far for a positive mGlu5 receptor modulator), although the EC_{50} values were at best in the same range as that for ADX47273. It is noteworthy that one negative mGlu5 receptor modulator also showed up in this series—the first example of a switch in pharmacological activity in a non-MPEP chemotype.

Agonist stimulation of mGlu5 receptors induces oscillatory changes in cytosolic Ca^{2+} concentrations; the frequency of these oscillations elicited by glutamate or quisqualate in CHO cells expressing mGlu5 receptors was increased by all four positive allosteric modulators described above (Bradley et al., 2009). The effects of DFB, CDPPB, and ADX47273, but not CPPHA, were antagonized by 5MPEP. CDPPB also increased calcium oscillations at low glutamate concentrations in a native preparation, rat cerebrocortical astrocytes. Using the same preparation, Zhang et al. (2005) found that although both DFB and CPPHA enhanced agonist-stimulated intracellular calcium mobilization, the two modulators had qualitatively different effects on DHPG-induced ERK1/2 phosphorylation. Whereas DFB induced a shift of the concentration-response curve (CRC) for DHPG similar to that seen in the calcium assay, CPPHA enhanced basal activity and the effects of low agonist concentrations but decreased ERK phosphorylation at high agonist concentrations. The phenomenon of "ligand-directed trafficking" might account for this discrepancy, related to the fact that DFB and CPPHA interact with different sites on the mGlu5 receptor. The effect of CPPHA on ERK phosphorylation at subthreshold DHPG concentrations was confirmed in cortical and hippocampal slices (Liu et al., 2006) and extended to the phosphorylation of cAMP-responsive element-binding protein (CREB) as well as that of the NR1 subunit of NMDA receptors. The latter finding seems likely to be important in the context of the presumed utility of positive mGlu5 receptor modulators in schizophrenia discussed in the next paragraph. ERK- and CREB-phosphorylation has also been used as a biochemical readout for glutamate-mediated signal transduction in vivo: at doses of 1 and 10 mg/kg i.p., ADX47273 increased the phosphorylation of extracellular ERK and CREB in the hippocampus and prefrontal cortex (Liu et al., 2008). These effects were antagonized by coadministration of MPEP.

At present, the main potential indication for positive allosteric mGlu5 receptor modulators doubtlessly is schizophrenia (Conn et al., 2009b; Marek et al., 2010). The glutamate hypothesis of schizophrenia assumes that hypofunction of NMDA receptors at critical sites in corticolimbic circuits is a major cause of the disease (for review, see Marek et al., 2010). Postsynaptic mGlu5 receptors are coupled to NMDA receptors through "Homer" scaffolding proteins, especially in certain GABAergic interneurons in the prefrontal cortex and in the hippocampal formation, which seem to be primarily involved in the etiology of schizophrenia (Belforte et al., 2010). The functional relationship between NMDA and mGlu5 receptors is one of synergism, hence the strategy of mGlu5 receptor activation as a treatment for schizophrenia (Conn et al., 2009b). At subthreshold DHPG concentrations, CPPHA has been found to enhance the phosphorylation of the NR1

subunit of NMDA receptors (Liu et al., 2006); in hippocampal slices, CPPHA (10 μM) potentiated NMDA receptor currents in the presence of the orthosteric mGlu5 receptor agonist DHPG at a threshold concentration but had no effect on these currents on its own (O'Brien et al., 2004). In primary neuronal cultures, ADX47273 enhanced intracellular Ca^{2+} mobilization induced by DHPG, as well as NMDA receptor-mediated Ca^{2+} influx in the presence of a subthreshold concentration of DHPG (Rosenbrock et al., 2010). In freely moving rats, CDPPB administered at 10 mg/kg i.p. counteracted the excessive firing of neurons in the medial prefrontal cortex induced by the NMDA receptor antagonist dizocilpine maleate (MK-801), presumably as a result of disinhibition ensuing from a lack of activity of GABAergic interneurons (Lecourtier et al., 2007). On the other hand, it had no effect on dopamine release in the medial prefrontal cortex and nucleus accumbens, as measured by in vivo microdialysis. Positive allosteric mGlu5 receptor modulators (VU-29 and ADX47273; Table 5) enhanced both LTP and long-term depression at the Schaffer collateral-CA1 synapse in the hippocampus (Ayala et al., 2009; Rosenbrock et al., 2010). In the study by Ayala et al. (2009), two systemically active positive mGlu5 receptor modulators (CDPPB and ADX47273, both at 10 mg/kg i.p.) enhanced the performance of mice in the Morris water maze, a measure of hippocampus-dependent spatial learning. Moreover, CDPPB also had beneficial effects on the performance of rats in a "set-shifting" task by reducing its impairment induced by the NMDA receptor antagonist MK-801 (Darrah et al., 2008). These results support the notion that positive mGlu5 receptor modulators improve cognitive function, which is impaired in schizophrenia. Several positive allosteric mGlu5 receptor modulators have shown in vivo activity in various animal models that are frequently used as antipsychotic screening tests: CDPPB, ADX47273, and compound **V** in Table 5 reversed phencyclidine (PCP)- or amphetamine-induced hyperlocomotion at doses in the range of 3–100 mg/kg i.p. or s.c. (Kinney et al., 2005; Liu et al., 2008; Sharma et al., 2009). ADX47273 also reduced conditioned avoidance responding in rats at a minimal effective dose of 30 mg/kg i.p. (Liu et al., 2008). A classic model in preclinical schizophrenia research is the prepulse inhibition (PPI) of the startle response in rodents, which is believed to reflect sensory gating and is disrupted by psychotomimetic drugs such as amphetamine or PCP. Compounds with clinical antipsychotic efficacy restore disrupted PPI. Knockout animals lacking mGlu5 receptors have deficits in PPI (Kinney et al., 2003; Brody et al., 2004), and MPEP potentiates the disruption of PPI by PCP (Henry et al., 2002; Kinney et al., 2003). On the other hand, CDPPB in the dose range of 3–30 mg/kg s.c. showed good activity in reversing amphetamine-induced deficits in PPI in rats (Kinney et al., 2005). Based on these promising results from animal models, clinical trials in humans are warranted and awaited with great expectations. However, to date only four chemotypes of positive allosteric mGlu5 receptor modulators are available, and improvement of their physicochemical properties (such as solubility in acceptable vehicles) seems highly desirable. In the search for novel types of positive mGlu5 receptor modulators, Mueller et al. (2010) performed an iterative virtual high-throughput screen using the experimentally determined potencies of primary hits for training artificial neuronal network quantitative SAR models ("machine learning"). With this procedure, they have identified approximately 200 active compounds, approximately 70% of which were close derivatives of known positive modulators, and approximately 30% were nontrivial analogs. Moreover, Hammond et al. (2010) have very recently identified a novel benzamide scaffold for positive mGlu5 receptor modulators (Table 5, compound **VII**), which at the same time also yielded the first neutral allosteric mGlu5 receptor ligand not binding to the MPEP site (Table 3, compound **XIII**). The situation regarding patents on positive allosteric mGlu5 receptor modulators has been reviewed elsewhere (Kanuma et al., 2010).

3. Allosteric Modulators of the mGlu1 Receptor

a. Negative Allosteric Modulators of the mGlu1 Receptor. The structure and function of the other group I mGlu receptor subtype, the mGlu1 receptor, has recently been the subject of a comprehensive review (Ferraguti et al., 2008). The discovery and development of allosteric modulators for the mGlu1 receptor mirrors that of its mGlu5 receptor counterpart, although it may seem to have been somewhat in its shadow. Nevertheless, the history of group I mGlu receptor modulators started with the compound CPCCOEt (Table 6), which was originally synthesized and described as a selective mGlu1 receptor antagonist by Annoura et al. (1996). Later, Hermans et al. (1998) and Litschig et al. (1999) observed a seemingly noncompetitive mechanism of action of this compound. CPCCOEt inhibited glutamate-induced increases in intracellular calcium levels in a recombinant cell line expressing human mGlu1 receptors with an IC_{50} of 6.5 μM, without having any activity at other mGlu receptor subtypes tested. CPCCOEt acted by decreasing the maximal levels of quisqualate or glutamate responses, in this case stimulating phosphoinositol metabolism (inositol monophosphate formation). In addition, CPCCOEt did not inhibit the binding of [^{3}H]glutamate to membranes from mGlu1 receptor-expressing cells. Although CPCCOEt became a useful tool for in vitro studies because of its selectivity for the mGlu1 receptor, the availability of more potent molecules with better properties for in vivo activity was needed. A large choice of negative allosteric mGlu1 receptor modulators with much structural diversity became available over the subsequent years. A few examples are shown in Table 6. They were all discovered and characterized with the help of essentially the same techniques: functional assays (inositol monophosphate formation, intracellular calcium mobilization) in recombinant cell lines, Schild analysis to demonstrate the apparent noncompetitive mechanism of action, [^{3}H]glu-

tamate or [^{3}H]quisqualate binding assays to exclude any direct interaction with the orthosteric agonist binding site, and functional assays for other mGlu receptor subtypes to demonstrate selectivity. A first improvement in potency compared with CPCCOEt was achieved with (3*aS*,6*aS*)-hexahydro-5-methylene-6*a*-(2-naphthalenylmethyl)-1*H*-cyclopenta[*c*]furan-1-one (BAY36-7620), which had an IC_{50} of 160 nM and acted as an inverse agonist at mGlu1 receptors (Carroll et al., 2001) (Table 6, compound **II**). EM-TBPC (Malherbe et al., 2003a) (Table 6, compound **VI**) had a higher potency in a functional assay (IC_{50} = 15 nM) and displayed a high binding affinity, making it suitable as a radioligand (K_D = 6.6 nM). With its help, mutational analysis identified several critical residues in the binding pocket in the 7TM domain. It is noteworthy that the rat mGlu1 receptor, but not the human receptor, has a high affinity for EM-TBPC. One of the critical amino acids in the rat, Val757, is replaced by leucine in the human receptor. A site-directed L757V "back"-mutation confers high binding affinity for EM-TBPC to the human mGlu1 receptor. This is a nice example of species differences in the effects of allosteric modulators arising from the fact that the 7TM region of mGlu receptors is less conserved than the orthosteric ligand binding site. Subsequently, several novel negative allosteric mGlu1 receptor modulators (Table 6) were described that in functional in vitro assays had high potencies with IC_{50} values in the low nanomolar range: (3,4-dihydro-2*H*-pyrano[2,3-*b*]quinolin-7-yl)-(*cis*-4-methoxycyclohexyl)-methanone (JNJ16259685) (Lavreysen et al., 2004), 6-amino-*N*-cyclohexyl-*N*,3-dimethylthiazolo[3,2-*a*]benzimidazole-2-carboxamide hydrochloride (YM-298198) (Kohara et al., 2005), 9-(dimethylamino)-3-(hexahydro-1*H*-azepin-1-yl)pyrido[3′,2′:4,5]thieno[3,2-*d*]pyrimidin-4(3*H*)-one (A-841720) (El-Kouhen et al., 2006), 4-(cycloheptylamino)-*N*-[[(2*R*)-tetrahydro-2-furanyl]methyl]-thieno[2,3-*d*]pyrimidine-6-methanamine (YM-230888) (Kohara et al., 2007), FTIDC (Suzuki et al., 2007a), 3-cyclohexyl-5-fluoro-6-methyl-7-(2-morpholin-4-ylethoxy)-4*H*-chromen-4-one (Fukuda et al., 2009) and compounds **IX** (Micheli et al., 2003) and **X** (Zheng et al., 2005) in Table 6. A number of additional negative allosteric mGlu1 receptor modulators (not shown in Table 6) have been described; their structures are given in the respective references (Mabire et al., 2005; Micheli et al., 2006; Di Fabio et al., 2007; Owen et al., 2007; Vanejevs et al., 2008; Sasikumar et al., 2009; Satoh et al., 2009). Some of these molecules showed good in vivo-activity, especially in models for chronic pain (see below).

Some of the compounds above that have high potencies at the mGlu1 receptor have been used in tritiated form as radioligands. [^{3}H]YM-298198 bound with high affinity to membranes from an mGlu1 receptor expressing cell line (K_D = 32 nM) and from rat cerebellum. It was displaced competitively by CPCCOEt and unlabeled YM-298198, but not by orthosteric mGlu1 receptor agonists (Kohara et al., 2005). Likewise, [^{3}H]1-(3,4-dihydro-2*H*-pyrano[2,3-*b*]quinolin-7-yl)-2-phenyl-1-ethanone (R214127; compound **XII** in Table 6), a derivative of JNJ16259685, is a high-affinity radioligand (K_D = 0.9 nM) labeling mGlu1 receptors in recombinant and native membrane preparations (Lavreysen et al., 2003). It has also been used in autoradiographic experiments in rat brain, showing highest mGlu1 receptor densities in the cerebellum; moderate labeling in the substantia nigra, thalamus, and dentate gyrus of the hippocampus; and lower binding in the cerebral cortex, caudate putamen, and nucleus accumbens. In membrane preparations, [^{3}H]R214127 binding was completely blocked by *N*-(1-adamantyl)quinoxaline-2-carboxamide (NPS2390), BAY36-7620, and CPCCOEt; likewise, the binding of the radioligand [^{3}H]2-[(4-indan-2-ylamino)-5,6,7,8-tetrahydroquinazolin-2-ylsulphanyl]-ethanol ([^{3}H]IPTE) (Johnson et al., 2004) to membranes from recombinant mGlu1 receptor cells was displaced by CPCCOEt, R214127 and BAY36-7620. Taken together, these findings strongly suggest that all these compounds interact with a common binding site shared by multiple negative allosteric modulators.

The effects of mGlu1 receptor antagonists in animal models for psychiatric diseases have recently been reviewed in detail by Lesage and Steckler (2010). mGlu1 receptors are expressed in key regions involved in anxiety (hippocampus, hypothalamus, periaqueductal gray, amygdala), where they are localized postsynaptically and believed to facilitate NMDA receptor-mediated responses and, at the same time, attenuate GABAergic transmission. This context points to a potential anxiolytic activity of mGlu1 receptor antagonists or negative allosteric modulators. The effects of such molecules in animal models of anxiety are conflicting, however. For example, anxiolytic-like activity was detected in the lick suppression test with JNJ16259685 (2.5 and 5 mg/kg i.p.) (Steckler et al., 2005a) and with FTIDC (3–30 mg/kg i.p.) in the maternal deprivation-induced ultrasonic vocalization and the stress-induced hyperthermia (SIH) models (Satow et al., 2008). On the other hand, FTIDC and JNJ16259685 were found to be inactive in the elevated plus maze (Steckler et al., 2005a; Satow et al., 2008). Only little (and conflicting) information is available about the effects of negative allosteric mGlu1 receptor modulators in animal models of depression (Lesage and Steckler, 2010). Hikichi et al. (2010b) have shown that negative mGlu1 receptor modulators improve PPI in mice, a model of impaired sensorimotor gating in schizophrenia, whereas various allosteric ligands for other mGlu receptor subtypes were inactive.

A coherent picture emerges from results obtained with negative mGlu1 receptor modulators in vivo in models of chronic pain (Schkeryantz et al., 2007). Like their mGlu5 receptor counterpart, mGlu1 receptors are localized at critical sites in nociceptive pathways. Clinical pain (chronic inflammatory pain, neuropathic pain) is due to long-term hypersensitivity as a consequence of peripheral or central sensitization processes. Its hallmarks are allodynia (pain induced by a stimulus that

TABLE 6
Negative allosteric mGlu1 receptor modulators
The potencies of the compounds have been obtained in functional assays under different conditions and should therefore be compared with caution.

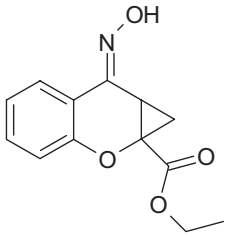
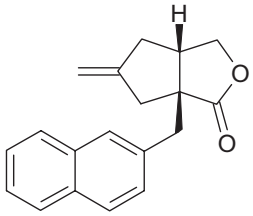
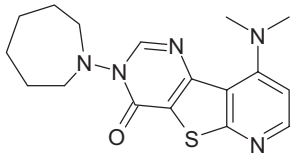
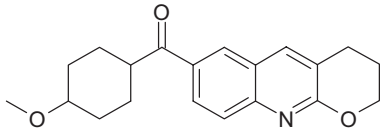
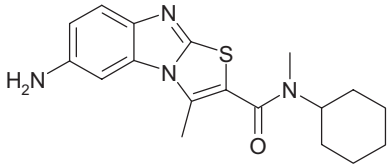
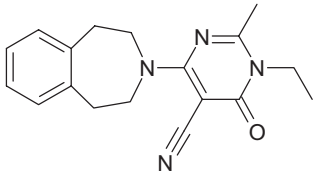
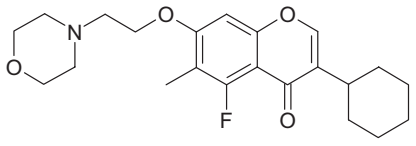
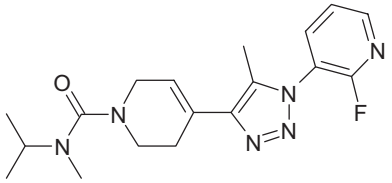
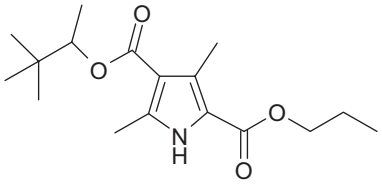
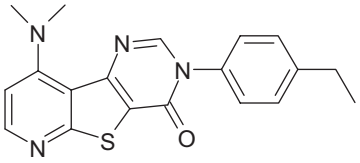
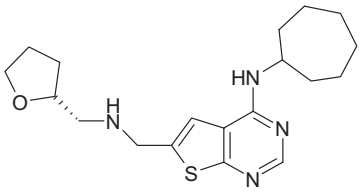
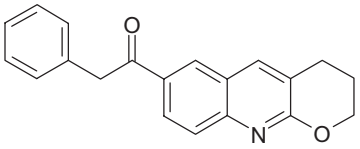
Compound	Structure	Potency	Comments	References
I: CPCCOEt		IC_{50} = 6.5 μM	First allosteric mGlu receptor modulator	Annoura et al., 1996; Hermans et al., 1998; Litschig et al., 1999
II: BAY36-7620		IC_{50} = 160 nM	Inverse allosteric agonist	Carroll et al., 2001
III: A841720		IC_{50} = 11 nM	Analgesic activity and disruption of cognitive function in rats in vivo	El-Kouhen et al., 2006
IV: JNJ16259685		IC_{50} = 3.2 nM	High occupancy of mGlu1 receptors in rat brain after systemic application	Lavreysen et al., 2004
V: YM-298198		IC_{50} = 16 nM	Analgesic activity in mice after oral application	Kohara et al., 2005
VI: EM-TBPC		IC_{50} = 15 nM K_D = 6.6 nM	Used as a radioligand in its tritiated form for mapping studies; has high affinity for rat but not for human receptor	Malherbe et al., 2003
VII: CFMMC		IC_{50} = 50 nM		Fukuda et al., 2009
VIII: FTIDC		IC_{50} = 6 nM	Active in formalin pain model	Suzuki et al., 2007a

TABLE 6—*Continued.*

Compound	Structure	Potency (IC_{50})	Comments	References
IX:		IC_{50} = 16 nM	Reduces "wind up." Analgesic activity in vivo	Micheli et al., 2003
X:		IC_{50} = 3 nM	Analgesic activity in various pain models	Zheng et al., 2005
XI: YM-230888		IC_{50} = 13 nM	Analgesic activity in several animal models	Kohara et al., 2007
XII: R214127		K_D = 0.9 nM	Good radioligand for mGlu1 receptors	Lavreysen et al., 2003

normally is not painful, such as touch) or hyperalgesia (exaggerated response to a noxious stimulus). Several animal models are frequently used to assess the efficacy of therapeutic approaches against different manifestations of chronic pain. Whereas formalin-induced pain in its early phase represents acute pain, in the late phase it is considered a model for inflammatory pain. Carrageenan- or complete Freund's adjuvant-induced hyperalgesia are also classic models for chronic inflammatory pain. On the other hand, models such as sciatic nerve constriction injury or spinal nerve ligation are used to test drug efficacy against neuropathic pain. Structurally different negative allosteric mGlu1 receptor modulators have shown antinociceptive efficacy in various pain models. Compound **X** in Table 6 counteracted hyperalgesia in formalin- (late phase), carrageenan-, and Freund's adjuvant-induced inflammatory pain models in the dose range of 10 to 20 μmol/kg i.p. (Zheng et al., 2005). FTIDC (3 and 10 mg/kg i.p.) significantly reduced licking behavior after formalin injection into the hind paw in mice in both the early and the late phases (Satow et al., 2008). YM-230888 reduced hyperalgesia in a Freund's adjuvant-induced arthritic pain model at 30 mg/kg p.o. in rats. This compound also recovered mechanical allodynia in a spinal nerve ligation model of neuropathic pain in the dose range of 3 to 30 mg/kg p.o. in rats (Kohara et al., 2007). Compound **IX** in Table 6 was also antinociceptive in models of acute (formalin early phase) and inflammatory pain (formalin late phase) in mice (ED_{50}, ~0.3 mg/kg p.o.) and in the carrageenan model in rats (ED_{50}, ~3 mg/kg i.p.) (Micheli et al., 2003). Moreover, in the chronic constriction injury model of neuropathic pain in rats, the compound completely reverted hyperalgesia at a dose of 10 mg/kg i.p., with a duration of action of at least 4 h. The compound was also tested in an in vitro electrophysiological model of the nociceptive pathway using a preparation of baby rat spinal cord. Most interestingly, the "wind-up" phenomenon (a summation of ventral root potentials) produced by multiple stimulation of C-fibers, an index of central sensitization, was reduced by compound **IX** (Table 6) at 1 μM (Micheli et al., 2003). The negative mGlu1 receptor modulator A-841720 in Table 6 significantly attenuated postoperative pain after paw skin incision in rats with an ED_{50} of 10 μmol/kg i.p.; some close derivatives of the compound were also active in that model (Zhu et al., 2008). Motor side effects were observed at 3- to 10-fold higher doses. A-841720 was also active against Freund's adjuvant-induced inflammatory pain with an ED_{50} of 23 μmol/kg i.p. and reduced mechanical allodynia in sciatic nerve constriction and spinal nerve ligation models of neuropathic pain in the same dose range (El-Kouhen et al., 2006). However, significant motor side effects occurred at analgesic doses, and the compound also impaired cognitive function in Y-maze and water maze tests. These findings suggest that a lack of therapeutic window might preclude the clinical use of mGlu1 receptor antagonists in pain indications. It remains to be seen

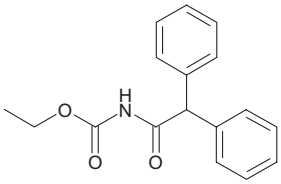
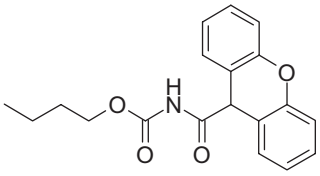
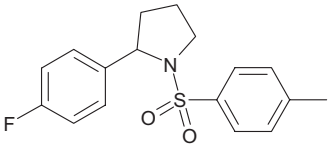
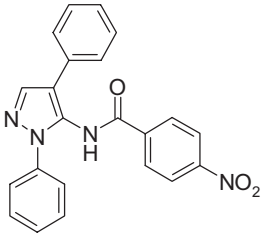
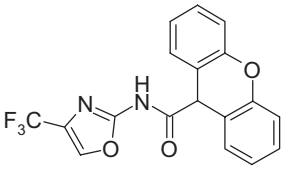
whether these side effects are mechanism-related or it is possible to find negative mGlu1 receptor modulators with a better side-effect profile.

b. Positive Allosteric Modulators of the mGlu1 Receptor. The first positive allosteric modulators for mGlu receptors ever were discovered and described by Knoflach et al. (2001). Three compounds of two different chemotypes [diphenylacetyl-carbamic acid ethyl ester (Ro 01-6128), butyl (9*H*-xanthene-9-carbonyl)carbamate (Ro 67-4853), and (*S*)-2-(4-fluorophenyl)-1-(toluene-4-sulfonyl)pyrrolidine (Ro 67-7476) (**I–III** in Table 7)] were identified by using high-throughput fluorometric imaging techniques. In HEK293 cells transiently transfected with rat mGlu1a receptors, these molecules enhanced the increase in intracellular calcium concentrations evoked by a threshold concentration of glutamate without producing such a calcium response on their own. Only in cells expressing very high mGlu1 receptor levels (i.e., in a system having a receptor reserve) did the three compounds produce an increase in intracellular calcium levels in the absence of exogenous glutamate. Ro 67-7476 and Ro 01-6128 both enhanced the binding affinity of [^{3}H]quisqualate for recombinant rat mGlu1, but not mGlu5, receptors, without affecting the maximal binding capacity. In kinetic experiments, it was shown that a decrease in the dissociation rate constant at least partly contributed to the increase in [^{3}H]quisqualate affinity for mGlu1 receptors. In CHO cells stably expressing inwardly rectifying potassium channels (GIRKs) and transiently transfected with rat mGlu1a receptor cDNA, agonists such as glutamate or DHPG produced inward currents that were enhanced by Ro 01-6128 (EC_{50} = 200 nM), Ro 67-4853 (EC_{50} = 63 nM), and Ro 67-7476 (EC_{50} = 158 nM). When concentration-response curves were measured with DHPG, 3 μM Ro 01-6128 increased the potency of the agonist from an EC_{50} of 7.9 μM to one of 1.6 μM. Ro 01-6128 and Ro 67-7476 did not enhance glutamate-induced GIRK currents in cells expressing rat mGlu5 receptors; only with Ro 67-4853 (10 μM) was a small poten-

TABLE 7

Positive allosteric mGlu1 receptor modulators

Information about compound potencies in vitro in one representative assay is given as an indication only. Because of the complexity of allosteric interactions and the different possibilities of measuring modulation, information on cooperativity is not summarized here but can be found in section III.A.3.b.

Compound	Structure	Potency/Affinity (EC_{50})	Comments	References
I: Ro 01-6128		200 nM		Knoflach et al., 2001
II: Ro 67-4853		69 nM	First positive mGlu receptor modulators ever described. Only **II** is active at both rat and human receptors; **I** and **III** do not modulate human mGlu1	Knoflach et al., 2001
III: Ro 67-7476		174 nM		Knoflach et al., 2001
IV: VU-71		2.4 μM		Hemstapat et al., 2006
V: Ro 07-11401		56 nM	Active in EAE-model for multiple sclerosis in mice	Vieira et al., 2009

EAE, experimental autoimmune encephalomyelitis.

tiating effect observed at this receptor. All three compounds were also devoid of any enhancing effect at mGlu2, mGlu4, mGlu8, $GABA_B$, and a large number of other receptors in different assay systems. It is noteworthy that when human instead of rat mGlu1 receptor cDNA was transfected into GIRK-expressing CHO cells, an enhancement of glutamate-induced currents was observed only with Ro 67-4853, not with the two other compounds, indicating the existence of a species difference for those. The new positive allosteric mGlu1 receptor modulators were also tested in native receptor systems. In freshly dissociated CA3 neurons, depolarization-evoked currents through voltage-gated calcium channels (VGCCs) were inhibited by the mGlu1 receptor agonist DHPG; this inhibition was more pronounced in the presence of Ro 01-6128 (EC_{50} = 1 μM) and Ro 67-4853 (EC_{50} = 100 nM). The enhancing effect of Ro 01-6128 was also seen in the presence of antagonists for ionotropic glutamate receptors. In this preparation, Ro 01-6128 had no effect on VGCC currents when applied alone and no effect on the inhibition of VGCC activity by the selective group II mGlu receptor agonist (+)-2-aminobicyclo[3.1.0]hexane-2,6-dicarboxylic acid (LY354740) in cerebellar Golgi cells. mGlu1 receptors are expressed at high levels in cerebellar Purkinje cells. Using a greased-gap recording technique in cerebellar slices, Knoflach et al. (2001) observed a significant enhancement of DHPG-induced depolarizations by Ro 67-7476 at 3 μM, whereas control AMPA responses remained unaffected. When synaptically evoked responses were measured under conditions likely to involve mGlu1 receptors, a marked enhancement of postsynaptic current amplitude and duration was observed in the presence of 3 μM Ro 67-7476. A few other chemotypes of positive allosteric mGlu1 receptor modulators, some of which are derivatives of Ro 67-4853, have been reported by the Roche group in subsequent years (Wichmann et al., 2002; Vieira et al., 2005, 2009).

Sheffler and Conn (2008) have observed differential modulation of independent mGlu1 receptor-mediated signaling pathways in baby hamster kidney (BHK) cells by Ro 01-6128, Ro 67-4853, and Ro 67-7476. On the one hand, they were able to confirm the effects of these modulators on intracellular calcium mobilization reported previously by Knoflach et al. (2001) using HEK cells. However, they observed that all three compounds were able to efficiently activate ERK 1/2 phosphorylation in the absence of an exogenously added agonist. These effects were fully antagonized by an orthosteric antagonist [(2*S*)-2-amino-2-(1*S*,2*S*-2-carboxycyclopronan-1-yl-3-(xanth-9-yl)propanoic acid (LY341495)] as well as by a negative allosteric mGlu1 receptor modulator (R214127; **XII** in Table 6). Because the three positive modulators tested bind to an allosteric site distinct from that for R214127 (see section III.A.4), this finding suggests a considerable degree of cooperativity between different orthosteric and allosteric ligand recognition sites. In some in vitro systems, mGlu1 receptors can also couple to $G\alpha_s$ and thereby stimulate cAMP formation. In BHK cells, the three Ro modulators enhanced the stimulation of adenylyl cyclase activity by glutamate but also had some intrinsic activity as low-efficacy allosteric partial agonists. This was again inhibited by both LY341495 and R214127. It is self-evident that such "ligand-induced differential signaling" (Urban et al., 2007) by allosteric compounds could have dramatic physiological and/or therapeutic consequences.

Little information is available on the in vivo effects of positive allosteric mGlu1 receptor modulators. A reduced expression of mGlu1a receptors in cerebellar Purkinje cells was found in seven of nine patients with multiple sclerosis (MS), along with an increase in mGlu5 receptors (Fazio et al., 2008). These changes were reproduced in mice developing experimental autoimmune encephalomyelitis, considered an animal model of human MS. In these mice, the positive allosteric mGlu1 receptor modulator Ro 07-11401 (compound **V** in Table 7) (Vieira et al., 2009) significantly improved motor coordination and performance (rotarod, paw print tests) at a dose of 10 mg/kg i.p., whereas the mGlu5 receptor antagonists MPEP and SIB-1757 had no effect. It thus seems that enhancement of mGlu1 receptor function is able to compensate for the reduced number of mGlu1 receptor in experimental autoimmune encephalomyelitis and might therefore be a promising therapeutic principle for the treatment of patients with MS. Based on the cognitive impairment produced by negative allosteric mGlu1 receptor modulators (Steckler et al., 2005b; El-Kouhen et al., 2006), it can be hypothesized that positive allosteric mGlu1 receptor modulators might be beneficial in disorders of learning and memory. In addition, because activation of the mGlu1 receptor, like that of the mGlu5 receptor, facilitates NMDA receptor responses, positive mGlu1 receptor modulators might have a therapeutic potential in schizophrenia (Lesage and Steckler, 2010).

4. Localization of Allosteric Binding Sites at Group I mGlu Receptors and Molecular Mechanisms of Modulation. A large number of studies using mutational analysis and/or chimeric or truncated constructs have shed light on the molecular mechanisms of allosteric modulation of group I mGlu receptors. Radioligand binding experiments suggest that the allosteric mGlu1 receptor antagonists known so far bind to a common site (see section III.A.3.a). This notion has essentially been confirmed in molecular studies. Experiments with chimeric mGlu1/5 receptor-constructs demonstrated the importance of transmembrane regions 4 to 7 for the selective inhibition of mGlu1 receptors by CPCCOEt (Litschig et al., 1999). Site-directed mutagenesis revealed that this molecule interacts with Thr815 and Ala818 located relatively close to the extracellular surface of transmembrane helix 7 (Litschig et al., 1999), and its benzene ring has been proposed to be positioned between transmembrane segments 3 and 7 (Pagano et al., 2000). Malherbe

et al. (2003a) have identified, also by mutational analysis, Phe801 and Tyr805 in transmembrane region 6 and again Thr815 in transmembrane segment 7 to be essential for the binding of EM-TBPC in addition to Val757 in transmembrane region 5. The aromatic amino acid cluster formed by Phe801, Tyr805, and Thr815 also seems to be essential for the activity of JNJ16259685, FTIDC, and 3-cyclohexyl-5-fluoro-6-methyl-7-(2-morpholin-4-ylethoxy)-4*H*-chromen-4-one (Suzuki et al., 2007a; Fukuda et al., 2009). Its interaction with antagonists has been proposed to prevent the movement of Trp798 in transmembrane helix 6, which is essential for receptor activation (Malherbe et al., 2003b). Figure 10 qualitatively illustrates the localization of critical amino acids for allosteric modulation at group I mGlu receptors.

Regarding the localization of the binding site for the first three positive allosteric mGlu1 receptor modulators (Ro compounds **I–III** in Table 7), Knoflach et al. (2001), by using chimeric receptor constructs and site-directed mutagenesis, came to the conclusion that critical amino acid residues in transmembrane regions 3 and 7 form an overlapping binding pocket in a region homologous to that which binds MPEP in mGlu5 receptors (Fig. 10). Hemstapat et al. (2006) have characterized a series of derivatives of the positive allosteric mGlu5 receptor modulator CDPPB (Table 5), some of which also served as positive modulators of mGlu1 receptors. Among these, VU-71 (Table 7) was selective for the mGlu1 receptor. Neither of these novel CDPPB analogs nor the three Ro compounds mentioned above displaced the radioligand [^{3}H]R214127 (a negative modulator) from its allosteric binding site on the mGlu1 receptor, indicating that positive allosteric mGlu1 receptor modulators and their negatively modulating counterparts bind to distinct sites in the 7TM domain. As reported previously by Knoflach et al. (2001), Hemstapat et al. (2006) also found valine at position 757 in transmembrane region 5 of mGlu1a receptors to be crucial for the activity of positive modulators.

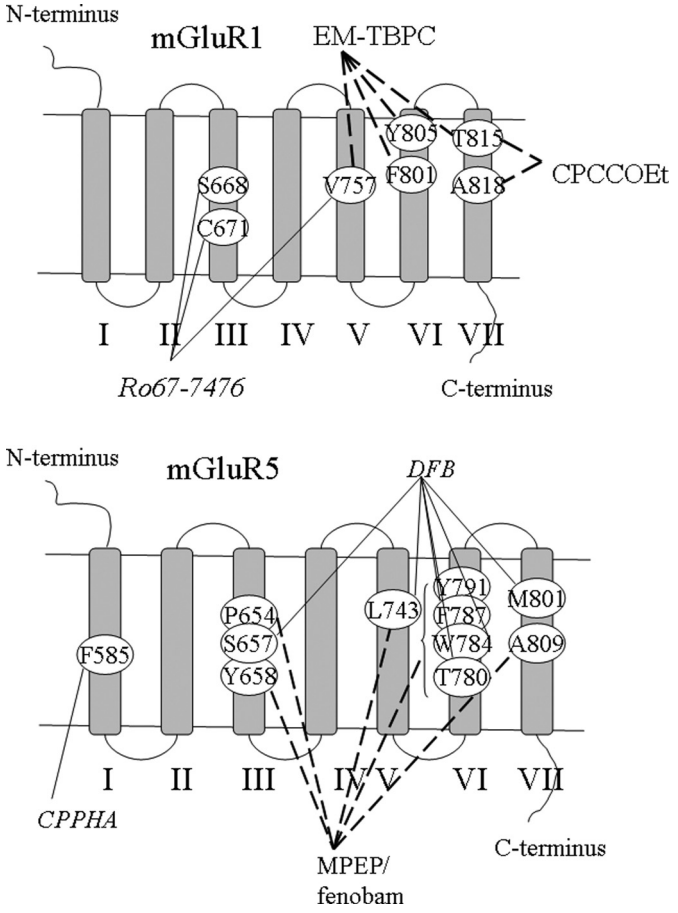


FIG. 10. Localization of amino acid residues in the 7TM-domain that are critical for allosteric modulation at group I mGlu receptors. Top, the amino acids that have been found on the basis of mutational analysis to interact with positive modulators (*italic*, solid lines) and with negative modulators (dashed lines) are shown. It is noteworthy that Val757 in transmembrane region 5 is important for both positive and negative allosteric modulation. Bottom, most positive modulators (represented by *DFB*, solid lines) and negative modulators (MPEP-type and fenobam, dashed lines) interact with largely overlapping clusters of amino acids in transmembrane regions 3, 5, 6 and 7 of the mGlu5 receptor. The positive modulator *CPPHA* is a noteworthy exception that binds to another site in the 7TM domain. Phe585 in transmembrane region 1 has been found to be critical for the effects of CPPHA. See section III.A.4 for further explanations.

Using site-directed mutagenesis and rhodopsin-based homology modeling, Malherbe et al. (2003b, 2006) identified a cluster of eight amino acid residues that are important for MPEP binding to rat mGlu5 receptors (Fig. 10). Their model proposes that MPEP, by interacting with a network of aromatic residues in transmembrane regions 3, 6, and 7, prevents the movement of transmembrane segment 6 relative to segment 3 that is involved in receptor activation, thus stabilizing the inactive conformation of the mGlu5 receptor. Subsequent work by the same group showed that the structurally unrelated antagonist fenobam binds in a very similar way to the same region of the 7TM domain as MPEP (with the exception of interaction with Leu743) (Malherbe et al., 2006).

Various experimental approaches have yielded important information on the mode of action of positive allosteric mGlu5 receptor modulators. Goudet et al. (2004) showed that DFB, contrary to its effect on wild-type receptors, was able to activate N-terminally truncated mGlu5 receptors, whereas MPEP acted as an inverse agonist in assays for the PLC-pathway in cells transfected with this construct. The same agonist activity was later also found with VU-29 (Chen et al., 2007). Thus, these constructs lacking the extracellular domain obviously function like rhodopsin-like class A GPCRs, which are activated by agonist binding to a site in the 7TM domain. Apparently, the removal of the large extracellular domain typical for family C GPCRs releases a kind of "allosteric brake" that prevents the direct activation of the wild-type 7TM domain by most positive allosteric modulators, such as, for example, DFB or VU-29. The location of the binding site for positive mGlu5 receptor modulators in the 7TM domain has been confirmed by mutational analysis (Mühlemann et al., 2006). Based on functional calcium mobilization assays in recombinant

cell lines expressing a series of mutant mGlu5 receptor constructs, six key residues in transmembrane segments 3, 5, 6, and 7 were found necessary for the modulation of mGlu5 receptors by DFB (Fig. 10). Based on a comparison with the residues essential for MPEP binding (Malherbe et al., 2003b) (Fig. 10) and the crystal structure of bovine rhodopsin, Mühlemann et al. (2006) proposed a homology model suggesting that DFB and MPEP bind to overlapping but slightly distinct parts of the same binding pocket. On the other hand, a mutation (A809V) that is critical for the binding of MPEP also abolished the allosteric effect of VU-29 but not that of CPPHA, whereas the mutation F585I in transmembrane region 1 eliminated the response to CPPHA but left positive modulation by VU-29 intact (Chen et al., 2008). These results show that CPPHA binds to a site distinct from that for the other mGlu5 receptor allosteric modulators known so far and are in line with the fact that CPPHA, unlike the other modulators, does not inhibit the binding of MPEP-like radioligands (see section III.A.2). Most interestingly, however, there seems to be an (allosteric!) interaction between these two allosteric sites on mGlu5 receptors: whereas in a functional assay the neutral MPEP-site antagonist 5MPEP at increasing concentrations up to 10 μM produced progressive parallel rightward shifts of the CRC for VU-29, it also inhibited positive modulation by CPPHA by progressively decreasing the maximal potentiation produced by CPPHA (Chen et al., 2008)!

Comparisons of the corresponding binding pockets in mGlu1 and mGlu5 receptors have also shed light on the interaction of allosteric modulators with group I mGlu receptors. In an early site-directed mutagenesis study using the radioligand [^{3}H]M-MPEP as a probe, Pagano et al. (2000) replaced the three nonconserved amino acids Ala810 in transmembrane segment 7, or Pro655 and Ser658 in transmembrane region 3 of the human mGlu5a receptor by the homologous residues of the human mGlu1b receptor, thus abolishing ligand binding. This finding explains the selectivity of MPEP and its analogs for mGlu5 over mGlu1 receptors. On the other hand, the reciprocal human mGlu1 receptor mutant bearing these three residues of the human mGlu5 receptor displayed a high binding affinity for the radioligand. Malherbe et al. (2003b) and Mühlemann et al. (2006) have aligned the critical residues in the binding pockets of mGlu1a and mGlu5a receptors for positive and negative allosteric modulators. Several homologous amino acids, especially in transmembrane region 6, are common to both. In particular, Trp798 in the mGlu1 receptor, critical for the inhibition by EM-TBPC (see above), is also essential in the homologous position 784 in the mGlu5 receptor (Fig. 10), not only for antagonism by MPEP but also for positive modulation by DFB. Apparently, DFB stabilizes the active conformation of the mGlu5 receptor by facilitating the movement of this tryptophan in transmembrane region 6, whereas negative modulators prevent the movement of transmembrane region 6 relative to region 3. The positive mGlu5 receptor modulator CPPHA (Table 5) also has an enhancing effect at mGlu1 receptors, albeit at approximately 10-fold higher concentrations (Chen et al., 2008). Whereas the mutation F585I in transmembrane region 1 abolishes the effect of CPPHA at mGlu5 receptors, the corresponding mutation F599I also does this at the mGlu1 receptor, without affecting positive modulation by Ro 67-7476. On the other hand, the mutation V757L in the mGlu1 receptor, which eliminates its modulation by Ro 67-7476, does not change the effect of CPPHA at this receptor. Thus, just as CPPHA has been found to interact with an allosteric site distinct from that for other modulators at mGlu5 receptors, it also does so at the mGlu1 receptor (Chen et al., 2008).

In a most interesting study using different mutants and chimeric constructs, Goudet et al. (2005) were able to show that the binding of a single molecule of a positive modulator (Ro 01-6128 for mGlu1 and DFB for mGlu5 receptors) to an mGlu receptor dimer is sufficient to provide full enhancement of receptor activity. This asymmetric functioning may seem surprising given the fact that mGlu receptors are symmetrical homodimers. On the other hand, when using an mGlu1 receptor mutant able to bind MPEP, Hlavackova et al. (2005) observed no decrease in receptor activity when a single 7TM domain was blocked in its inactive state by MPEP. This finding indicates that two molecules of a negative modulator per dimer are required to block receptor activation.

B. Allosteric Modulators of Group II Metabotropic Glutamate Receptors

Group II metabotropic glutamate receptors comprise mGlu2 and mGlu3 subtypes. They are located mainly presynaptically to inhibit glutamate release (Cartmell and Schoepp, 2000), but they are also found to a certain extent postsynaptically and on glia cells. Group II mGlu receptor ligands seem to have a great therapeutic potential in various central nervous system indications (for review, see Swanson et al., 2005; Linden and Schoepp, 2006). In particular, the selective mGlu2/3 receptor agonists LY354740 and (−)-(1*R*,4*S*,5*S*,6*S*)-4-amino-2-sulfonylbicyclo[3.1.0]-hexane-4,6-dicarboxylic acid (LY404039) and their respective prodrugs have been found to have anxiolytic and antischizophrenic activity, respectively, not only in preclinical animal models, but also in human trials (Grillon et al., 2003; Patil et al., 2007). Because these orthosteric agonists are not selective for either of the two group II mGlu receptor subtypes, it seems not clear whether the mGlu2, the mGlu3 receptor, or both are responsible for these effects. However, studies using knockout animals point to the conclusion that mostly the mGlu2 receptor is required for obtaining antipsychotic-like activity in animal models (Spooren et al., 2000a; Fell et al., 2008; Woolley et al., 2008).

Group II mGlu receptor modulators are striking examples of the subtype selectivity that can be achieved by targeting allosteric rather than orthosteric binding sites. Although the orthosteric ligand binding site of group II mGlu receptors has been highly conserved during evolution, some key differences between residues in the 7TM region allow for the discrimination of the two by allosteric agents (Schaffhauser et al., 2003). In fact, substitution of Ser688 and/or Gly689 in transmembrane region 4 along with Asn735 in transmembrane region 5 with the homologous amino acids of the mGlu3 receptor abolished allosteric modulation of mGlu2 receptors by 2,2,2-trifluoro-*N*-[4-(2-methoxyphenoxy)phenyl]-*N*-(3-pyridinylmethyl)-ethanesulfonamide (LY487379) and other positive modulators (Rowe et al., 2008) (see below). Of a great number of positive allosteric group II modulators known to date, only a very few enhance both mGlu2 and mGlu3 receptor responses with more or less similar potencies (Bonnefous et al., 2005; Govek et al., 2005), whereas most of them are selective for the mGlu2 receptor subtype.

Two main chemical classes of positive allosteric mGlu2 receptor modulators were originally described and have become standard tool compounds (Table 8): pyrimidylsulfonamides from Eli Lilly (represented by LY487379, compounds **I–VI** in Table 8) and indanone compounds (Merck), represented by [biphenylindanone A (BINA), compounds **IX–XI** in Table 8]. Their extensively explored medicinal chemistry has been comprehensively reviewed by Rudd and McCauley (2005). The first positive allosteric mGlu2 receptor modulator was found in an HTS program using a fluorescence imaging plate reader (FLIPR) assay in a cell line stably expressing human mGlu2 receptors together with the promiscuous G-protein $G\alpha_{15}$ coupling the receptor to intracellular calcium mobilization (Johnson et al., 2003). The pyridylmethyl-sulfonamide *N*-(4-phenoxyphenyl)-*N*-(3-pyridinylmethyl)ethanesulfonamide (LY181837, compound **I**, Table 8) enhanced the signal produced by 1 μM glutamate with an EC_{50} of 1.5 μM but showed no response in the absence of glutamate. It did not enhance the signals elicited by glutamate on the seven mGlu receptor subtypes other than mGlu2. This lead compound was then used as a starting point for further optimization and SAR studies (Barda et al., 2004; Hu et al., 2004; Rudd and McCauley, 2005). One important finding was that replacement of the ethyl sulfonamide group by trifluoroethyl sulfonamide increased potency in many cases, such as for example in the compound LY487379 (compound **II**, Table 8). Another important SAR finding was that replacement of the biphenyl ether moiety by an alkyl-phenyl ether containing a bulky alkyl group significantly increased potency, resulting in the compounds cyPPTS and 2,2,2-trifluoroethyl [3-(1-methyl-butoxy)-phenyl]-pyridin-3-ylmethyl-sulfonamide (compounds **IV** and **V**, Table 8) with EC_{50} values of 24 and 14 nM, respectively (Barda et al., 2004).

The pharmacological properties of these compounds in vitro contain all the typical attributes of positive allosteric modulators (Schaffhauser et al., 2003; Johnson et al., 2005). In radioligand binding assays for native receptors in rat brain membranes (Schaffhauser et al., 2003), LY487379 did not displace the group II-selective radioligand [^{3}H]DCG-IV from the glutamate recognition site; rather, it increased the binding of this agonist ligand, indicating that it binds to a distinct site, thereby increasing the affinity of [^{3}H]DCG-IV. The same was found with 3-MPPTS (compound **III**, Table 8) in membranes containing recombinant mGlu2 receptors (Johnson et al., 2005). Moreover, 3-MPPTS increased the potencies of the orthosteric agonists glutamate, (1*S*, 3*R*)-1-aminocyclopentane-1,3-dicarboxylic acid, DCG-IV, and LY354740 as displacers of [^{3}H]DCG-IV at the mGlu2 receptor. These results obtained in radioligand binding assays were further corroborated in functional tests. 3-MPPTS, cyPPTS, CBiPES, and LY487379 selectively enhanced glutamate-stimulated transient increases in intracellular calcium levels in a cell line coexpressing a promiscuous G-protein together with the human mGlu2 receptor. In addition, LY487379 enhanced the stimulation of GTPγ^{35}S binding via mGlu2, but not mGlu3 receptors, by the agonists glutamate, DCG-IV, (2*S*,1′*S*,2′*S*)-2-(carboxycyclopropyl)glycine, and (−)-2-thia-4-aminobicyclo[3.1.0]hexane-4,6-dicarboxylate (LY379268) by increasing their potencies, and in some cases their maximal responses as well, without having any intrinsic activity on its own (Schaffhauser et al., 2003). A synergistic increase in GTPγ^{35}S binding stimulated by the orthosteric agonist LY379268 produced by 3-MPPTS could also be observed by autoradiographic techniques in rat or mouse brain slices (Johnson et al., 2004). The regional distribution of this response corresponded to the distribution of mGlu2 receptors in rodent brain (Johnson et al., 2004). At a more physiological level, in rat hippocampal slices, LY487379 strengthened the inhibition of excitatory synaptic transmission produced by the mGlu2/3 receptor agonist DCG-IV at the medial perforant path—dentate gyrus synapse (Schaffhauser et al., 2003). Most interestingly, in striatal brain slices, cyPPTS, unlike the orthosteric agonist LY354740, inhibited excitatory postsynaptic potentials (EPSPs) evoked by the stimulation of corticostriatal afferents only at high (2 and 4Hz) and not at lower frequencies of presynaptic stimulation (Fig. 11) (Johnson et al., 2005). The frequency-dependent suppression of EPSPs in striatal neurons by cyPPTS was reverted by the competitive antagonist LY341495, indicating its dependence on concomitant activation of the orthosteric site. This state-dependent activity beautifully illustrates the differences between the ways in which orthosteric agonists and positive allosteric modulators exert their effects. Whereas the former directly stimulate presynaptic receptors independently of the amount of glutamate released, the latter will only produce an inhibition of presynaptic

TABLE 8

Allosteric modulators of group II metabotropic glutamate receptors

Compounds **I**–**XI** are first generation, **XII**–**XVI** recently discovered selective positive mGlu2 receptor modulators. MNI-136 and MNI-137 (**XVII** and **XVIII**) are from a series of nonselective negative mGlu2/3 receptor modulators. Most compounds shown are single representatives from a chemical series. Allosteric modulator potencies are dependent on assay conditions and should be compared with caution. Information on the degrees of cooperativity is not summarized here but can be found in section III.B.

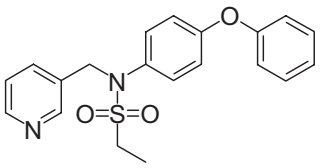
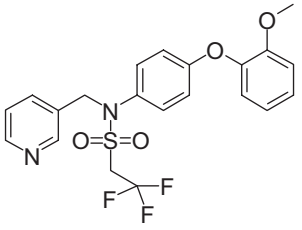
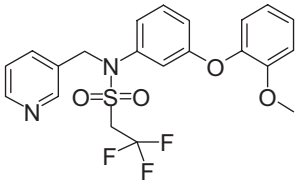
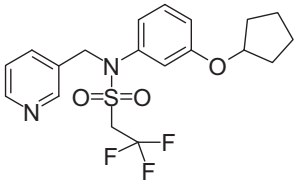
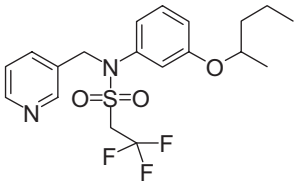
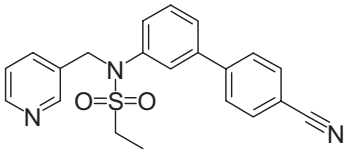
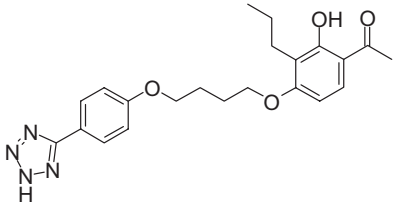
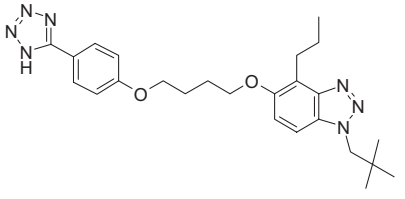
Compound	Structure	Potency in Vitro	In Vivo Activity	References
I: LY181837		EC_{50} mGlu2 = 1.5 μM		Johnson et al., 2003
II: LY487379 (4-MPPTS)		EC_{50} mGlu2 = 270 nM	Anxiolytic-like activity (FPS, SIH); reversal of amphetamine-induced locomotion and disruption of PPI	Johnson et al., 2003, 2005; Galici et al., 2005
III: 3-MPPTS		EC_{50} mGlu2 = 154 nM		Johnson et al., 2005
IV: cyPPTS		EC_{50} mGlu2 = 24 nM		Barda et al., 2004; Johnson et al., 2005
V: 2,2,2-TEMPS		EC_{50} mGlu2 = 14 nM		Barda et al., 2004
VI: CBiPES		EC_{50} mGlu2 = 93 nM	Inhibition of PCP-induced hyperlocomotion in mice	Johnson et al., 2005
VII		EC_{50} mGlu2 = 0.35 μM	Inhibits ketamine-induced NE release and hyperactivity in vivo	Pinkerton et al., 2004
VIII		EC_{50} mGlu2 = 188 nM mGlu3 = 936 nM		Govek et al., 2005

TABLE 8—*Continued.*

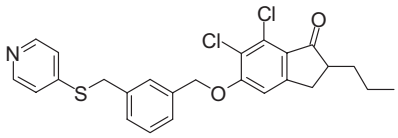
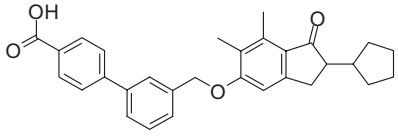
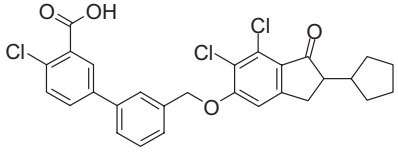
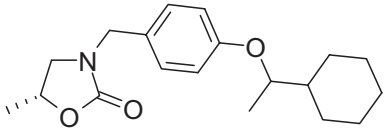
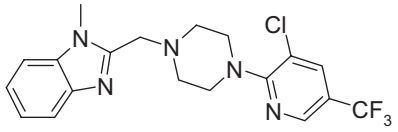
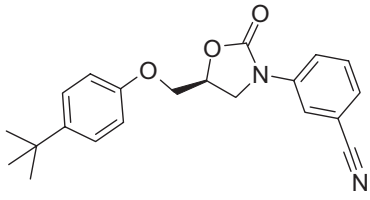
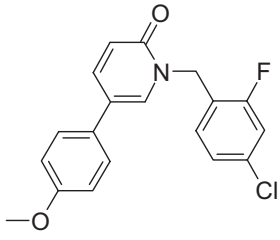
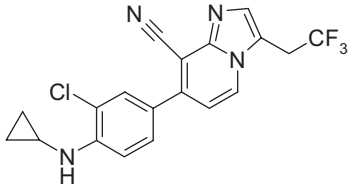
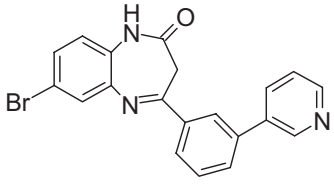
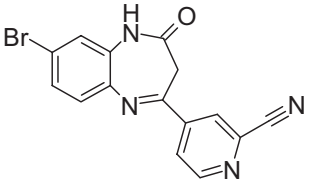
Compound	Structure	Potency (IC_{50})	Comments	References
IX		EC_{50} mGlu2 = 186 nM	Inhibits ketamine-induced hyperactivity in rats	Pinkerton et al., 2005
X: BINA		EC_{50} mGlu2 = 111 nM	Inhibits PCP-induced locomotion and disruption of PPI; anxiolytic-like activity in EPM and SIH	Bonnefous et al., 2005; Galici et al., 2006
XI		EC_{50} mGlu2 = 5 nM		Bonnefous et al., 2005
XII		EC_{50} mGlu2 = 5 nM		Duplantier et al., 2009
XIII: GSK1331258		EC_{50} mGlu2 = 79 nM		D'Alessandro et al., 2010
XIV		EC_{50} mGlu2 = 82 nM	Inhibits ketamine-induced psychomotor activity (locomotion) in rats	Brnardic et al., 2010
XV		EC_{50} mGlu2 = 525 nM	Inhibits PCP-induced locomotion in mice	Cid et al., 2010
XVI		EC_{50} mGlu2 = 141 nM		Tresadern et al., 2010
XVII: MNI-136		IC_{50} mGlu2 = 20 nM mGlu3 = 21 nM		Hemstapat et al., 2007; Woltering et al., 2007

TABLE 8—*Continued.*

Compound	Structure	Potency (IC_{50})	Comments	References
XVIII: MNI-137		IC_{50} mGlu2 = 13 nM mGlu3 = 20 nM		Hemstapat et al., 2007; Woltering et al., 2007

NE, norepinephrine; FPS, fear-potentiated startle; EPM, elevated plus-maze.

glutamate release (and thereby a decrease in postsynaptic EPSPs) in the presence of sufficiently high levels of glutamate (i.e., at higher stimulation frequencies) (Johnson et al., 2004, 2005).

In vivo, these mGlu2 receptor-selective positive modulators have effects in animal models of anxiolytic-like and antipsychotic activity very similar to those of orthosteric dual mGlu2/3 receptor agonists such as LY354740 and LY379268 (Swanson et al., 2005; Linden and Schoepp, 2006). LY487379 was shown to be active in the rat fear-potentiated startle model of anxiety at surprisingly low doses (0.1 mg/kg s.c.). CBiPES was also found to be active in SIH, a less sensitive anxiety model, although at considerably higher doses (Johnson et al., 2005). In terms of antipsychotic-like activity, CBiPES inhibited PCP-induced hyperlocomotion in mice in the same way as the orthosteric agonist LY379268; the effect of the modulator was reverted by pretreatment with the competitive antagonist LY341495 (Johnson et al., 2005). Likewise, both PCP- and amphetamine-induced locomotion in mice were dose dependently inhibited by the modulator LY487379 and the agonist LY379268, without an effect of these compounds on spontaneous locomotor activity. The effects of both compounds were reversed by the orthosteric antagonist LY341495, the latter case reflecting direct competition and the former, the dependence of the modulator on receptor activation by endogenous glutamate (Galici et al., 2005). In the same study, it was also shown that LY487379, but not LY379268, restored PPI disrupted by amphetamine administration, an observation also thought of as predictive of antipsychotic activity (Galici et al., 2005). A recent study by Nikiforuk et al. (2010) has shown that LY487379 improves cognitive flexibility and inhibitory control of impulsive behavior in rats, paralleled by increases in extracellular levels of serotonin and norepinephrine in the prefrontal cortex. These findings might be relevant for the treatment of cognitive deficits associated with schizophrenia.

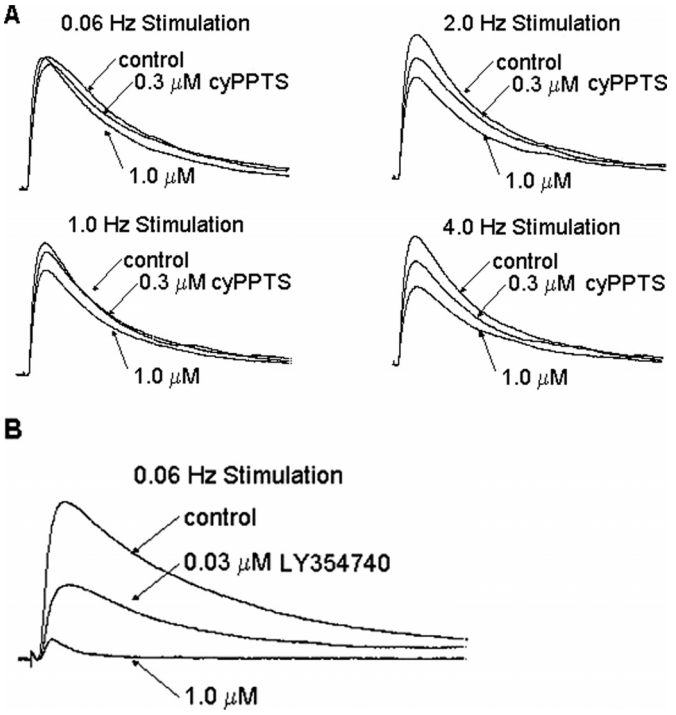


FIG. 11. The inhibition of corticostriatal EPSPs by the positive allosteric mGlu2 receptor modulator cyPPTS (Table 8) is frequency-dependent. A, responses from an individual striatal neuron to 0.06 to 4 Hz stimulation of glutamatergic inputs in the presence of different concentrations of cyPPTS. Inhibition is seen only at higher stimulation frequencies. B, the orthosteric mGlu2 receptor agonist LY354740 inhibits EPSPs already at the low stimulation frequency of 0.06 Hz. [Reproduced from Johnson MP, Barda D, Britton TC, Emkey R, Hornback WJ, Jagdmann GE, McKinzie DL, Nisenbaum ES, Tizzano JP, and Schoepp DD (2005) Metabotropic glutamate 2 receptor potentiators: receptor modulation, frequency-dependent synaptic activity, and efficacy in preclinical anxiety and psychosis model(s). *Psychopharmacology* **179:**271–283. Copyright © 2005 Springer Science + Business Media. Used with permission.]

Another class of positive allosteric mGlu2 receptor modulators, phenyl-tetrazolyl acetophenones, was discovered at Merck research laboratories also by HTS using a FLIPR calcium assay in a cell line coexpressing the human mGlu2 receptor and the promiscuous G-protein $G\alpha_{16}$ (Pinkerton et al., 2004b). In a secondary confirmation assay, the lead compound (compound **VII** in Table 8) increased the potency (and slightly the maximal response) of glutamate in stimulating GTPγ^{35}S binding via mGlu2 receptors, but not mGlu3 or other mGlu receptors. In the absence of glutamate, it did not stimulate mGlu2 receptors. When given intracerebroventricularly (due to poor brain penetration) at a dose of 100 nmol, compound **VII** (Table 8) inhibited ketamine-induced norepinephrine release in vivo. This effect was reversed by coapplication of the competitive antagonist LY341495 and therefore mediated via the mGlu2 receptor. Likewise, the compound inhibited ketamine-induced hyper-

activity in rats at doses of 100 and 300 nmol i.c.v. Extensive derivatization of this lead compound has demonstrated the difficulties in optimizing allosteric modulator compounds. Small structural modifications can result in substantial changes in activity, without the emergence of clear structure activity relationships (Rudd and McCauley, 2005). Nevertheless, one series using benzazoles as acetophenone replacements was relatively successful (Govek et al., 2005). The best compound in this series, an indole, reached relevant brain levels after systemic administration and inhibited ketamine-induced hyperactivity in rats. It is noteworthy that one compound, a benzotriazole analog (compound **VIII**, Table 8), also enhanced mGlu3 receptor activity with a potency only a 5-fold weaker than that at the mGlu2 receptor. Moreover, replacement of the phenyltetrazol by a thiopyridyl or a phenylpropanoic acid end group resulted in compounds with improved brain penetration (Pinkerton et al., 2004a; Cube et al., 2005).

Another lead structure emerging from this HTS was a phenyltetrazolindanone (rather than -acetophenone) compound (Pinkerton et al., 2005). Optimization of this lead, taking into account SAR information from the acetophenone series, on the one hand led to a dichloroindanone analog with a thiopyridyl end group (compound **IX** in Table 8). This compound had good potency and cooperativity in vitro, and brain levels (after a dose active in the ketamine model in vivo) corresponding to the active concentration range in vitro (Pinkerton et al., 2005). On the other hand, this route has led to a series of biphenyl-indanones (Bonnefous et al., 2005) resulting in the best in vitro and in vivo properties yet found in positive mGlu2 receptor modulators. In fact, compound **XI** in Table 8 had an EC_{50} value of 5 nM in the GTPγ^{35}S assay, and BINA (compound **X** in Table 8) (EC_{50} in vitro, 111 nM) was found to have good pharmacokinetic properties in vivo [i.e., high plasma exposure (16 μM at 2 h after 20 mg/kg i.p.) and good brain penetration (brain/plasma ratio = 0.75 at 2 h) (Bonnefous et al., 2005)], making it a useful tool for in vivo studies.

The pharmacological properties of BINA in vitro and in vivo (Galici et al., 2006) are very similar to those of the pyridylmethylsulfonamide series described above. The amino acid residues in transmembrane regions 4 and 5 that are essential for the effects of LY487379 (Schaffhauser et al., 2003) are also critical for allosteric modulation by BINA, strongly suggesting that the two modulators interact with the same binding site (Hemstapat et al., 2007). BINA (300 nM) increased the potency of glutamate in a calcium mobilization assay (in CHO cells coexpressing the human mGlu2 receptor and the chimeric G-protein G_{iq5}) by approximately 10-fold without changing the maximal effect of glutamate. In the presence of a low concentration of glutamate (EC_{20}), EC_{50} values for BINA were found to be 33 and 98 nM for the human and rat receptors, respectively. Using this assay system, BINA did not enhance the effects of glutamate at mGlu1, mGlu5 and mGlu4 receptors at all. Likewise, 300 nM BINA produced a leftward shift in the glutamate-stimulated GTPγ^{35}S binding by approximately a 10-fold at mGlu2, but not at mGlu3 receptors. As described previously for LY487379 (see above), BINA strengthened the inhibition of excitatory synaptic transmission produced by the mGlu2/3 receptor agonist DCG-IV at the medial perforant path-dentate gyrus synapse, which is believed to be involved in mechanisms of fear and anxiety (Bergink et al., 2004).

BINA produces robust and long-lasting effects in behavioral models of antipsychotic- and anxiolytic-like activity in mice, much like the findings previously reported for group II mGlu receptor agonists (Galici et al., 2006). At a dose of 32 mg/kg i.p., BINA blocked PCP-induced hyperlocomotion, with a duration of action up to 8 h after administration. It is noteworthy, however, that BINA had no effect on amphetamine-induced hyperlocomotion, unlike the effects of mGlu2/3 receptor agonists and the allosteric modulator LY487379 (see above). In another model of antipsychotic-like activity, BINA blocked PCP-induced disruption of PPI in mice. Moreover, at doses of 10 and 32 mg/kg i.p., BINA had anxiolytic-like activity in the elevated plus maze and SIH models in mice. In a subsequent study (Benneyworth et al., 2007), BINA was found to be active in another drug model of psychosis, namely to inhibit the in vivo effects of the hallucinogenic $5HT_{2A/2C}$ agonist (−)-2,5-dimethoxy-4-bromoamphetamine. This went in parallel with an inhibition of spontaneous EPSPs and (−)-2,5-dimethoxy-4-bromoamphetamine-induced expression of Fos in the medial prefrontal cortex, a brain area believed to be involved in the pathophysiology of schizophrenia. In all these animal models, the effects of BINA were antagonized by the selective group II competitive antagonist LY341495, demonstrating their pharmacological specificity and dependence on activation of mGlu2 receptors by endogenous glutamate. Hackler et al. (2010) have confirmed the inhibitory effect of BINA (32 mg/kg i.p.) on PCP-induced locomotion in rats; with the use of blood oxygenation level-dependent pharmacological magnetic resonance imaging, they found that BINA attenuated the amplitude of the blood oxygenation level-dependent response to PCP in specific brain areas such as the prefrontal cortex, caudate-putamen, nucleus accumbens and mediodorsal thalamus.

Taken together, the effects of allosteric mGlu2 receptor modulators strengthen the notion of the importance of these receptors in clinical indications such as anxiety and schizophrenia. The finding that the effects of orthosteric dual mGlu2/3 receptor agonists have also been found with mGlu2 receptor-selective positive modulators confirms the findings from studies with knockout animals that this subtype alone is sufficient for their mediation. Some remarkable differences between the actions of orthosteric agonists and modulators have also been found, however, such as a lack of effect of the

former in the antipsychotic model of amphetamine-induced disruption of prepulse inhibition. Most recently, Jin et al. (2010) have reported that BINA inhibited the reinforcing and rewarding effects of cocaine and decreased cue-induced cocaine-seeking behavior in rats, suggesting that positive mGlu2 receptor modulators might also have a potential in the treatment of addiction to cocaine and possibly other drugs of abuse.

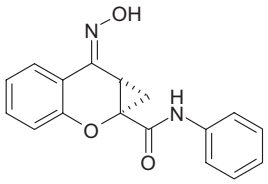
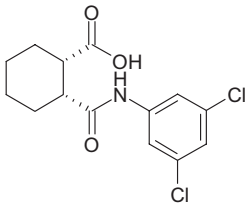
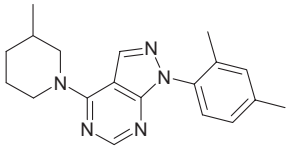
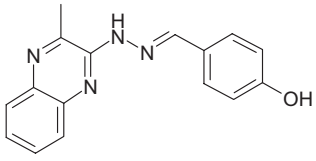
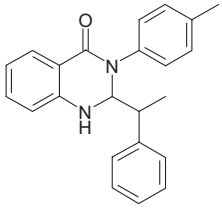
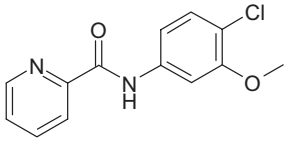
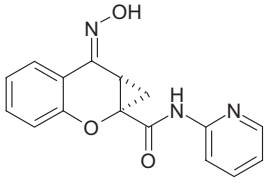
Although several novel types of positive allosteric modulators have been discovered (**XII–XVI** in Table 8) (Zhang et al., 2008b; Duplantier et al., 2009; Brnardic et al., 2010; Cid et al., 2010; D'Alessandro et al., 2010; Tresadern et al., 2010), only few negative allosteric modulators of group II mGlu receptors are known. For two non–amino acid-based classes of group II antagonists (Kolczewski et al., 1999; Wichmann et al., 1999), it has not been clearly established whether they are competitive or allosteric. Dihydro-benzo[*b*][1,4]diazepin-2-one derivatives (represented by compounds **XVII** and **XVIII** in Table 8) were found as group II mGlu receptor antagonists in an HTS campaign (Woltering et al., 2007) and characterized in more detail by Hemstapat et al. (2007). These compounds inhibit glutamate responses at both mGlu2 and mGlu3 receptors in calcium mobilization and GTPγ^{35}S assays but have no effect at group I and III mGlu receptors. The best compounds in this series (MNI-136 and MNI-137, Table 8) have potencies in the range of 10 to 100 nM. They lower the maximal effects of glutamate, but not its binding affinity, indicating that their mechanism of action is negative allosteric modulation of efficacy. They also potently reverse the mGlu2/3 receptor-mediated inhibition of EPSPs at the perforant path synapse in the hippocampus. These compounds do not displace the competitive antagonist radioligand [^{3}H]LY341495 from the orthosteric binding site. It is noteworthy that point mutations in transmembrane regions 4 and 5, which abolished the effects of positive allosteric mGlu2 receptor modulators, did not affect the inhibitory effects of these new compounds, pointing to the possible existence of separate binding sites for positive and negative allosteric group II mGlu receptor modulators (Hemstapat et al., 2007). The somewhat unusual lack of subtype-selectivity of these agents strongly suggests that this separate binding site probably would be common to mGlu2 and mGlu3 receptors. Schann et al. (2010) have used a truncated construct of the mGlu2 receptor, in which the N-terminal region was replaced by green fluorescent protein, for a FRET-based binding assay. In this way they discovered a close analog of the positive mGlu4 receptor modulator PHCCC (Table 9), which bound to the 7TM domain of the mGlu2 receptor, but was inactive in subsequent functional tests. Surprisingly, when making simple derivatives of this neutral allosteric ligand, they obtained the first compounds with opposite activities at mGlu2 (negative allosteric modulation) and mGlu3 receptors (positive allosteric modulation) (see the article by Schann et al. (2010) for structures).

C. Allosteric Ligands Acting at Group III Metabotropic Glutamate Receptors

Many more allosteric modulators have been described so far for group I and II mGlu receptors than for their counterparts belonging to group III. To date, within group III, allosteric ligands have mainly been found for mGlu4 and mGlu7 receptors. Some of them have most interesting and unique properties. It is remarkable that some compounds known as allosteric modulators for group I mGlu receptors have opposite effects on group III mGlu receptors (for detailed review, see Mathiesen and Ramirez, 2006). For example, the positive allosteric mGlu5 receptor modulators CPPHA and DFB (Table 5) have been found to have at the same time some negative allosteric effects on mGlu4, mGlu8, and (DFB only) mGlu7 receptors in calcium assays (O'Brien et al., 2003, 2004). This is even more striking in light of the fact that CPPHA and DFB exert their allosteric effects through distinct sites in the 7TM region of the mGlu5 receptor (see section III.A.4). Their binding sites on group III mGlu receptors have not yet been identified. On the other hand, in a GTPγ^{35}S assay using membranes from BHK cells expressing mGlu4 receptors, the negative allosteric mGlu5 receptor modulators MPEP and SIB-1893 (Table 2) both increased the maximal response and potency of L-AP4 (Mathiesen et al., 2003). MPEP and SIB-1893 had no effect on their own, and the enhancement of the stimulation produced by the agonist L-AP4 was abolished by the competitive antagonist (*R*,*S*)-α-cyclopropyl-4-phosphonophenyl glycine, indicating an allosteric mechanism of action, dependent on concomitant activation of the orthosteric site by an agonist. Both compounds did not displace the agonist radioligand [^{3}H]L-AP4 from its orthosteric binding site; on the contrary, [^{3}H]L-AP4 binding was increased by SIB-1893 but not MPEP. This finding indicates that SIB-1893 has positive binding cooperativity with respect to L-AP4, whereas MPEP has neutral affinity cooperativity. In a cellular system, both modulators enhanced the potency and maximal response of L-AP4 to inhibit forskolin-stimulated cAMP production in a BHK cell line expressing mGlu4 receptors. However, in this assay system both MPEP and SIB-1893 had an effect on their own. It was not clear whether this was due to the presence of endogenous glutamate or a true intrinsic efficacy of the modulators, which may become visible in sensitive systems with high receptor expression levels. In another cellular system, SIB-1893 and MPEP enhanced the Ca^{2+} mobilization response evoked by L-AP4 in a HEK cell line expressing the mGlu4 receptor, which had been cotransfected with the chimeric G-protein $G\alpha_{qo5}$, thus coupling mGlu4 receptors to the phospholipase C pathway (Marino et al., 2003). Of course, these mixed effects of compounds on group I and III mGlu receptors may be confounding in test systems on native receptors in vitro and in vivo.

TABLE 9
Positive allosteric mGlu4 receptor modulators

Compound potencies in vitro in one representative assay are given as an indication only. Because of the complexity of allosteric interactions, information on the positive modulation is not summarized here but can be found in section III.C.

Compound	Structure	Potency	Comments	References
I: (−)-PHCCC		EC_{50} = 4 μM	Close derivative of CPCCOEt (Table 6); has inhibitory activity at other mGlu receptor subtypes; decreases reserpine-induced akinesia in rats	Maj et al., 2003; Marino et al., 2003
II: VU0155041		EC_{50} = 750 nM	Decreases haloperidol-induced catalepsy and reserpine-induced akinesia in rats	Niswender et al., 2008a; Williams et al., 2009a
III: VU0080241		EC_{50} = 5 μM		Williams et al., 2009b
IV: VU0001171		EC_{50} = 1.7 μM		Williams et al., 2009b
V: VU0092145		EC_{50} = 3 μM		Williams et al., 2009b
VI:		EC_{50} = 200 nM		Engers et al., 2009a
VII: VU0359516		EC_{50} = 380 nM		Williams et al., 2010

Very similar effects have been found for the compound PHCCC (compound **I** in Table 9), which was described previously as a noncompetitive mGlu1 receptor antagonist (Annoura et al., 1996) [it is a structural analog of the negative allosteric mGlu1 receptor modulator CPCCOEt (Table 6)]. The compound increased both the potencies and maximal effects (more than 2-fold) of glutamate and L-AP4 in a GTPγ^{35}S assay (EC_{50} in the low micromolar range, depending on the agonist concentration) (Maj et al., 2003). The activity was found solely in the (−)-enantiomer of PHCCC. In a recombinant cell line expressing the human mGlu4 receptor, PHCCC enhanced agonist-induced inhibition of forskolin-stimulated cAMP formation (EC_{50} = 2.8 μM at 5 μM L-glutamate) (Maj et al., 2003). In the calcium mobilization assay described above, PHCCC also enhanced the effect of L-AP4 in a mGlu4 receptor expressing cell line with an EC_{50} of approximately 4 μM (Marino et al., 2003). The

structurally related compound CPCCOEt (Table 6) did not show positive mGlu4 receptor modulation. However, PHCCC has non-negligible antagonist effects at some of the other mGlu receptor subtypes (Marino et al., 2003), and it activates mGlu6 receptor as an agonist (Beqollari and Kammermeier, 2008). Using receptor chimeras, the binding site for PHCCC was shown to be localized, as expected, in the mGlu4 receptor 7TM domain (Maj et al., 2003).

Activation of mGlu4 receptors, and its positive allosteric modulation, have recently attracted great interest as a possible new strategy for the treatment of Parkinson's disease (Lavreysen and Dautzenberg, 2008; Lindsley et al., 2009) because of the observation that group III mGlu receptors modulate the striatopallidal synapse; application of the agonist L-AP4 inhibits GABAergic inhibitory postsynaptic currents via a presynaptic mechanism. This was not observed in mGlu4 receptor knockout mice, strongly indicating that this effect is mediated by presynaptic mGlu4 heteroreceptors located on GABAergic terminals (Valenti et al., 2003). The inhibition of the GABAergic striatopallidal synapse may well restore the balance in the basal ganglia motor circuit, which is believed to be impaired in Parkinson's disease (excessive inhibition of the globus pallidus). Accordingly, intracerebroventricular injections of L-AP4 produced beneficial effects in rodent models of Parkinson's disease (Valenti et al., 2003). In electrophysiological experiments in vitro, PHCCC has been shown to enhance the inhibition of GABAergic transmission produced by L-AP4 but has no effect on its own (Marino et al., 2003). On the other hand, PHCCC did not strengthen effects of L-AP4 on signaling at synapses expressing mGlu7 or mGlu8 receptors. In vivo, PHCCC also showed activity in a rodent model of Parkinsonism, namely a marked antiakinetic effect in reserpinized rats when administered on its own (Marino et al., 2003). The structurally closely related compound CPCCOEt, which does not enhance mGlu4 receptor function, had no effect in this model. The more recent mGlu4 receptor positive allosteric modulator *cis*-2-[[(3,5-dichlorophenyl)amino]carbonyl]cyclohexanecarboxylic acid (VU0155041; see below) has also been reported to have antiparkinsonian-like effects in rats (antagonism of haloperidol-induced catalepsy and reserpine-induced akinesia) (Niswender et al., 2008a). Finally, PHCCC has been found not only to reverse reserpine-induced akinesia but also to have neuroprotective effects by reducing nigrostriatal degeneration in the MPTP model of Parkinson's disease (Battaglia et al., 2006). This protective effect was not obtained in mGlu4 receptor knockout mice.

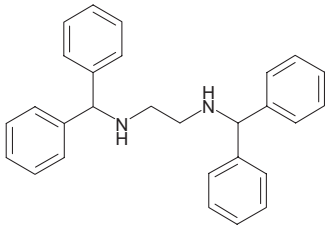
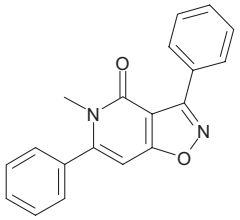
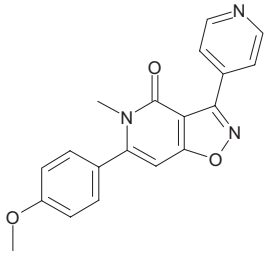
In models of NMDA- and β-amyloid-induced neurotoxicity in vitro, (−)-PHCCC enhanced the neuroprotective effect of L-AP4 (Maj et al., 2003). It also had such an effect when applied alone; this was most likely due to an increase in extracellular glutamate concentrations induced by the NMDA challenge. In fact, the neuroprotection provided by PHCCC alone was reversed by competitive (orthosteric) group III mGlu receptor antagonists (Maj et al., 2003). mGlu4 receptor modulators may well have a potential in further clinical indications as well (Lavreysen and Dautzenberg, 2008). PHCCC, when administered locally into the basolateral amygdala, displayed significant anticonflict effects in the Vogel punished drinking test, suggesting anxiolytic activity (Stachowicz et al., 2004). Intrathecal application of (−)-PHCCC reduces mechanical hyperalgesia in inflammatory and neuropathic pain models (Goudet et al., 2008). PHCCC also enhances the number of spike and wave discharges in WAG/Rij rats, which develop spontaneous absence seizures (Ngomba et al., 2008). Absence-like seizures in pentylenetetrazole-treated mice were also enhanced by PHCCC. These findings suggest that mGlu4 receptor activation plays a role in the generation of absence seizures and that negative modulators might have a place in this indication. However, before valid conclusions regarding the therapeutic potential of mGlu4 receptor modulators can be drawn, these results need to be confirmed with positive allosteric modulators, which are more selective than PHCCC, in addition to having better potency and bioavailability.

Big efforts to find improved mGlu4 receptor positive modulators were undertaken at Vanderbilt University. The compound VU0155041 (Table 9, compound **II**) (Niswender et al., 2008a; Williams et al., 2009a) is the result of a first attempt to optimize the PHCCC scaffold. However, little if any improvement in potency and cooperative effects compared with PHCCC was obtained from a large series of derivatives because of the "flat" SAR around this lead, as is often observed with allosteric modulators. Nevertheless, VU0155041, which also has some partial agonist activity, represents an improvement over PHCCC in terms of aqueous solubility and selectivity for the mGlu4 receptor. Subsequent HTS efforts then yielded novel structures of positive mGlu4 receptor modulators (Table 9, **III**–**V**) (Niswender et al., 2008b; Williams et al., 2009b), but little or no tractable SAR were again encountered. In a series of heterobiarylamides, some compounds with submicromolar potency and relatively good central penetration were found (Engers et al., 2009a). In Table 9, one of the two best compounds of the series is shown (compound **VI**). Very recently, Williams et al. (2010) have taken up the PHCCC lead structure again. The compound VU0359516 (Table 9, compound **VII**) has an improved potency (~4-fold) and cooperativity (2-fold) compared with (−)-PHCCC; moreover, unlike PHCCC, it is selective versus other mGlu receptors. In addition, novel chemotypes of positive mGlu4 receptor modulators have recently been disclosed by Merck and by ADDEX Pharmaceuticals. More structural information is given in the reviews by Lindsley et al. (2009) and East and Gerlach (2010).

A most interesting drug is the selective allosteric mGlu7 receptor agonist *N*,*N*'-dibenzhydrylethane-1,2-diamine dihydrochloride (AMN082) (Table 10) (Mitsukawa et al., 2005). This compound directly activates

TABLE 10
Allosteric ligands at the mGlu7 receptor

See section III.C for further information and references.

Compound	Structure	Potencies	Comments	References
I: AMN082		EC_{50} = 260 nM	Allosteric agonist, the first selective agonist for the mGlu7 receptor; active in vivo in various animal models.	Mitsukawa et al., 2005
II: MDIP		IC_{50} = 20 nM	Negative allosteric mGlu7 receptor modulator	Suzuki et al., 2007
III: MMPIP		IC_{50} = 26 nM	Negative allosteric mGlu7 receptor modulator with inverse agonist activity Inhibits effects of AMN082 in vitro	

MDIP, 5-methyl-3,6-diphenylisoxazolo[4,5-*c*]pyridin-4(5*H*)-one.

mGlu7 receptor signaling via an allosteric site in the 7TM domain. In the original characterization, AMN082 stimulated GTPγ^{35}S binding to membranes from mGlu7 receptor-expressing cells with a relatively high potency (EC_{50} = 260 nM) and a maximal response comparable with that of L-AP4 but clearly superior to that of glutamate. Likewise, AMN082 inhibited forskolin-stimulated cAMP formation with an IC_{50} of 54 nM. It was inactive in either stimulating or inhibiting GTPγ^{35}S binding or inositol phosphate hydrolysis in assays for all other mGlu receptor subtypes. It is noteworthy that adding fixed concentrations of glutamate only marginally, if at all, increased the potency of AMN082 in the GTPγ^{35}S assay and vice versa. Moreover, addition of 3 μM AMN082 did not appreciably affect the affinities of the agonists L-serine-*o*-phosphate, L-AP4, and glutamate as displacers of the radioligand [^{3}H]LY341495 from the orthosteric binding site. AMN082 did not directly displace the radioligand either. Thus, there are obviously no significant cooperative interactions between orthosteric agonists and AMN082. Experiments using chimeric mGlu6/7 receptor constructs allowed to localize the binding site for AMN082 to the 7TM domain. Taken together, these results clearly show that AMN082 is an allosteric mGlu7 receptor agonist (i.e., a compound that is able to stimulate the receptor on its own via a site distinct from the orthosteric agonist site) (Mitsukawa et al., 2005). It has no cooperative effects on the activation of that site by agonists. Subsequently, Suzuki et al. (2007b) have reported that although AMN087 inhibits forskolin-stimulated cAMP accumulation in CHO cells expressing rat or human mGlu7 receptors, it did not induce intracellular Ca^{2+} mobilization in CHO cells coexpressing the rat mGlu7 receptor together with the promiscuous G-protein Gα_{15}. In line with this finding, AMN082 has also been found, unlike L-AP4, not to activate GIRK potassium channels through mGlu7 receptors coexpressed in HEK293 cells and not to stimulate mGlu7 receptors at the Schaffer collateral-CA1 synapse in the adult rat hippocampus (Ayala et al., 2008). This is a nice example of "agonist-directed trafficking" (reviewed by Urban et al., 2007)—in this case via an allosteric site! This must be kept in mind when AMN082 is used as a pharmacological tool to elucidate the role of mGlu7 receptors in a given physiological context.

Using immunofluorescence and other labeling techniques, Pelkey et al. (2007) have demonstrated that exposure of dissociated hippocampal cultured neurons to AMN082 leads to robust mGlu7 receptor internalization, much like the receptor endocytosis induced by the orthosteric mGlu7 receptor agonist L-AP4. This shows that AMN082 not only activates the mGlu7 receptor intracellular signal transduction cascade (inhibition of cAMP formation), but also the receptor endocytosis/desensitization pathway. Although this might make it a

useful tool for the study of the mGlu7 receptor internalization process, it seems that this allosteric agonist does not share the advantage of the $GABA_B$ positive modulator GS39783, which allows receptor activation without leading to desensitization (Gjoni and Urwyler, 2008) (see section IV.D).

AMN082 is also a striking example of how better bioavailability and brain penetration can be obtained with an allosteric drug, compared with its orthosteric, amino acid-like counterparts. In fact, 1 h after oral administration of 10 mg/kg to mice or rats, pharmacologically relevant concentrations of AMN082 were found in total brain tissue (Mitsukawa et al., 2005). Because the mGlu7 receptor plays a role in the regulation of hormonal responses to stress situations (Mitsukawa et al., 2006), the effect of oral administration of AMN082 on serum levels of the stress hormones adrenocorticotrophin and corticosterone was analyzed. A dose of 6 mg/kg increased the levels of both hormones by approximately 2-fold in wild type but not in mGlu7 receptor-deficient knockout mice (Mitsukawa et al., 2005). This finding strongly suggests that mGlu7 receptors are involved in conditions involving chronic stress (e.g., anxiety and depression), an interpretation that is in line with the anxiolytic-like phenotype of mGlu7 receptor knockout animals (Callaerts-Vegh et al., 2006) (for review, see O'Connor et al., 2010). Therefore, the anxiolytic effects of AMN082 described below are unexpected and seem paradoxical.

The fact that AMN082 is the first selective mGlu7 receptor agonist has raised great hopes in this compound as a pharmacological tool to elucidate the physiological role of this receptor. However, the effects of this compound have turned out to be complex, and its property of pathway-selective agonism outlined above adds a caveat in the interpretation of experimental data: a lack of effect does not necessarily mean that the mGlu7 receptor is not involved in a given function under study. mGlu7 receptors are widely distributed throughout the brain and mainly localized as presynaptic receptors at the active zones mainly of GABAergic and glutamatergic neurons. Li et al. (2008) have interpreted the results from their in vivo microdialysis study such that mGlu7 receptor activation by AMN082 in the nucleus accumbens modulates nonvesicular GABA and glutamate release by a G_i protein-coupled mechanism. In the hands of Ugolini et al. (2008), AMN082 inhibited afferent glutamatergic transmission in a slice preparation of the basolateral amygdala, a brain region known to be involved in the generation of anxiety. This inhibition was observed only at high (2 Hz) and not at lower (0.05 Hz) stimulation frequencies, suggesting that AMN082 actually acts as an allosteric modulator (its effect being dependent on the amount of glutamate released) rather than an allosteric agonist (in which case its effect would be frequency-independent; see Fig. 11). This interpretation is supported by the observation that the orthosteric agonist L-AP4 inhibited synaptic transmission also at the lower stimulation frequency, and this effect was enhanced by AMN082. In the context of effects on synaptic signaling in the amygdala, AMN082 inhibits long-term potentiation (LTP) in this brain region (Fendt et al., 2008). Accordingly, AMN082 also blocks the acquisition of conditioned fear [measured in the fear-potentiated startle paradigm (Fendt et al., 2008; Siegl et al., 2008)], which is believed to be an example of amygdala-dependent learning. Rather paradoxically, however, AMN082 at the same time facilitated the extinction of aversive memories, which is also considered an active learning process (Fendt et al., 2008). O'Connor et al. (2010) have suggested that this observation might be related to different roles of mGlu7 receptors in different brain regions, the amygdala being responsible for fear acquisition and the prefrontal cortex, on the other hand, for extinction learning. In any case, the combination of inhibition of fear acquisition and the facilitation of its extinction predicts anxiolytic effects of AMN082, which were actually found by Stachowicz et al. (2008) in wild-type, but not in mGlu7 receptor knockout mice. Likewise, antidepressant activity was seen with AMN082 administered intraperitoneally in the forced swim and the tail suspension tests only in wild type but not in mice lacking mGlu7 receptors (Palucha et al., 2007). However, significant inhibition of spontaneous locomotion was observed at higher doses of AMN082 also in knockout animals, suggesting off-target effects of the compound. As mentioned above, the anxiolytic-like activity of AMN082 seems to be in contradiction with the anxiolytic-like phenotype of mGlu7 receptor knockout animals. Of course, however, the possibility of developmental adaptations occurring in knockout animals should always be considered in such situations. Moreover, one could also speculate about a possible role of receptor desensitization (internalization) induced by AMN082 in its anxiolytic-like effects.

The prominent expression of mGlu7 receptors in the basal ganglia has prompted Greco et al. (2010) to investigate effects of AMN082 in animal models of Parkinson's disease. They observed a reversal of haloperidol-induced catalepsy in rats at only one low (5 mg/kg p.o.), but not at higher doses, and a seemingly paradoxical inhibition of apomorphine-induced circling behavior in animals with unilateral 6-OHDA lesions, again with an inverse dose-response relationship. It is noteworthy that AMN082 also improved the reaction time of bilaterally 6-OHDA-lesioned rats in a more complex task involving responding to a visual cue, which is impaired in akinetic animals. The utility of mGlu7 receptor activation as a therapeutical basis in Parkinsonism awaits further investigation. In the prospect of other potential indications, AMN082 (1 or 5 mg/kg i.p.) inhibited thermal hyperalgesia in inflammatory and surgical pain models (Dolan et al., 2009). Finally, AMN082 has been reported to inhibit the rewarding effects of cocaine

and cocaine self-administration in a dose-dependent manner upon peripheral or central administration (Li et al., 2009).

The isoxazolopyridone compounds II/III shown in Table 10 have been characterized extensively by Suzuki et al. (2007b). 5-Methyl-3,6-diphenylisoxazolo[4,5-*c*]pyridin-4(5*H*)-one and MMPIP inhibited L-AP4-induced Ca^{2+} mobilization in CHO cells coexpressing the rat mGlu7 receptor with the promiscuous G-protein $G\alpha_{15}$, with IC_{50} values of 20 to 30 nM. The maximum agonist response was reduced in the presence of MMPIP; and this compound did not inhibit the binding of the orthosteric antagonist [^{3}H]LY341495, two typical signs of negative allosteric modulation. Both compounds also reverted L-AP4-induced inhibition of forskolin-stimulated cAMP production in mGlu7 receptor-expressing cells. MMPIP had no effect in functional assays for the other mGlu receptor subtypes. Most interestingly, MMPIP was able to antagonize the inhibition of cAMP formation produced by the allosteric agonist AMN082. Given the available data, it was unclear whether this inhibition was competitive versus AMN082 (i.e., whether the two compounds bind to the same allosteric site). When applied alone, MMPIP caused a further increase in forskolin-stimulated cAMP levels in mGlu7 receptor-expressing cells, strongly suggesting allosteric inverse agonist activity, which was not seen with the competitive antagonist LY341495. Very much like in the situation with AMN082, the effects of MMPIP seem to be context-dependent. In the hands of Niswender et al. (2010), MMPIP inhibited L-AP4-induced intracellular calcium mobilization in a BHK cell line coexpressing the mGlu7 receptor and the promiscuous G-protein $G\alpha_{15}$. On the other hand, MMPIP was clearly less active in inhibiting mGlu7 receptor-mediated regulation of GIRK channels, with a potency in the low micromolar range. Moreover, contrary to the findings reported by Suzuki et al. (2007b), who used CHO cells, it did not reverse the L-AP4-induced inhibition of cAMP formation in mGlu7 receptor-expressing HEK cells. Finally, MMPIP did also not block the mGlu7 receptor-mediated reduction of synaptic signaling at the Schaffer Collateral-CA1 synapse (of note, AMN082 did not have agonistic activity in this paradigm, see above). This is a nice example of "permissive antagonism," meaning that a compound can block one or more particular signaling pathways, but not others (Kenakin, 2005; Leach et al., 2007; Kenakin and Miller, 2010).

Up to now, very few studies have addressed the in vivo effects of negative allosteric mGlu7 receptor modulators. First, local coadministration of MMPIP blocks the effects of AMN082 on cocaine reward and self-administration described above (Li et al., 2009). Very recently, MMPIP at doses ranging from 3 to 30 mg/kg s.c. has been reported to impair cognitive performance in mice (object recognition test, object location test) and to reduce social exploration in rats but to have no effects in a number of other behavioral (locomotor, sensory processing, muscle relaxant, anxiolytic, antidepressant, analgesic, seizure threshold) models (Hikichi et al., 2010a).

L-AP4, the prototypical mGlu7 receptor agonist available so far, activates this receptor only at concentrations much higher than those required to stimulate its closest relatives, the other group III mGlu receptors (mGlu4, -6, and -8). Therefore, it has been impossible to elucidate the physiological role of mGlu7 receptors with this type of pharmacological tool without confounding effects induced by activation of these other receptors (Conn and Niswender, 2006). For this reason, the availability of the highly potent, selective and (at least proven for AMN082) bioavailable allosteric tool compounds AMN082 (agonist) and 5-methyl-3,6-diphenylisoxazolo[4,5-*c*]pyridin-4(5*H*)-one/MMPIP (antagonist/inverse agonist) should represent a breakthrough in the study of the physiological function of this seemingly most important receptor. A true positive modulator would be a useful add-on to the pharmacological mGlu7 receptor toolbox. The same holds of course true for the other subtypes of group III mGlu receptors (i.e., mGlu6 and mGlu8).

IV. $GABA_B$ Receptor Function Involves Allosteric Interactions across a Heterodimer

A. $GABA_B$ Receptor Structure and Function

Baclofen [i.e., racemic *β-p*-chlorophenyl-GABA (Lioresal)], a lipophilic, brain-penetrating analog of GABA, was introduced in the beginning of the 1970s as a muscle-relaxant drug used in the treatment of spasticity in patients suffering from, e.g., multiple sclerosis or spinal injury. Only several years later, its use as a pharmacological tool in neurotransmitter release and radioligand binding assays led to the discovery of a novel, bicuculline-insensitive GABA receptor (Bowery et al., 1980; Hill and Bowery, 1981). It was termed the "$GABA_B$" receptor, as opposed to its bicuculline-sensitive, baclofen-insensitive "$GABA_A$" counterpart. Subsequently, it became clear from numerous biochemical and physiological studies that the $GABA_B$ receptor has an inhibitory nature but, unlike the ionotropic $GABA_A$ receptor, coupled to G-protein–mediated intracellular pathways. Over time, a great number of useful pharmacological tools, potent and selective agonists and antagonists, for the $GABA_B$ receptor also became available (for review, see Bowery et al., 2002). Nevertheless, the agonist baclofen remains the only therapeutically used drug acting via $GABA_B$ receptors.

Although the existence and the properties of the $GABA_B$ receptor were well established on pharmacological and physiological grounds, its molecular entity remained elusive for a long time. It was not until 1997 that a complementary DNA, encoding a $GABA_B$ receptor protein, was successfully cloned for the first time (Kaupmann et al., 1997). It was found to resemble mGlu re-

ceptors and to exist in two distinct variants, named $GABA_{B1a}$ (containing 960 amino acids) and $GABA_{B1b}$ (844 amino acids), which differ in the presence or the absence, respectively, of an N-terminal complement protein sequence ("Sushi repeats" [Hawrot et al., 1998]). These isoforms arise from the initiation of transcription at different sites, under the control of distinct promoters. The existence of true splice variants of the $GABA_{B1}$ protein has also been shown (summarized by Bettler et al., 2004). Surprisingly, although heterologous expression of either $GABA_B$ receptor protein in a recombinant cell line allowed measuring the binding of high-affinity antagonist radioligands, a considerably lower binding affinity for agonists compared with native receptors was found, and it did not make possible the measurement of robust functional responses (Kaupmann et al., 1997). This finding remained enigmatic until several groups reported at the same time the cloning of a second $GABA_B$ receptor protein [$GABA_{B2}$] containing 941 amino acids (110 kDa) (Jones et al., 1998; Kaupmann et al., 1998; White et al., 1998; Kuner et al., 1999). $GABA_{B1}$ and $GABA_{B2}$ are derived from different genes and share 35% sequence identity and approximately 50% similarity. The coexpression of $GABA_{B1}$ and $GABA_{B2}$ to form heterodimeric assemblies (Fig. 12), at that time a unique feature among GPCRs, is a prerequisite to form functional $GABA_B$ receptor entities.

Both $GABA_B$ receptor proteins are part of "family C" GPCRs. Unlike most other GPCRs belonging to this class; however, the $GABA_B$ receptor does not have an extracellular cysteine-rich sequence between the ligand-binding and the 7TM domains. Although both $GABA_B$ receptor subunits contain the VFTM (Fig. 12), only $GABA_{B1}$ can bind GABA and other agonist or competitive antagonist ligands, because amino acids critical for ligand binding are not conserved in the flytrap module of $GABA_{B2}$ (Galvez et al., 2000a). Despite the inability of the $GABA_{B2}$ subunit to bind orthosteric ligands, it is essential for $GABA_B$ receptor expression and function in several ways. $GABA_{B1}$ alone cannot be transported to the cell surface, because a retention signal at its C terminus prevents its release from the endoplasmic reticulum. The interaction of the C-terminal parts of $GABA_{B1}$ and $GABA_{B2}$, forming a coiled-coil domain, masks this retention signal, thus making possible the transport of the heterodimeric complex to the plasma membrane. Moreover, the extracellular N-terminal domain of $GABA_{B2}$ interacts allosterically with its $GABA_{B1}$ counterpart and thus facilitates agonist binding, resulting in the agonist affinities that have been observed for native $GABA_B$ receptors (Galvez et al., 2001). Finally, whereas receptor activation is initiated by agonist binding to the VFTM of $GABA_{B1}$, it is the $GABA_{B2}$ subunit that transmits the signal to the intracellular effector systems via an interaction of its second intracellular loop with matching G-proteins (Fig. 12) (Margeta-Mitrovic et al., 2001; Robbins et al., 2001; Grünewald et al., 2003; Havlickova et al., 2003). $GABA_B$ receptors couple to different intracellular effector systems. Their activation produces a robust, pertussis toxin-sensitive inhibition of forskolin-stimulated adenylyl cyclase activity in native and recombinant receptor assay systems (Wojcik and Neff, 1984; Cunningham and Enna, 1996; Knight and Bowery, 1996; Wise et al., 1999; Hirst et al., 2003). On the other hand, baclofen has been reported to enhance the activity of adenylyl cyclase stimulated by corticotropin-releasing hormone, pituitary adenylate cyclase-activating peptide, or noradrenaline in native systems (Knight and Bowery, 1996; Olianas and Onali, 1999; Onali and Olianas, 2001). These effects are most likely due to receptor-receptor interactions ("cross-talk") and seem, like the stimulation of basal adenylyl cyclase activity, to involve signaling by β,γ–G-protein subunits (Olianas and Onali, 1999; Onali and Olianas, 2001). Also via this pathway, $GABA_B$ receptors are linked to G-protein coupled inwardly rectifying potassium channels (Kir3-type, "GIRK") (Lüscher et al., 1997). The activation of these channels is responsible for the late, long-lasting component of the inhibitory postsynaptic potentials. The ionotropic $GABA_A$ receptor, on the other hand, mediates the fast, short-lasting component of inhibitory postsynaptic potentials. Furthermore, presumably via the inhibition of voltage-sensitive calcium channels (Takahashi et al., 1998), presynaptic $GABA_B$ auto- and heteroreceptors inhibit the depolarization-induced release of a number of neurotransmitters, such as GABA itself or various neuropeptides, catecholamines, acetylcholine, or glutamate (for review, see Bettler et al., 2004).

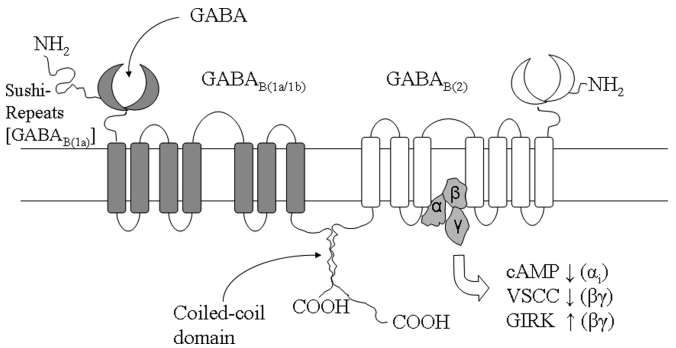


FIG. 12. Schematic representation of the structure of the $GABA_B$ receptor. The receptor consists of a heterodimeric assembly of two distinct subunits, $GABA_{(B1)}$ and $GABA_{(B2)}$. The ligand binding domain is formed by two VFTM lobes in $GABA_{(B1)}$ that close upon agonist binding. Competitive antagonists bind to the same site without closing the VFTM. The homologous part in $GABA_{(B2)}$ is unable to bind orthosteric ligands. On the other hand, the $GABA_{(B2)}$ subunit couples to heterotrimeric G-proteins made up of α-, β-, and γ-subunits. Upon receptor activation, the α_i-subunit inhibits cAMP formation, whereas $\beta\gamma$-signaling inhibits presynaptic voltage-sensitive calcium channels (VSCC) and activates postsynaptic GIRKs. Moreover, the $GABA_{(B2)}$ subunit is essential for the expression of the receptor at the cell surface by masking a retention signal in the C-terminal part of $GABA_{(B1)}$ via a coiled-coil interaction in the intracellular domains of the two subunits.

B. The Discovery and Early Characterization of Allosteric $GABA_B$ Receptor Modulators

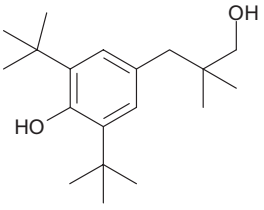
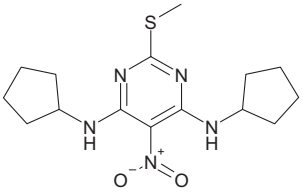
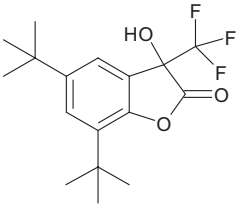
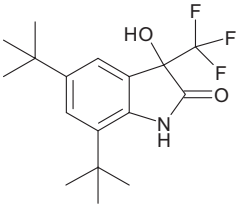
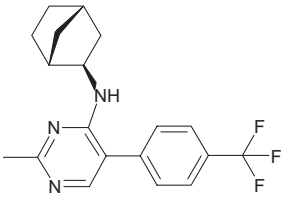
The first positive allosteric $GABA_B$ receptor modulators were discovered by compound screening using a $GTP\gamma^{35}S$ assay and membranes from a CHO cell line stably expressing the $GABA_B$ receptor (Urwyler et al., 2001, 2003) and have since then been the subject of numerous in vitro and in vivo studies (for review, see Urwyler, 2006; Adams and Lawrence, 2007, Pin and Prézeau, 2007). The two compounds CGP7930 and GS39783 (Table 11), and a number of analogs thereof, enhance the stimulation of $GTP\gamma^{35}S$ binding by GABA or baclofen without activating it by themselves. No enhancement of GABA-stimulated $GTP\gamma^{35}S$ binding by CGP7930 or GS39783 was observed in the presence of the competitive antagonist [3-[1-(*R*)-[[3-cyclohexylmethyl)hydroxyphosphinyl]-2-(*S*)-hydroxypropyl]amino]ethyl]-benzoic acid, monolithium salt (CGP56999A), further confirming that the effects of these compounds are dependent on the simultaneous activation of the receptor by an agonist. The results obtained with both modulators in $GTP\gamma^{35}S$ assays using the recombinant $GABA_B$ receptor-expressing cell line were very similar to those found with native receptors in rat brain membranes (Urwyler et al., 2001, 2003) or in samples from human postmortem brain tissue (Olianas et al., 2005).

A striking characteristic of the potentiating effects of CGP7930 and GS39783 on agonist stimulation of native and recombinant $GABA_B$ receptors is the finding that both the potency and the maximal response of GABA are

TABLE 11

Positive allosteric $GABA_B$ receptor modulators

Information about compound potencies in vitro were obtained in different assay systems and should be compared with caution. Information on the cooperative effects of the compounds is given in section IV. No negative allosteric $GABA_B$ receptor modulators have been described to date.

Compound	Structure	Potencies (EC_{50})	Comments	References
CGP7930		4.6 μM	The first positive $GABA_B$ allosteric modulator reported, together with its aldehyde analog CGP13501; in vivo activity in models of anxiety and pain	Urwyler et al., 2001; Jacobson and Cryan, 2008; Brusberg et al., 2009
GS39783		3.5 μM	Several analogs are also active in vitro; GS39783 is active in biochemical and behavioral models in vivo (anxiolytic-like, drug abuse)	Urwyler et al., 2003; Cryan et al., 2004; Gjoni et al., 2006; Lhuillier et al., 2007
rac-BHFF		234 nM	Anxiolytic-like activity in the SIH model in mice	Malherbe et al., 2008
BHFI		289 nM	Nonhydrolyzable, more stable analog of rac-BHFF	Malherbe et al., 2008
BHF177		1.7 μM		Guery et al., 2007

BHFI, 5,7-di-*tert*-butyl-3-hydroxy-3-trifluoromethyl-1,3-dihydro-indol-2-one.

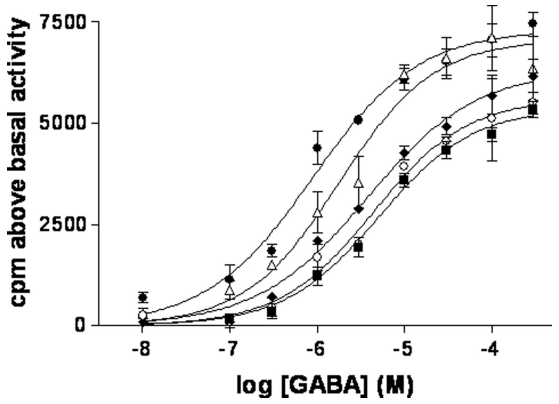


FIG. 13. CGP7930 enhances at the same time the potency and the maximal efficacy of GABA as an agonist at the $GABA_B$ receptor. A GTPγ[^{35}S] binding assay was conducted with membranes from a recombinant $GABA_B$ expressing CHO cell line. GABA concentration-response curves were measured in the absence (■) and in the presence (○, 1 μM; ◆, 3 μM; △, 10 μM; ●, 30 μM) of CGP7930. [Reproduced from Urwyler S, Mosbacher J, Lingenhoehl K, Heid J, Hofstetter K, Froestl W, Bettler B, and Kaupmann K (2001) Positive allosteric modulation of native and recombinant, γ-aminobutyric $acid_B$ receptors by 2,6-di-*tert*-butyl-4-(3-hydroxy-2,2-dimethyl-propyl)-phenol (CGP7930) and its aldehyde analog CGP13501. *Mol Pharmacol* **60:**963–971. Copyright © 2001 American Society for Pharmacology and Experimental Therapeutics. Used with permission.]

enhanced (Fig. 13). Whereas the EC_{50} values for GABA were decreased 5- to 10-fold in the presence of maximally active concentrations of the two modulators, the maximal stimulation of GTPγ^{35}S binding at saturating GABA concentrations was 1.5- to 2.0-fold of that obtained in the absence of modulators (Urwyler et al., 2001, 2003). This dual action was subsequently confirmed in other assay systems as well.

The phenomenon that some allosteric modulators enhance not only agonist affinity but also efficacy is explained by recently developed theoretical receptor models (see *Introduction*) (Hall, 2000; Christopoulos and Kenakin, 2002). These models are basically applicable to all kinds of orthosteric ligands, independently of their intrinsic properties. This implies that partial agonists, inverse agonists, and competitive antagonists should a priori be as much amenable to allosteric modulation as full agonists. In fact, we have found that in a GTPγ^{35}S assay, the maximal response of the partial agonist CGP47656 was enhanced by approximately 4-fold by CGP7930 and GS39783, compared with only approximately 1.5- to 2-fold increases found with GABA (Urwyler et al., 2005). Mannoury la Cour et al. (2008) have made similar observations with regard to the partial agonist 3-aminopropyl(methyl)phosphinic acid (SKF97541). Obviously, the less efficacious receptor activation with a partial agonist leaves more room for an increase in the maximal agonist response. Further in line with this concept, two compounds [(3-aminopropyl)(diethoxymethyl) phosphinic acid (CGP35348) and 2-hydroxy-saclofen], which have previously been considered neutral (or "silent") $GABA_B$ receptor antagonists (Bowery et al., 2002) did not stimulate GTPγ^{35}S binding at all on their own but became partial agonists in the presence of CGP7930 or GS39783. Thus, apparently the modulators amplified hidden, marginal agonistic properties of these two orthosteric ligands that could normally not be detected in the insufficiently sensitive GTPγ^{35}S assay. On the other hand, we have also found in the same study (Urwyler et al., 2005) that the binding affinities of a number of competitive $GABA_B$ receptor antagonists were decreased in the presence of CGP7930 or GS39783—a good example of the probe dependence of allosteric interactions.

Onali et al. (2003) have confirmed the allosteric effects of CGP7930 on native $GABA_B$ receptors by cAMP measurements in membranes from different rat brain regions. Their experiments comprised the study of basal and forskolin-, corticotropin-releasing hormone-, and Ca^{2+}/calmodulin-stimulated adenylyl cyclase activity and thus demonstrated that both signaling pathways via the α- and the β/γ- subunits of $G_{i/o}$-proteins are affected by the allosteric modulator.

Mannoury la Cour et al. (2008) have investigated the influence of positive allosteric modulators on $GABA_B$ receptor coupling to different G-protein subtypes with the use of an immunocapture scintillation proximity assay system (DeLapp, 2004). GABA and (*R*)-baclofen activated $G\alpha_o$ and $G\alpha_{i1/3}$ in rat cortex, hippocampus, and cerebellum, whereas $G\alpha_q$ and $G\alpha_{s/olf}$ were unaffected. CGP7930, 3,5-*bis*(1,1-dimethylethyl)-4-hydroxy-α,α-dimethylbenzenepropanal (CGP13501) and, more markedly, GS39783 enhanced agonist potencies and maximal responses for the stimulation of GTPγ^{35}S binding to $G\alpha_o$ in all three brain regions. On their own, the three modulators were inactive. On the other hand, they did not modify agonist-induced $GABA_B$ receptor coupling to $G\alpha_{i1/3}$. In recombinant HEK293 cells expressing $GABA_{B(1a + 2)}$ or $GABA_{B(1b + 2)}$, however, the modulators massively (almost up to 3 log units for GS39783) enhanced the potency but not the maximal effect of GABA to stimulate GTPγ^{35}S binding to $G\alpha_{i1/3}$. The lack of effects on the maximal response, however, might be due to a low level of $G\alpha_{i1/3}$ protein available for coupling, being the limiting factor. This might also apply to the situation in native brain tissue. These results illustrate the dependence of ligand effects on the system used to measure responses (see section I.D). Nevertheless, they are also indicative of the possibility that positive allosteric modulators might differentially affect the coupling of $GABA_B$ receptors to specific G-protein subtypes.

Several chemistry groups have attempted to improve on CGP7930 and GS39783, mostly by taking these two lead molecules as starting points for derivatization. Kerr et al. (2006, 2007) made a series of analogs of CGP7930, none of which, however, surpassed the lead compound in terms of potency and degree of cooperativity. Malherbe et al. (2008) have identified rac-BHFF (Table 11), which is structurally related to CGP7930, as a positive allosteric modulator at recombinant $GABA_B$ receptors. Similar to results obtained with CGP7930, this compound enhanced both the potency and maximal effect of GABA in

GTPγ^{35}S binding and intracellular Ca^{2+} mobilization (FLIPR) assays. (+)-BHFF was more potent than the (−)-enantiomer, the first example of stereoselectivity at the $GABA_B$ allosteric modulator binding site. Rac-BHFF was quickly hydrolyzed in vivo; for this reason, the authors also synthesized the nonhydrolyzable lactam analog 5,7-di-*tert*-butyl-3-hydroxy-3-trifluoromethyl-1,3-dihydroindol-2-one (BHFI; Table 11). Unlike CGP7930, BHFF and 5,7-di-*tert*-butyl-3-hydroxy-3-trifluoromethyl-1,3-dihydroindol-2-one also significantly stimulated GTPγ^{35}S binding in the absence of GABA; i.e., they acted as allosteric agonists. In an effort to obtain molecules without the genotoxic potential of GS39783, Guery et al. (2007) made a number of analogs lacking the nitro group of the lead compound, first by replacing it with a nitro-mimetic such as a trifluoromethyl group, but then also by making molecules more distantly related to GS39783. The most active compound from their series is BHF177 (Table 11), with a potency and cooperativity in the GTPγ^{35}S binding assay similar to those of GS39783. Other structural classes have been claimed as positive allosteric $GABA_B$ receptor modulators in a few patent applications; the current patent situation is reviewed elsewhere (Froestl, 2010). Apparently, no negative allosteric $GABA_B$ receptor modulators are known at present.

C. Interactions between Allosteric and Orthosteric Ligand Binding Sites: Mapping of the Allosteric Modulator Site on $GABA_B$ Receptors

Radioligand-binding experiments provide a powerful tool to analyze the mechanisms underlying allosteric receptor modulation. In saturation experiments with the agonist radioligand [^{3}H]3-aminopropylphosphinic acid, CGP7930 produced an increase of its binding affinity to $GABA_B$ receptors in rat cortical membranes (Urwyler et al., 2001). At the same time, a small increase in the maximal binding capacity was also observed. An increase of agonist affinity produced by allosteric modulators was also visible in displacement experiments. The curves describing the inhibition of the binding of the antagonist radioligand [^{3}H]3-[(1*R*)-1-[[(2*S*)-2-hydroxy-3-[hydroxy-[(4-methoxyphenyl)methyl]phosphoryl]propyl]-methylamino]ethyl]benzoic acid (CGP62349) to native $GABA_B$ receptors by agonists were biphasic, consisting of a high-affinity and a low-affinity component, most likely reflecting the G-protein-coupled and -uncoupled states of the $GABA_B$ receptor (Urwyler et al., 2003, 2004). The allosteric modulators CGP7930 and GS39783 enhanced the agonist affinities of both components. Moreover, the two modulators also increased the relative proportion of high agonist affinity sites. This finding strongly suggests that the allosteric agents not only promote agonist binding to the orthosteric site but also strengthen the interaction of $GABA_B$ receptors with their associated G-proteins. The increase in receptor-G-protein coupling produced by CGP7930 explains the increase in the number of binding sites for [^{3}H]3-aminopropylphosphinic acid, which only labels the high agonist affinity (G-protein-coupled) receptor state. On the other hand, the antagonist radioligand [^{3}H]CGP62349 labels both receptor forms, which are visible in agonist-displacement experiments. Accordingly, the maximal binding capacity for this radioligand remained unchanged in the presence of CGP7930 or GS39783 (Urwyler et al., 2005). Complex results were obtained from radioligand-binding experiments performed with recombinant $GABA_B$ receptor preparations (Urwyler et al., 2001). In membranes from CHO cells expressing the $GABA_{B1}$ subunit only, the binding of the radioligand [^{3}H]CGP62349 was not inhibited by CGP7930, confirming that this compound does not bind to the orthosteric receptor site. GABA displaced the binding of [^{3}H]CGP62349 with low affinity (IC_{50} ~ 100 μM), which remained unchanged in the presence of CGP7930. On the other hand, when the same experiments were performed using membranes from cells expressing both $GABA_B$ receptor subunits, biphasic displacement curves were obtained with GABA. The IC_{50} values of the low-affinity component were close to those found with the $GABA_{B1}$ monomer and remained unchanged in the presence of CGP7930, whereas the IC_{50} of the high-affinity component was decreased by CGP7930 by 2.5-fold. The low-affinity component comprised approximately 70% of the total ligand binding and in this case was attributed to an overexpression of the $GABA_{B1}$ subunit in the stable CHO cell line. These results demonstrate that the presence of the $GABA_{B2}$ subunit is essential for the modulation of agonist-binding affinity by CGP7930. Binet et al. (2004) have confirmed and extended this interpretation by demonstrating that CGP7930 binds to and directly activates the heptahelical domain of $GABA_{B2}$ (see section IV.D). The *Drosophila melanogaster* $GABA_B$ receptor is not amenable to allosteric modulation by GS39783 (Dupuis et al., 2006). However, coexpression of *D. melanogaster* $GABA_{B1}$ with rat $GABA_{B2}$ resulted in an enhancement of $GABA_B$ receptor function by GS39783. Data obtained with rat/*D. melanogaster* $GABA_{B2}$ subunit chimeras and point mutations further indicated a critical role of the $GABA_{B2}$ transmembrane region for positive modulation. Two key mutations (G706T and A708P) were necessary and sufficient for direct activation of the rat $GABA_{B2}$ subunit by GS39783 (Dupuis et al., 2006).

Matsushita et al. (2010) have recently shed light on the mechanism of $GABA_B$ receptor activation from a different angle. To clarify agonist-induced structural changes, each receptor subunit was fused with either Cerulean or enhanced yellow fluorescent protein at intracellular loops, and fluorescence resonance energy transfer (FRET) changes after agonist application were recorded. FRET decreases were observed between $GABA_{B1a}$ loop 2 and $GABA_{B2}$ loop 2, and also between $GABA_{B1a}$ loop 2 and $GABA_{B2}$ loop 1. These FRET decreases were more pronounced when CGP7930 (100 μM) was added together with 3 or 10 μM GABA, whereas CGP7930 alone had no effect. On the other hand, when intrasubunit FRET constructs carrying Cerulean at the C terminus and enhanced yellow

fluorescent protein at the intracellular loop 1 of either the $GABA_{B1}$ or $GABA_{B2}$ subunit were studied, GABA application did not evoke any FRET change, with or without CGP7930. This apparent lack of activation-induced structural changes in the individual subunits is unexpected especially with regard to $GABA_{B2}$, because the allosteric modulators are believed to act through this subunit. Matsushita et al. (2010) have proposed a model involving a widening of the cleft between the two subunits occurring upon agonist stimulation, keeping the helical configuration of each subunit unchanged. CGP7930 would possibly bind in the cleft, thereby further enhancing the separation between the two subunits.

D. $GABA_B$ Receptor Modulation in Cellular and Physiological Assay Systems: Effects of Allosteric Modulators on Receptor Desensitization

CGP7930 and GS39783 are effective not only in membrane, but also in cellular or intact tissue preparations. For example, the two positive modulators enhance the $GABA_B$ receptor-mediated inhibition of adenylyl cyclase activity induced by a water-soluble forskolin analog in intact CHO cells (Urwyler et al., 2005). This experimental system turned out to be highly sensitive, the potency of GABA being considerably higher than that previously observed in GTPγ^{35}S assays, suggesting the existence of a significant number of spare receptors. The high sensitivity of the cAMP assay in the CHO cell line stably expressing the $GABA_B$ receptor also enabled us to demonstrate that CGP35348 and 2-hydroxy-saclofen do in fact have some weak intrinsic agonist efficacy that was enhanced by CGP7930 and GS39783, thus confirming previous results obtained in the GTPγ^{35}S assay (see section IV.B). Further in line with a high assay sensitivity, a modest inhibition of cAMP formation produced by CGP7930 and GS39783 on their own was observed, indicating a low degree of partial agonistic activity, via the allosteric site, of the two modulators. A similar observation was also made by Binet et al. (2004), who also used an assay system with a high degree of receptor reserve, and by Tu et al. (2007), who have shown that CGP7930 alone, like GABA and baclofen, induces ERK1/2 phosphorylation in cultured cerebellar granule neurons. These findings are in line with recent theoretical receptor models allowing for allosteric modulators to have some intrinsic efficacy of their own via the allosteric-binding site (Hall, 2000). Gjoni and Urwyler (2009) have also observed that GS39783 became an allosteric agonist at desensitized $GABA_B$ receptors in which the activation mechanisms of the receptor and the interactions between its structural modules have probably undergone some fundamental changes.

In *X. laevis* oocytes injected with mRNA for the two $GABA_B$ receptor subunits and for inwardly rectifying (Kir3) potassium channels, CGP7930 and GS39783 enhanced the stimulation of potassium currents by GABA (Urwyler et al., 2001, 2003). No potassium currents were elicited in these oocytes in the presence of either of the modulators alone. In cell lines transiently transfected with $GABA_B$ receptors and an appropriate chimeric G-protein, $GABA_B$ receptor-mediated intracellular calcium mobilization via the phospholipase C pathway was enhanced by CGP7930 and GS39783 (Urwyler et al., 2001, 2003). Again, no intrinsic agonistic activity was observed with both modulators. In both assay systems, essentially the same results were obtained with $GABA_{B1a}$ and $GABA_{B1b}$ receptor isoforms. Baclofen reduces the frequency of synchronized intracellular calcium oscillations in rat cortical neurons in primary culture. CGP7930, at a concentration at which it had no effect on its own, further reduced the calcium oscillation frequency in the presence of L-baclofen (Urwyler et al., 2001). In a hippocampal slice preparation, GS39783, like baclofen, reversed paired pulse inhibition (Urwyler et al., 2003). The competitive antagonist CGP55845A counteracted the effects of both compounds. Therefore, the effect of GS39783 on paired pulse inhibition was most likely due to a potentiation of the activity of endogenous GABA rather than to a direct activation of $GABA_B$ receptors (Urwyler et al., 2001). Chen et al. (2006) have reported that CGP7930 enhances the inhibitory effects of baclofen on synaptic inhibition in the hippocampal CA1 area but not that on synaptic excitation. However, in the latter, a clear trend was observed, although it did not reach statistical significance.

Interesting neuroprotective effects of CGP7930 have recently been reported by Tu et al. (2010). Activation of $GABA_B$ receptors can protect neurons from apoptosis through a mechanism that involves transactivation of the insulin-like growth factor 1 receptor, which is a tyrosine kinase-type receptor. This neuroprotection via $GABA_B$/insulin-like growth factor 1 receptor transactivation involves G-protein $\beta\gamma$ subunit-signaling and goes through the phosphatidylinositol 3 kinase pathway and Akt phosphorylation, leading to inhibition of caspase-3 activity. Using potassium deprivation to induce apoptosis in cerebellar granule neurons, Tu et al. (2010) found that baclofen (30 μM) significantly decreased the number of apoptotic neurons. The positive allosteric modulator CGP7930 (0.3–30 μM), on its own, also decreased the number of apoptotic neurons and caspase-3 activity induced by potassium deprivation. An inhibitor of phosphatidylinositol 3 kinase abolished the neuroprotective effects and inhibition of caspase-3 activity produced by baclofen and CGP7930. Both baclofen and CGP7930 induced Akt phosphorylation; the competitive $GABA_B$ receptor antagonist [*S*-(*R**,*R**)]-[3-[[1-(3,4-dichlorophenyl)ethyl-]amino]-2-hydroxypropyl](cyclohexylmethyl) phosphinic acid (CGP54626) blocked the effect of baclofen, but not CGP7930, on Akt phosphorylation.

Persistent activation by agonists frequently results in receptor desensitization, which may be a mechanism involved in the development of tolerance (Gainetdinov et al., 2004). Rapid development of tolerance is indeed one of the major clinical shortcomings of continuous treat-

ment with baclofen. Because of their use-dependent mechanism, positive allosteric modulators are expected to have a lower propensity for inducing receptor desensitization than orthosteric agonists. Gjoni and Urwyler (2008) set out to test this hypothesis by using both recombinant and native cellular assay systems. The strategy was to persistently activate $GABA_B$ receptors on the one hand with desensitizing agonist concentrations and, on the other hand, with combinations of low agonist concentrations and GS39783 that activated the receptor to the same extent. The potency of GABA to inhibit cAMP formation in a recombinant cell line decreased after exposure to a saturating GABA concentration, but not after pretreatment with the combination of low agonist and the modulator. Similar observations were made in primary neurons for baclofen-induced inhibition of spontaneous Ca^{2+} oscillations (Gjoni and Urwyler, 2008). These findings indicate that, in the presence of GS39783, the degree of occupancy of the orthosteric site with an agonist that is needed to produce a full functional response is not sufficient to induce receptor desensitization. Thus, in this respect, positive allosteric modulators primarily affecting agonist efficacy would have an ideal profile. On the other hand, a modulator that acts by enhancing primarily agonist affinity would increase the occupancy of the orthosteric site, which could potentially lead to receptor desensitization. In addition, these findings are somewhat reminiscent of the phenomenon of "agonist-directed trafficking" (Urban et al., 2007); whereas the continued presence of an agonist both stimulates the receptor and promotes desensitization, the change in the receptor conformation induced by the allosteric modulator GS39783 apparently enhances only the functional $GABA_B$ receptor response but not its desensitization pathway. It is, however, entirely feasible that opposite could be true with another allosteric modulator. The important conclusion here is that it is possible, with the help of positive allosteric modulators, to uncouple functional receptor response from the desensitization process.

E. Modulation of $GABA_B$ Receptor Function by Other Mechanisms and Other Agents

The extracellular Ca^{2+}-sensing receptor, which also belongs to family C GPCRs and shares sequence similarity with the $GABA_B$ receptor, is allosterically modulated by amino acids and arylalkylamine-like molecules (Hammerland et al., 1998; Nemeth et al., 1998; Conigrave et al., 2000). This has prompted Kerr et al. (2002) and Kerr and Ong (2003) to examine several arylalkylamines, amino acids, and dipeptides for their actions on $GABA_B$ receptor-mediated responses. They found that these agents enhanced baclofen-induced field potentials in rat neocortical slices. Because the compounds had no effect when applied alone, the authors suggested that they would act as allosteric modulators at $GABA_B$ receptors. However, we have failed to see any enhancement of GABA affinity or maximal effect when we tested the same arylalkylamines, amino acids, and dipeptides at native and recombinant $GABA_B$ receptors in different biochemical assay systems (Urwyler et al., 2004). Olianas et al. (2005) also failed to see an enhancing effect of the arylalkylamine fendiline in a $GTP\gamma^{35}S$ assay, but in their hands, it counteracted the allosteric effect of CGP7930 on the maximal effect of GABA (but not its potency). The precise nature of the interactions of amino acids and arylalkylamines with $GABA_B$ receptor function remains unclear (Kerr and Ong, 2006; Urwyler, 2006).

Certain mGlu receptor subtypes have been shown to be modulated by Ca^{2+} (Kubo et al., 1998; Saunders et al., 1998). Ca^{2+}, at micromolar concentrations, was also found to enhance the potency, but not the maximal effect, of GABA at native and recombinant $GABA_B$ receptors in functional assays (Wise et al., 1999; Galvez et al., 2000b). Moreover, we found Ca^{2+} to enhance the binding affinities of GABA, but not baclofen, for native and recombinant $GABA_B$ receptors (Galvez et al., 2000b). Unlike CGP7930, calcium also increased the binding affinity of GABA in membranes from CHO cells expressing the $GABA_{B1}$ subunit only. Mutagenesis experiments showed Ser269 in the $GABA_{B1}$ subunit to be critical for the effect of calcium (Galvez et al., 2000b). The fact that Ser269 is located near the GABA-binding site (Bessis et al., 2000; Galvez et al., 1999, 2000a) strongly suggests that Ca^{2+} might enhance the binding affinity of GABA by the formation of a complex, with one or two amino acid residues (Ser269, possibly also Tyr366) and GABA being bound as ligands around Ca^{2+}as the central atom. In this case, the mechanism of the effects of Ca^{2+} would actually not be allosteric at all (in contrast to the modulation of mGlu receptors by calcium). This hypothesis might also explain the lack of calcium sensitivity of baclofen, which docks into the $GABA_B$ receptor cavity in a manner somewhat different from that of GABA (Costantino et al., 2001).

F. Effects of Allosteric $GABA_B$ Receptor Modulators In Vivo; Possible Therapeutic Applications

Gjoni et al. (2006) have shown in a microdialysis study for the first time at the biochemical-mechanistic level that GS39783 enhances $GABA_B$ receptor-mediated responses in vivo (Fig. 14). Orally applied GS39783 dose dependently inhibited cAMP formation in rat striatum only in conjunction with a threshold concentration of locally administered baclofen but not on its own. The inhibition of forskolin-stimulated cAMP production by GS39783 and baclofen was reversed by a competitive $GABA_B$ receptor antagonist, proving the dependence of GS39783 effects on the concomitant activation of the orthosteric agonist site.

Investigations on the role of $GABA_B$ receptors in behavioral processes have hitherto mostly relied on the use of the prototype agonist baclofen. However, baclofen induces sedation, hypothermia, and muscle relaxation,

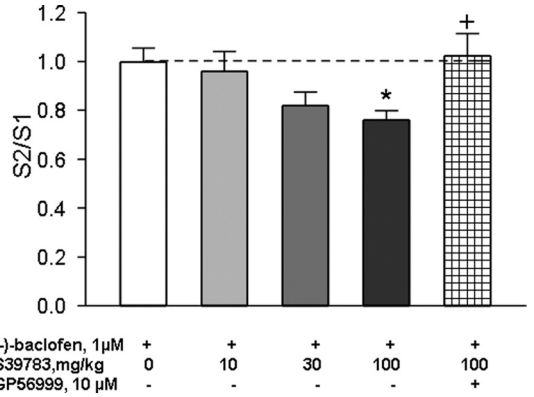


FIG. 14. Orally administered GS39783 enhances the inhibition of cAMP formation by the $GABA_B$ agonist baclofen in vivo. cAMP concentrations were measured in rat brain striatum by in vivo microdialysis. Adenylyl cyclase was stimulated by two consecutive administrations of a water-soluble forskolin-analog through the dialysis probe. Drugs were administered before the second stimulation, and drug effects were calculated as the ratio between the areas under the two cAMP peaks (S2/S1). Baclofen was administered through the dialysis probe at a threshold concentration (1 μM), which by itself did not evoke a detectable inhibition of cAMP formation (S2/S1 = 1). However, in conjunction with the positive allosteric modulator GS39783, a dose-dependent inhibition of cAMP production could be measured. This inhibition was reverted by coapplication of the competitive antagonist CGP56999A, indicating the dependence of the effects of GS39783 on the presence of an orthosteric agonist. GS39783 alone had no effect (not shown). [Reproduced from Gjoni T, Desrayaud S, Imobersteg S, and Urwyler S (2006) The positive allosteric modulator GS39783 enhances $GABA_B$ receptor-mediated inhibition of cyclic AMP formation in rat striatum in vivo. *J Neurochem* **96:**1416–1422. Copyright © 2006 Blackwell Publishing. Used with permission.]

which may significantly interfere with behavioral measurements in animals. The effectiveness of CGP7930 in vivo has in fact been demonstrated by its ability to enhance the sedative/hypnotic effects (loss of righting reflex) of threshold doses of the $GABA_B$ receptor agonists baclofen and γ-hydroxybutyrate (Kaupmann et al., 2003) in DBA mice (Carai et al., 2004). On the other hand, in the dose range tested, CGP7930 did not induce any loss of righting reflex when administered alone. Koek et al. (2010) have recently extended these findings by showing that CGP7930 and rac-BHFF enhanced baclofen- and γ-hydroxybutyrate-induced loss of righting reflex but not hypothermia. These results have increased the interest in studying allosteric modulators, which are potentially devoid of the side effects caused by such agonists as baclofen, in behavioral paradigms.

The established role of GABAergic neurotransmission in anxiety prompted Cryan et al. (2004) to study the effects of GS39783 in various animal models for anxiety and depression. Short- and long-term treatment with GS39783 decreased anxiety in the light-dark box and elevated zero maze anxiolysis tests, but no effect of GS39783 was found in the forced swim test for antidepressant activity. The positive modulator had none of the side effects associated with baclofen and/or benzodiazepines, such as muscle relaxation, sedation, impairment of motor ability or cognitive function, and potentiation of the effects of ethanol. A similar profile was more recently reported for CGP7930, which showed anxiolytic-like activity in the staircase, stress-induced hyperthermia, and elevated zero maze tests in mice without inducing motor impairment or hypothermia (Jacobson and Cryan, 2008).

$GABA_B$ receptors seem to play a role in drug addiction (Heidbreder, 2005; Filip and Frankowska, 2008). Baclofen has been shown to reduce the consumption of drugs, such as cocaine or nicotine (Brebner et al., 2000; Shoptaw et al., 2003; Paterson et al., 2004) not only in laboratory animals but also in preliminary clinical trials. It inhibits nicotine-, cocaine-, and opiate-induced release of dopamine in the nucleus accumbens (Xi and Stein, 1999; Fadda et al., 2003), which is believed to mediate the rewarding effects of drugs of abuse (Robbins and Everitt, 1999). Xi et al. (2003) found that after repeated cocaine administration to rats, $GABA_B$ receptor function in the nucleus accumbens, assessed by the stimulation of $GTP\gamma^{35}S$ binding by baclofen, was reduced, without a concomitant reduction in the level of $GABA_{B1}$ or $GABA_{B2}$ proteins. Likewise, Amantea et al. (2004) have reported that on repeated administration of nicotine to rats, $GABA_B$ receptor density and affinity were not altered in different brain regions. However, the level of G-protein coupling to the receptor was reduced in the prefrontal cortex and the nucleus accumbens. It is exactly in such a situation that one would expect positive allosteric modulators to be of clinical benefit, because they precisely act by improving the efficiency of the coupling of the $GABA_B$ receptor to its G-proteins. On the basis of the established role of $GABA_B$ receptors in the reinforcing and sensitizing effects of cocaine (Roberts et al., 1996; Xi et al., 2003; Jayaram and Steketee, 2004), Smith et al. (2004) set out to examine the effects of CGP7930 and GS39783 on cocaine intake in rats. Both drugs reduced cocaine self-administration under various schedules of reinforcement, without producing sedation or motor impairment. Slattery et al. (2005) have come to the same conclusion using an intracranial self-stimulation paradigm in rats. Both GS39783 and baclofen counteracted the threshold-lowering effect of cocaine administration in a dose-dependent manner. However, whereas GS39783 had no intrinsic effect on intracranial self-stimulation reward thresholds, baclofen elevated them. Filip and Frankowska (2007) found that CGP7930 reduced cocaine-seeking behavior without affecting food-seeking; in this respect it was more selective than the direct $GABA_B$ receptor agonists baclofen and SKF97541. Chen et al. (2005) found that $GABA_B$ receptor modulators enhance baclofen-induced inhibition of dopaminergic activity in the rat ventral tegmental area, which may well provide a mechanistic basis for these different observations. In a further very informative study, behavioral effects of short- and long-term cocaine administration were aligned with several biochemical correlates (Lhuillier et al., 2007). GS39783 counteracted the increase in both locomotion and in striatal expression of the immediate early gene c-*fos* produced by acutely administered cocaine. Most

interestingly, GS39783 also somewhat attenuated the behavioral sensitization seen after long-term cocaine treatment and at the same time the concomitant up-regulation of the transcription factors ΔFosB and CREB and the protein phosphatase 1 inhibitor dopamine- and cAMP-regulated phosphoprotein of 32 kDa. These findings strongly suggest that the positive $GABA_B$ receptor modulator prevents long-term adaptive changes in dopaminergic signaling pathways induced by long-term cocaine intake (Lhuillier et al., 2007). In a similar study, it was shown that GS39783 blocked the rewarding effects of nicotine in a conditioned place preference paradigm in rats (Mombereau et al., 2007). At the same time, GS39783 completely counteracted nicotine-induced accumulation of ΔFosB in the nucleus accumbens of these animals. Paterson et al. (2008) reported that positive $GABA_B$ receptor modulators, similarly to agonists, attenuated the reinforcing and reward-enhancing effects of nicotine. It is noteworthy that in these studies, the effects of GS39783 were observed in a dose range that exactly matched active doses producing the biochemical effects in in vivo microdialysis experiments (Gjoni et al., 2006). The $GABA_B$ receptor also seems to play a role in alcohol-seeking and drinking behavior (Maccioni and Colombo, 2009). Both CGP7930 and GS39783, like baclofen, reduced ethanol-intake in alcohol-preferring rats in free choice or operant responding paradigms, without having an effect on water or sucrose consumption (Orrù et al., 2005; Liang et al., 2006; Maccioni et al., 2007). More recently, the capacity of allosteric $GABA_B$ receptor modulators to specifically suppress the reinforcing properties of alcohol was extended to *N*-[(1*R*,2*R*,4*S*)-bicyclo[2.2.1]hept-2-yl]-2-methyl-5-[4-(trifluoromethyl) phenyl]-4-pyrimidinamine (BHF177) (Maccioni et al., 2009).

Baclofen is effective in animal models of somatic pain, and its antinociceptive effects have also been demonstrated in humans. However, its clinical use as an analgesic drug is limited by its side effects. Brusberg et al. (2009) reported that systemic CGP7930, like baclofen, has analgesic properties in a mechanically evoked visceral pain model. Both drugs reduced colorectal distension-induced visceromotor and cardiovascular responses in conscious rats. The authors concluded that $GABA_B$ receptors might be a promising target for the treatment of painful gastrointestinal disorders, such as irritable bowel syndrome. In fact, peripheral $GABA_B$ receptors are localized on nerve endings innervating different regions of the gastrointestinal tract. Thus, allosteric modulators devoid of blood-brain barrier permeability would selectively target these peripheral receptors.

Although CGP7930 and GS39783 are useful prototype compounds to investigate mechanisms of allosteric $GABA_B$ receptor modulation, their use as in vivo tools is limited by their relatively low potency and rather unfavorable pharmacokinetic properties. Attempts to optimize these existing molecules have not been very successful (see section IV.B), so the discovery and optimization of novel types of molecules acting as $GABA_B$ receptor modulators with high potency, selectivity, and brain permeability remains an important challenge for pharmacologists and medicinal chemists. The clinical indications for which a therapy with allosteric $GABA_B$ receptor modulators might be successful include anxiety, epilepsy, pain, drug abuse, gastrointestinal disorders, and possibly more. The $GABA_B$ receptor agonist baclofen has been in clinical use as an antispastic agent for several decades. However, the strong muscle-relaxant property of this drug also precludes its therapeutical application in other indications, in which it would be an unwanted side effect. On the other hand, the fact that allosteric modulators are, as should be expected on theoretical grounds, devoid of the side effects associated with the $GABA_B$ agonist baclofen in animal experiments means that spasticity will not be a potential indication for such compounds. Thus, it seems that $GABA_B$ receptor agonists and positive modulators might well cover complementary sets of clinical indications.

V. Allosteric Modulators of the Calcium-Sensing Receptor: the First Clinical Success

A. The Role of the Calcium-Sensing Receptor in Calcium Homeostasis in Health and Disease

Calcium is not only an important extra- and intracellular signaling agent but also plays a major role in the metabolic dynamics and function of various tissues (e.g., in bone, kidney, parathyroid gland, and intestine). The extracellular CaSR is a key regulator of calcium homeostasis that acts by detecting changes in ambient calcium concentrations and triggering tissue responses that restore these to normal (Fig. 15) (for reviews, see Tfelt-Hansen and Brown, 2005; Brown, 2007; Brennan and Conigrave, 2009). Its identification by molecular cloning (Brown et al., 1993) has fostered the elucidation of the physiology of calcium homeostasis and the pathophysiology of its associated diseases. Calcium homeostasis is maintained by several organs and tissues, such as the kidneys, intestines, and bones, in which calcium translocation is regulated by hormones such as parathyroid hormone (PTH) or calcitonin, or in direct response to extracellular calcium levels (Brown, 2007). The CaSR mediates monitoring of extracellular calcium levels in parathyroid cells, calcitonin-secreting thyroid "C cells," kidney cells, and possibly bone-derived and intestinal cells. In the kidney, extracellular Ca^{2+} directly enhances urinary calcium excretion by inhibiting distal tubular calcium reabsorption. In C cells, activation of the CaSR by high Ca^{2+} levels stimulates the release of calcitonin, which inhibits bone resorption and stimulates renal calcium excretion. In the parathyroid gland, the CaSR regulates cellular proliferation as well as the synthesis and release of PTH, a calcium-elevating hormone. Thus, a decrease in serum calcium levels will result in increased secretion of PTH, which in turn will trigger increased calcium reabsorption in the kidney and

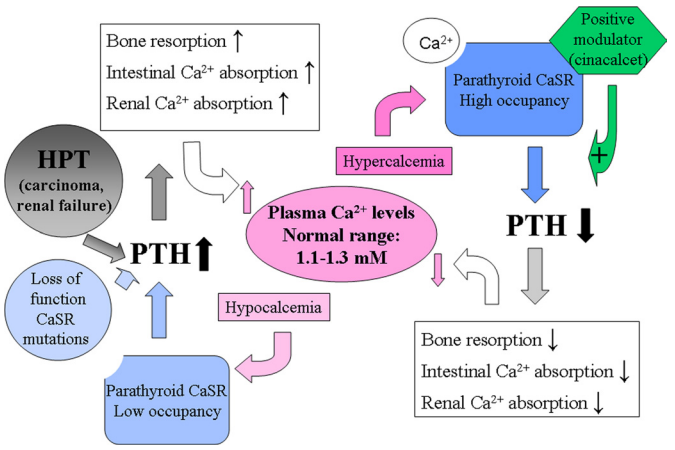


FIG. 15. Physiological mechanisms of calcium homeostasis. Free plasma calcium levels are kept within a narrow range. An increase in plasma Ca^{2+} concentrations results in activation of the parathyroid CaSR and subsequent suppression of PTH release, leading to reduced Ca^{2+} absorption/mobilization from bone, kidney, and intestine. The opposite mechanisms will come into play under hypocalcemic conditions. Primary or secondary hyperparathyroidism (HPT; parathyroid gland hypertrophy) can be a consequence for example of parathyroid carcinoma or chronic renal failure. This will result in pathologically increased PTH levels and can lead to loss of bone mass and blood vessel calcification. Inactivating CaSR mutations can lead to a similar pathology. Positive allosteric CaSR modulators (e.g., cinacalcet) can compensate for pathologically increased PTH levels by rendering the CaSR more sensitive to Ca^{2+} and more efficacious.

calcium release from bone tissue, whereas increased calcium levels will decrease PTH secretion (Fig. 15). This role of the CaSR is confirmed in humans who are homozygous carriers of CaSR-disabling mutations (Pollak et al., 1994) and in CaSR knockout mice (Ho et al., 1995); in both cases strongly elevated PTH levels are observed despite high plasma calcium concentrations.

There are a number of diseases in which inherited or acquired malfunction of the CaSR is at the origin or otherwise essentially involved (Tfelt-Hansen and Brown, 2005; Brown, 2007). Heterozygous loss of function mutations can lead to familial hypocalciuric hypercalcemia; homozygous inactivating mutations can result in life-threatening neonatal severe hyperparathyroidism. Activating mutations in the CaSR, on the other hand, will result in hypocalcemia and hypercalciuria. Similar consequences arise from activating or inhibiting autoantibodies against the CaSR (for detailed review, see Tfelt-Hansen and Brown, 2005). Primary hyperparathyroidism results from parathyroid adenoma, hyperplasia, or carcinoma and is characterized by oversecretion of PTH. Secondary hyperparathyroidism is a consequence of advanced stage renal failure. In both cases, down-regulation of the parathyroid CaSR and a concomitant change of the calcium set point toward higher concentrations (resistance to extracellular calcium) are observed. The elevated levels of PTH can lead to bone loss, cardiovascular complications, and gastrointestinal distress. Until recently, there was no pharmacological treatment for hyperparathyroidism, the only therapy being surgical removal of the parathyroid gland, thus leaving a clear medical need for drugs normalizing the PTH secretion (Nemeth et al., 1996; Nemeth and Fox, 1999). The "calcimimetic" compound cinacalcet, a positive allosteric modulator of the CaSR, has now filled this gap (for review, see Barman Balfour and Scott, 2005).

On the other hand, there is also a clear medical need for an anabolic treatment stimulating bone formation, which would be a significant progress in the therapy of osteoporosis, a major public health problem in an ageing general population. Current treatments (e.g., estrogen) instead prevent further bone loss without increasing bone mass. Because plasma calcium concentrations are a key regulatory factor in the complex interplay between bone formation and bone resorption, compounds targeting the CaSR might also have a therapeutic potential for the treatment of osteoporosis. The consequences of PTH plasma levels on bone metabolism result from a complex regulation of the temporal pattern of the secretion of the hormone, which is released intermittently physiologically. Whereas sustained elevation of PTH in the blood results in an increase of plasma Ca^{2+} levels from bone resorption, a transient increase in PTH concentrations leads to activation of osteoblasts and stimulation of bone formation (Dempster et al., 1993; Kimmel et al., 1993). Therefore, an antagonist blocking the CaSR and thereby transiently increasing plasma PTH levels stimulating new bone formation might be a promising novel approach in the treatment of this disease (Nemeth et al., 2001; Nemeth, 2002; Arey et al., 2005; Deal, 2009). Novel drugs that indeed produce such a transient PTH increase upon oral administration have been described in recent years (see section V.D).

B. The Structure and Function of the Calcium Sensing Receptor: interaction of the Receptor with Divalent Cations and Amino Acids

Like that of other family C GPCRs, the structure of the CaSR consists of a large N-terminal extracellular domain (containing 612 amino acid residues), a 250-residue 7TM domain, and an intracellular C-terminal tail containing 216 amino acids (Brown et al., 1993; Aida et al., 1995; Garrett et al., 1995). The CaSR forms a homodimeric complex at the cell surface; the two monomers are linked by disulfide bridges as well as by noncovalent interactions (Bai et al., 1998; Fan et al., 1998; Pace et al., 1999; Ray et al., 1999; Zhang et al., 2001; Hu and Spiegel, 2003). In addition, as in other family C GPCRs, the binding site for endogenous and other cation agonists (Ca^{2+}, Mg^{2+}, Gd^{3+}, neomycin) has been shown to be located in the extracellular VFTM domain (Bräuner-Osborne et al., 1999); a CaSR construct lacking the ECD is activated only by Gd^{3+} and not by the natural agonist Ca^{2+} (Hammerland et al., 1999). Using a homology model based on mGlu1 receptor X-ray structure and mutagenesis studies (see section III), Silve et al. (2005) identified critical amino acids delineating the Ca^{2+} binding pocket. These Ca^{2+}-contacting amino acid residues turned out to be well conserved within the

VFTM of family C GPCRs and located adjacent to the amino acid binding site in mGlu receptors and the $GABA_B$ receptor. In fact, aromatic and other amino acids enhance the function of the CaSR via a site near the calcium binding site (Conigrave et al., 2000; Zhang et al., 2002b; Mun et al., 2005). Thus, the amino acid-sensing function of the calcium receptor on the one hand and the enhancement of mGlu and $GABA_B$ receptor function by calcium (Kubo et al., 1998; Galvez et al., 2000b) on the other seem to have a common structural basis. It seems tempting to speculate that at some point during evolution, the interplay between amino acids and calcium shifted from one to the other as the actual endogenous agonist for a given receptor. However, in some situations, amino acids, in the presence of calcium, might also well be activators of the calcium-sensing receptors in their own right (e.g., in the gastrointestinal tract) (Conigrave et al., 2004; Conigrave and Brown, 2006).

The CaSR has a rather low affinity for its natural ligand; in fact, it senses calcium at millimolar concentrations. The activity of the receptor is characterized by a marked degree of cooperativity with Hill coefficients of 3 to 4 (Bräuner-Osborne et al., 1999; Hammerland et al., 1999), which enables it to react to very small changes in surrounding calcium concentrations. The CaSR couples to different types of G-proteins and activates a number of second messenger systems such as phospholipases A2, C and D (Kifor et al., 1997) as well as mitogen-activated protein kinase pathways in native and in recombinant expression systems (for review, see Brennan and Conigrave, 2009; Brown and MacLeod, 2001). In parathyroid cells, the CaSR also negatively couples to adenylyl cyclase activity via the inhibitory G_i protein.

C. Allosteric Modulators of the Calcium Sensing Receptor: "Calcimimetics" and "Calcilytics"

The CaSR is obviously a most difficult drug target, the natural ligand being an inorganic ion that cannot be mimicked by synthetic organic molecules (apart from some organic polycations such as spermine or neomycin, which are not selective and do not have drug-like properties). The only way out of this dilemma seems to be allosteric agonism or modulation. The history and the mechanisms of allosteric modulation of the CaSR by "calcimimetics" and "calcilytics" have recently been the subject of several reviews (Jensen and Bräuner-Osborne, 2007; Hu, 2008; Saidak et al., 2009).

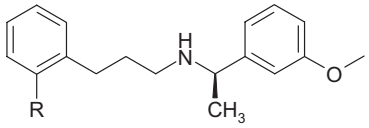
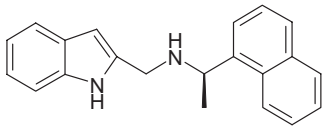
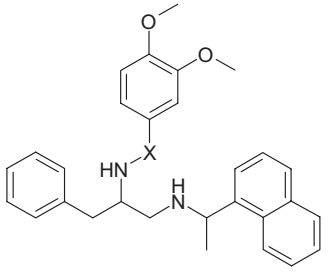
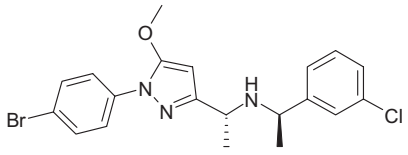
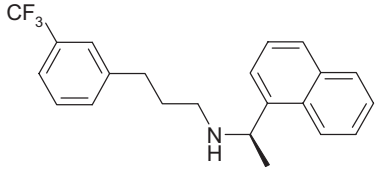
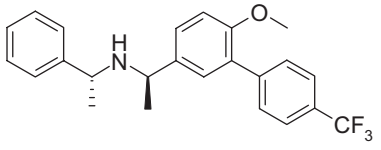
The medicinal chemistry of calcimimetics has been reviewed by Harrington and Fotsch (2007). The first positive allosteric modulators of the CaSR were the phenylalkylamines NPS568 and its deschloro-derivative NPS467 (Table 12, compound **I**) which were obtained in a derivatization program using fendiline as a starting point (Nemeth et al., 1996, 1998). They were called "calcimimetics", a somewhat misleading term because the presence of Ca^{2+} is required for their activity. In bovine parathyroid cells or in HEK293 cells transfected with the human parathyroid CaSR (but not in wild-type HEK293 cells), (*R*)-*N*-(3-methoxy-α-phenylethyl)-3-(2′-chlorphenyl)-1-(propylamine hydrochloride) (NPS568) and *N*-(3-methoxy-α-phenylethyl)-3-phenyl-1-propylamine (NPS467) enhanced intracellular calcium levels at a fixed extracellular calcium concentration in a concentration-dependent fashion, with EC_{50} values in the low micromolar range. These effects were stereospecific, the *R*-enantiomers being 10- to 100-fold more potent than the *S*-enantiomers. In bovine parathyroid cells, NPS-568 inhibited PTH secretion at concentrations below 1 μM. The intracellular calcium and PTH responses were not observed in the absence of extracellular calcium. NPS568 also inhibited PTH secretion from pathological human parathyroid cells. Both compounds failed to affect intracellular calcium responses mediated by other GPCRs. NPS467 and NPS568 also stereoselectively increased the potency of extracellular Ca^{2+} to stimulate chloride currents in *Xenopus laevis* oocytes expressing the bovine or human calcium sensing receptors, respectively (Hammerland et al., 1998). This assay system is suitable for characterizing receptors mobilizing intracellular calcium, which then in turn stimulates calcium-activated chloride channels. The stimulation of the PLC pathway by the CaSR recombinantly expressed in CCL39 hamster fibroblast cells was studied by Mailland et al. (1997). A concentration-dependent increase in the potency and, contrary to the previous study, also of the maximal level of Ca^{2+}-stimulated inositol phosphate formation was produced by the *R*-enantiomer of NPS-568. It is noteworthy that a stimulation of DNA synthesis and cell proliferation by calcium was also observed in this study. Both responses were strongly enhanced by NPS568. In a recent report (Lu et al., 2009), it was shown that NPS568 can correct inactivating CaSR mutations in two different assay systems in recombinant cell lines. This important finding that loss of function CaSR mutants are still responsive to positive allosteric modulators may give hope for the potential use of calcimimetics to treat hereditary diseases involving the CaSR, such as familial hypocalciuric hypercalcemia and neonatal severe hyperparathyroidism. In fact, at least one clinical report describes the successful treatment of a patient with such a disease (see section V.E).

Most interestingly, these "calcimimetic" agents act quite selectively upon the CaSR in the parathyroid gland, leaving its counterparts (e.g., in C cells or in distal tubules in the kidney) rather unaffected (Lavigne et al., 1998; Nemeth and Fox, 1999). The reason for this tissue selectivity is not clear at present but may be linked to different intracellular signal transduction pathways in different organs (this would then be another nice example of context-dependent pharmacology or ligand-directed trafficking). Alternatively, a higher degree of receptor expression and thereby receptor reserve in the parathyroid cells might be at the origin of this phenomenon, implying that a small occupancy of calcium sensing receptors in the parathyroid gland

TABLE 12

Positive allosteric modulators of the calcium sensing receptor ("calcimimetics")

EC_{50} values depend on the assay conditions and should be compared with caution. Information about cooperativity is given in section V.C.

Compound	Structure	Potencies (EC_{50})	Comments	References
I: R = H, NPS467		4.8 μM	The prototypical "calcimimetics"	Nemeth et al., 1998; Nemeth and Fox, 1999
R = Cl, NPS568		0.6 μM		
II Calindol		4.8 μM		Kessler et al., 2004
III: X = CH2 or SO2		None Given		Dauban et al., 2000
IV		23 nM	Orally active in rats to lower PTH levels	Poon et al., 2009
V: Cinacalcet		28 nM	On the market for HPT caused by chronic renal failure and parathyroid cancer	Nemeth et al., 2004
VI		17 nM	Orally active in rats to lower PTH levels	Harrington et al., 2010

HPT, hyperparathyroidism.

would be sufficient to affect PTH secretion maximally (Nemeth and Fox, 1999).

Screening and optimization efforts in other chemical series have produced novel types of CaSR modulators, which are the subject of a large number of patents and publications (for review, see Harrington and Fotsch, 2007 and references therein). A few examples are shown in Table 12. Most calcimimetic molecules are α-methylbenzylamines with linked aryl rings (e.g., compounds **I**, **III**, **V**, or the recently reported dibenzylamine **VI** in Table 12) (Dauban et al., 2000; Kessler et al., 2004; Harrington et al., 2010). Some of the later derivatives of this prototype template are conformationally restricted by the insertion of different ring systems, such as in compound **IV** in Table 12 (Poon et al., 2009) or in calindol [compound **II** (Kessler et al., 2004)] (see these references or Harrington and Fotsch, 2007 for further examples). Only a few non α-methylbenzylamine-type calcimimetics have been reported (see Harrington and Fotsch, 2007 and references therein for further information, also on new structures disclosed in patents). The most important progress, however, was the development of cinacalcet (compound **V**, Table 12), which is also based on the open-chain α-methylbenzylamine template but has an improved in vitro- and in vivo- profile compared with its predecessors NPS467 and NPS568 (Nemeth et al., 2004). Cinacalcet stimulated the increase of intracellular cytosolic Ca^{2+} concentrations in HEK293 cells expressing the human parathyroid CaSR, but not in nontransfected cells, with an EC_{50} of 28 nM at an extracellular calcium concentration of 0.5 mM. This effect was dependent on extracellular calcium and abolished in the presence of EGTA

TABLE 13

Negative allosteric modulators of the calcium sensing receptor ("calcilytics")

The potencies of the compounds have indicative value only and should be compared with caution. Most compounds shown are representative examples of a chemical series.

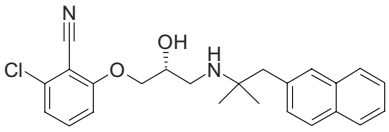
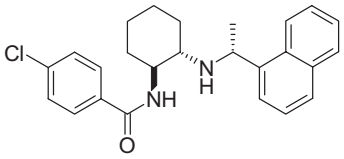
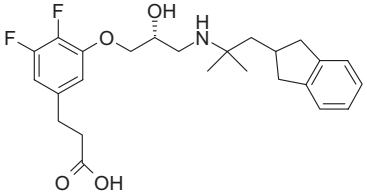
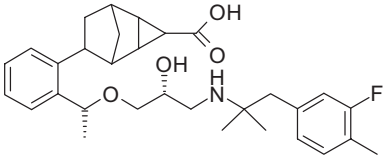
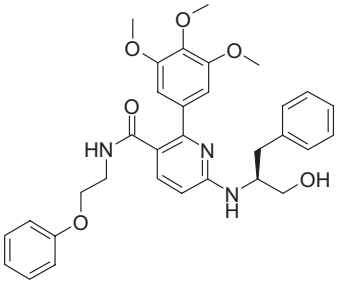
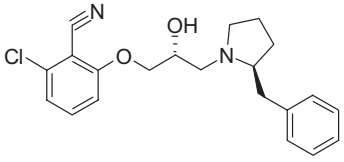
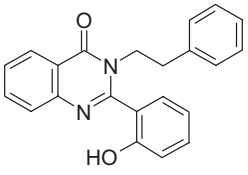
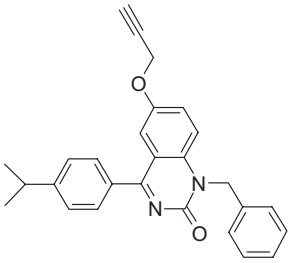
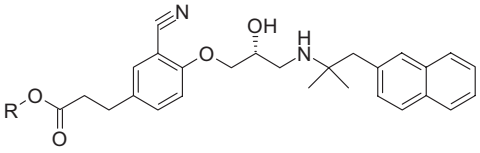
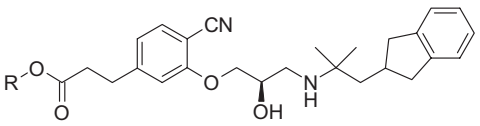
Compound	Structure	Potency (IC_{50})	Comments	References
I: NPS 2143		43 nM	The first calcilytic compound	Nemeth et al., 2001
II: Calhex 231		390 nM		Petrel et al., 2003; Kessler et al., 2006
III: Ronacaleret		150 μM	First results in humans negative	Fitzpatrick et al., 2009; Trivedi et al., 2009
IV		25 nM	Mixture of two diastereoisomers; robust PTH response in rats at 10 mg/kg p.o.	Balan et al., 2009
V		76 nM	Transient stimulation of PTH secretion in vivo	Arey et al., 2005; Yang et al., 2009
VI		120 nM		Yang et al., 2005
VII		300 nM		Shcherbakova et al., 2005
VIII		4.4 nM	Good in vivo activity in rats and dogs with a close analog	Widler et al., 2010

TABLE 13—*Continued.*

Compound	Structure	Potency (IC_{50})	Comments	References
IX: R = H R = Et		R = H: 200 nM	Drug (R = H)/prodrug (R = Et) pairs. Prodrugs transiently increase PTH in vivo, SB-423557 stimulates bone formation	Marquis et al., 2009b
X: R = H, SB-423562 R = Et, SB-423557		R = H: 73 nM		Kumar et al., 2010

(Nemeth et al., 2004). Likewise, cinacalcet inhibited the release of PTH from cultured bovine parathyroid cells (IC_{50} = 28 nM) and produced an increase in calcitonin secretion from rat medullary thyroid carcinoma cells expressing the CaSR (EC_{50} = 34 nM). All these effects were brought about by a concentration-dependent increase of the potency, but not the maximal effect, of calcium. They were stereoselective, the *R*-enantiomer of cinacalcet being at least 75-fold more potent than the *S*-enantiomer (Nemeth et al., 2004).

Despite its structural resemblance to the phenylalkylamine calcimimetics (Table 12), the first negative allosteric modulator of the CaSR was not obtained by direct derivatization based on these, but by optimization of a hit found in a high-throughput screening campaign (Nemeth et al., 2001; Nemeth, 2002). Because no orthosteric CaSR antagonists were known previously, 2-chloro-6-[3-[1,1-dimethyl-2-(2-naphthyl)ethylamino]-2(*R*)-hydroxypropoxy-]benzonitrile (NPS2143; compound **I** in Table 13) is the first calcilytic compound ever. As expected, it was found to have effects in cellular in vitro assays opposite to those of the calcimimetics described above: NPS2143 inhibited increases in intracellular calcium concentrations elicited by stimulation of the CaSR recombinantly expressed in HEK293 cells (IC_{50} = 43 nM), and it stimulated PTH secretion from bovine parathyroid cells in the presence of extracellular calcium. In particular, NPS2143 also counteracted the effects of the calcimimetic NPS467 in these assay systems (Nemeth et al., 2001). In the intracellular calcium mobilization assay, NPS2143 shifted the CRC for extracellular calcium toward higher concentrations without reducing its maximal effect. However, NPS2143 has unfavorable pharmacokinetic properties in vivo (see section V.D). Later on, several HTS and lead optimization programs have yielded a number of negative allosteric CaSR modulators from various chemical classes: compounds relatively closely related to NPS2143 (Gavai et al., 2005), but also different molecules, such as Calhex 231 (compound **II** in Table 13) and derivatives thereof (Petrel et al., 2003; Kessler et al., 2006), trisubstituted pyridines/pyrimidines (compound **V** in Table 13) (Arey et al., 2005; Yang et al., 2009), benzyloxy analogs (compound **IV** in Table 13) (Balan et al., 2009), 2-benzylpyrrolidine-substituted aryloxypropanols (compound **VI** in Table 13) (Yang et al., 2005), *3H*-quinazolin-4-ones (compound **VII** in Table 13) (Shcherbakova et al., 2005), and 4-aryl-quinazolin-2-ones (compound **VIII** in Table 13) (Widler et al., 2010) were identified as novel calcilytics, having essentially similar effects on intracellular calcium mobilization and inositol formation in cellular assay systems, some of them also being active in vivo. More compounds having in vivo activity are discussed below.

The binding site for NPS467 has been shown in a mutagenesis study to be distinct from that of the amino acid modulator L-phenylalanine (Zhang et al., 2002a), which is in line with the expectation that non-amino acid allosteric CaSR modulators, like those of other family C GPCRs, bind in the 7TM region. This has been confirmed in several mutagenesis studies based on homology models of the 7TM domain using the crystal structure of rhodopsin as a template (Miedlich et al., 2004; Petrel et al., 2003, 2004). Because the 7TM region constitutes the agonist binding site of rhodopsin-like receptors, the question arises whether calcimimetic compounds might have agonist properties in the absence of calcium, as has been observed with allosteric compounds acting at mGlu or $GABA_B$ receptors (see above) (Binet et al., 2004; Goudet et al., 2004; Mitsukawa et al., 2005). Calindol has indeed been found to directly activate a construct of the CaSR truncated at both the extracellular and the intracellular domains (Ray et al., 2005), further strengthening the notion of a close evolutionary relationship between the agonist binding site in rhodopsin-like receptors and allosteric modulatory sites in family C GPCRs. Whereas the structurally related phenylalkylamine calcimimetics and calcilytics seem to share a common allosteric binding site, a structurally distinct calcilytic from Bristol-Myers Squibb (Stamford, CT) has been found not to displace a tritiated NPS2143 analog from its binding site (Arey et al., 2005), strongly suggesting that this compound acts via a distinct site. Thus, as in mGlu receptors (see sections III.A.4 and III.B), more than one distinct allosteric binding sites on the CaSR seem to exist. Also of interest is the finding that a mutation of Glu837 (E837A), which is a most critical amino acid for the binding of phenylalkylamine modulators, converts the Bristol-Myers

Squibb compound into a positive modulator (Hu et al., 2006).

D. In Vivo Effects of Allosteric Calcium Sensing Receptor Modulators in Animals

The prototype *calcimimetic* NPS568 was shown to rapidly reduce plasma levels of parathyroid hormone, and subsequently calcium, upon oral administration in the dose range from 3.3 to 100 mg/kg to rats (Fox et al., 1999a). The hypocalcemic response was unaffected by nephrectomy but abolished by parathyroidectomy, indicating that it was due to inhibition of PTH release by activation of the parathyroid Ca^{2+} receptor, but not by acting on renal calcium receptors to increase Ca^{2+} secretion. An additional mechanism causing hypocalcemia involves the activation of calcium sensing receptors on C cells resulting in increased plasma calcitonin levels. However, this required approximately 40-fold higher doses than those needed for reducing PTH levels; thus, NPS568 quite selectively targets parathyroid gland calcium-sensing receptors in rats (Fox et al., 1999b). The compound was also effective in decreasing PTH levels in rats with renal or dietary secondary hyperparathyroidism (Fox et al., 1999). Moreover, it suppressed parathyroid cell proliferation in rats with renal insufficiency (Wada et al., 1997). Essentially the same results were later reported for the second-generation calcimimetic cinacalcet, i.e., a dose-dependent reduction of serum PTH and calcium concentrations and an increase in calcitonin levels at approximately a 30-fold higher oral doses (Nemeth et al., 2004).

In secondary hyperparathyroidism, elevated PTH levels play a role in several sequelae of renal failure, such as bone disease or cardiovascular complications. Ogata et al. (2003) have observed beneficial effects of NPS568 on the progression of renal failure and on cardiovascular risk factors. CaSR expression is reduced in the arteries of uremic patients, and the progression of vascular calcification is accelerated in these compared with the general population. In fact, cardiovascular disease is the leading cause of mortality in patients with chronic kidney failure. In a uremic mouse model, NPS568 delayed the progression of aortic calcification and atherosclerosis (Joki et al., 2009). This effect might have been produced indirectly via modulation of parathyroid gland CaSRs and the reduction of hyperparathyroidism, as well as directly via modulation of CaSRs in vascular walls (Joki et al., 2009) and thereby inhibition of calcium deposition. Such beneficial effects of calcimimetics have also been found in clinical studies (see section V.E).

It is basically to be expected that pharmacodynamic effects of *calcilytic* agents would be the opposite to those of calcimimetics. In fact, intravenous infusion of the calcilytic NPS2143 to normal rats resulted in a rapid 3- to 4-fold increase in plasma PTH levels, which was sustained during the 120-min infusion and then returned to baseline. It was accompanied by a transient, somewhat delayed 2-fold increase in plasma Ca^{2+} concentrations (Nemeth et al., 2001). A sustained elevation of PTH levels leads to increased bone turnover by activating both osteoblasts and osteoclasts, rather than a net increase in bone mass. To achieve a net anabolic effect on bone by selective activation of osteoblasts, the increase in circulating PTH must be transient (Nemeth, 2002). This is in line with the finding that stimulation of bone formation can be obtained by a pulse administration of exogenous PTH (Fox et al., 1997; Gowen et al., 2000). However, although acute PTH is an effective bone anabolic agent, it cannot be administered orally. If the same effect could be obtained by an orally active small molecule, this would represent a major progress in the treatment of osteoporosis. Daily oral treatment with NPS2143 for 5 weeks in ovariectomized rats, a model of osteoporosis, resulted in a sustained increase in plasma PTH levels and bone turnover, with a trend to a decrease in bone mineral density. However, when the animals were given a combination of NPS2143 and the antiresorptive agent 17β-estradiol, a net increase in bone mineral density was observed (Gowen et al., 2000; Nemeth, 2002). Whereas the effects of NPS2143 on PTH levels are undesirably long-lasting, compound **V** in Table 13 stimulates PTH secretion in vivo in a more transient fashion (Arey et al., 2005). The reason for the sustained action of NPS2143 is most likely its large volume of distribution (11 l/kg in the rat), resulting in prolonged drug exposure and elevation of PTH levels (Marquis et al., 2009a). The addition of a carboxylic acid functionality to the amino alcohol NPS2143-template resulted in the zwitterionic compound **IX** (Table 13, r = H) with an approximately 10-fold lower volume of distribution and a shorter half-life (Marquis et al., 2009b). An improved bioavailability was obtained with the corresponding ester prodrug (**IX**, r = ethyl), which elicited a rapid and transient increase in PTH levels after oral administration in the dog (Marquis et al., 2009b). With the very closely related drug/prodrug pair **X** in Table 13 (SB-423562 and its orally bioavailable precursor SB-423557), Kumar et al. (2010) have then also found a transient increase in circulating PTH levels in several species, including humans. In an ovariectomized rat model of bone loss, daily oral administration of the prodrug SB-423557 then indeed increased bone formation and bone strength (Kumar et al., 2010). Long-term administration of the drug did not increase parathyroid cell proliferation, a theoretically possible side effect of calcilytic drugs. These results foster the hope for success with this novel approach in the prevention and treatment of osteoporosis.

E. The Effects of Calcimimetics and Calcilytics in Humans: from Proof of Concept to Clinical Application

"Proof of concept" was obtained in a small single-dose study showing that oral administration of the prototype calcimimetic NPS568 lowered the plasma levels of PTH and calcium in postmenopausal women with mild primary hyperparathyroidism (Silverberg et al., 1997). In

addition, in a dose-finding study in patients with kidney failure who had secondary hyperparathyroidism, NPS568 caused a rapid decrease in plasma PTH levels (Antonsen et al., 1998). This was then confirmed in a repeated-dosing study over a period of 15 days (Goodman et al., 2000). However, NPS568 was not further developed because of its unfavorable pharmacokinetic properties, leaving the stage to the second generation calcimimetic cinacalcet.

The clinical studies with cinacalcet have been reviewed elsewhere (Franceschini et al., 2003; Barman Balfour and Scott, 2005; Brown, 2007; Trivedi et al., 2008; Drüeke and Ritz, 2009) and are summarized only briefly here. The ability of cinacalcet to lower PTH levels in patients receiving hemodialysis who had secondary hyperparathyroidism was first demonstrated in several placebo-controlled studies on a rather low number of patients (Franceschini et al., 2003). The pooled data from three key phase III clinical trials have been reported by Block et al. (2004), see also the review by Barman Balfour and Scott (2005). More than 1000 patients with chronic kidney disease on dialysis with uncontrolled secondary hyperparathyroidism were enrolled in these studies. Significantly more patients receiving cinacalcet at oral doses ranging from 30 to 180 mg daily achieved the defined endpoints (i.e., target reductions in plasma PTH levels compared with the placebo group). Cinacalcet significantly decreased plasma PTH (by more than 30%) and serum calcium, phosphorus, and calcium × phosphorus levels, whereas these levels showed no changes in placebo recipients. The beneficial effects of cinacalcet on serum PTH, calcium, and phosphorus levels have been shown in long-term studies to be sustained (Moe et al., 2005; Sprague et al., 2009). The drug was generally well tolerated, with only relatively minor gastrointestinal side effects.

While biochemical parameters such as PTH levels are valid surrogate markers, the ultimate criteria for a drug's value is its efficacy on clinical outcome (i.e., morbidity and mortality). A combined analysis of safety data from four controlled clinical trials encompassing more than 1000 patients revealed significant reductions in the risk of parathyroidectomy, bone fractures, and cardiovascular complications, along with self-reported diminished pain and general well being (Cunningham et al., 2005). A recent report by Aladrén Regidor (2009) has confirmed that cinacalcet reduces cardiovascular complications (calcification) in secondary hyperparathyroidism in patients receiving hemodialysis.

Parathyroid carcinoma causes excess secretion of PTH, resulting in severe hypercalcemia that can lead to renal, cardiovascular, and skeletal complications and is a main component of morbidity. The cancer is usually resistant to chemotherapy and radiotherapy, and parathyroidectomy is often not successful because recurrence and metastases are common. Cinacalcet was tested in an open-label, single-arm multicenter study in patients with inoperable parathyroid carcinoma (Silverberg et al., 2007). Mean calcium and PTH levels were markedly elevated at baseline. At the end of the titration period, serum calcium was effectively reduced in approximately two thirds of the patients, mostly so in patients with highest baseline levels. At the same time, a small, nonsignificant reduction in PTH levels was also observed. Thus, cinacalcet offers benefits in the management of hypercalcemia in patients with parathyroid cancer, although the progression of the disease will eventually overwhelm the drug's efficacy (Brown, 2007).

Cinacalcet is the first positive allosteric GPCR modulator to be marketed (Sensipar/Mimpara; Amgen, Thousand Oaks, CA). It is approved for the treatment of secondary hyperparathyroidism in patients with chronic renal insufficiency who are receiving dialysis, and in patients with hyperparathyroidism due to parathyroid cancer. The approval for other forms of hyperparathyroidism seems possible; the drug has also been shown in double-blind, placebo-controlled studies to be active in primary hyperparathyroidism (Shoback et al., 2003; Peacock et al., 2005). Timmers et al. (2006) have reported the case of a patient with hypercalcemia as a result of an inactivating mutation of the calcium-sensing receptor who was successfully treated with cinacalcet. Current issues and future prospects for the treatment with cinacalcet have recently been reviewed (Drüeke and Ritz, 2009).

On the other hand, "calcilytic" antagonists of the CaSR seem likely to be useful in disorders such as osteoporosis (Deal, 2009; Trivedi et al., 2009) or different forms of hypocalcemia that are due to increased sensitivity of the CaSR to extracellular calcium (Brown, 2007). Little information is available on human studies with the calcilytic ronacaleret (compound **III** in Table 13). To date, the results of a controlled phase II study in patients with osteoporosis have only been reported in abstract form (Fitzpatrick et al., 2009). Although bone formation markers were increased, the desired net gain in bone mineral density was not achieved. Moreover, ronacaleret administration was associated with a state of mild hyperparathyroidism. It remains to be seen whether a second-generation calcilytic such as SB-423557 (the orally active precursor for SB-423562, compound **X** in Table 13), which is short-acting and elicits transient increases in PTH levels in healthy male volunteers (Kumar et al., 2010), would result in a better outcome. Thus, the strategy of promoting bone formation in osteoporosis by antagonizing the parathyroid CaSR awaits further clinical validation (Marie, 2010).

VI. Summary, Highlights, and Outlook

The study of the mechanisms of allosteric modulation has taught us a lot about the functioning of family C GPCRs. The sites to which allosteric modulators bind are sites of pharmacological action in their own right, to which the theoretical concepts of affinity and full, partial, neutral, or inverse efficacy fully apply, enriched by

TABLE 14

Possible and confirmed clinical indications for allosteric modulators of family C GPCRS

This table is not exhaustive. It lists only the clinical indications that are confirmed or those most likely on the basis of physiological mechanisms, receptor localization, or data in animal models. Much more information on possible clinical indications is discussed in the text.

Type of clinical indication	Taste Receptors	Metabotropic Glutamate Receptors			$GABA_B$ Receptor	CaSR
		Group I	Group II	Group III		
Metabolic, endocrinological, gastrointestinal	*Allosteric agonists or positive modulators* in **diabetes, obesity**	*Negative mGlu5 modulators* in **GERD** (animal data, clinical trials)			Possibly *positive modulators* in **IBS**, based on receptor localization and activity in one animal model for visceral pain.	*Positive modulators*: **HPT** due to chronic renal failure, carcinoma or loss of function CaSR mutations. Cinacalcet marketed. *Negative modulators*: possibly in **osteoporosis** based on animal data
Psychiatric (anxiety, schizophrenia, mental retardation, drug abuse)		*Negative mGlu5 modulators*: **Anxiety** (animal data, clinical results with fenobam). **Fragile X syndrome** (data with FMRP KO animals, fenobam in clinical trials). *mGlu5 positive modulators*: **Schizophrenia** (based on animal data and glutamate hypothesis of schizophrenia)	*Positive modulators* in **anxiety** and **schizophrenia**, based on animal models and clinical results with agonists	Possibly *positive mGlu7 modulators* in **anxiety**. Animal data are conflicting, however.	Possibly *positive modulators* in **anxiety**, based on data from animal models. Potential for *positive modulators* in **drug abuse**, based on data from animal models for cocaine, nicotine, and alcohol abuse.	
Geriatric and Parkinson's disease	*Positive modulators* (taste enhancers) against **nutritional deficits due to loss of appetite** in elderly people	*Negative mGlu5 modulators* in clinical trials for **L-DOPA-induced tardive dyskinesias in Parkinson's disease**		*Positive mGlu4 receptor modulators* for **Parkinson's** disease based on their localization in basal ganglia and results from animal models		
Pain conditions including migraine		Group I mGlu receptors are localized in **pain** pathways. Activity of *negative mGlu1 and mGlu5 modulators* in various animal pain models. Clinical trials in **migraine** patients with ADX10059 promising, stopped because of side effects.			Possibly *positive modulators* for **visceral pain** (see above)	

IBS, irritable bowel syndrome; HPT, hyperparathyroidism.

the complex aspects of cooperative interactions occurring between the orthosteric and allosteric ligand binding sites. Allosteric sites have become most attractive drug targets in recent years for various reasons, such as a lesser propensity for side effects and tolerance development due to their activity-dependent mechanism, bet-

ter receptor subtype selectivity, and better chemical accessibility. The development of allosteric modulators for family C GPCRs is not in its infancy anymore. While taste-enhancing compounds may be considered a special case because many of them bind to "atypical" sites on their receptors (opening region of the VFTM, cysteine-rich domain), the clinical success of the calcimimetic drug cinacalcet has definitely opened the stage for compounds acting through the 7TM domain of the different receptors, which are members of this family. Cinacalcet is a beautiful example, showing how it is possible to target a receptor with a poorly accessible orthosteric ligand binding site, in this case recognizing an inorganic ion that is difficult to mimic with a synthetic molecule. Similar obstacles are posed by metabotropic glutamate receptors because of their narrow binding cleft for the highly polar, charged natural ligand. A large number of existing allosteric ligands with positive, negative, and even neutral cooperativity or with intrinsic agonist efficacy have circumvented this problem and in addition provided subtype selectivity among the members in the three groups of the mGlu receptor family with their highly conserved orthosteric binding site. Numerous allosteric modulators have proven efficacy in vitro and in vivo and confirmed most of their predicted advantages. For example, positive allosteric $GABA_B$ receptor modulators lack the side effects of their orthosteric agonist counterpart baclofen and have been shown not to induce receptor desensitization, at least in vitro. Numerous clinical indications seem to be amenable to treatment with allosteric family C GPCR modulators on the basis of promising preclinical findings (Table 14). Whereas the calcimimetic drug cinacalcet has already entered the market, a success of a negative allosteric CaSR modulator (a calcilytic drug) in an indication such as osteoporosis seems rather likely. The nonsensory family C GPCRs for glutamate and GABA are mostly located in the nervous system and therefore open up promising perspectives for neurological and psychiatric diseases. The most advanced candidates are probably mGlu5 receptor modulators, which have a strong preclinical basis for positive modulators in schizophrenia and already positive clinical data for antagonists to treat L-DOPA-induced dyskinesias in Parkinsonism. PET ligands for labeling allosteric sites on the mGlu5 receptor have made it possible to correlate receptor occupancy in vivo with the therapeutic effects of a novel drug. Orthosteric group II mGlu receptor agonists have already shown anxiolytic and antischizophrenic activity in man. Unlike orthosteric agonists, positive modulators allow the selective targeting of mGlu2 receptors. Positive mGlu2 receptor modulators show the same effects in animal models as orthosteric mGlu2/3 receptor agonists, which is in line with data from knockout animals, demonstrating that the mGlu2 receptor is responsible for mediating activity in these models. This fosters the hope that such drugs will successfully follow-up their agonist predecessors in man. Thus, group I and II mGlu receptor modulators seem to be good candidates for following cinacalcet as the next representatives of family C GPCR modulators in clinical application. On the other hand, allosteric drugs modulating group III mGlu receptors and $GABA_B$ receptors might represent a drug generation of a somewhat more distant future. Given the variety of clinical indications that seem to be related to these targets based on preclinical data, and given the therapeutic importance of the $GABA_B$ agonist baclofen, but also its shortcomings, allosteric modulators with better potency and pharmacokinetic properties than the ones currently available for group III mGlu and $GABA_B$ receptors are highly desirable.

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References

Adams CL and Lawrence AJ (2007) CGP7930: a positive allosteric modulator of the GABA(B) receptor. *CNS Drug Rev* **13:**308–316.

Aida K, Koishi S, Tawata M, and Onaya T (1995) Molecular cloning of a putative Ca^{2+}-sensing receptor cDNA from human kidney. *Biochem Biophys Res Commun* **214:**524–529.

Aladrén Regidor MJ (2009) Cinacalcet reduces vascular and soft tissue calcification in secondary hyperparathyroidism (SHPT) in hemodialysis patients. *Clin Nephrol* **71:**207–213.

Amantea D, Tessari M, and Bowery NG (2004) Reduced G-protein coupling to the $GABA_B$ receptor in the nucleus accumbens and the medial prefrontal cortex of the rat after chronic treatment with nicotine. *Neurosci Lett* **355:**161–164.

Ametamey SM, Kessler LJ, Honer M, Wyss MT, Buck A, Hintermann S, Auberson YP, Gasparini F, and Schubiger PA (2006) Radiosynthesis and preclinical evaluation of ^{11}C-ABP688 as a probe for imaging the metabotropic glutamate receptor subtype 5. *J Nucl Med* **47:**698–705.

Ametamey SM, Treyer V, Streffer J, Wyss MT, Schmidt M, Blagoev M, Hintermann S, Auberson Y, Gasparini F, Fischer UC, et al. (2007) Human PET studies of metabotropic glutamate receptor subtype 5 with ^{11}C-ABP688. *J Nucl Med* **48:**247–252.

Anderson JJ, Rao SP, Rowe B, Giracello DR, Holtz G, Chapman DF, Tehrani L, Bradbury MJ, Cosford ND, and Varney MA (2002) [^{3}H]Methoxymethyl-3-[(2-methyl-1,3-thiazol-4-yl)ethynyl]pyridine binding to metabotropic glutamate receptor subtype 5 in rodent brain: in vitro and in vivo characterization. *J Pharmacol Exp Ther* **303:**1044–1051.

Annoura H, Fukunaga A, Uesugi M, Tatsuoka T, and Horikawa Y (1996) A novel class of antagonists for metabotropic glutamate receptors, 7-(hydroxyimino)cyclopropa[*b*]chromen-la-carboxylates. *Bioorg Med Chem Lett* **6:**763–766.

Antonsen JE, Sherrard DJ, and Andress DL (1998) A calcimimetic agent acutely suppresses parathyroid hormone levels in patients with chronic renal failure. Rapid communication. *Kidney Int* **53:**223–227.

Arey BJ, Seethala R, Ma Z, Fura A, Morin J, Swartz J, Vyas V, Yang W, Dickson JK Jr, and Feyen JH (2005) A novel calcium-sensing receptor antagonist transiently stimulates parathyroid hormone secretion in vivo. *Endocrinology* **146:**2015–2022.

Assadi-Porter FM, Maillet EL, Radek JT, Quijada J, Markley JL, and Max M (2010) Key amino acid residues involved in multi-point binding interactions between brazzein, a sweet protein, and the T1R2–T1R3 human sweet receptor. *J Mol Biol* **398:**584–599.

Ayala JE, Chen Y, Banko JL, Sheffler DJ, Williams R, Telk AN, Watson NL, Xiang Z, Zhang Y, Jones PJ, et al. (2009) mGluR5 positive allosteric modulators facilitate both hippocampal LTP and LTD and enhance spatial learning. *Neuropsychopharmacology* **34:**2057–2071.

Ayala JE, Niswender CM, Luo Q, Banko JL, and Conn PJ (2008) Group III mGluR regulation of synaptic transmission at the SC-CA1 synapse is developmentally regulated. *Neuropharmacology* **54:**804–814.

Bai M, Trivedi S, and Brown EM (1998) Dimerization of the extracellular calcium-sensing receptor (CaR) on the cell surface of CaR-transfected HEK293 cells. *J Biol Chem* **273:**23605–23610.

Balan G, Bauman J, Bhattacharya S, Castrodad M, Healy DR, Herr M, Humphries P, Jennings S, Kalgutkar AS, Kapinos B, et al. (2009) The discovery of novel calcium sensing receptor negative allosteric modulators. *Bioorg Med Chem Lett* **19:**3328–3332.
Barda DA, Wang ZQ, Britton TC, Henry SS, Jagdmann GE, Coleman DS, Johnson MP, Andis SL, and Schoepp DD (2004) SAR study of a subtype-selective allosteric potentiator of metabotropic glutamate 2 receptor, *N*-(4-phenoxyphenyl) *N*-(3-pyridinylmethyl)ethanesulfonamide. *Bioorg Med Chem Lett* **14:**3099–3102.
Barman Balfour JA and Scott LJ (2005) Cinacalcet hydrochloride. *Drugs* **65:**271–281.
Battaglia G, Busceti CL, Molinaro G, Biagioni F, Traficante A, Nicoletti F, and Bruno V (2006) Pharmacological activation of mGlu4 metabotropic glutamate receptors reduces nigrostriatal degeneration in mice treated with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. *J Neurosci* **26:**7222–7229.
Bear MF, Huber KM, and Warren ST (2004) The mGluR theory of fragile X mental retardation. *Trends Neurosci* **27:**370–377.
Belforte JE, Zsiros V, Sklar ER, Jiang Z, Yu G, Li Y, Quinlan EM, and Nakazawa K (2010) Postnatal NMDA receptor ablation in corticolimbic interneurons confers schizophrenia-like phenotypes. *Nat Neurosci* **13:**76–83.
Benneyworth MA, Xiang Z, Smith RL, Garcia EE, Conn PJ, and Sanders-Bush E (2007) A selective positive allosteric modulator of metabotropic glutamate receptor subtype 2 blocks a hallucinogenic drug model of psychosis. *Mol Pharmacol* **72:**477–484.
Beqollari D and Kammermeier PJ (2008) The mGlu(4) receptor allosteric modulator *N*-phenyl-7-(hydroxyimino)cyclopropa[*b*]chromen-1*a*-carboxamide acts as a direct agonist at mGlu(6) receptors. *Eur J Pharmacol* **589:**49–52.
Bergink V, van Megen HJ, and Westenberg HG (2004) Glutamate and anxiety. *Eur Neuropsychopharmacol* **14:**175–183.
Berry-Kravis E, Hessl D, Coffey S, Hervey C, Schneider A, Yuhas J, Hutchison J, Snape M, Tranfaglia M, Nguyen DV, et al. (2009) A pilot open label, single dose trial of fenobam in adults with fragile X syndrome. *J Med Genet* **46:**266–271.
Bessis AS, Bertrand HO, Galvez T, De Colle C, Pin JP, and Acher F (2000) Three-dimensional model of the extracellular domain of the type 4a metabotropic glutamate receptor: new insights into the activation process. *Protein Sci* **9:**2200–2209.
Bessis AS, Bolea C, Bonnet B, Epping-Jordan M, Poirier N, Poli SM, Rocher JP, and Thollon Y (2005), inventors; Bessis AS, Bolea C, Bonnet B, Epping-Jordan M, Poirier N, Poli SM, Rocher JP, Thollon, and Addex Pharmaceuticals SA, assignees. Novel alkynyl derivatives as modulators of metabotropic glutamate receptors. World patent WO/2005/123703. 2005 Jun 17.
Bessis AS, Rondard P, Gaven F, Brabet I, Triballeau N, Prezeau L, Acher F, and Pin JP (2002) Closure of the venus flytrap module of mGlu8 receptor and the activation process: insights from mutations converting antagonists into agonists. *Proc Natl Acad Sci USA* **99:**11097–11102.
Bettler B, Kaupmann K, Mosbacher J, and Gassmann M (2004) Molecular structure and physiological functions of $GABA_B$ receptors. *Physiol Rev* **84:**835–867.
Bhave G, Karim F, Carlton SM, and Gereau RW 4th (2001) Peripheral group I metabotropic glutamate receptors modulate nociception in mice. *Nat Neurosci* **4:**417–423.
Binet V, Brajon C, Le Corre L, Acher F, Pin JP, and Prézeau L (2004) The heptahelical domain of $GABA_{B2}$ is activated directly by CGP7930, a positive allosteric modulator of the $GABA_B$ receptor. *J Biol Chem* **279:**29085–29091.
Birdsall NJ, Browning C, Hern J, and Lazareno S (2004) Allosteric regulation of binding and function at GPCRS. *Med Chem Res* **13:**52–62.
Block GA, Martin KJ, de Francisco AL, Turner SA, Avram MM, Suranyi MG, Hercz G, Cunningham J, Abu-Alfa AK, Messa P, et al. (2004) Cinacalcet for secondary hyperparathyroidism in patients receiving hemodialysis. *N Engl J Med* **350:**1516–1525.
Bockaert J and Pin JP (1999) Molecular tinkering of G protein-coupled receptors: an evolutionary success. *EMBO J* **18:**1723–1729.
Bolea C, Mutel V, Rocher JP, Bessis AS, and Le Poul E (2004), inventors; Bolea C, Mutel V, Rocher JP, Bessis AS, Le Poul E, and Addex Pharmaceuticals SA, assignees. Novel aminopyridine derivatives as mGluR5 antagonists. World patent no. WO/2004/078728. 2004 Mar 4.
Bonnefous C, Vernier JM, Hutchinson JH, Gardner MF, Cramer M, James JK, Rowe BA, Daggett LP, Schaffhauser H, and Kamenecka TM (2005) Biphenyl-indanones: allosteric potentiators of the metabotropic glutamate subtype 2 receptor. *Bioorg Med Chem Lett* **15:**4354–4358.
Bowery NG, Bettler B, Froestl W, Gallagher JP, Marshall F, Raiteri M, Bonner TI, and Enna SJ (2002) International Union of Pharmacology. XXXIII. Mammalian γ-aminobutyric $acid_B$ receptors: structure and function. *Pharmacol Rev* **54:**247–264.
Bowery NG, Hill DR, Hudson AL, Doble A, Middlemiss DN, Shaw J, and Turnbull M (1980) (−)Baclofen decreases neurotransmitter release in the mammalian CNS by an action at a novel GABA receptor. *Nature* **283:**92–94.
Bräuner-Osborne H, Jensen AA, Sheppard PO, O'Hara P, and Krogsgaard-Larsen P (1999) The agonist-binding domain of the calcium-sensing receptor is located at the amino-terminal domain. *J Biol Chem* **274:**18382–18386.
Bräuner-Osborne H, Wellendorph P, and Jensen AA (2007) Structure, pharmacology and therapeutic prospects of family C G-protein coupled receptors. *Current Drug Targets* **8:**169–184.
Bradley SJ, Watson JM, and Challiss RA (2009) Effects of positive allosteric modulators on single-cell oscillatory Ca^{2+} signaling initiated by the type 5 metabotropic glutamate receptor. *Mol Pharmacol* **76:**1302–1313.
Brebner K, Phelan R, and Roberts DC (2000) Effect of baclofen on cocaine self-administration in rats reinforced under fixed-ratio 1 and progressive-ratio schedules. *Psychopharmacology* **148:**314–321.
Brennan SC and Conigrave AD (2009) Regulation of cellular signal transduction pathways by the extracellular calcium-sensing receptor. *Curr Pharm Biotechnol* **10:**270–281.
Breysse N, Baunez C, Spooren W, Gasparini F, and Amalric M (2002) Chronic but not acute treatment with a metabotropic glutamate 5 receptor antagonist reverses the akinetic deficits in a rat model of parkinsonism. *J Neurosci* **22:**5669–5678.
Brink CB, Harvey BH, Bodenstein J, Venter DP, and Oliver DW (2004) Recent advances in drug action and therapeutics: relevance of novel concepts in G-protein-coupled receptor and signal transduction pharmacology. *Br J Clin Pharmacol* **57:**373–387.
Brnardic EJ, Fraley ME, Garbaccio RM, Layton ME, Sanders JM, Culberson C, Jacobson MA, Magliaro BC, Hutson PH, O'Brien JA, et al. (2010) 3-Aryl-5-phenoxymethyl-1,3-oxazolidin-2-ones as positive allosteric modulators of mGluR2 for the treatment of schizophrenia: hit-to-lead efforts. *Bioorg Med Chem Lett* **20:**3129–3133.
Brody SA, Conquet F, and Geyer MA (2004) Effect of antipsychotic treatment on the prepulse inhibition deficit of mGluR5 knockout mice. *Psychopharmacology* **172:**187–195.
Brown AK, Kimura Y, Zoghbi SS, Siméon FG, Liow JS, Kreisl WC, Taku A, Fujita M, Pike VW, and Innis RB (2008) Metabotropic glutamate subtype 5 receptors are quantified in the human brain with a novel radioligand for PET. *J Nucl Med* **49:**2042–2048.
Brown EM (2007) Clinical lessons from the calcium-sensing receptor. *Nat Clin Pract Endocrinol Metab* **3:**122–133.
Brown EM, Gamba G, Riccardi D, Lombardi M, Butters R, Kifor O, Sun A, Hediger MA, Lytton J, and Hebert SC (1993) Cloning and characterization of an extracellular Ca^{2+}-sensing receptor from bovine parathyroid. *Nature* **366:**575–580.
Brown EM and MacLeod RJ (2001) Extracellular calcium sensing and extracellular calcium signaling. *Physiol Rev* **81:**239–297.
Brusberg M, Ravnefjord A, Martinsson R, Larsson H, Martinez V, and Lindström E (2009) The $GABA_B$ receptor agonist, baclofen, and the positive allosteric modulator, CGP7930, inhibit visceral pain-related responses to colorectal distension in rats. *Neuropharmacology* **56:**362–367.
Burdi DF, Hunt R, Fan L, Hu T, Wang J, Guo Z, Huang Z, Wu C, Hardy L, Detheux M, et al. (2010) Design, synthesis, and structure-activity relationships of novel bicyclic azole-amines as negative allosteric modulators of metabotropic glutamate receptor 5. *J Med Chem* **53:**7107–7118.
Callaerts-Vegh Z, Beckers T, Ball SM, Baeyens F, Callaerts PF, Cryan JF, Molnar E, and D'Hooge R (2006) Concomitant deficits in working memory and fear extinction are functionally dissociated from reduced anxiety in metabotropic glutamate receptor 7-deficient mice. *J Neurosci* **26:**6573–6582.
Carai MA, Colombo G, Froestl W, and Gessa GL (2004) In vivo effectiveness of CGP7930, a positive allosteric modulator of the $GABA_B$ receptor. *Eur J Pharmacol* **504:**213–216.
Carroll FY, Stolle A, Beart PM, Voerste A, Brabet I, Mauler F, Joly C, Antonicek H, Bockaert J, Müller T, et al. (2001) BAY36-7620: a potent non-competitive mGlu1 receptor antagonist with inverse agonist activity. *Mol Pharmacol* **59:**965–973.
Cartmell J and Schoepp DD (2000) Regulation of neurotransmitter release by metabotropic glutamate receptors. *J Neurochem* **75:**889–907.
Chandrashekar J, Hoon MA, Ryba NJ, and Zuker CS (2006) The receptors and cells for mammalian taste. *Nature* **444:**288–294.
Chandrashekar J, Mueller KL, Hoon MA, Adler E, Feng L, Guo W, Zuker CS, and Ryba NJ (2000) T2Rs function as bitter taste receptors. *Cell* **100:**703–711.
Chen Y, Goudet C, Pin JP, and Conn PJ (2008) N-{4-Chloro-2-[(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)methyl]phenyl}-2-hydroxybenzamide (CPPHA) acts through a novel site as a positive allosteric modulator of group 1 metabotropic glutamate receptors. *Mol Pharmacol* **73:**909–918.
Chen Y, Menendez-Roche N, and Sher E (2006) Differential modulation by the $GABA_B$ receptor allosteric potentiator 2,6-di-tert-butyl-4-(3-hydroxy-2,2-dimethylpropyl)-phenol (CGP7930) of synaptic transmission in the rat hippocampal CA1 area. *J Pharmacol Exp Ther* **317:**1170–1177.
Chen Y, Nong Y, Goudet C, Hemstapat K, de Paulis T, Pin JP, and Conn PJ (2007) Interaction of novel positive allosteric modulators of metabotropic glutamate receptor 5 with the negative allosteric antagonist site is required for potentiation of receptor responses. *Mol Pharmacol* **71:**1389–1398.
Chen Y, Phillips K, Minton G, and Sher E (2005) $GABA_B$ receptor modulators potentiate baclofen-induced depression of dopamine neuron activity in the rat ventral tegmental area. *Br J Pharmacol* **144:**926–932.
Christopoulos A and Kenakin T (2002) G protein-coupled receptor allosterism and complexing. *Pharmacol Rev* **54:**323–374.
Cid JM, Duvey G, Cluzeau P, Nhem V, Macary K, Raux A, Poirier N, Muller J, Boléa C, Finn T, et al. (2010) Discovery of 1,5-disubstituted pyridones: a new class of positive allosteric modulators of the metabotropic glutamate 2 receptor. *ACS Chem Neurosci* **1:**788–795.
Conigrave AD and Brown EM (2006) Taste receptors in the gastrointestinal tract. II. L-amino acid sensing by calcium-sensing receptors: implications for GI physiology. *Am J Physiol Gastrointest Liver Physiol* **291:**G753–G761.
Conigrave AD, Mun HC, Delbridge L, Quinn SJ, Wilkinson M, and Brown EM (2004) L-Amino acids regulate parathyroid hormone secretion. *J Biol Chem* **279:**38151–38159.
Conigrave AD, Quinn SJ, and Brown EM (2000) L-Amino acid sensing by the extracellular Ca^{2+} sensing receptor. *Proc Natl Acad Sci USA* **97:**4814–4819.
Conn PJ, Christopoulos A, and Lindsley CW (2009a) Allosteric modulators of GPCRs: a novel approach for the treatment of CNS disorders. *Nat Rev Drug Discov* **8:**41–54.
Conn PJ, Lindsley CW, and Jones CK (2009b) Activation of metabotropic glutamate receptors as a novel approach for the treatment of schizophrenia. *Trends Pharmacol Sci* **30:**25–31.
Conn PJ and Niswender CM (2006) mGluR7's lucky number. *Proc Natl Acad Sci USA* **103:**251–252.
Cosford ND, Tehrani L, Roppe J, Schweiger E, Smith ND, Anderson J, Bristow L, Brodkin J, Jiang X, McDonald I, et al. (2003a) 3-[(2-Methyl-1,3-thiazol-4-yl)ethynyl]-pyridine: a potent and highly selective metabotropic glutamate subtype 5 receptor antagonist with anxiolytic activity. *J Med Chem* **46:**204–206.
Cosford ND, Roppe J, Tehrani L, Schweiger EJ, Seiders TJ, Chaudary A, Rao S, and

Varney MA (2003b) [^{3}H]-methoxymethyl-MTEP and [^{3}H]-methoxy-PEPy: potent and selective radioligands for the metabotropic glutamate subtype 5 (mGlu5) receptor. *Bioorg Med Chem Lett* **13:**351–354.
Costantino G (2006) In silico approaches towards the understanding of the structure-function relationships in metabotropic glutamate receptors (mGluRs) and other family C GPRCs. *Curr Pharm Des* **12:**2159–2173.
Costantino G, Macchiarulo A, Entrena Guadix A, and Pellicciari R (2001) QSAR and molecular modeling studies of baclofen analogues as $GABA_B$ agonists. Insights into the role of the aromatic moiety in $GABA_B$ binding and activation. *J Med Chem* **44:**1827–1832.
Cryan JF, Kelly PH, Chaperon F, Gentsch C, Mombereau C, Lingenhoehl K, Froestl W, Bettler B, Kaupmann K, and Spooren WP (2004) Behavioral characterization of the novel $GABA_B$ receptor-positive modulator GS39783 (*N,N′*-dicyclopentyl-2-methylsulfanyl-5-nitro-pyrimidine-4,6-diamine): anxiolytic-like activity without side effects associated with baclofen or benzodiazepines. *J Pharmacol Exp Ther* **310:**952–963.
Cube RV, Vernier JM, Hutchinson JH, Gardner MF, James JK, Rowe BA, Schaffhauser H, Daggett L, and Pinkerton AB (2005) 3-(2-Ethoxy-4-{4-[3-hydroxy-2-methyl-4-(3-methylbutanoyl)phenoxy]butoxy}phenyl)propanoic acid: a brain penetrant allosteric potentiator at the metabotropic glutamate receptor 2 (mGluR2). *Bioorg Med Chem Lett* **15:**2389–2393.
Cui M, Jiang P, Maillet E, Max M, Margolskee RF, and Osman R (2006) The heterodimeric sweet taste receptor has multiple potential ligand binding sites. *Curr Pharm Des* **12:**4591–4600.
Cunningham J, Danese M, Olson K, Klassen P, and Chertow GM (2005) Effects of the calcimimetic cinacalcet HCl on cardiovascular disease, fracture, and health-related quality of life in secondary hyperparathyroidism. *Kidney Int* **68:**1793–1800.
Cunningham MD and Enna SJ (1996) Evidence for pharmacologically distinct $GABA_B$ receptors associated with cAMP production in rat brain. *Brain Res* **720:**220–224.
D'Alessandro PL, Corti C, Roth A, Ugolini A, Sava A, Montanari D, Bianchi F, Garland SL, Powney B, Koppe EL, et al. (2010) The identification of structurally novel, selective, orally bioavailable positive modulators of mGluR2. *Bioorg Med Chem Lett* **20:**759–762.
Darrah JM, Stefani MR, and Moghaddam B (2008) Interaction of *N*-methyl-D-aspartate and group 5 metabotropic glutamate receptors on behavioral flexibility using a novel operant set-shift paradigm. *Behav Pharmacol* **19:**225–234.
Dauban P, Ferry S, Faure H, Ruat M, and Dodd RH (2000) N1-Arylsulfonyl-N2-(1-aryl)ethyl-3-phenylpropane-1,2-diamines as novel calcimimetics acting on the calcium sensing receptor. *Bioorg Med Chem Lett* **10:**2001–2004.
De Amici M, Dallanoce C, Holzgrabe U, Tränkle C, and Mohr K (2010) Allosteric ligands for G protein-coupled receptors: a novel strategy with attractive therapeutic opportunities. *Med Res Rev* **30:**463–549.
De Lean A, Stadel JM, and Lefkowitz RJ (1980) A ternary complex model explains the agonist-specific binding properties of the adenylate cyclase-coupled beta-adrenergic receptor. *J Biol Chem* **255:**7108–7117.
de Paulis T, Hemstapat K, Chen Y, Zhang Y, Saleh S, Alagille D, Baldwin RM, Tamagnan GD, and Conn PJ (2006) Substituent effects of *N*-(1,3-diphenyl-1*H*-pyrazol-5-yl)benzamides on positive allosteric modulation of the metabotropic glutamate-5 receptor in rat cortical astrocytes. *J Med Chem* **49:**3332–3344.
Deal C (2009) Future therapeutic targets in osteoporosis. *Curr Opin Rheumatol* **21:**380–385.
DeLapp NW (2004) The antibody-capture [^{35}S]GTP[γ]S scintillation proximity assay: a powerful emerging technique for analysis of GPCR pharmacology. *Trends Pharmacol Sci* **25:**400–401.
Dempster DW, Cosman F, Parisien M, Shen V, and Lindsay R (1993) Anabolic actions of parathyroid hormone on bone. *Endocr Rev* **14:**690–709.
Di Fabio R, Micheli F, Alvaro G, Cavanni P, Donati D, Gagliardi T, Fontana G, Giovannini R, Maffeis M, Mingardi A, et al. (2007) From pyrroles to 1-oxo-2,3,4,9-tetrahydro-1*H*-beta-carbolines: a new class of orally bioavailable mGluR1 antagonists. *Bioorg Med Chem Lett* **17:**2254–2259.
Dolan S, Gunn MD, Biddlestone L, and Nolan AM (2009) The selective metabotropic glutamate receptor 7 allosteric agonist AMN082 inhibits inflammatory pain-induced and incision-induced hypersensitivity in rat. *Behav Pharmacol* **20:**596–604.
Dölen G, Osterweil E, Rao BS, Smith GB, Auerbach BD, Chattarji S, and Bear MF (2007) Correction of fragile X syndrome in mice. *Neuron* **56:**955–962.
Drüeke TB and Ritz E (2009) Treatment of secondary hyperparathyroidism in CKD patients with cinacalcet and/or vitamin D derivatives. *Clin J Am Soc Nephrol* **4:**234–241.
Duplantier AJ, Efremov I, Candler J, Doran AC, Ganong AH, Haas JA, Hanks AN, Kraus KG, Lazzaro JT Jr, Lu J, et al. (2009) 3-Benzyl-1,3-oxazolidin-2-ones as mGluR2 positive allosteric modulators: hit-to lead and lead optimization. *Bioorg Med Chem Lett* **19:**2524–2529.
Dupuis DS, Relkovic D, Lhuillier L, Mosbacher J, and Kaupmann K (2006) Point mutations in the transmembrane region of $GABA_{B2}$ facilitate activation by the positive modulator *N,N′*-dicyclopentyl-2-methylsulfanyl-5-nitro-pyrimidine-4,6-diamine (GS39783) in the absence of the $GABA_{B1}$ subunit. *Mol Pharmacol* **70:**2027–2036.
East SP and Gerlach K (2010) mGluR4 positive allosteric modulators with potential for the treatment of Parkinson's disease: WO09010455. *Expert Opin Ther Patents* **20:**441–445.
El-Kouhen O, Lehto SG, Pan JB, Chang R, Baker SJ, Zhong C, Hollingsworth PR, Mikusa JP, Cronin EA, Chu KL, et al. (2006) Blockade of mGluR1 receptor results in analgesia and disruption of motor and cognitive performances: effects of A-841720, a novel non-competitive mGluR1 receptor antagonist. *Br J Pharmacol* **149:**761–774.
Engers DW, Niswender CM, Weaver CD, Jadhav S, Menon UN, Zamorano R, Conn PJ, Lindsley CW, and Hopkins CR (2009a) Synthesis and evaluation of a series of heterobiarylamides that are centrally penetrant metabotropic glutamate receptor 4 (mGluR4) positive allosteric modulators (PAMs). *J Med Chem* **52:**4115–4118.
Engers DW, Rodriguez AL, Williams R, Hammond AS, Venable D, Oluwatola O, Sulikowski GA, Conn PJ, and Lindsley CW (2009b) Synthesis, SAR and unanticipated pharmacological profiles of analogues of the mGluR5 ago-potentiator ADX-47273. *ChemMedChem* **4:**505–511.
Fadda P, Scherma M, Fresu A, Collu M, and Fratta W (2003) Baclofen antagonizes nicotine-, cocaine-, and morphine-induced dopamine release in the nucleus accumbens of rat. *Synapse* **50:**1–6.
Fan GF, Ray K, Zhao XM, Goldsmith PK, and Spiegel AM (1998) Mutational analysis of the cysteines in the extracellular domain of the human Ca^{2+} receptor: effects on cell surface expression, dimerization and signal transduction. *FEBS Lett* **436:**353–356.
Fazio F, Notartomaso S, Aronica E, Storto M, Battaglia G, Vieira E, Gatti S, Bruno V, Biagioni F, Gradini R, et al. (2008) Switch in the expression of mGlu1 and mGlu5 metabotropic glutamate receptors in the cerebellum of mice developing experimental autoimmune encephalomyelitis and in autoptic cerebellar samples from patients with multiple sclerosis. *Neuropharmacology* **55:**491–499.
Fell MJ, Svensson KA, Johnson BG, and Schoepp DD (2008) Evidence for the role of metabotropic glutamate (mGlu)2 not mGlu3 receptors in the preclinical antipsychotic pharmacology of the mGlu2/3 receptor agonist (−)-(1*R*,4*S*,5*S*,6*S*)-4-amino-2-sulfonylbicyclo[3.1.0]hexane-4,6-dicarboxylic acid (LY404039). *J Pharmacol Exp Ther* **326:**209–217.
Felts AS, Saleh SA, Le U, Rodriguez AL, Weaver CD, Conn PJ, Lindsley CW, and Emmitte KA (2009) Discovery and SAR of 6-substituted-4-anilinoquinazolines as non-competitive antagonists of mGlu5. *Bioorg Med Chem Lett* **19:**6623–6626.
Fendt M, Schmid S, Thakker DR, Jacobson LH, Yamamoto R, Mitsukawa K, Maier R, Natt F, Hüsken D, Kelly PH, et al. (2008) mGluR7 facilitates extinction of aversive memories and controls amygdala plasticity. *Mol Psychiatry* **13:**970–979.
Ferraguti F, Crepaldi L, and Nicoletti F (2008) Metabotropic glutamate 1 receptor: current concepts and perspectives. *Pharmacol Rev* **60:**536–581.
Filip M and Frankowska M (2007) Effects of $GABA_B$ receptor agents on cocaine priming, discrete contextual cue and food induced relapses. *Eur J Pharmacol* **571:**166–173.
Filip M and Frankowska M (2008) GABA(B) receptors in drug addiction. *Pharmacol Rep* **60:**755–770.
Fitzpatrick L, Dabrowski C, Cicconetti G, Fuerst T, Engelke K, and Genant H (2009) The calcium-sensing receptor antagonist, ronacaleret (SB-751689) causes modest increases in trabecular but not cortical BMD by QCT in postmenopausal women (Abstract). *J Bone Miner Res* **24 (Suppl 1):**A09001332.
Fox J, Lowe SH, Conklin RL, and Nemeth EF (1999) The calcimimetic NPS R-568 decreases plasma PTH in rats with mild and severe renal or dietary secondary hyperparathyroidism. *Endocrine* **10:**97–103.
Fox J, Lowe SH, Conklin RL, Petty BA, and Nemeth EF (1999b) Calcimimetic compound NPS R-568 stimulates calcitonin secretion but selectively targets parathyroid gland Ca^{2+} receptor in rats. *J Pharmacol Exp Ther* **290:**480–486.
Fox J, Lowe SH, Petty BA, and Nemeth EF (1999a) NPS R-568: a type II calcimimetic compound that acts on parathyroid cell calcium receptor of rats to reduce plasma levels of parathyroid hormone and calcium. *J Pharmacol Exp Ther* **290:**473–479.
Fox J, Miller MA, Stroup GB, Nemeth EF, and Miller SC (1997) Plasma levels of parathyroid hormone that induce anabolic effects in bone of ovariectomized rats can be achieved by stimulation of endogenous hormone secretion. *Bone* **21:**163–169.
Franceschini N, Joy MS, and Kshirsagar A (2003) Cinacalcet HCl: a calcimimetic agent for the management of primary and secondary hyperparathyroidism. *Expert Opin Investig Drugs* **12:**1413–1421.
Frauli M, Neuville P, Vol C, Pin JP, and Prézeau L (2006) Among the twenty classical L-amino acids, only glutamate directly activates metabotropic glutamate receptors. *Neuropharmacology* **50:**245–253.
Fredriksson R, Lagerström MC, Lundin LG, and Schiöth HB (2003) The G-protein-coupled receptors in the human genome form five main families. Phylogenetic analysis, paralogon groups, and fingerprints. *Mol Pharmacol* **63:**1256–1272.
Friedmann CT, Davis LJ, Ciccone PE, and Rubin RT (1980) Phase II double blind controlled study of a new anxiolytic, fenobam (McN-3377) vs. placebo. *Curr Ther Res* **27:**144–151.
Frisby CL, Mattsson JP, Jensen JM, Lehmann A, Dent J, and Blackshaw LA (2005) Inhibition of transient lower esophageal sphincter relaxation and gastroesophageal reflux by metabotropic glutamate receptor ligands. *Gastroenterology* **129:**995–1004.
Froestl W (2010) Novel $GABA_B$ receptor positive modulators: a patent survey. *Expert Opin Ther Pat* **20:**1007–1017.
Fukuda J, Suzuki G, Kimura T, Nagatomi Y, Ito S, Kawamoto H, Ozaki S, and Ohta H (2009) Identification of a novel transmembrane domain involved in the negative modulation of mGluR1 using a newly discovered allosteric mGluR1 antagonist, 3-cyclohexyl-5-fluoro-6-methyl-7-(2-morpholin-4-ylethoxy)-4H-chromen-4-one. *Neuropharmacology* **57:**438–445.
Gainetdinov RR, Premont RT, Bohn LM, Lefkowitz RJ, and Caron MG (2004) Desensitization of G protein-coupled receptors and neuronal functions. *Annu Rev Neurosci* **27:**107–144.
Galici R, Echemendia NG, Rodriguez AL, and Conn PJ (2005) A selective allosteric potentiator of metabotropic glutamate (mGlu) 2 receptors has effects similar to an orthosteric mGlu2/3 receptor agonist in mouse models predictive of antipsychotic activity. *J Pharmacol Exp Ther* **315:**1181–1187.
Galici R, Jones CK, Hemstapat K, Nong Y, Echemendia NG, Williams LC, de Paulis T, and Conn PJ (2006) Biphenyl-indanone A, a positive allosteric modulator of the metabotropic glutamate receptor subtype 2, has antipsychotic- and anxiolytic-like effects in mice. *J Pharmacol Exp Ther* **318:**173–185.
Galvez T, Duthey B, Kniazeff J, Blahos J, Rovelli G, Bettler B, Prézeau L, and Pin JP (2001) Allosteric interactions between GB1 and GB2 subunits are required for optimal $GABA_B$ receptor function. *EMBO J* **20:**2152–2159.

Galvez T, Parmentier ML, Joly C, Malitschek B, Kaupmann K, Kuhn R, Bittiger H, Froestl W, Bettler B, and Pin JP (1999) Mutagenesis and modeling of the $GABA_B$ receptor extracellular domain support a venus flytrap mechanism for ligand binding. *J Biol Chem* **274:**13362–13369.

Galvez T, Prezeau L, Milioti G, Franek M, Joly C, Froestl W, Bettler B, Bertrand HO, Blahos J, and Pin JP (2000a) Mapping the agonist-binding site of $GABA_B$ type 1 subunit sheds light on the activation process of $GABA_B$ receptors. *J Biol Chem* **275:**41166–41174.

Galvez T, Urwyler S, Prézeau L, Mosbacher J, Joly C, Malitschek B, Heid J, Brabet I, Froestl W, Bettler B, et al. (2000b) Ca^{2+} requirement for high-affinity gamma-aminobutyric acid (GABA) binding at $GABA_B$ receptors: involvement of serine 269 of the $GABA_B$R1 subunit. *Mol Pharmacol* **57:**419–426.

Garrett JE, Capuano IV, Hammerland LG, Hung BC, Brown EM, Hebert SC, Nemeth EF, and Fuller F (1995) Molecular cloning and functional expression of human parathyroid calcium receptor cDNAs. *J Biol Chem* **270:**12919–12925.

Gasparini F, Andres H, Flor PJ, Heinrich M, Inderbitzin W, Lingenhöhl K, Müller H, Munk VC, Omilusik K, Stierlin C, et al. (2002) [^{3}H]-M-MPEP, a potent, subtype-selective radioligand for the metabotropic glutamate receptor subtype 5. *Bioorg Med Chem Lett* **12:**407–409.

Gasparini F, Bilbe G, Gomez-Mancilla B, and Spooren W (2008) mGluR5 antagonists: discovery, characterization and drug development. *Curr Opin Drug Discov Devel* **11:**655–665.

Gasparini F, Lingenhöhl K, Stoehr N, Flor PJ, Heinrich M, Vranesic I, Biollaz M, Allgeier H, Heckendorn R, Urwyler S, et al. (1999) 2-Methyl-6-(phenylethynyl)-pyridine (MPEP), a potent, selective and systemically active mGlu5 receptor antagonist. *Neuropharmacology* **38:**1493–1503.

Gavai AV, Vaz RJ, Mikkilineni AB, Roberge JY, Liu Y, Lawrence RM, Corte JR, Yang W, Bednarz M, Dickson JK Jr, et al. (2005) Discovery of novel 1-arylmethyl pyrrolidin-2-yl ethanol amines as calcium-sensing receptor antagonists. *Bioorg Med Chem Lett* **15:**5478–5482.

Gjoni T, Desrayaud S, Imobersteg S, and Urwyler S (2006) The positive allosteric modulator GS39783 enhances $GABA_B$ receptor-mediated inhibition of cyclic AMP formation in rat striatum in vivo. *J Neurochem* **96:**1416–1422.

Gjoni T and Urwyler S (2009) Changes in the properties of allosteric and orthosteric $GABA_B$ receptor ligands after a continuous, desensitizing agonist pretreatment. *Eur J Pharmacol* **603:**37–41.

Gjoni T and Urwyler S (2008) Receptor activation involving positive allosteric modulation, unlike full agonism, does not result in $GABA_B$ receptor desensitization. *Neuropharmacology* **55:**1293–1299.

Goodman WG, Frazao JM, Goodkin DA, Turner SA, Liu W, and Coburn JW (2000) A calcimimetic agent lowers plasma parathyroid hormone levels in patients with secondary hyperparathyroidism. *Kidney Int* **58:**436–445.

Goudet C, Chapuy E, Alloui A, Acher F, Pin JP, and Eschalier A (2008) Group III metabotropic glutamate receptors inhibit hyperalgesia in animal models of inflammation and neuropathic pain. *Pain* **137:**112–124.

Goudet C, Gaven F, Kniazeff J, Vol C, Liu J, Cohen-Gonsaud M, Acher F, Prézeau L, and Pin JP (2004) Heptahelical domain of metabotropic glutamate receptor 5 behaves like rhodopsin-like receptors. *Proc Natl Acad Sci USA* **101:**378–383.

Goudet C, Kniazeff J, Hlavackova V, Malhaire F, Maurel D, Acher F, Blahos J, Prézeau L, and Pin JP (2005) Asymmetric functioning of dimeric metabotropic glutamate receptors disclosed by positive allosteric modulators. *J Biol Chem* **280:**24380–24385.

Govek SP, Bonnefous C, Hutchinson JH, Kamenecka T, McQuiston J, Pracitto R, Zhao LX, Gardner MF, James JK, Daggett LP, et al. (2005) Benzazoles as allosteric potentiators of metabotropic glutamate receptor 2 (mGluR2): efficacy in an animal model for schizophrenia. *Bioorg Med Chem Lett* **15:**4068–4072.

Gowen M, Stroup GB, Dodds RA, James IE, Votta BJ, Smith BR, Bhatnagar PK, Lago AM, Callahan JF, DelMar EG, et al. (2000) Antagonizing the parathyroid calcium receptor stimulates parathyroid hormone secretion and bone formation in osteopenic rats. *J Clin Invest* **105:**1595–1604.

Greco B, Lopez S, van der Putten H, Flor PJ, and Amalric M (2010) Metabotropic glutamate 7 receptor subtype modulates motor symptoms in rodent models of parkinson's disease. *J Pharmacol Exp Ther* **332:**1064–1071.

Gregory KJ, Dong EN, Meiler J, and Conn PJ (2011) Allosteric modulation of metabotropic glutamate receptors: structural insights and therapeutic potential. *Neuropharmacology* **60:**66–81

Grillon C, Cordova J, Levine LR, and Morgan CA 3rd (2003) Anxiolytic effects of a novel group II metabotropic glutamate receptor agonist (LY354740) in the fear-potentiated startle paradigm in humans. *Psychopharmacology* (*Berl*) **168:**446–454.

Grünewald S, Schupp BJ, Ikeda SR, Kuner R, Steigerwald F, Kornau HC, and Köhr G (2003) Importance of the γ-aminobutyric acid B receptor C-termini for G-protein coupling. *Mol Pharmacol* **61:**1070–1080.

Guery S, Floersheim P, Kaupmann K, and Froestl W (2007) Syntheses and optimization of new GS39783 analogues as positive allosteric modulators of $GABA_B$ receptors. *Bioorg Med Chem Lett* **17:**6206–6211.

Hackler EA, Byun NE, Jones CK, Williams JM, Baheza R, Sengupta S, Grier MD, Avison M, Conn PJ, and Gore JC (2010) Selective potentiation of the metabotropic glutamate receptor subtype 2 blocks phencyclidine-induced hyperlocomotion and brain activation. *Neuroscience* **168:**209–218.

Hall DA (2000) Modeling the functional effects of allosteric modulators at pharmacological receptors: an extension of the two-state model of receptor activation. *Mol Pharmacol* **58:**1412–1423.

Hamill TG, Krause S, Ryan C, Bonnefous C, Govek S, Seiders TJ, Cosford ND, Roppe J, Kamenecka T, Patel S, et al. (2005) Synthesis, characterization, and first successful monkey imaging studies of metabotropic glutamate receptor subtype 5 (mGluR5) PET radiotracers. *Synapse* **56:**205–216.

Hammerland LG, Garrett JE, Hung BC, Levinthal C, and Nemeth EF (1998) Allosteric activation of the Ca^{2+} receptor expressed in xenopus laevis oocytes by NPS 467 or NPS 568. *Mol Pharmacol* **53:**1083–1088.

Hammerland LG, Krapcho KJ, Garrett JE, Alasti N, Hung BC, Simin RT, Levinthal C, Nemeth EF, and Fuller FH (1999) Domains determining ligand specificity for Ca^{2+} receptors. *Mol Pharmacol* **55:**642–648.

Hammond AS, Rodriguez AL, Townsend SD, Niswender CM, Gregory KJ, Lindsley CW, and Conn PJ (2010) Discovery of a novel chemical class of mGlu(5) allosteric ligands with distinct modes of pharmacology. *ACS Chem Neurosci* **1:**702–716.

Harrington PE and Fotsch C (2007) Calcium sensing receptor activators: calcimimetics. *Curr Med Chem* **14:**3027–3034.

Harrington PE, St Jean DJ Jr, Clarine J, Coulter TS, Croghan M, Davenport A, Davis J, Ghiron C, Hutchinson J, Kelly MG, Lott F, Lu JY, Martin D, Morony S, Poon SF, Portero-Larragueta E, Reagan JD, Regal KA, Tasker A, Wang M, Yang Y, Yao G, Zeng Q, Henley C 3rd, and Fotsch C.Jean Jr DJ, Clarine J, Coulter TS, Croghan M, Davenport A, Davis J, Ghiron C, Hutchinson J, Kelly MG, Lott F, Lu JYL, Martin D, Morony S, Poon SF, Portero-Larragueta E, Reagan JD, Regal KA, Tasker A, Wang M, Yang Y, Yao G, Zeng Q, Henley III C, and Fotsch C (2010) The discovery of an orally efficacious positive allosteric modulator of the calcium sensing receptor containing a dibenzylamine core. *Bioorg Med Chem Lett* **20:**5544–5547.

Havlickova M, Blahos J, Brabet I, Liu J, Hruskova B, Prézeau L, and Pin JP (2003) The second intracellular loop of metabotropic glutamate receptors recognizes C termini of G-protein α-subunits. *J Biol Chem* **278:**35063–35070.

Hawrot E, Xiao Y, Shi QL, Norman D, Kirkitadze M, and Barlow PN (1998) Demonstration of a tandem pair of complement protein modules in $GABA_B$ receptor 1a. *FEBS Lett* **432:**103–108.

Heidbreder C (2005) Novel pharmacotherapeutic targets for the management of drug addiction. *Eur J Pharmacol* **526:**101–112.

Hemstapat K, Da Costa H, Nong Y, Brady AE, Luo Q, Niswender CM, Tamagnan GD, and Conn PJ (2007) A novel family of potent negative allosteric modulators of group II metabotropic glutamate receptors. *J Pharmacol Exp Ther* **322:**254–264.

Hemstapat K, de Paulis T, Chen Y, Brady AE, Grover VK, Alagille D, Tamagnan GD, and Conn PJ (2006) A novel class of positive allosteric modulators of metabotropic glutamate receptor subtype 1 interact with a site distinct from that of negative allosteric modulators. *Mol Pharmacol* **70:**616–626.

Henry SA, Lehmann-Masten V, Gasparini F, Geyer MA, and Markou A (2002) The mGluR5 antagonist MPEP, but not the mGluR2/3 agonist LY314582, augments PCP effects on prepulse inhibition and locomotor activity. *Neuropharmacology* **43:**1199–1209.

Hermans E, Nahorski SR, and Challiss RA (1998) Reversible and non-competitive antagonist profile of CPCCOEt at the human type 1α metabotropic glutamate receptor. *Neuropharmacology* **37:**1645–1647.

Hikichi H, Murai T, Okuda S, Maehara S, Satow A, Ise S, Nishino M, Suzuki G, Takehana H, Hata M, et al. (2010a) Effects of a novel metabotropic glutamate receptor 7 negative allosteric modulator, 6-(4-methoxyphenyl)-5-methyl-3-pyridin-4-ylisoxazonolo[4,5-c]pyridin-4
(5*H*)-one (MMPIP),on the central nervous system in rodents. *Eur J Pharmacol* **639:**106–114.

Hikichi H, Nishino M, Fukushima M, Satow A, Maehara S, Kawamoto H, and Ohta H (2010b) Pharmacological effects of metabotropic glutamate receptor ligands on prepulse inhibition in DBA/2J mice. *Eur J Pharmacol* **639:**99–105.

Hill DR and Bowery NG (1981) ^{3}H-baclofen and ^{3}H-GABA bind to bicuculline-insensitive GABA B sites in rat brain. *Nature* **290:**149–152.

Hintermann S, Vranesic I, Allgeier H, Brülisauer A, Hoyer D, Lemaire M, Moenius T, Urwyler S, Whitebread S, Gasparini F, et al. (2007) ABP688, a novel selective and high affinity ligand for the labeling of mGlu5 receptors: identification, in vitro pharmacology, pharmacokinetic and biodistribution studies. *Bioorg Med Chem* **15:**903–914.

Hirst WD, Babbs AJ, Green A, Minton JA, Shaw TE, Wise A, Rice SQ, Pangalos MN, and Price GW (2003) Pharmacological characterisation of a cell line expressing $GABA_{B1b}$ and $GABA_{B2}$ receptor subunits. *Biochem Pharmacol* **65:**1103–1113.

Hlavackova V, Goudet C, Kniazeff J, Zikova A, Maurel D, Vol C, Trojanova J, Prézeau L, Pin JP, and Blahos J (2005) Evidence for a single heptahelical domain being turned on upon activation of a dimeric GPCR. *EMBO J* **24:**499–509.

Ho C, Conner DA, Pollak MR, Ladd DJ, Kifor O, Warren HB, Brown EM, Seidman JG, and Seidman CE (1995) A mouse model of human familial hypocalciuric hypercalcemia and neonatal severe hyperparathyroidism. *Nat Genet* **11:**389–394.

Hopkins AL and Groom CR (2002) The druggable genome. *Nat Rev Drug Discov* **1:**727–730.

Hu E, Chua PC, Tehrani L, Nagasawa JY, Pinkerton AB, Rowe BA, Vernier JM, Hutchinson JH, and Cosford ND (2004) Pyrimidine methyl anilines: selective potentiators for the metabotropic glutamate 2 receptor. *Bioorg Med Chem Lett* **14:**5071–5074.

Hu J (2008) Allosteric modulators of the human calcium-sensing receptor: structures, sites of action, and therapeutic potentials. *Endocr Metab Immune Disord Drug Targets* **8:**192–197.

Hu J, Jiang J, Costanzi S, Thomas C, Yang W, Feyen JH, Jacobson KA, and Spiegel AM (2006) A missense mutation in the seven-transmembrane domain of the human Ca^{2+} receptor converts a negative allosteric modulator into a positive allosteric modulator. *J Biol Chem* **281:**21558–21565.

Hu J and Spiegel AM (2003) Naturally occurring mutations of the extracellular Ca^{2+}-sensing receptor: implications for its structure and function. *Trends Endocrinol Metab* **14:**282–288.

Huber KM, Gallagher SM, Warren ST, and Bear MF (2002) Altered synaptic plasticity in a mouse model of fragile X mental retardation. *Proc Natl Acad Sci USA* **99:**7746–7750.

Jacob W, Gravius A, Pietraszek M, Nagel J, Belozertseva I, Shekunova E, Malyshkin A, Greco S, Barberi C, and Danysz W (2009) The anxiolytic and analgesic properties of fenobam, a potent mGlu5 receptor antagonist, in relation to the impairment of learning. *Neuropharmacology* **57:**97–108.

Jacobson LH and Cryan JF (2008) Evaluation of the anxiolytic-like profile of the $GABA_B$ receptor positive modulator CGP7930 in rodents. *Neuropharmacology* **54:**854–862.

Jaeschke G, Wettstein JG, Nordquist RE, and Spooren W (2008) mGlu5 receptor antagonists and their therapeutic potential. *Expert Opin Ther Patents* **18:**123–142.
Jayaram P and Steketee JD (2004) Effects of repeated cocaine on medial prefrontal cortical $GABA_B$ receptor modulation of neurotransmission in the mesocorticolimbic dopamine system. *J Neurochem* **90:**839–847.
Jensen AA and Bräuner-Osborne H (2007) Allosteric modulation of the calcium-sensing receptor. *Current Neuropharmacology* **5:**180–186.
Jensen J, Lehmann A, Uvebrant A, Carlsson A, Jerndal G, Nilsson K, Frisby C, Blackshaw LA, and Mattsson JP (2005) Transient lower esophageal sphincter relaxations in dogs are inhibited by a metabotropic glutamate receptor 5 antagonist. *Eur J Pharmacol* **519:**154–157.
Jiang P, Cui M, Zhao B, Liu Z, Snyder LA, Benard LM, Osman R, Margolskee RF, and Max M (2005a) Lactisole interacts with the transmembrane domains of human T1R3 to inhibit sweet taste. *J Biol Chem* **280:**15238–15246.
Jiang P, Cui M, Zhao B, Snyder LA, Benard LM, Osman R, Max M, and Margolskee RF (2005b) Identification of the cyclamate interaction site within the transmembrane domain of the human sweet taste receptor subunit T1R3. *J Biol Chem* **280:**34296–34305.
Jiang P, Ji Q, Liu Z, Snyder LA, Benard LM, Margolskee RF, and Max M (2004) The cysteine-rich region of T1R3 determines responses to intensely sweet proteins. *J Biol Chem* **279:**45068–45075.
Jin X, Semenova S, Yang L, Ardecky R, Sheffler DJ, Dahl R, Conn PJ, Cosford ND, and Markou A (2010) The mGluR2 positive allosteric modulator BINA decreases cocaine self-administration and cue-induced cocaine-seeking and counteracts cocaine-induced enhancement of brain reward function in rats. *Neuropsychopharmacology* **35:**2021–2036.
Johnson MP, Baez M, Jagdmann GE, Jr., Britton TC, Large TH, Callagaro DO, Tizzano JP, Monn JA, and Schoepp DD (2003) Discovery of allosteric potentiators for the metabotropic glutamate 2 receptor: synthesis and subtype selectivity of *N*-(4-(2-methoxyphenoxy)phenyl)-*N*-(2,2,2-trifluoroethylsulfonyl)pyrid-3-ylmethylamine. *J Med Chem* **46:**3189–3192.
Johnson MP, Barda D, Britton TC, Emkey R, Hornback WJ, Jagdmann GE, McKinzie DL, Nisenbaum ES, Tizzano JP, and Schoepp DD (2005) Metabotropic glutamate 2 receptor potentiators: receptor modulation, frequency-dependent synaptic activity, and efficacy in preclinical anxiety and psychosis model(s). *Psychopharmacology* **179:**271–283.
Johnson MP, Nisenbaum ES, Large TH, Emkey R, Baez M, and Kingston AE (2004) Allosteric modulators of metabotropic glutamate receptors: lessons learnt from mGlu1, mGlu2 and mGlu5 potentiators and antagonists. *Biochem Soc Trans* **32:**881–887.
Johnston TH, Fox SH, McIldowie MJ, Piggott MJ, and Brotchie JM (2010) Reduction of L-DOPA-induced dyskinesia by the selective metabotropic glutamate receptor 5 antagonist 3-[(2-methyl-1,3-thiazol-4-yl)ethynyl]pyridine in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-lesioned macaque model of Parkinson's disease. *J Pharmacol Exp Ther* **333:**865–873.
Joki N, Nikolov IG, Caudrillier A, Mentaverri R, Massy ZA, and Drüeke TB (2009) Effects of calcimimetic on vascular calcification and atherosclerosis in uremic mice. *Bone* **45:**S30–S34.
Jones KA, Borowsky B, Tamm JA, Craig DA, Durkin MM, Dai M, Yao WJ, Johnson M, Gunwaldsen C, Huang LY, et al. (1998) $GABA_B$ receptors function as a heteromeric assembly of the subunits $GABA_BR1$ and $GABA_BR2$. *Nature* **396:**674–679.
Kanuma K, Aoki T, and Shimazaki Y (2010) Recent patents on positive allosteric modulators of the metabotropic glutamate 5 receptor as a potential treatment for schizophrenia. *Recent Pat CNS Drug Discov* **5:**23–34.
Kaupmann K, Cryan JF, Wellendorph P, Mombereau C, Sansig G, Klebs K, Schmutz M, Froestl W, van der Putten H, Mosbacher J, et al. (2003) Specific γ-hydroxybutyrate-binding sites but loss of pharmacological effects of γ-hydroxybutyrate in $GABA_{B(1)}$-deficient mice. *Eur J Neurosci* **18:**2722–2730.
Kaupmann K, Huggel K, Heid J, Flor PJ, Bischoff S, Mickel SJ, McMaster G, Angst C, Bittiger H, Froestl W, et al. (1997) Expression cloning of $GABA_B$ receptors uncovers similarity to metabotropic glutamate receptors. *Nature* **386:**239–246.
Kaupmann K, Malitschek B, Schuler V, Heid J, Froestl W, Beck P, Mosbacher J, Bischoff S, Kulik A, Shigemoto R, et al. (1998) $GABA_B$-receptor subtypes assemble into functional heteromeric complexes. *Nature* **396:**683–687.
Kenakin T (2005) New concepts in drug discovery: collateral efficacy and permissive antagonism. *Nat Rev Drug Discov* **4:**919–927.
Kenakin T and Miller LJ (2010) Seven transmembrane receptors as shapeshifting proteins: the impact of allosteric modulation and functional selectivity on new drug discovery. *Pharmacol Rev* **62:**265–304.
Kerr DI, Khalafy J, Ong J, Perkins MV, Prager RH, Puspawati NM, and Rimaz M (2006) Synthesis and biological activity of allosteric modulators of $GABA_B$ receptors, part 2. 3-(2,6-bis-tert-butyl-4-hydroxyphenyl)propanols. *Aust J Chem* **59:**457–462.
Kerr DI, Khalafy J, Ong J, Prager RH, and Rimaz M (2007) Synthesis and biological activity of allosteric modulators of GABA B receptors part 3. 3-(2,6-bis-iso-propyl-4-hydroxyphenyl)propanols. *J Braz Chem Soc* **18:**721–727.
Kerr DI and Ong J (2003) Potentiation of metabotropic $GABA_B$ receptors by L-amino acids and dipeptides in rat neocortex. *Eur J Pharmacol* **468:**103–108.
Kerr DI and Ong J (2006) Allosteric interactions at $GABA_B$ and related G-protein-coupled receptors, in *Allosteric Receptor Modulation in Drug Targeting* (Bowery NG ed) pp 259–285, Taylor & Francis, New York.
Kerr DI, Ong J, Puspawati NM, and Prager RH (2002) Arylalkylamines are a novel class of positive allosteric modulators at $GABA_B$ receptors in rat neocortex. *Eur J Pharmacol* **451:**69–77.
Kessler A, Faure H, Petrel C, Rognan D, Césario M, Ruat M, Dauban P, and Dodd RH (2006) N1-Benzoyl-N2-[1-(1-naphthyl)ethyl]-trans-1,2-diaminocyclohexanes: development of 4-chlorophenylcarboxamide (calhex 231) as a new calcium sensing receptor ligand demonstrating potent calcilytic activity. *J Med Chem* **49:**5119–5128.
Kessler A, Faure H, Petrel C, Ruat M, Dauban P, and Dodd RH (2004) N2-benzyl-N1-(1-(1-naphthyl)ethyl)-3-phenylpropane-1,2-diamines and conformationally restrained indole analogues: development of calindol as a new calcimimetic acting at the calcium sensing receptor. *Bioorg Med Chem Lett* **14:**3345–3349.
Keywood C, Wakefield M, and Tack J (2009) A proof-of-concept study evaluating the effect of ADX10059, a metabotropic glutamate receptor-5 negative allosteric modulator, on acid exposure and symptoms in gastro-oesophageal reflux. *Gut* **58:**1192–1199.
Kifor O, Diaz R, Butters R, and Brown EM (1997) The Ca^{2+}-sensing receptor (CaR) activates phospholipases C, A2, and D in bovine parathyroid and CaR-transfected, human embryonic kidney (HEK293) cells. *J Bone Mineral Res* **12:**715–725.
Kim YH, Park CK, Back SK, Lee CJ, Hwang SJ, Bae YC, Na HS, Kim JS, Jung SJ, and Oh SB (2009) Membrane-delimited coupling of TRPV1 and mGluR5 on presynaptic terminals of nociceptive neurons. *J Neurosci* **29:**10000–10009.
Kimmel DB, Bozzato RP, Kronis KA, Coble T, Sindrey D, Kwong P, and Recker RR (1993) The effect of recombinant human (1–84) or synthetic human (1–34) parathyroid hormone on the skeleton of adult osteopenic ovariectomized rats. *Endocrinology* **132:**1577–1584.
Kinney GG, Burno M, Campbell UC, Hernandez LM, Rodriguez D, Bristow LJ, and Conn PJ (2003) Metabotropic glutamate subtype 5 receptors modulate locomotor activity and sensorimotor gating in rodents. *J Pharmacol Exp Ther* **306:**116–123.
Kinney GG, O'Brien JA, Lemaire W, Burno M, Bickel DJ, Clements MK, Chen TB, Wisnoski DD, Lindsley CW, Tiller PR, et al. (2005) A novel selective positive allosteric modulator of metabotropic glutamate receptor subtype 5 has in vivo activity and antipsychotic-like effects in rat behavioral models. *J Pharmacol Exp Ther* **313:**199–206.
Kitagawa M, Kusakabe Y, Miura H, Ninomiya Y, and Hino A (2001) Molecular genetic identification of a candidate receptor gene for sweet taste. *Biochem Biophys Res Comm* **283:**236–242.
Kniazeff J, Saintot PP, Goudet C, Liu J, Charnet A, Guillon G, and Pin JP (2004) Locking the dimeric $GABA_B$ G-protein-coupled receptor in its active state. *J Neurosci* **24:**370–377.
Knight AR and Bowery NG (1996) The pharmacology of adenylyl cyclase modulation by $GABA_B$ receptors in rat brain slices. *Neuropharmacology* **35:**703–712.
Knoflach F, Mutel V, Jolidon S, Kew JN, Malherbe P, Vieira E, Wichmann J, and Kemp JA (2001) Positive allosteric modulators of metabotropic glutamate 1 receptor: characterization, mechanism of action, and binding site. *Proc Natl Acad Sci USA* **98:**13402–13407.
Koek W, France CP, Cheng K, and Rice KC (2010) $GABA_B$ receptor-positive modulators: enhancement of $GABA_B$ receptor agonist effects in vivo. *J Pharmacol Exp Ther* **335:**163–171.
Kohara A, Nagakura Y, Kiso T, Toya T, Watabiki T, Tamura S, Shitaka Y, Itahana H, and Okada M (2007) Antinociceptive profile of a selective metabotropic glutamate receptor 1 antagonist YM-230888 in chronic pain rodent models. *Eur J Pharmacol* **571:**8–16.
Kohara A, Toya T, Tamura S, Watabiki T, Nagakura Y, Shitaka Y, Hayashibe S, Kawabata S, and Okada M (2005) Radioligand binding properties and pharmacological characterization of 6-amino-N-cyclohexyl-N,3-dimethylthiazolo[3,2-a]benzimidazole-2-carboxamide (YM-298198), a high-affinity, selective, and non-competitive antagonist of metabotropic glutamate receptor type 1. *J Pharmacol Exp Ther* **315:**163–169.
Koizumi A, Nakajima K, Asakura T, Morita Y, Ito K, Shmizu-Ibuka A, Misaka T, and Abe K (2007) Taste-modifying sweet protein, neoculin, is received at human T1R3 amino terminal domain. *Biochem Biophys Res Comm* **358:**585–589.
Kolczewski S, Adam G, Stadler H, Mutel V, Wichmann J, and Woltering T (1999) Synthesis of heterocyclic enol ethers and their use as group 2 metabotropic glutamate receptor antagonists. *Bioorg Med Chem Lett* **9:**2173–2176.
Kubo Y, Miyashita T, and Murata Y (1998) Structural basis for a Ca^{2+}-sensing function of the metabotropic glutamate receptors. *Science* **279:**1722–1725.
Kuhn C, Bufe B, Winnig M, Hofmann T, Frank O, Behrens M, Lewtschenko T, Slack JP, Ward CD, and Meyerhof W (2004) Bitter taste receptors for saccharin and acesulfame K. *J Neurosci* **24:**10260–10265.
Kulkarni SS, Zou MF, Cao J, Deschamps JR, Rodriguez AL, Conn PJ, and Newman AH (2009) Structure-activity relationships comparing N-(6-methylpyridin-yl)-substituted aryl amides to 2-methyl-6-(substituted-arylethynyl)pyridines or 2-methyl-4-(substituted-arylethynyl)thiazoles as novel metabotropic glutamate receptor subtype 5 antagonists. *J Med Chem* **52:**3563–3575.
Kumar S, Matheny CJ, Hoffman SJ, Marquis RW, Schultz M, Liang X, Vasko JA, Stroup GB, Vaden VR, Haley H, et al. (2010) An orally active calcium-sensing receptor antagonist that transiently increases plasma concentrations of PTH and stimulates bone formation. *Bone* **46:**534–542.
Kuner R, Köhr G, Grünewald S, Eisenhardt G, Bach A, and Kornau HC (1999) Role of heteromer formation in $GABA_B$ receptor function. *Science* **283:**74–77.
Kunishima N, Shimada Y, Tsuji Y, Sato T, Yamamoto M, Kumasaka T, Nakanishi S, Jingami H, and Morikawa K (2000) Structural basis of glutamate recognition by a dimeric metabotropic glutamate receptor. *Nature* **407:**971–977.
Kusakabe Y, Yasuoka A, Asano-Miyoshi M, Iwabuchi K, Matsumoto I, Arai S, Emori Y, and Abe K (2000) Comprehensive study on G protein α-subunits in taste bud cells, with special reference to the occurrence of Gαi2 as a major Gα species. *Chem Senses* **25:**525–531.
Lagerström MC and Schiöth HB (2008) Structural diversity of G protein-coupled receptors and significance for drug discovery. *Nat Rev Drug Discov* **7:**339–357.
Langmead CJ and Christopoulos A (2006) Allosteric agonists of 7TM receptors: expanding the pharmacological toolbox. *Trends Pharmacol Sci* **27:**475–481.
Lavigne JR, Zahradnik RJ, Conklin RL, Lambert LD, Logan MA, Parihar A, and Fox J (1998) Stimulation of calcitonin secretion by calcium receptor activators: evaluation using a new, highly sensitive, homologous immunoradiometric assay for rat calcitonin. *Endocrine* **9:**293–301.
Lavreysen H and Dautzenberg FM (2008) Therapeutic potential of group III metabotropic glutamate receptors. *Curr Med Chem* **15:**671–684.
Lavreysen H, Janssen C, Bischoff F, Langlois X, Leysen JE, and Lesage AS (2003) [^{3}H]R214127: a novel high-affinity radioligand for the mGlu1 receptor reveals a

common binding site shared by multiple allosteric antagonists. *Mol Pharmacol* **63:**1082–1093.
Lavreysen H, Wouters R, Bischoff F, Nóbrega Pereira S, Langlois X, Blokland S, Somers M, Dillen L, and Lesage AS (2004) JNJ16259685, a highly potent, selective and systemically active mGlu1 receptor antagonist. *Neuropharmacology* **47:**961–972.
Leach K, Sexton PM, and Christopoulos A (2007) Allosteric GPCR modulators: taking advantage of permissive receptor pharmacology. *Trends Pharmacol Sci* **28:**382–389.
Lecourtier L, Homayoun H, Tamagnan G, and Moghaddam B (2007) Positive allosteric modulation of metabotropic glutamate 5 (mGlu5) receptors reverses *N*-methyl-D-aspartate antagonist-induced alteration of neuronal firing in prefrontal cortex. *Biol Psychiatry* **62:**739–746.
Leff P (1995) The two-state model of receptor activation. *Trends Pharmacol Sci* **16:**89–97.
Lesage A and Steckler T (2010) Metabotropic glutamate mGlu1 receptor stimulation and blockade: therapeutic opportunities in psychiatric illness. *Eur J Pharmacol* **639:**2–16.
Lhuillier L, Mombereau C, Cryan JF, and Kaupmann K (2007) $GABA_B$ receptor-positive modulation decreases selective molecular and behavioral effects of cocaine. *Neuropsychopharmacology* **32:**388–398.
Li JQ, Chen SR, Chen H, Cai YQ, and Pan HL (2010) Regulation of increased glutamatergic input to spinal dorsal horn neurons by mGluR5 in diabetic neuropathic pain. *J Neurochem* **112:**162–172.
Li X, Gardner EL, and Xi ZX (2008) The metabotropic glutamate receptor 7 (mGluR7) allosteric agonist AMN082 modulates nucleus accumbens GABA and glutamate, but not dopamine, in rats. *Neuropharmacology* **54:**542–551.
Li X, Li J, Peng XQ, Spiller K, Gardner EL, and Xi ZX (2009) Metabotropic glutamate receptor 7 modulates the rewarding effects of cocaine in rats: involvement of a ventral pallidal GABAergic mechanism. *Neuropsychopharmacology* **34:**1783–1796.
Li X, Li W, Wang H, Cao J, Maehashi K, Huang L, Bachmanov AA, Reed DR, Legrand-Defretin V, Beauchamp GK, et al. (2005) Pseudogenization of a sweet-receptor gene accounts for cats' indifference toward sugar. *PLOS genetics* **1:**27–35.
Li X, Staszewski L, Xu H, Durick K, Zoller M, and Adler E (2002) Human receptors for sweet and umami taste. *Proc Natl Acad Sci USA* **99:**4692–4696.
Liang JH, Chen F, Krstew E, Cowen MS, Carroll FY, Crawford D, Beart PM, and Lawrence AJ (2006) The $GABA_B$ receptor allosteric modulator CGP7930, like baclofen, reduces operant self-administration of ethanol in alcohol-preferring rats. *Neuropharmacology* **50:**632–639.
Lindemann B (2001) Receptors and transduction in taste. *Nature* **413:**219–225.
Linden AM and Schoepp DD (2006) Metabotropic glutamate receptor targets for neuropsychiatric disorders. *Drug Discov Today Ther Strateg* **3:**507–517.
Lindsley CW and Emmitte KA (2009) Recent progress in the discovery and development of negative allosteric modulators of mGluR5. *Curr Opin Drug Discov Devel* **12:**446–457.
Lindsley CW, Niswender CM, Engers DW, and Hopkins CR (2009) Recent progress in the development of mGluR4 positive allosteric modulators for the treatment of Parkinson's disease. *Curr Top Med Chem* **9:**949–963.
Lindsley CW, Wisnoski DD, Leister WH, O'brien JA, Lemaire W, Williams DL Jr, Burno M, Sur C, Kinney GG, Pettibone DJ, et al. (2004) Discovery of positive allosteric modulators for the metabotropic glutamate receptor subtype 5 from a series of *N*-(1,3-diphenyl-1*H*-pyrazol-5-yl)benzamides that potentiate receptor function in vivo. *J Med Chem* **47:**5825–5828.
Lindström E, Brusberg M, Hughes PA, Martin CM, Brierley SM, Phillis BD, Martinsson R, Abrahamsson C, Larsson H, Martinez V, et al. (2008) Involvement of metabotropic glutamate 5 receptor in visceral pain. *Pain* **137:**295–305.
Litschig S, Gasparini F, Rueegg D, Stoehr N, Flor PJ, Vranesic I, Prézeau L, Pin JP, Thomsen C, and Kuhn R (1999) CPCCOEt, a noncompetitive metabotropic glutamate receptor 1 antagonist, inhibits receptor signaling without affecting glutamate binding. *Mol Pharmacol* **55:**453–461.
Liu F, Grauer S, Kelley C, Navarra R, Graf R, Zhang G, Atkinson PJ, Popiolek M, Wantuch C, Khawaja X, et al. (2008) ADX47273 [*S*-(4-fluoro-phenyl)-{3-[3-(4-fluoro-phenyl)-[1,2,4]-oxadiazol-5-yl]-piperidin-1-yl}-methanone]: a novel metabotropic glutamate receptor 5-selective positive allosteric modulator with preclinical antipsychotic-like and procognitive activities. *J Pharmacol Exp Ther* **327:**827–839.
Liu F, Zhang G, Hornby G, Vasylyev D, Bowlby M, Park K, Gilbert A, Marquis K, and Andree TH (2006) The effect of mGlu5 receptor positive allosteric modulators on signaling molecules in brain slices. *Eur J Pharmacol* **536:**262–268.
Lu JY, Yang Y, Gnacadja G, Christopoulos A, and Reagan JD (2009) Effect of the calcimimetic R-568 [3-(2-chlorophenyl)-*N*-((1R)-1-(3-methoxyphenyl)ethyl)-1-propanamine] on correcting inactivating mutations in the human calcium-sensing receptor. *J Pharmacol Exp Ther* **331:**775–786.
Lundstrom K (2005) The future of G protein-coupled receptors as targets in drug discovery. *IDrugs* **8:**909–913.
Lüscher C, Jan LY, Stoffel M, Malenka RC, and Nicoll RA (1997) G protein-coupled inwardly rectifying K^+ channels (GIRKs) mediate postsynaptic but not presynaptic transmitter actions in hippocampal neurons. *Neuron* **19:**687–695.
Mabire D, Coupa S, Adelinet C, Poncelet A, Simonnet Y, Venet M, Wouters R, Lesage AS, Van Beijsterveldt L, and Bischoff F (2005) Synthesis, structure-activity relationship, and receptor pharmacology of a new series of quinoline derivatives acting as selective, noncompetitive mGlu1 antagonists. *J Med Chem* **48:**2134–2153.
Maccioni P, Carai MA, Kaupmann K, Guery S, Froestl W, Leite-Morris KA, Gessa GL, and Colombo G (2009) Reduction of alcohol's reinforcing and motivational properties by the positive allosteric modulator of the $GABA_B$ receptor, BHF177, in alcohol-preferring rats. *Alcohol Clin Exp Res* **33:**1749–1756.
Maccioni P and Colombo G (2009) Role of the $GABA_B$ receptor in alcohol-seeking and drinking behavior. *Alcohol* **43:**555–558.
Maccioni P, Pes D, Orrù A, Froestl W, Gessa GL, Carai MA, and Colombo G (2007) Reducing effect of the positive allosteric modulator of the $GABA_B$ receptor, GS39,783, on alcohol self-administration in alcohol-preferring rats. *Psychopharmacology* **193:**171–178.
Mailland M, Waelchli R, Ruat M, Boddeke HG, and Seuwen K (1997) Stimulation of cell proliferation by calcium and a calcimimetic compound. *Endocrinology* **138:**3601–3605.
Maj M, Bruno V, Dragic Z, Yamamoto R, Battaglia G, Inderbitzin W, Stoehr N, Stein T, Gasparini F, Vranesic I, et al. (2003) (−)-PHCCC, a positive allosteric modulator of mGluR4: characterization, mechanism of action, and neuroprotection. *Neuropharmacology* **45:**895–906.
Malherbe P, Kratochwil N, Knoflach F, Zenner MT, Kew JN, Kratzeisen C, Maerki HP, Adam G, and Mutel V (2003a) Mutational analysis and molecular modeling of the allosteric binding site of a novel, selective, noncompetitive antagonist of the metabotropic glutamate 1 receptor. *J Biol Chem* **278:**8340–8347.
Malherbe P, Kratochwil N, Mühlemann A, Zenner MT, Fischer C, Stahl M, Gerber PR, Jaeschke G, and Porter RH (2006) Comparison of the binding pockets of two chemically unrelated allosteric antagonists of the mGlu5 receptor and identification of crucial residues involved in the inverse agonism of MPEP. *J Neurochem* **98:**601–615.
Malherbe P, Kratochwil N, Zenner MT, Piussi J, Diener C, Kratzeisen C, Fischer C, and Porter RH (2003b) Mutational analysis and molecular modeling of the binding pocket of the metabotropic glutamate 5 receptor negative modulator 2-methyl-6-(phenylethynyl)-pyridine. *Mol Pharmacol* **64:**823–832.
Malherbe P, Masciadri R, Norcross RD, Knoflach F, Kratzeisen C, Zenner MT, Kolb Y, Marcuz A, Huwyler J, Nakagawa T, et al. (2008) Characterization of (R,S)-5,7-di-tert-butyl-3-hydroxy-3-trifluoromethyl-3H-benzofuran-2-one as a positive allosteric modulator of $GABA_B$ receptors. *Br J Pharmacol* **154:**797–811.
Malitschek B, Schweizer C, Keir M, Heid J, Froestl W, Mosbacher J, Kuhn R, Henley J, Joly C, Pin JP, et al. (1999) The N-terminal domain of gamma-aminobutyric $acid_B$ receptors is sufficient to specify agonist and antagonist binding. *Mol Pharmacol* **56:**448–454.
Mannoury la Cour C, Herbelles C, Pasteau V, de Nanteuil G, and Millan MJ (2008) Influence of positive allosteric modulators on $GABA_B$ receptor coupling in rat brain: a scintillation proximity assay characterisation of G protein subtypes. *J Neurochem* **105:**308–323.
Marek GJ, Behl B, Bespalov AY, Gross G, Lee Y, and Schoemaker H (2010) Glutamatergic (N-methyl-D-aspartate receptor) hypofrontality in schizophrenia: too little juice or a miswired brain? *Mol Pharmacol* **77:**317–326.
Margeta-Mitrovic M, Jan YN, and Jan LY (2001) Function of GB1 and GB2 subunits in G protein coupling of $GABA_B$ receptors. *Proc Natl Acad Sci USA* **98:**14649–14654.
Marie PJ (2010) The calcium-sensing receptor in bone cells: a potential therapeutic target in osteoporosis. *Bone* **46:**571–576.
Marino MJ, Williams DL Jr, O'Brien JA, Valenti O, McDonald TP, Clements MK, Wang R, DiLella AG, Hess JF, Kinney GG, et al. (2003) Allosteric modulation of group III metabotropic glutamate receptor 4: a potential approach to parkinson's disease treatment. *Proc Natl Acad Sci USA* **100:**13668–13673.
Marinozzi M and Pellicciari R (2000) Novel enantioselective synthesis of (2*S*,2′*R*,3′*R*)-2-(2′,3′-dicarboxycyclopropyl)glycine (DCG-IV). *Tetrahedron Lett* **41:**9125–9128.
Marquis RW, Lago AM, Callahan JF, Rahman A, Dong X, Stroup GB, Hoffman S, Gowen M, DelMar EG, Van Wagenen BC, et al. (2009b) Antagonists of the calcium receptor. 2. Amino alcohol-based parathyroid hormone secretagogues. *J Med Chem* **52:**6599–6605.
Marquis RW, Lago AM, Callahan JF, Trout RE, Gowen M, DelMar EG, Van Wagenen BC, Logan S, Shimizu S, Fox J, et al. (2009a) Antagonists of the calcium receptor. I. Amino alcohol-based parathyroid hormone secretagogues. *J Med Chem* **52:**3982–3993.
Masu M, Tanabe Y, Tsuchida K, Shigemoto R, and Nakanishi S (1991) Sequence and expression of a metabotropic glutamate receptor. *Nature* **349:**760–765.
Mathiesen JM and Ramirez MT (2006) Allosteric modulators of group III metabotropic glutamate receptors, in *Allosteric Receptor Modulation in Drug Targeting* (Bowery NG ed) pp 207–234, Taylor & Francis, New York.
Mathiesen JM, Svendsen N, Bräuner-Osborne H, Thomsen C, and Ramirez MT (2003) Positive allosteric modulation of the human metabotropic glutamate receptor 4 (hmGluR4) by SIB-1893 and MPEP. *Br J Pharmacol* **138:**1026–1030.
Matsushita S, Nakata H, Kubo Y, and Tateyama M (2010) Ligand-induced rearrangements of the $GABA_B$ receptor revealed by fluorescence resonance energy transfer. *J Biol Chem* **285:**10291–10299.
Maudsley S, Martin B, and Luttrell LM (2005) The origins of diversity and specificity in G protein-coupled receptor signaling. *J Pharmacol Exp Ther* **314:**485–494.
Max M, Shanker YG, Huang L, Rong M, Liu Z, Campagne F, Weinstein H, Damak S, and Margolskee RF (2001) Tas1r3, encoding a new candidate taste receptor, is allelic to the sweet responsiveness locus sac. *Nat Genetics* **28:**58–63.
McLoughlin DJ, Bertelli F, and Williams C (2007) The A, B, Cs of G-protein-coupled receptor pharmacology in assay development for HTS. *Expert Opin Drug Discov* **2:**603–619.
Mela F, Marti M, Dekundy A, Danysz W, Morari M, and Cenci MA (2007) Antagonism of metabotropic glutamate receptor type 5 attenuates L-DOPA-induced dyskinesia and its molecular and neurochemical correlates in a rat model of Parkinson's disease. *J Neurochem* **101:**483–497.
Micheli F, Cavanni P, Di Fabio R, Marchioro C, Donati D, Faedo S, Maffeis M, Sabbatini FM, and Tranquillini ME (2006) From pyrroles to pyrrolo[1,2-*a*]pyrazinones: a new class of mGluR1 antagonists. *Bioorg Med Chem Lett* **16:**1342–1345.
Micheli F, Fabio RD, Cavanni P, Rimland JM, Capelli AM, Chiamulera C, Corsi M, Corti C, Donati D, Feriani A, et al. (2003) Synthesis and pharmacological characterisation of 2,4-dicarboxy-pyrroles as selective non-competitive mGluR1 antagonists. *Bioorg Med Chem* **11:**171–183.
Miedlich SU, Gama L, Seuwen K, Wolf RM, and Breitwieser GE (2004) Homology modeling of the transmembrane domain of the human calcium sensing receptor and localization of an allosteric binding site. *J Biol Chem* **279:**7254–7263.

Milbank JB, Knauer CS, Augelli-Szafran CE, Sakkab-Tan AT, Lin KK, Yamagata K, Hoffman JK, Zhuang N, Thomas J, Galatsis P, et al. (2007) Rational design of 7-arylquinolines as non-competitive metabotropic glutamate receptor subtype 5 antagonists. *Bioorg Med Chem Lett* **17:**4415–4418.
Mitsukawa K, Mombereau C, Lötscher E, Uzunov DP, van der Putten H, Flor PJ, and Cryan JF (2006) Metabotropic glutamate receptor subtype 7 ablation causes dysregulation of the HPA axis and increases hippocampal BDNF protein levels: implications for stress-related psychiatric disorders. *Neuropsychopharmacology* **31:**1112–1122.
Mitsukawa K, Yamamoto R, Ofner S, Nozulak J, Pescott O, Lukic S, Stoehr N, Mombereau C, Kuhn R, McAllister KH, et al. (2005) A selective metabotropic glutamate receptor 7 agonist: activation of receptor signaling via an allosteric site modulates stress parameters *in vivo*. *Proc Natl Acad Sci USA* **102:**18712–18717.
Moe SM, Cunningham J, Bommer J, Adler S, Rosansky SJ, Urena-Torres P, Albizem MB, Guo MD, Zani VJ, Goodman WG, et al. (2005) Long-term treatment of secondary hyperparathyroidism with the calcimimetic cinacalcet HCl. *Nephrol Dial Transplant* **20:**2186–2193.
Möhler H (2006) Allosteric modulation of $GABA_A$ receptors, in *Allosteric Receptor Modulation in Drug Targeting* (Bowery NG ed) pp 79–92, Taylor & Francis, New York.
Mombaerts P (2004) Genes and ligands for odorant, vomeronasal and taste receptors. *Nat Rev Neurosci* **5:**263–278.
Mombereau C, Lhuillier L, Kaupmann K, and Cryan JF (2007) $GABA_B$ receptor-positive modulation-induced blockade of the rewarding properties of nicotine is associated with a reduction in nucleus accumbens ΔFosB accumulation. *J Pharmacol Exp Ther* **321:**172–177.
Montana MC, Cavallone LF, Stubbert KK, Stefanescu AD, Kharasch ED, and Gereau RW 4th (2009) The metabotropic glutamate receptor subtype 5 antagonist fenobam is analgesic and has improved in vivo selectivity compared with the prototypical antagonist 2-methyl-6-(phenylethynyl)-pyridine. *J Pharmacol Exp Ther* **330:**834–843.
Montmayeur JP, Liberles SD, Matsunami H, and Buck LB (2001) A candidate taste receptor gene near a sweet taste locus. *Nat Neurosci* **4:**492–498.
Morin N, Grégoire L, Gomez-Mancilla B, Gasparini F, and Di Paolo T (2010) Effect of the metabotropic glutamate receptor type 5 antagonists MPEP and MTEP in parkinsonian monkeys. *Neuropharmacology* **58:**981–986.
Morini G, Bassoli A, and Temussi PA (2005) From small sweeteners to sweet proteins: anatomy of the binding sites of the human T1R2_T1R3 receptor. *J Med Chem* **48:**5520–5529.
Mueller R, Rodriguez AL, Dawson ES, Butkiewicz M, Nguyen TT, Oleszkiewicz S, Bleckmann A, Weaver CD, Lindsley CW, Conn PJ, et al. (2010) Identification of metabotropic glutamate receptor subtype 5 potentiators using virtual high-throughput screening. *ACS Chem Neurosci* **1:**288–305.
Mühlemann A, Ward NA, Kratochwil N, Diener C, Fischer C, Stucki A, Jaeschke G, Malherbe P, and Porter RH (2006) Determination of key amino acids implicated in the actions of allosteric modulation by 3,3′-difluorobenzaldazine on rat mGlu5 receptors. *Eur J Pharmacol* **529:**95–104.
Mun HC, Culverston EL, Franks AH, Collyer CA, Clifton-Bligh RJ, and Conigrave AD (2005) A double mutation in the extracellular Ca^{2+}-sensing receptor's venus flytrap domain that selectively disables L-amino acid sensing. *J Biol Chem* **280:**29067–29072.
Mustafi D and Palczewski K (2009) Topology of class A G protein - coupled receptors: insights gained from crystal structures of rhodopsins, adrenergic and adenosine receptors. *Mol Pharmacol* **75:**1–12.
Muto T, Tsuchiya D, Morikawa K, and Jingami H (2007) Structures of the extracellular regions of the group II/III metabotropic glutamate receptors. *Proc Natl Acad Sci USA* **104:**3759–3764.
Nelson G, Chandrashekar J, Hoon MA, Feng L, Zhao G, Ryba NJ, and Zuker CS (2002) An amino-acid taste receptor. *Nature* **416:**199–202.
Nelson G, Hoon MA, Chandrashekar J, Zhang Y, Ryba NJ, and Zuker CS (2001) Mammalian sweet taste receptors. *Cell* **106:**381–390.
Nemeth EF (2002) The search for calcium receptor antagonists (calcilytics). *J Mol Endocrinol* **29:**15–21.
Nemeth EF, Delmar EG, Heaton WL, Miller MA, Lambert LD, Conklin RL, Gowen M, Gleason JG, Bhatnagar PK, and Fox J (2001) Calcilytic compounds: potent and selective Ca^{2+} receptor antagonists that stimulate secretion of parathyroid hormone. *J Pharmacol Exp Ther* **299:**323–331.
Nemeth EF and Fox J (1999) Calcimimetic compounds: a direct approach to controlling plasma levels of parathyroid hormone in hyperparathyroidism. *Trends Endocrinol Metab* **10:**66–71.
Nemeth EF, Heaton WH, Miller M, Fox J, Balandrin MF, Van Wagenen BC, Colloton M, Karbon W, Scherrer J, Shatzen E, et al. (2004) Pharmacodynamics of the type II calcimimetic compound cinacalcet HCl. *J Pharmacol Exp Ther* **308:**627–635.
Nemeth EF, Steffey ME, and Fox J (1996) The parathyroid calcium receptor: a novel therapeutic target for treating hyperparathyroidism. *Pediatr Nephrol* **10:**275–279.
Nemeth EF, Steffey ME, Hammerland LG, Hung BC, Van Wagenen BC, DelMar EG, and Balandrin MF (1998) Calcimimetics with potent and selective activity on the parathyroid calcium receptor. *Proc Natl Acad Sci USA* **95:**4040–4045.
Ngomba RT, Ferraguti F, Badura A, Citraro R, Santolini I, Battaglia G, Bruno V, De Sarro G, Simonyi A, van Luijtelaar G, et al. (2008) Positive allosteric modulation of metabotropic glutamate 4 (mGlu4) receptors enhances spontaneous and evoked absence seizures. *Neuropharmacology* **54:**344–354.
Nicoletti F, Wroblewski JT, Novelli A, Alho H, Guidotti A, and Costa E (1986) The activation of inositol phospholipid metabolism as a signal-transduction system for excitatory amino acids in primary cultures of cerebellar granule cells. *J Neurosci* **6:**1905–1911.
Nie Y, Vigues S, Hobbs JR, Conn GL, and Munger SD (2005) Distinct contributions of T1R2 and T1R3 taste receptor subunits to the detection of sweet stimuli. *Curr Biol* **15:**1948–1952.
Nikiforuk A, Popik P, Drescher KU, van Gaalen M, Relo AL, Mezler M, Marek G, Schoemaker H, Gross G, and Bespalov A (2010) Effects of a positive allosteric modulator of group II metabotropic glutamate receptors, LY487379, on cognitive flexibility and impulse-like responding in rats. *J Pharmacol Exp Ther* **335:**665–673.
Niswender CM and Conn PJ (2010) Metabotropic glutamate receptors: physiology, pharmacology, and disease. *Annu Rev Pharmacol Toxicol* **50:**295–322.
Niswender CM, Johnson KA, Miller NR, Ayala JE, Luo Q, Williams R, Saleh S, Orton D, Weaver CD, and Conn PJ (2010) Context-dependent pharmacology exhibited by negative allosteric modulators of metabotropic glutamate receptor 7. *Mol Pharmacol* **77:**459–468.
Niswender CM, Johnson KA, Weaver CD, Jones CK, Xiang Z, Luo Q, Rodriguez AL, Marlo JE, de Paulis T, Thompson AD, et al. (2008a) Discovery, characterization, and antiparkinsonian effect of novel positive allosteric modulators of metabotropic glutamate receptor 4. *Mol Pharmacol* **74:**1345–1358.
Niswender CM, Lebois EP, Luo Q, Kim K, Muchalski H, Yin H, Conn PJ, and Lindsley CW (2008b) Positive allosteric modulators of the metabotropic glutamate receptor subtype 4 (mGluR4): part I. discovery of pyrazolo[3,4-d]pyrimidines as novel mGluR4 positive allosteric modulators. *Bioorg Med Chem Lett* **18:**5626–5630.
Noeske T, Gutcaits A, Parsons CG, and Weil T (2006) Allosteric modulation of family 3 GPCRs**.** *QSAR Comb Sci* **25:**134–146.
O'Brien JA, Lemaire W, Chen TB, Chang RS, Jacobson MA, Ha SN, Lindsley CW, Schaffhauser HJ, Sur C, Pettibone DJ, et al. (2003) A family of highly selective allosteric modulators of the metabotropic glutamate receptor subtype 5. *Mol Pharmacol* **64:**731–740.
O'Brien JA, Lemaire W, Wittmann M, Jacobson MA, Ha SN, Wisnoski DD, Lindsley CW, Schaffhauser HJ, Rowe B, Sur C, et al. (2004) A novel selective allosteric modulator potentiates the activity of native metabotropic glutamate receptor subtype 5 in rat forebrain. *J Pharmacol Exp Ther* **309:**568–577.
O'Connor RM, Finger BC, Flor PJ, and Cryan JF (2010) Metabotropic glutamate receptor 7: at the interface of cognition and emotion. *Eur J Pharmacol* **639:**123–131.
O'Hara PJ, Sheppard PO, Thøgersen H, Venezia D, Haldeman BA, McGrane V, Houamed KM, Thomsen C, Gilbert TL, and Mulvihill ER (1993) The ligand-binding domain in metabotropic glutamate receptors is related to bacterial periplasmic binding proteins. *Neuron* **11:**41–52.
Ogata H, Ritz E, Odoni G, Amann K, and Orth SR (2003) Beneficial effects of calcimimetics on progression of renal failure and cardiovascular risk factors. *J Am Soc Nephrol* **14:**959–967.
Olianas MC, Ambu R, Garau L, and Onali P (2005) Allosteric modulation of $GABA_B$ receptor function in human frontal cortex. *Neurochem Int* **46:**149–158.
Olianas MC and Onali P (1999) $GABA_B$ receptor-mediated stimulation of adenylyl cyclase activity in membranes of rat olfactory bulb. *Br J Pharmacol* **126:**657–664.
Onali P, Mascia FM, and Olianas MC (2003) Positive regulation of $GABA_B$ receptors dually coupled to cyclic AMP by the allosteric agent CGP7930. *Eur J Pharmacol* **471:**77–84.
Onali P and Olianas MC (2001) $\beta\gamma$-mediated enhancement of corticotropin-releasing hormone-stimulated adenylyl cyclase activity by activation of γ-aminobutyric $acid_B$ receptors in membranes of rat frontal cortex. *Biochem Pharmacol* **62:**183–190.
O'Neill MJ, Fell MJ, Svensson KA, Witkin JM, and Mitchell SN (2010) Recent developments in metabotropic glutamate receptors as novel drug targets. *Drugs Future* **35:**307–324.
Orrù A, Lai P, Lobina C, Maccioni P, Piras P, Scanu L, Froestl W, Gessa GL, Carai MA, and Colombo G (2005) Reducing effect of the positive allosteric modulators of the $GABA_B$ receptor, CGP7930 and GS39783, on alcohol intake in alcohol-preferring rats. *Eur J Pharmacol* **525:**105–111.
Ossowska K, Konieczny J, Wolfarth S, and Pilc A (2005) MTEP, a new selective antagonist of the metabotropic glutamate receptor subtype 5 (mGluR5), produces antiparkinsonian-like effects in rats. *Neuropharmacology* **49:**447–455.
Ouattara B, Grégoire L, Morissette M, Gasparini F, Vranesic I, Bilbe G, Johns DR, Rajput A, Hornykiewicz O, Rajput AH, et al. (2009) Metabotropic glutamate receptor 5 in levodopa-induced motor complications. *Neurobiol Aging*. doi:10.1016/j.neurobiolaging.2009.07.014.
Overington JP, Al-Lazikani B, and Hopkins AL (2006) How many drug targets are there? *Nat Rev Drug Discov* **5:**993–996.
Owen DR, Dodd PG, Gayton S, Greener BS, Harbottle GW, Mantell SJ, Maw GN, Osborne SA, Rees H, Ringer TJ, et al. (2007) Structure-activity relationships of novel non-competitive mGluR1 antagonists: a potential treatment for chronic pain. *Bioorg Med Chem Lett* **17:**486–490.
Ozeck M, Brust P, Xu H, and Servant G (2004) Receptors for bitter, sweet and umami taste couple to inhibitory G protein signaling pathways. *Eur J Pharmacol* **489:**139–149.
Pace AJ, Gama L, and Breitwieser GE (1999) Dimerization of the calcium-sensing receptor occurs within the extracellular domain and is eliminated by Cys → Ser mutations at Cys^{101} and Cys^{236}. *J Biol Chem* **274:**11629–11634.
Pagano A, Ruegg D, Litschig S, Stoehr N, Stierlin C, Heinrich M, Floersheim P, Prezèau L, Carroll F, Pin JP, et al. (2000) The non-competitive antagonists 2-methyl-6-(phenylethynyl)pyridine and 7-hydroxyiminocyclopropan [*b*]chromen-1α-carboxylic acid ethyl ester interact with overlapping binding pockets in the transmembrane region of group I metabotropic glutamate receptors. *J Biol Chem* **275:**33750–33758.
Palmer RK (2007) The pharmacology and signaling of bitter, sweet, and umami taste sensing. *Mol Interv* **7:**87–98.
Palucha A, Klak K, Branski P, van der Putten H, Flor PJ, and Pilc A (2007) Activation of the mGlu7 receptor elicits antidepressant-like effects in mice. *Psychopharmacology* **194:**555–562.
Parmentier ML, Prézeau L, Bockaert J, and Pin JP (2002) A model for the functioning of family 3 GPCRs. *Trends Pharmacol Sci* **23:**268–274.
Paterson NE, Froestl W, and Markou A (2004) The $GABA_B$ receptor agonists baclofen and CGP44532 decreased nicotine self-administration in the rat *Psychopharmacology* **172:**179–186.

Paterson NE, Vlachou S, Guery S, Kaupmann K, Froestl W, and Markou A (2008) Positive modulation of $GABA_B$ receptors decreased nicotine self-administration and counteracted nicotine-induced enhancement of brain reward function in rats. *J Pharmacol Exp Ther* **326:**306–314.
Patil ST, Zhang L, Martenyi F, Lowe SL, Jackson KA, Andreev BV, Avedisova AS, Bardenstein LM, Gurovich IY, Morozova MA, et al. (2007) Activation of mGlu2/3 receptors as a new approach to treat schizophrenia: a randomized phase 2 clinical trial. *Nat Med* **13:**1102–1107.
Peacock M, Bilezikian JP, Klassen PS, Guo MD, Turner SA, and Shoback D (2005) Cinacalcet hydrochloride maintains long-term normocalcemia in patients with primary hyperparathyroidism. *J Clin Endocrinol Metab* **90:**135–141.
Pecknold JC, McClure DJ, Appeltauer L, Wrzesinski L, and Allan T (1982) Treatment of anxiety using fenobam (a nonbenzodiazepine) in a double-blind standard (diazepam) placebo-controlled study. *J Clin Psychopharmacol* **2:**129–133.
Pelkey KA, Yuan X, Lavezzari G, Roche KW, and McBain CJ (2007) mGluR7 undergoes rapid internalization in response to activation by the allosteric agonist AMN082. *Neuropharmacology* **52:**108–117.
Pellicciari R, Marinozzi M, Natalini B, Costantino G, Luneia R, Giorgi G, Moroni F, and Thomsen C (1996) Synthesis and pharmacological characterization of all sixteen stereoisomers of 2-(2′-carboxy-3′-phenylcyclopropyl)glycine. Focus on (2*S*,1′*S*,2′*S*,3′*R*)-2-(2′-carboxy-3′-phenylcyclopropyl)glycine, a novel and selective group II metabotropic glutamate receptors antagonist. *J Med Chem* **39:**2259–2269.
Petrel C, Kessler A, Dauban P, Dodd RH, Rognan D, and Ruat M (2004) Positive and negative allosteric modulators of the Ca^{2+}-sensing receptor interact within overlapping but not identical binding sites in the transmembrane domain. *J Biol Chem* **279:**18990–18997.
Petrel C, Kessler A, Maslah F, Dauban P, Dodd RH, Rognan D, and Ruat M (2003) Modeling and mutagenesis of the binding site of Calhex 231, a novel negative allosteric modulator of the extracellular Ca^{2+}-sensing receptor. *J Biol Chem* **278:**49487–49494.
Pin JP, Galvez T, and Prézeau L (2003) Evolution, structure, and activation mechanism of family 3/C G-protein-coupled receptors. *Pharmacol Ther* **98:**325–354.
Pin JP and Prézeau L (2007) Allosteric modulators of $GABA_B$ receptors: mechanism of action and therapeutic perspective. *Curr Neuropharmacol* **5:**195–201.
Pinkerton AB, Cube RV, Hutchinson JH, James JK, Gardner MF, Rowe BA, Schaffhauser H, Rodriguez DE, Campbell UC, Daggett LP, et al. (2005) Allosteric potentiators of the metabotropic glutamate receptor 2 (mGlu2). Part 3: Identification and biological activity of indanone containing mGlu2 receptor potentiators. *Bioorg Med Chem Lett* **15:**1565–1571.
Pinkerton AB, Cube RV, Hutchinson JH, James JK, Gardner MF, Schaffhauser H, Rowe BA, Daggett LP, and Vernier JM (2004a) Allosteric potentiators of the metabotrophic glutamate receptor 2 (mGlu2). Part 2: 4-Thiopyridyl acetophenones as non-tetrazole containing mGlu2 receptor potentiators. *Bioorg Med Chem Lett* **14:**5867–5872.
Pinkerton AB, Vernier JM, Schaffhauser H, Rowe BA, Campbell UC, Rodriguez DE, Lorrain DS, Baccei CS, Daggett LP, and Bristow LJ (2004b) Phenyl-tetrazolyl acetophenones: discovery of positive allosteric potentiators for the metabotropic glutamate 2 receptor. *J Med Chem* **47:**4595–4599.
Pollak MR, Chou YH, Marx SJ, Steinmann B, Cole DE, Brandi ML, Papapoulos SE, Menko FH, Hendy GN, and Brown EM (1994) Familial hypocalciuric hypercalcemia and neonatal severe hyperparathyroidism. Effects of mutant gene dosage on phenotype. *J Clin Invest* **93:**1108–1112.
Poon SF, St Jean DJ Jr, Harrington PE, Henley C 3rd, Davis J, Morony S, Lott FD, Reagan JD, Lu JY, Yang Y, and Fotsch C.Jean DJ Jr, Harrington JF, Henley III C, Davis J, Morony S, Lott FD, Reagan JD, Ying-Lin Lu J, Yang Y, and Fotsch C (2009) Discovery and optimization of substituted 1-(1-phenyl-1*H*-pyrazol-3-yl)methanamines as potent and efficacious type II calcimimetics. *J Med Chem* **52:**6535–6538.
Porter RH, Jaeschke G, Spooren W, Ballard TM, Büttelmann B, Kolczewski S, Peters JU, Prinssen E, Wichmann J, Vieira E, et al. (2005) Fenobam: a clinically validated nonbenzodiazepine anxiolytic is a potent, selective, and noncompetitive mGlu5 receptor antagonist with inverse agonist activity. *J Pharmacol Exp Ther* **315:**711–721.
Raddatz R, Schaffhauser H, and Marino MJ (2007) Allosteric approaches to the targeting of G-protein-coupled receptors for novel drug discovery: a critical assessment. *Biochem Pharmacol* **74:**383–391.
Ray K, Hauschild BC, Steinbach PJ, Goldsmith PK, Hauache O, and Spiegel AM (1999) Identification of the cysteine residues in the amino-terminal extracellular domain of the human Ca^{2+} receptor critical for dimerization. Implications for function of monomeric Ca^{2+} receptor. *J Biol Chem* **274:**27642–27650.
Ray K, Tisdale J, Dodd RH, Dauban P, Ruat M, and Northup JK (2005) Calindol, a positive allosteric modulator of the human Ca^{2+} receptor, activates an extracellular ligand-binding domain-deleted rhodopsin-like seven-transmembrane structure in the absence of Ca^{2+}. *J Biol Chem* **280:**37013–37020.
Rees S, Morrow D, and Kenakin T (2002) GPCR drug discovery through the exploitation of allosteric drug binding sites. *Receptors Channels* **8:**261–268.
Riedel G, Casabona G, Platt B, Macphail EM, and Nicoletti F (2000) Fear conditioning-induced time- and subregion-specific increase inexpression of mGlu5 receptor protein in rat hippocampus. *Neuropharmacology* **39:**1943–1951.
Riera CE, Vogel H, Simon SA, Damak S, and le Coutre J (2008) The capsaicin receptor participates in artificial sweetener aversion. *Biochem Biophys Res Comm* **376:**653–657.
Ritzén A, Sindet R, Hentzer M, Svendsen N, Brodbeck RM, and Bundgaard C (2009) Discovery of a potent and brain penetrant mGluR5 positive allosteric modulator. *Bioorg Med Chem Lett* **19:**3275–3278.
Robbins MJ, Calver AR, Filippov AK, Hirst WD, Russell RB, Wood MD, Nasir S, Couve A, Brown DA, Moss SJ, et al. (2001) $GABA_{B2}$ is essential for G-protein coupling of the $GABA_B$ receptor heterodimer. *J Neurosci* **21:**8043–8052.
Robbins TW and Everitt BJ (1999) Drug addiction: bad habits add up. *Nature* **398:**567–570.
Roberts DC, Andrews MM, and Vickers GJ (1996) Baclofen attenuates the reinforcing effects of cocaine in rats. *Neuropsychopharmacology* **15:**417–423.
Rodrigues SM, Bauer EP, Farb CR, Schafe GE, and LeDoux JE (2002) The group I metabotropic glutamate receptor mGluR5 is required for fear memory formation and long-term potentiation in the lateral amygdala. *J Neurosci* **22:**5219–5229.
Rodriguez AL, Grier MD, Jones CK, Herman EJ, Kane AS, Smith RL, Williams R, Zhou Y, Marlo JE, Days EL, et al. (2010) Discovery of novel allosteric modulators of metabotropic glutamate receptor subtype 5 reveals chemical and functional diversity and in vivo activity in rat behavioral models of anxiolytic and antipsychotic activity. *Mol Pharmacol* **78:**1105–1123.
Rodriguez AL, Nong Y, Sekaran NK, Alagille D, Tamagnan GD, and Conn PJ (2005) A close structural analog of 2-methyl-6-(phenylethynyl)-pyridine acts as a neutral allosteric site ligand on metabotropic glutamate receptor subtype 5 and blocks the effects of multiple allosteric modulators. *Mol Pharmacol* **68:**1793–1802.
Rodriguez AL and Williams R (2007) Recent progress in the development of allosteric modulators of mGluR5. *Curr Opin Drug Discov Devel* **10:**715–722.
Rodriguez AL, Williams R, Zhou Y, Lindsley SR, Le U, Grier MD, Weaver CD, Conn PJ, and Lindsley CW (2009) Discovery and SAR of novel mGluR5 non-competitive antagonists not based on an MPEP chemotype. *Bioorg Med Chem Lett* **19:**3209–3213.
Rondard P, Goudet C, Kniazeff J, Pin JP, and Prézeau L (2011) The complexity of their activation mechanism opens new possibilities for the modulation of mGlu and $GABA_B$ class C G protein-coupled receptors. *Neuropharmacology* **60:**82–92.
Rosenbrock H, Kramer G, Hobson S, Koros E, Grundl M, Grauert M, Reymann KG, and Schröder UH (2010) Functional interaction of metabotropic glutamate receptor 5 and NMDA-receptor by a metabotropic glutamate receptor 5 positive allosteric modulator. *Eur J Pharmacol* **639:**40–46.
Rowe BA, Schaffhauser H, Morales S, Lubbers LS, Bonnefous C, Kamenecka TM, McQuiston J, and Daggett LP (2008) Transposition of three amino acids transforms the human metabotropic glutamate receptor (mGluR)-3-positive allosteric modulation site to mGluR2, and additional characterization of the mGluR2-positive allosteric modulation site. *J Pharmacol Exp Ther* **326:**240–251.
Rudd MT and McCauley JA (2005) Positive allosteric modulators of the metabotropic glutamate receptor subtype 2 (mGluR2). *Curr Top Med Chem* **5:**869–884.
Rylander D, Iderberg H, Li Q, Dekundy A, Zhang J, Li H, Baishen R, Danysz W, Bezard E, and Cenci MA (2010) A mGluR5 antagonist under clinical development improves L-DOPA–induced dyskinesia in parkinsonian rats and monkeys. *Neurobiol Dis* **39:**352–361.
Saidak Z, Brazier M, Kamel S, and Mentaverri R (2009) Agonists and allosteric modulators of the calcium-sensing receptor and their therapeutic applications. *Mol Pharmacol* **76:**1131–1144.
Sainz E, Korley JN, Battey JF, and Sullivan SL (2001) Identification of a novel member of the T1R family of putative taste receptors. *J Neurochem* **77:**896–903.
Samadi P, Grégoire L, Morissette M, Calon F, Hadj Tahar A, Bélanger N, Dridi M, Bédard PJ, and Di Paolo T (2008) Basal ganglia group II metabotropic glutamate receptors specific binding in non-human primate model of L-Dopa-induced dyskinesias. *Neuropharmacology* **54:**258–268.
Sasikumar TK, Qiang L, Burnett DA, Greenlee WJ, Li C, Heimark L, Pramanik B, Grilli M, Bertorelli R, Lozza G, et al. (2009) Tricyclic thienopyridine-pyrimidones/thienopyrimidine-pyrimidones as orally efficacious mGluR1 antagonists for neuropathic pain. *Bioorg Med Chem Lett* **19:**3199–3203.
Satoh A, Nagatomi Y, Hirata Y, Ito S, Suzuki G, Kimura T, Maehara S, Hikichi H, Satow A, Hata M, et al. (2009) Discovery and in vitro and in vivo profiles of 4-fluoro-N-[4-[6-(isopropylamino)pyrimidin-4-yl]-1,3-thiazol-2-yl]-N-methylbenzamide as novel class of an orally active metabotropic glutamate receptor 1 (mGluR1) antagonist. *Bioorg Med Chem Lett* **19:**5464–5468.
Satow A, Maehara S, Ise S, Hikichi H, Fukushima M, Suzuki G, Kimura T, Tanak T, Ito S, Kawamoto H, et al. (2008) Pharmacological effects of the metabotropic glutamate receptor 1 antagonist compared with those of the metabotropic glutamate receptor 5 antagonist and metabotropic glutamate receptor 2/3 agonist in rodents: detailed investigations with a selective allosteric metabotropic glutamate receptor 1 antagonist, FTIDC [4-[1-(2-fluoropyridine-3-yl)-5-methyl-1*H*-1,2,3-triazol-4-yl]-*N*-isopropyl-*N*-methyl-3,6-dihydropyridine-1(2*H*)-carboxamide]. *J Pharmacol Exp Ther* **326:**577–586.
Saunders R, Nahorski SR, and Challiss RA (1998) A modulatory effect of extracellular Ca^{2+} on type 1α metabotropic glutamate receptor-mediated signalling. *Neuropharmacology* **37:**273–276.
Schaffhauser H, Rowe BA, Morales S, Chavez-Noriega LE, Yin R, Jachec C, Rao SP, Bain G, Pinkerton AB, Vernier JM, et al. (2003) Pharmacological characterization and identification of amino acids involved in the positive modulation of metabotropic glutamate receptor subtype 2. *Mol Pharmacol* **64:**798–810.
Schann S, Mayer S, Franchet C, Frauli M, Steinberg E, Thomas M, Baron L, and Neuville P (2010) Chemical switch of a metabotropic glutamate receptor 2 silent allosteric modulator into dual metabotropic glutamate receptor 2/3 negative/positive allosteric modulators. *J Med Chem* **53:**8775–8779.
Schkeryantz JM, Kingston AE, and Johnson MP (2007) Prospects for metabotropic glutamate 1 receptor antagonists in the treatment of neuropathic pain. *J Med Chem* **50:**2563–2568.
Schlyer S and Horuk R (2006) I want a new drug: G-protein-coupled receptors in drug development. *Drug Discov Today* **11:**481–493.
Schwartz TW and Holst B (2006) Ago-allosteric modulation and other types of allostery in dimeric 7TM receptors. *J Recept Signal Transduct Res* **26:**107–128.
Scott K (2004) The sweet and the bitter of mammalian taste. *Curr Opin Neurobiol* **14:**423–427.
Servant G, Tachdjian C, Tang XQ, Werner S, Zhang F, Li X, Kamdar P, Petrovic G, Ditschun T, Java A, et al. (2010) Positive allosteric modulators of the human sweet taste receptor enhance sweet taste. *Proc Natl Acad Sci USA* **107:**4746–4751.
Sharma S, Kedrowski J, Rook JM, Smith RL, Jones CK, Rodriguez AL, Conn PJ, and Lindsley CW (2009) Discovery of molecular switches that modulate modes of metabotropic glutamate receptor subtype 5 (mGlu5) pharmacology in vitro and in

vivo within a series of functionalized, regioisomeric 2- and 5-(phenylethynyl) pyrimidines. *J Med Chem* **52:**4103–4106.
Sharma S, Rodriguez AL, Conn PJ, and Lindsley CW (2008) Synthesis and SAR of a mGluR5 allosteric partial antagonist lead: unexpected modulation of pharmacology with slight structural modifications to a 5-(phenylethynyl)pyrimidine scaffold. *Bioorg Med Chem Lett* **18:**4098–4101.
Shcherbakova I, Balandrin MF, Fox J, Ghatak A, Heaton WL, and Conklin RL (2005) ^{3}H-Quinazolin-4-ones as a new calcilytic template for the potential treatment of osteoporosis. *Bioorg Med Chem Lett* **15:**1557–1560.
Sheffler DJ and Conn PJ (2008) Allosteric potentiators of metabotropic glutamate receptor subtype 1a differentially modulate independent signaling pathways in baby hamster kidney cells. *Neuropharmacology* **55:**419–427.
Shoback DM, Bilezikian JP, Turner SA, McCary LC, Guo MD, and Peacock M (2003) The calcimimetic cinacalcet normalizes serum calcium in subjects with primary hyperparathyroidism. *J Clin Endocrinol Metab* **88:**5644–5649.
Shoptaw S, Yang X, Rotheram-Fuller EJ, Hsieh YC, Kintaudi PC, Charuvastra VC, and Ling W (2003) Randomized placebo-controlled trial of baclofen for cocaine dependence: preliminary effects for individuals with chronic patterns of cocaine use. *J Clin Psychiatry* **64:**1440–1448.
Siegl S, Flor PJ, and Fendt M (2008) Amygdaloid metabotropic glutamate receptor subtype 7 is involved in the acquisition of conditioned fear. *Neuroreport* **19:**1147–1150.
Silve C, Petrel C, Leroy C, Bruel H, Mallet E, Rognan D, and Ruat M (2005) Delineating a Ca^{2+} binding pocket within the venus flytrap module of the human calcium-sensing receptor. *J Biol Chem* **280:**37917–37923.
Silverberg SJ, Bone HG 3rd, Marriott TB, Locker FG, Thys-Jacobs S, Dziem G, Kaatz S, Sanguinetti EL, and Bilezikian JP (1997) Short-term inhibition of parathyroid hormone secretion by a calcium-receptor agonist in patients with primary hyperparathyroidism. *N Engl J Med* **337:**1506–1511.
Silverberg SJ, Rubin MR, Faiman C, Peacock M, Shoback DM, Smallridge RC, Schwanauer LE, Olson KA, Klassen P, and Bilezikian JP (2007) Cinacalcet hydrochloride reduces the serum calcium concentration in inoperable parathyroid carcinoma. *J Clin Endocrinol Metab* **92:**3803–3808.
Siméon FG, Brown AK, Zoghbi SS, Patterson VM, Innis RB, and Pike VW (2007) Synthesis and simple 18F-labeling of 3-fluoro-5-(2-(2-(fluoromethyl)thiazol-4-yl)ethynyl)benzonitrile as a high affinity radioligand for imaging monkey brain metabotropic glutamate subtype-5 receptors with positron emission tomography. *J Med Chem* **50:**3256–3266.
Sladeczek F, Pin JP, Récasens M, Bockaert J, and Weiss S (1985) Glutamate stimulates inositol phosphate formation in striatal neurones. *Nature* **317:**717–719.
Slassi A, Isaac M, Edwards L, Minidis A, Wensbo D, Mattsson J, Nilsson K, Raboisson P, McLeod D, Stormann TM, et al. (2005) Recent advances in non-competitive mGluR5 receptor antagonists and their potential therapeutic applications. *Curr Top Med Chem* **5:**897–911.
Slattery DA, Markou A, Froestl W, and Cryan JF (2005) The $GABA_B$ receptor-positive modulator GS39783 and the $GABA_B$ receptor agonist baclofen attenuate the reward-facilitating effects of cocaine: intracranial self-stimulation studies in the rat. *Neuropsychopharmacology* **30:**2065–2072.
Smith MA, Yancey DL, Morgan D, Liu Y, Froestl W, and Roberts DC (2004) Effects of positive allosteric modulators of the $GABA_B$ receptor on cocaine self-administration in rats. *Psychopharmacology* **173:**105–111.
Spanka C, Glatthar R, Desrayaud S, Fendt M, Orain D, Troxler T, and Vranesic I (2010) Piperidyl amides as novel, potent and orally active mGlu5 receptor antagonists with anxiolytic-like activity. *Bioorg Med Chem Lett* **20:**184–188.
Spooren WP, Gasparini F, van der Putten H, Koller M, Nakanishi S, and Kuhn R (2000a) Lack of effect of LY314582 (a group 2 metabotropic glutamate receptor agonist) on phencyclidine-induced locomotor activity in metabotropic glutamate receptor 2 knockout mice. *Eur J Pharmacol* **397:**R1–R2.
Spooren WP, Vassout A, Neijt HC, Kuhn R, Gasparini F, Roux S, Porsolt RD, and Gentsch C (2000b) Anxiolytic-like effects of the prototypical metabotropic glutamate receptor 5 antagonist 2-methyl-6-(phenylethynyl)pyridine in rodents. *J Pharmacol Exp Ther* **295:**1267–1275.
Sprague SM, Evenepoel P, Curzi MP, González MT, Husserl FE, Kopyt N, Sterling LR, Mix C, and Wong G (2009) Simultaneous control of PTH and CaxP is sustained over three years of treatment with cinacalcet HCl. *Clin J Am Soc Nephrol* **4:**1465–1476.
Stachowicz K, Brański P, Kłak K, van der Putten H, Cryan JF, Flor PJ, and Andrzej P (2008) Selective activation of metabotropic G-protein-coupled glutamate 7 receptor elicits anxiolytic-like effects in mice by modulating GABAergic neurotransmission. *Behav Pharmacol* **19:**597–603.
Stachowicz K, Kłak K, Kłodzińska A, Chojnacka-Wojcik E, and Pilc A (2004) Anxiolytic-like effects of PHCCC, an allosteric modulator of mGlu4 receptors, in rats. *Eur J Pharmacol* **498:**153–156.
Steckler T, Lavreysen H, Oliveira AM, Aerts N, Van Craenendonck H, Prickaerts J, Megens A, and Lesage AS (2005a) Effects of mGlu1 receptor blockade on anxiety-related behaviour in the rat lick suppression test. *Psychopharmacology* **179:**198–206.
Steckler T, Oliveira AF, Van Dyck C, Van Craenendonck H, Mateus AM, Langlois X, Lesage AS, and Prickaerts J (2005b) Metabotropic glutamate receptor 1 blockade impairs acquisition and retention in a spatial water maze task. *Behav Brain Res* **164:**52–60.
Surgand JS, Rodrigo J, Kellenberger E, and Rognan D (2006) A chemogenomic analysis of the transmembrane binding cavity of human G-protein-coupled receptors. *Proteins* **62:**509–538.
Suzuki G, Kimura T, Satow A, Kaneko N, Fukuda J, Hikichi H, Sakai N, Maehara S, Kawagoe-Takaki H, Hata M, et al. (2007a) Pharmacological characterization of a new, orally active and potent allosteric metabotropic glutamate receptor 1 antagonist, 4-[1-(2-fluoropyridin-3-yl)-5-methyl-1*H*-1,2,3-triazol-4-yl]-*N*-isopropyl-*N*-methyl-3,6-dihydropyridine-1(2*H*)-carboxamide (FTIDC). *J Pharmacol Exp Ther* **321:**1144–1153.
Suzuki G, Tsukamoto N, Fushiki H, Kawagishi A, Nakamura M, Kurihara H, Mitsuya M, Ohkubo M, and Ohta H (2007b) In vitro pharmacological characterization of novel isoxazolopyridone derivatives as allosteric metabotropic glutamate receptor 7 antagonists. *J Pharmacol Exp Ther* **323:**147–156.
Swanson CJ, Bures M, Johnson MP, Linden AM, Monn JA, and Schoepp DD (2005) Metabotropic glutamate receptors as novel targets for anxiety and stress disorders. *Nat Rev Drug Discov* **4:**131–144.
Takahashi T, Kajikawa Y, and Tsujimoto T (1998) G-protein-coupled modulation of presynaptic calcium currents and transmitter release by a $GABA_B$ receptor. *J Neurosci* **18:**3138–3146.
Takeda S, Kadowaki S, Haga T, Takaesu H, and Mitaku S (2002) Identification of G protein-coupled receptor genes from the human genome sequence. *FEBS Lett* **520:**97–101.
Temussi PA (2002) Why are sweet proteins sweet? Interaction of brazzein, monellin and thaumatin with the T1R2–T1R3 receptor. *FEBS Lett* **526:**1–4.
Temussi PA (2009) Sweet, bitter and umami receptors: a complex relationship. *Trends Biochem Sci* **34:**296–302.
Tfelt-Hansen J and Brown EM (2005) The calcium-sensing receptor in normal physiology and pathophysiology: a review. *Crit Rev Clin Lab Sci* **42:**35–70.
Timmers HJ, Karperien M, Hamdy NA, de Boer H, and Hermus AR (2006) Normalization of serum calcium by cinacalcet in a patient with hypercalcemia due to a de novo inactivating mutation of the calcium-sensing receptor. *J Intern Med* **260:**177–182.
Tomchik SM, Berg S, Kim JW, Chaudhari N, and Roper SD (2007) Breadth of tuning and taste coding in mammalian taste buds. *J Neurosci* **27:**10840–10848.
Tresadern G, Cid JM, Macdonald GJ, Vega JA, de Lucas AI, García A, Matesanz E, Linares ML, Oehlrich D, Lavreysen H, et al. (2010) Scaffold hopping from pyridones to imidazo[1,2-*a*]pyridines. New positive allosteric modulators of metabotropic glutamate 2 receptor. *Bioorg Med Chem Lett* **20:**175–179.
Triballeau N, Acher F, Brabet I, Pin JP, and Bertrand HO (2005) Virtual screening workflow development guided by the "receiver operating characteristic" curve approach. Application to high-throughput docking on metabotropic glutamate receptor subtype 4. *J Med Chem* **48:**2534–2547.
Trivedi R, Mithal A, and Chattopadhyay N (2008) Recent updates on the calcium-sensing receptor as a drug target. *Curr Med Chem* **15:**178–186.
Trivedi R, Mithal A, and Chattopadhyay N (2009) Pharmacological manipulation of calcium-sensing receptor: prospect as anabolic therapy for postmenopausal osteoporosis. *Drugs Future* **34:**803–809.
Tsuchiya D, Kunishima N, Kamiya N, Jingami H, and Morikawa K (2002) Structural views of the ligand-binding cores of a metabotropic glutamate receptor complexed with an antagonist and both glutamate and Gd^{3+}. *Proc Natl Acad Sci USA* **99:**2660–2665.
Tu H, Rondard P, Xu C, Bertaso F, Cao F, Zhang X, Pin JP, and Liu J (2007) Dominant role of GABAB2 and Gbetagamma for GABAB receptor-mediated-ERK1/2/CREB pathway in cerebellar neurons. *Cell Signal* **19:**1996–2002.
Tu H, Xu C, Zhang W, Liu Q, Rondard P, Pin JP, and Liu J (2010) $GABA_B$ receptor activation protects neurons from apoptosis via IGF-1 receptor transactivation. *J Neurosci* **30:**749–759.
Ugolini A, Large CH, and Corsi M (2008) AMN082, an allosteric mGluR7 agonist that inhibits afferent glutamatergic transmission in rat basolateral amygdala. *Neuropharmacology* **55:**532–536.
Urban JD, Clarke WP, von Zastrow M, Nichols DE, Kobilka B, Weinstein H, Javitch JA, Roth BL, Christopoulos A, Sexton PM, et al. (2007) Functional selectivity and classical concepts of quantitative pharmacology. *J Pharmacol Exp Ther* **320:**1–13.
Urwyler S (2006) Allosteric modulation of $GABA_B$ receptors, in *Allosteric Receptor Modulation in Drug Targeting* (Bowery NG ed) pp 235–257, Taylor & Francis, New York.
Urwyler S, Gjoni T, Kaupmann K, Pozza MF, and Mosbacher J (2004) Selected amino acids, dipeptides and arylalkylamine derivatives do not act as allosteric modulators at $GABA_B$ receptors. *Eur J Pharmacol* **483:**147–153.
Urwyler S, Gjoni T, Koljatić J, and Dupuis DS (2005) Mechanisms of allosteric modulation at $GABA_B$ receptors by CGP7930 and GS39783: effects on affinities and efficacies of orthosteric ligands with distinct intrinsic properties. *Neuropharmacology* **48:**343–353.
Urwyler S, Mosbacher J, Lingenhoehl K, Heid J, Hofstetter K, Froestl W, Bettler B, and Kaupmann K (2001) Positive allosteric modulation of native and recombinant, gamma-aminobutyric $acid_B$ receptors by 2,6-di-*tert*-butyl-4-(3-hydroxy-2,2-dimethyl-propyl)-phenol (CGP7930) and its aldehyde analog CGP13501. *Mol Pharmacol* **60:**963–971.
Urwyler S, Pozza MF, Lingenhoehl K, Mosbacher J, Lampert C, Froestl W, Koller M, and Kaupmann K (2003) *N*,*N*′-dicyclopentyl-2-methylsulfanyl-5-nitro-pyrimidine-4,6-diamine (GS39783) and structurally related compounds: novel allosteric enhancers of γ-aminobutyric $Acid_B$ receptor function. *J Pharmacol Exp Ther* **307:**322–330.
Valenti O, Marino MJ, Wittmann M, Lis E, DiLella AG, Kinney GG, and Conn PJ (2003) Group III metabotropic glutamate receptor - mediated modulation of the striatopallidal synapse. *J Neurosci* **23:**7218–7226.
Vanejevs M, Jatzke C, Renner S, Müller S, Hechenberger M, Bauer T, Klochkova A, Pyatkin I, Kazyulkin D, Aksenova E, et al. (2008) Positive and negative modulation of group I metabotropic glutamate receptors. *J Med Chem* **51:**634–647.
Varney MA, Cosford ND, Jachec C, Rao SP, Sacaan A, Lin FF, Bleicher L, Santori EM, Flor PJ, Allgeier H, et al. (1999) SIB-1757 and SIB-1893: selective, noncompetitive and antagonists of metabotropic glutamate receptor type 5. *J Pharmacol Exp Ther* **290:**170–181.
Vieira E, Huwyler J, Jolidon S, Knoflach F, Mutel V, and Wichmann J (2005) 9*H*-Xanthene-9-carboxylic acid [1,2,4]oxadiazol-3-yl- and (2*H*-tetrazol-5-yl)-amides as potent, orally available mGlu1 receptor enhancers. *Bioorg Med Chem Lett* **15:**4628–4631.
Vieira E, Huwyler J, Jolidon S, Knoflach F, Mutel V, and Wichmann J (2009) Fluorinated 9H-xanthene-9-carboxylic acid oxazol-2-yl-amides as potent, orally available mGlu1 receptor enhancers. *Bioorg Med Chem Lett* **19:**1666–1669.
Wada M, Furuya Y, Sakiyama J, Kobayashi N, Miyata S, Ishii H, and Nagano N

(1997) The calcimimetic compound NPS R-568 suppresses parathyroid cell proliferation in rats with renal insufficiency. Control of parathyroid cell growth via a calcium receptor. *J Clin Invest* **100:**2977–2983.
Walker K, Bowes M, Panesar M, Davis A, Gentry C, Kesingland A, Gasparini F, Spooren W, Stoehr N, Pagano A, et al. (2001a) Metabotropic glutamate receptor subtype 5 (mGlu5) and nociceptive function I. selective blockade of mGlu5 receptors in models of acute, persistent and chronic pain. *Neuropharmacology* **40:**1–9.
Walker K, Reeve A, Bowes M, Winter J, Wotherspoon G, Davis A, Schmid P, Gasparini F, Kuhn R, and Urban L (2001b) mGlu5 receptors and nociceptive function II. mGlu5 receptors functionally expressed on peripheral sensory neurones mediate inflammatory hyperalgesia. *Neuropharmacology* **40:**10–19.
Weis WI and Kobilka BK (2008) Structural insights into G-protein-coupled receptor activation. *Curr Opin Struct Biol* **18:**734–740.
Wellendorph P, Johansen LD, and Bräuner-Osborne H (2009) Molecular pharmacology of promiscuous seven transmembrane receptors sensing organic nutrients. *Mol Pharmacol* **76:**453–465.
White JH, Wise A, Main MJ, Green A, Fraser NJ, Disney GH, Barnes AA, Emson P, Foord SM, and Marshall FH (1998) Heterodimerization is required for the formation of a functional $GABA_B$ receptor. *Nature* **396:**679–682.
Wichmann J, Adam G, Kolczewski S, Mutel V, and Woltering T (1999) Structure-activity relationships of substituted 5H-thiazolo [3,2-a]pyrimidines as group 2 metabotropic glutamate receptor antagonists. *Bioorg Med Chem Lett* **9:**1573–1576.
Wichmann J, Bleicher K, Vieira E, Woltering T, Knoflach F, and Mutel V (2002) Alkyl diphenylacetyl, 9H-xanthene- and 9H-thioxanthene-carbonyl carbamates as positive allosteric modulators of mGlu1 receptors. *Il Farmaco* **57:**989–992.
Widler L, Altmann E, Beerli R, Breitenstein W, Bouhelal R, Buhl T, Gamse R, Gerspacher M, Halleux C, John MR, et al. (2010) 1-Alkyl-4-phenyl-6-alkoxy-1*H*-quinazolin-2-ones: a novel series of potent calcium-sensing receptor antagonists. *J Med Chem* **53:**2250–2263.
Williams R, Johnson KA, Gentry PR, Niswender CM, Weaver CD, Conn PJ, Lindsley CW, and Hopkins CR (2009a) Synthesis and SAR of a novel positive allosteric modulator (PAM) of the metabotropic glutamate receptor 4 (mGluR4). *Bioorg Med Chem Lett* **19:**4967–4970.
Williams R, Niswender CM, Luo Q, Le U, Conn PJ, and Lindsley CW (2009b) Positive allosteric modulators of the metabotropic glutamate receptor subtype 4 (mGluR4). Part II: Challenges in hit-to-lead. *Bioorg Med Chem Lett* **19:**962–966.
Williams R, Zhou Y, Niswender CM, Luo Q, Conn PJ, Lindsley CW, and Hopkins CR (2010) Re-exploration of the PHCCC scaffold: discovery of improved positive allosteric modulators of mGluR4. *ACS Chem Neurosci* **1:**411–419.
Winkel C, de Klerk A, Visser J, de Rijke E, Bakker J, Koenig T, and Renes H (2008) New developments in umami (enhancing) molecules. *Chem Biodivers* **5:**1195–1203.
Winnig M, Bufe B, and Meyerhof W (2005) Valine 738 and lysine 735 in the fifth transmembrane domain of rTas1r3 mediate insensitivity towards lactisole of the rat sweet taste receptor. *BMC Neuroscience* **6:**22.
Wise A, Green A, Main MJ, Wilson R, Fraser N, and Marshall FH (1999) Calcium sensing properties of the $GABA_B$ receptor. *Neuropharmacology* **38:**1647–1656.
Wojcik WJ and Neff NH (1984) γ-Aminobutyric acid B receptors are negatively coupled to adenylate cyclase in brain, and in the cerebellum these receptors may be associated with granule cells. *Mol Pharmacol* **25:**24–28.
Woltering TJ, Adam G, Alanine A, Wichmann J, Knoflach F, Mutel V, and Gatti S (2007) Synthesis and characterization of 8-ethynyl-1,3-dihydro-benzo[*b*][1,4]diazepin-2-one derivatives: new potent non-competitive metabotropic glutamate receptor 2/3 antagonists. part 1. *Bioorg Med Chem Lett* **17:**6811–6815.
Woolley ML, Pemberton DJ, Bate S, Corti C, and Jones DN (2008) The mGlu2 but not the mGlu3 receptor mediates the actions of the mGluR2/3 agonist, LY379268, in mouse models predictive of antipsychotic activity. *Psychopharmacology* **196:**431–440.
Xi ZX, Ramamoorthy S, Shen H, Lake R, Samuvel DJ, and Kalivas PW (2003) GABA transmission in the nucleus accumbens is altered after withdrawal from repeated cocaine. *J Neurosci* **23:**3498–3505.
Xi ZX and Stein EA (1999) Baclofen inhibits heroin self-administration behavior and mesolimbic dopamine release. *J Pharmacol Exp Ther* **290:**1369–1374.
Xu H, Staszewski L, Tang H, Adler E, Zoller M, and Li X (2004) Different functional roles of T1R subunits in the heteromeric taste receptors. *Proc Natl Acad Sci USA* **101:**14258–14263.
Yan QJ, Rammal M, Tranfaglia M, and Bauchwitz RP (2005) Suppression of two major fragile X syndrome mouse modelphenotypes by the mGluR5 antagonist MPEP. *Neuropharmacology* **49:**1053–1066.
Yang W, Ruan Z, Wang Y, Van Kirk K, Ma Z, Arey BJ, Cooper CB, Seethala R, Feyen JH, and Dickson JK Jr (2009) Discovery and structure-activity relationships of trisubstituted pyrimidines/pyridines as novel calcium-sensing receptor antagonists. *J Med Chem* **52:**1204–1208.
Yang W, Wang Y, Roberge JY, Ma Z, Liu Y, Michael Lawrence R, Rotella DP, Seethala R, Feyen JH, and Dickson JK Jr (2005) Discovery and structure-activity relationships of 2-benzylpyrrolidine-substituted aryloxypropanols as calcium-sensing receptor antagonists. *Bioorg Med Chem Lett* **15:**1225–1228.
Young RL, Page AJ, O'Donnell TA, Cooper NJ, and Blackshaw LA (2007) Peripheral versus central modulation of gastric vagal pathways bymetabotropic glutamate receptor 5. *Am J Physiol Gastrointest Liver Physiol* **292:**G501–G511.
Yu M (2007) Recent developments of the PET imaging agents for metabotropic glutamate receptor subtype 5. *Curr Top Med Chem* **7:**1800–1805.
Zerbib F, Keywood C, and Strabach G (2010) Efficacy, tolerability and pharmacokinetics of a modified release formulation of ADX10059, a negative allosteric modulator of metabotropic glutamate receptor 5: an esophageal pH-impedance study in healthy subjects. *Neurogastroenterol Motil* **22:**859–865.
Zhang F, Klebansky B, Fine RM, Liu H, Xu H, Servant G, Zoller M, Tachdjian C, and Li X (2010a) Molecular mechanism of the sweet taste enhancers. *Proc Natl Acad Sci USA* **107:**4752–4757.
Zhang F, Klebansky B, Fine RM, Xu H, Pronin A, Liu H, Tachdjian C, and Li X (2008a) Molecular mechanism for the umami taste synergism. *Proc Natl Acad Sci USA* **105:**20930–20934.
Zhang L, Rogers BN, Duplantier AJ, McHardy SF, Efremov I, Berke H, Qian W, Zhang AQ, Maklad N, Candler J, et al. (2008b) 3-(Imidazolyl methyl)-3-azabicyclo[3.1.0]hexan-6-yl)methyl esters: a novel series of mGluR2 positive allosteric modulators. *Bioorg Med Chem Lett* **18:**5493–5496.
Zhang P, Zou MF, Rodriguez AL, Conn PJ, and Newman AH (2010b) Structure-activity relationships in a novel series of 7-substituted-arylquinolines and 5-substituted-aryl benzothiazoles at the metabotropic glutamate receptor subtype 5. *Bioorg Med Chem* **18:**3026–3035.
Zhang Y, Hoon MA, Chandrashekar J, Mueller KL, Cook B, Wu D, Zuker CS, and Ryba NJ (2003) Coding of sweet, bitter and umami tastes: different receptor cells sharing similar signaling pathways. *Cell* **112:**293–301.
Zhang Y, Rodriguez AL, and Conn PJ (2005) Allosteric potentiators of metabotropic glutamate receptor subtype 5 have differential effects on different signaling pathways in cortical astrocytes. *J Pharmacol Exp Ther* **315:**1212–1219.
Zhang Z, Jiang Y, Quinn SJ, Krapcho K, Nemeth EF, and Bai M (2002a) L-phenylalanine and NPS R-467 synergistically potentiate the function of the extracellular calcium-sensing receptor through distinct sites. *J Biol Chem* **277:**33736–33741.
Zhang Z, Qiu W, Quinn SJ, Conigrave AD, Brown EM, and Bai M (2002b) Three adjacent serines in the extracellular domains of the CaR are required for L-amino acid-mediated potentiation of receptor function. *J Biol Chem* **277:**33727–33735.
Zhang Z, Sun S, Quinn SJ, Brown EM, and Bai M (2001) The extracellular calcium-sensing receptor dimerizes through multiple types of intermolecular interactions. *J Biol Chem* **276:**5316–5322.
Zhao GQ, Zhang Y, Hoon MA, Chandrashekar J, Erlenbach I, Ryba NJ, and Zuker CS (2003) The receptors for mammalian sweet and umami taste. *Cell* **115:**255–266.
Zhao Z, Wisnoski DD, O'Brien JA, Lemaire W, Williams DL Jr, Jacobson MA, Wittman M, Ha SN, Schaffhauser H, Sur C, et al. (2007) Challenges in the development of mGluR5 positive allosteric modulators: the discovery of CPPHA. *Bioorg Med Chem Lett* **17:**1386–1391.
Zheng GZ, Bhatia P, Daanen J, Kolasa T, Patel M, Latshaw S, El Kouhen OF, Chang R, Uchic ME, Miller L, et al. (2005) Structure-activity relationship of triazafluorenone derivatives as potent and selective mGluR1 antagonists. *J Med Chem* **48:**7374–7388.
Zhou Y, Rodriguez AL, Williams R, Weaver CD, Conn PJ, and Lindsley CW (2009) Synthesis and SAR of novel, non-MPEP chemotype mGluR5 NAMs identified by functional HTS. *Bioorg Med Chem Lett* **19:**6502–6506.
Zhu CZ, Baker S, El-Kouhen O, Lehto SG, Hollingsworth PR, Gauvin DM, Hernandez G, Zheng G, Chang R, Moreland RB, et al. (2008) Analgesic activity of metabotropic glutamate receptor 1 antagonists on spontaneous post-operative pain in rats. *Eur J Pharmacol* **580:**314–321.
Zhu CZ, Hsieh G, Ei-Kouhen O, Wilson SG, Mikusa JP, Hollingsworth PR, Chang R, Moreland RB, Brioni J, Decker MW, et al. (2005) Role of central and peripheral mGluR5 receptors in post-operative pain in rats. *Pain* **114:**195–202.