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REVIEW

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Recent Progress on the Identification of Metabotropic Glutamate 4 Receptor Ligands and Their Potential Utility as CNS Therapeutics

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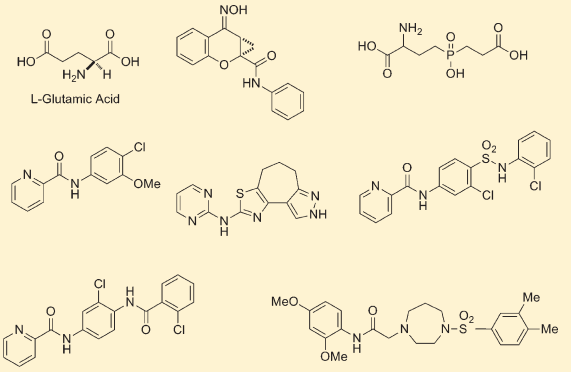
ABSTRACT: This Review describes recent activity in the advancement of ligands for the metabotropic glutamate 4 receptor subtype and their potential utility as central nervous system (CNS) therapeutics. Until recently, there was a paucity of compounds with suitable selectivity and druglike properties to elucidate the value of this target. The search for selective entities has led several groups to the investigation of allosteric modulators as a path to optimization of potential ligands. Recent efforts, discussed here, have afforded a variety of derivatives with improvements in potency, solubility, and pharmacokinetic properties that garner support for continued investigation and optimization.

KEYWORDS: Metabotropic glutamate receptor 4, orthosteric ligand, allosteric modulator, Parkinson's disease, anxiety, pain, cognitive disorders, psychiatric disorders, neurodegenerative disorders, Class C GPCR



L-Glutamic acid (glutamate) **1** (Figure 1), the most abundant excitatory neurotransmitter in the vertebrate nervous system,[1] and the receptor systems it impacts have been the target of much research in the past two decades.[2−10] Based upon the mechanism of action, the receptors are divided into two classes, ionotropic and metabotropic.[11] The receptors of the ionotropic class (iGluRs) are ligand gated ion channels that mediate fast synaptic transmission, while the metabotropic receptors (mGluRs) are G-protein coupled receptors (GPCRs) that modulate the release or postsynaptic effects of glutamate.

The mGluRs belong to Class C of the GPCR superfamily and are further divided into three subgroups containing eight known receptor subtypes.[12,13] Group I contains mGlu1 and mGlu5, group II contains mGlu2 and mGlu3, and group III contains mGlu4, mGlu6, mGlu7, and mGlu8.[14] The group III mGluRs are all found within the basal ganglia circuitry of the brain and are negatively coupled to adenylate cyclase through Gi/Go proteins.[15] They are found primarily on presynaptic terminals of GABAergic and glutamatergic neurons responsible for regulation of synaptic transmission through inhibition of voltage-gated calcium flow across the cell membrane.[16,17] They have also been identified postsynaptically in various brain regions producing membrane hyperpolarization through activation of G-protein coupled inwardly rectifying potassium channels.[18]

Class C GPCRs are somewhat unique among the family of GPCRs. Found typically as dimers, they contain two distinct regions; the classical seven transmembrane domain, ubiquitous among the GPCRs, along with a large extracellular N-terminus (Figure 2).[19]

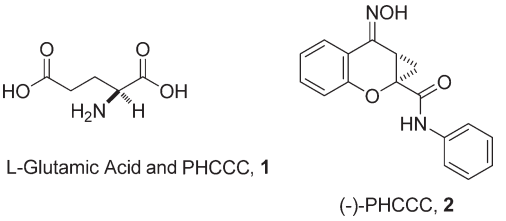


Figure 1. Glutamate and PHCCC.

This extracellular domain of the mGluR contains the endogenous ligand binding site, formed by two hinged globular domains, typically referred to as the Venus Flytrap domain (VFD).[20] Once glutamate binds, it causes the two domains to close, providing the required structural transformation that triggers intracellular G-protein activation. Not surprisingly, the level of homology that exists among the subtypes of the mGluRs in this VFD region is quite high.[21] Thus, affinity for glutamate is somewhat consistent among the majority of mGlu receptor subtypes (~3−60 uM), with the exception of mGlu7, which is much less potent, and mGlu3 which is much more potent (40 nM).[22] It follows that identifying ligands that would selectively bind to one receptor among this group at the endogenous site would pose a daunting challenge, one that has yet to afford significant entities.

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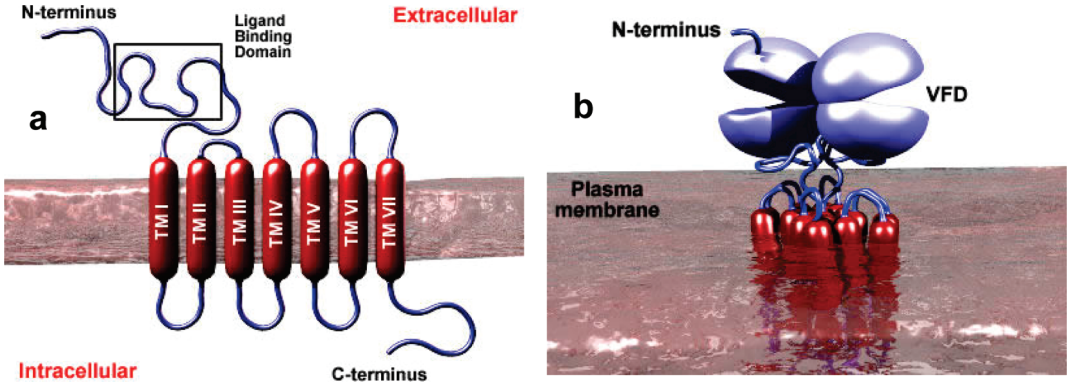


Figure 2. (a) Typical class A GPCR transmembrane domain structure and (b) Class C GPCR with dimeric transmembrane region and Venus Flytrap N-terminus.

1. ALLOSTERIC VERSUS ORTHOSTERIC MODULATION

It has long been a challenge in drug discovery to identify selective ligands for receptors, enzymes, and ion channel sites that are of therapeutic importance. Often times, the degree of homology among any particular class is such that there are minimal opportunities to design into the ligand the proper interactions with the peptide backbone, at the native or orthosteric site, to afford any real differential affinity. There are occasions when pan-modulators (nonspecific, ubiquitous affinity) can be considered as potential therapeutics, but more often than not off-target affinity even within the same class affords unwanted, or even contradictory, effects. An alternate strategy for the design of selective ligands might then focus on finding other sites, allosteric sites (from the Greek allos: "other"), to modulate the receptor that are not ubiquitous among the class.[23] There has been much research in the area of allosteric modulation, both positive (PAM) and negative (NAM) in the past decade.[24−28] Identification of a binding site which impacts receptor function that is specific for a subtype within the class has the potential to address the selectivity issues as well as afford molecular entities that have improved pharmacokinetic and physical chemical properties, a challenge in particular for certain targets within the neuroscience arena. Furthermore, the ability to impact the augmentation, or reduction, of the receptor function (with binding of the native ligand to the orthosteric site) exists with modulators that bind allosterically. Homotropic allosteric modulators are substrates for the target site as well as regulators of the sites function; that is, they can bind to the native site and act a classical agonist or antagonist or they can bind allosterically and affect the function of the native ligand. Heterotropic modulators are regulatory molecules that are not a substrate for the native function in the absence of the endogenous ligand. It is the latter that are of particular interest, due in part to the reasons stated above regarding selectivity and deleterious effects. There has been significant recent progress in designing allosteric modulators for a number of receptor classes. The utility of these is only recently being explored in the clinic.[29,30]

2. THERAPEUTIC POTENTIAL OF MGLU4 RECEPTOR LIGANDS

The therapeutic potential of the group III mGluRs is rapidly expanding and has become a focus for several groups. Recent efforts in this area are beginning to reveal a multitude of opportunities for regulation of glutamatergic dysfunction in the central compartment through the various receptor subtypes. Anxiety, pain, cognition, psychiatric, and neurodegenerative disorders have all been implicated as potential targets for mGluR modulation.

In particular, the mGlu4 receptor has received much interest following the discovery that PHCCC (*N*-phenyl-7-(hydroxyimino)-cyclopropa[*b*]chromen-1a-carboxamide) **2**, a partially selective mGlu4 PAM, showed activity in models of neuroprotection and Parkinson's disease (PD).[31,32] Subsequent results with several other tool compounds have reinforced the validation in various rodent and monkey models of PD. There are several key reviews describing the early in vivo and mechanistic work.[14,33−35] The progress made has significantly enabled the advancement of the chemical matter and provided key insights into the potential of mGlu4 modulation on neurodegeneration and PD in particular. However, until recently, the compounds utilized have been lacking the necessary potency and pharmacokinetic properties to test the hypothesis further. Nevertheless, the need for non-dopaminergic treatments for PD and the potential to identify a disease modifying therapeutic have spurred interest in mGlu4 modulators.

This Review will focus on recent efforts at identifying mGlu4-selective ligands with improved potency and druglike qualities. In all cases, the human and rat binding affinities are closely aligned, and for the purposes of the disclosures in this Review only the human data will be discussed. In addition, in the vast majority of cases, very little, if any, selectivity data is reported for much of the cited work. In some cases, a sampling of selectivity data for closely related mGlu receptors is given, but unless specifically stated the data is unavailable. One should note this when interpreting the various in vivo efficacy findings.

3. MGLU4 LIGANDS

Significant progress has been made since 2009, particularly in advancing the equity around mGlu4 positive allosteric modulators. To date, there are no known mGlu4 NAMs and the interest in identifying selective orthosteric agonists has been difficult and less attractive due to the various reasons described earlier. Thus, research on positive allosteric modulators for the mGlu4 receptor has received more attention and has made greater progress. As mentioned earlier, mGlu4 receptors are a member of the Class C GPCRs and as such are somewhat unique in their structure. With Class A and B GPCRs, the allosteric and orthosteric sites are found either within the membrane spanning 7-TM domain or

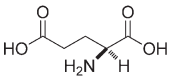
within the amino terminus or extracellular loops. However, studies with Class C GPCRs have shown significant deviation in that receptors in this class are dimeric in structure, suggesting potentially large structural deviations from the classical monomeric GPCR structure. In addition, the lack of a crystal structure for receptors of this type makes it difficult to quantify the differences. Furthermore the orthosteric binding site of Class C GPCRs is found within the VFD, unlike Class A and B GPCRs, and the allosteric binding sites are typically found in the 7-TM spanning region of the receptor (Figure 1).[14] Finally, allosteric modulators afford the obvious potential advantages for selectivity among closely related subtype receptors. These characteristics coupled with the structural deviations of the Class C GPCRs afford tremendous opportunity for the identification of selective mGlu4 modulators.

3.1. Orthosteric Ligands. The earliest work on modulators of the mGlu4 receptor centered around the endogenous ligand glutamate **1**. L-AP$_4$ **3**[17,36−38] and L-SOP **4**[39,40] along with attempts at constrained analogues such as **5**[41] and **6**[42,43] (Scheme 1) afforded interesting tool compounds, albeit with all the unwanted physicochemical issues associated with acids and acid-mimetics that make them undesirable druglike entities. However, these tool compounds were active in acute and chronic rodent models of PD, which has further bolstered the case for the utility of mGlu4 PAMs.

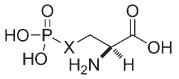
Although there has been a paucity of interest in orthosteric mGlu4 ligands, a modestly potent nonselective mGlu4 agonist lead was recently identified.[44] Through the use of a virtual screening effort, PCEP **7** (Scheme 2 and Table 1) was discovered to be a 7 μM ligand at the mGlu 4 receptor, as measured in an IP production assay. In addition the dicarboxylic acid showed modest selectivity over mGlu6 and mGlu8, with 32 and 20 μM potency, respectively. The eudismic ratio was approximately 3 with the (*S*)-enantiomer **8** being the more potent eutomer. A focused campaign to explore the breadth of the structure−activity relationship (SAR) was met with less than impressive results and further demonstrated the limited scope available at the orthosteric binding site for optimization. Closely related derivatives LSP1-3154 **10** and LSP1-3155 **11** were slightly more potent at the target receptor and **11** was only modestly more selective versus mGlu6 and mGlu8 (as measured in a Ca+^{2} FLIPR assay) compared with **8**.

In addition to these compounds, Acher, Pin, and co-workers have also developed more potent and selective orthosteric agonists that have been evaluated in in vivo models of both PD and anxiety.[45−47] The two highlighted compounds are LSP1-2111, **12**, and LSP1-3081, **13**, EC_{50} = 2.20 and 0.16 μM, respectively. Although LSP1-2111 is less potent versus mGlu4 compared to LSP1-3081 and L-AP4, it does show more selectivity versus mGlu8 (~30-fold). LSP1-3081 is equipotent to L-AP4 and shows a similar selectivity profile as the prototypical agonist. LSP1-2111 has been dosed both interpallidally and systemically and has been shown to counteract akinesia via inhibition of striatopallidal transmission.[46] This is the first report of an mGlu4 preferring orthosteric agonist to counteract akinesia. In addition to reversal of akinesia, LSP1-2111 was also found to produce anxiolytic-like effects in the stress-induced hyperthermia (SIH) and elevated plus maze assays in mice after systemic injection.[45] The authors also showed that the effects were inhibited by the benzodiazepine receptor antagonist (flumazenil) and by a 5-HT$_{1A}$ receptor antagonist (WAY100635). Lastly, a 5-HT2$_{A/C}$ antagonist (ritanserin) did not affect the results, nor was LSP1-2111 active in animals with a depleted 5-HT system. LSP1-2111 did not produce an antidepressant-like effect, which when taken all of the results together led the authors to summarize that the GABAergic and serotonergic systems are involved with the potential anxiolytic affects of LSP1-2111 and inferred that orthosteric agonists of mGlu4 can produce antianxiolytic but not

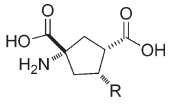
Scheme 1. Earliest mGlu4 Modulators



L-Glutamic Acid, **1**



X = CH_2, L-AP$_4$, **3**
X = O, L-SOP, **4**

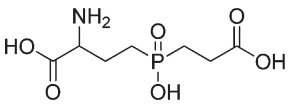


R = H, **5**
R = CO_2H, **6**

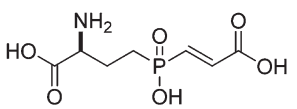
Table 1. Potency and Selectivity of PCEP and Analogues

compd	mGlu4 EC50 (μM)	mGlu6/mGlu4	mGlu8/mGlu4
PCEP **7**	7.1 ± 1.5	4.5	2.8
(*S*)-PCEP **8**	6.4 ± 1.4	4.8	1.2
(*R*)-PCEP **9**	17 ± 2	5.8	3.4
LSP1-3154 **10**	3.0 ± 1.0	1.1	5.1
LSP1-3155 **11**	3.8 ± 1.5	8.9	8.3
LSP1-2111 **12**	2.2 ± 0.3	1	30
LSP1-3081 **13**	0.16 ± 0.03	20.4	3.4

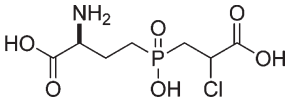
Scheme 2. PCEP and Closely Related Analogues



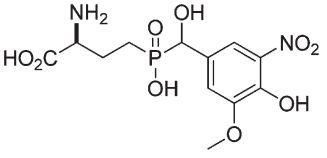
PCEP, **7**
(S)-PCEP, **8**
(R)-PCEP, **9**



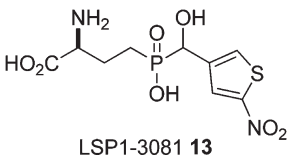
LSP1-3154 **10**



LSP1-3155 **11**



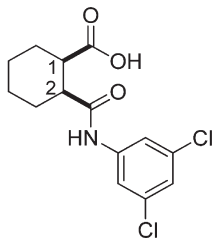
LSP1-2111 **12**



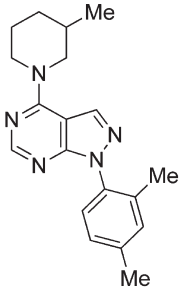
LSP1-3081 **13**

dx.doi.org/10.1021/cn200043e |*ACS Chem. Neurosci.* 2011, 2, 433–449

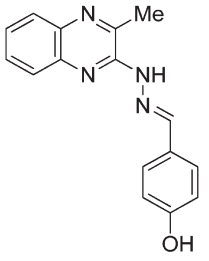
Scheme 3. Initial Vanderbilt University Leads



VU0003423, cis,trans, racemic, **14**
VU0155041, cis, racemic, **15**



VU0080241, **16**



VU0001171, **17**

antidepressant-like effects after systemic administration. LSP1-3081 has also recently been shown to negatively regulate glutamate and GABA synaptic release in the striatum.[47] The authors of this study also show that, by stimulating these receptors, LSP1-3081 improves akinesia in a rat model of PD. Although these orthosteric agonists do not possess favorable druglike characteristics, they have provided vital confirmation that activation of mGlu4 should be beneficial as a novel therapeutic for PD and, potentially, anxiety.

3.2. Allosteric Modulators. The identification of PHCCC **2** in 1996[48] and the subsequent revelation reported by Maj et al. and Marino et al. in 2003 that the (−)-enantiomer was a moderately potent mGlu4 positive allosteric modulator opened the door for continued validation and efforts to identify optimized allosteric entities.[31,32] Marino and co-workers showed that PHCCC was effective in acute models of PD with intracerebroventricular (icv) administration and, furthermore, demonstrated that PHCCC potentiated the effects of L-AP_4 on striatopallidal synapse function, supporting the connection to modulating antiparkinsonian effects. Subsequently, Battaglia and co-workers showed that PHCCC could prevent dopaminergic neuron degeneration induced by the toxin MPTP in mice.[49] Bolstered by these results, and the poor pharmacokinetic and SAR characteristics for this compound class, efforts to identify mGlu4 PAMs with improved druglike properties gained significant momentum. In particular, the group at Vanderbilt began in earnest the search for new chemotypes which would allow greater understanding of the potential of this approach to treat PD. From 2008 to 2009, Conn, Lindsley and co-workers reported several novel classes of mGlu 4 PAMs with varying degrees of efficacy.[34] In designing modulators, one must be aware that efficacy can be judged by different measures: the affinity of the modulator at the allosteric site (EC_{50}), the maximal effect compared to the native ligand (E_{max}), and/or the shift in potency of the native ligand in a functional assay (fold shift (FS)). Progress has been made on these varying degrees of efficacy; however, the optimal combination is not yet apparent from the current state of the art (vide supra). Indeed, the proper mix of EC_{50}, E_{max}, and FS may have vastly different requirements for different disease states, and a significant volume of data and progress are needed in the field before this important issue can be resolved.

Utilizing a high throughput screening (HTS) assay, Niswender and co-workers identified the racemic cyclohexylamide **14** (VU0003423) (Scheme 3), a novel ligand with good potency in both human and rat mGlu4 expressing cell lines (hEC_{50} 750 nM, FS 6.5).[50] Separation of the isomers showed that the cis-regioisomer **15** (VU0155041) was solely responsible for the potency. Subsequent chiral chromatographic separation of the cis-isomers apparently showed no enantiomeric preference for the receptor. However, this was later hypotheized to be due to an unusual racemization reaction, as resynthesis of the enantiomers by an independent group showed the activity to reside solely with one enantiomer ((1*R*, 2*S*)-**15**).[51] The SAR reported for this series was unremarkable, as little change was tolerated in various domains of the scaffold.[52] Interestingly, **15** has only partial agonist activity in the mGlu4 assay and does not compete with PHCCC binding. This suggests that **15** may bind to a different allosteric site than PHCCC as well as function as a dual agonist-PAM entity. Selectivity studies in a large panel screen showed **15** to be discriminative versus an array of GPCRs (including other mGluRs), ion channels, and transporters. In vivo efficacy studies (via icv administration) in two rat models of PD (reversal of haloperidol-induced catalepsy and reserpine-induced akinesia) demonstrated its antiparkinsonian potential, further supporting the therapeutic potential of mGlu4 PAMs.[50]

Subsequent work by the Vanderbilt group identified additional leads of varying chemical diversity (**16**, **17**) (Scheme 3) and potency at mGlu4.[53,54] The noteworthy feature of both of these ligands **16** VU0080241 and **17** VU0001171 was their rather large increase in fold shift (**16**, EC_{50} 4.6 μM, FS 28; **17**, EC_{50} 0.65 μM, FS 36), a feature not previously reported. Unfortunately, the ability to expand the genre was met with disappointment as the limited SAR trait seen with other series was shared by these scaffolds.

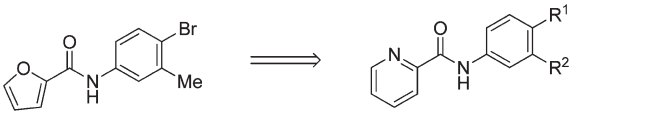
The understanding of the varying profiles (EC_{50} vs FS) and how this may impact efficacy in a mechanistic model of the disease is no doubt of paramount importance. The search for optimized entities continued as the potential impact has been underscored by the work of the Vanderbilt group. In the last 2−3 years, significant progress has been made in the identification of ligands with improved potency and pharmacokinetic (PK) properties. Hopkins and co-workers reported the first mGlu4 PAM that has sufficient PK properties to be administered peripherally without the need for high concentrations of dimethyl sulfoxide (DMSO).[55] During the course of their HTS campaign, a number of low molecular weight and moderately potent leads, such as the furylamide **18** (EC_{50} ~5 μM, FS 5), shown in Scheme 4, were identified. After an efficient library synthesis exercise was undertaken, this lead was optimized to pyridyl amides **17** (VU0361737) (EC_{50} 240 nM, FS 28) and **20** (EC_{50} 340 nM, FS 28). Characteristic among this scaffold was the tight SAR which

plagued all previous mGlu4 PAM explorations. A good example of this is exhibited by trifluoromethyl derivative **21**. The small change going from difluoromethoxy **20** to trifluoromethoxy **21** gives rise to potency exceeding 5 μM, roughly a 15-fold loss in affinity. Other very minor changes resulted in significant loss of activity as well. However, these compounds did have superior solubility and pharmacokinetic properties compared to previous tools, and allowed for examination of their exposure into the central nervous system (CNS) compartment when dosed more traditionally (i.e., intraperitoneal (ip) or oral as opposed to icv administration). Although **19** and **20** showed poor microsomal stability and thus were high clearance, short half-life compounds, they were both highly brain penetrant. When dosed ip at 10 mg/kg (aqueous 10% Tween-80), **20** showed >3 μM maximum concentration (C_{max}) in brain with a reported brain to plasma ratio (B/P) of ~10 (as measured by total concentration−time area (AUC 1−8 h)). Following the initial publication, the group at Vanderbilt evaluated the ability of VU0361737 (**19**) to reverse haloperidol-induced catalepsy, a preclinical rodent model of motor impairments associated with PD. In this model, rats are administered haloperidol, a dopamine D_2 antagonist which induces severe catalepsy, and then latency to withdrawal is evaluated at four doses (3, 10, 30, and 56.6 mg/kg).[56] VU0361737, **19**, was shown to reverse the catalepsy at the two top doses (30 and 56.6 mg/kg) and was as effective as a gold standard A_{2A} receptor antagonist[57] at the same dose. Thus, VU0361737 (**19**) is the first mGlu4 PAM to show efficacy in a preclinical PD model upon systemic dosing.[58]

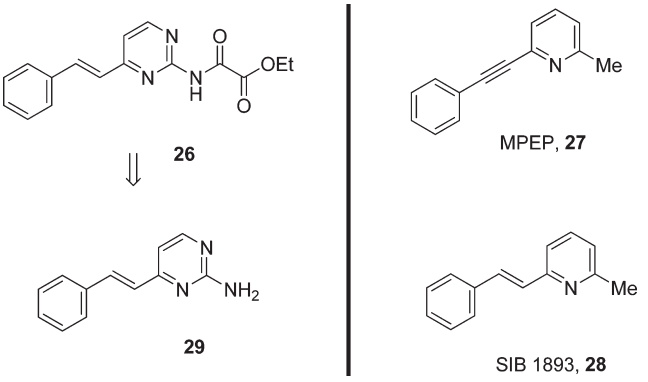
Following on another of the HTS small molecule leads, Hopkins and co-workers identified sulfonamide **22** (VU0130734) (Scheme 5), a close analogue to the previously reported amides (**18**−**21**), with modest 6 μM affinity at mGlu4.[59] Utilizing the SAR from the amide series, the furyl group was exchanged for the optimized 2-pyridyl moiety, providing **23** (VU0361591), with a concomitant improvement in activity of more than 30 fold (EC_{50} 159 nM). A focused SAR optimization of the remaining aryl groups afforded the 2-chlorophenyl derivative **24** (VU0364439), the most potent mGlu4 PAM reported at that time (EC_{50} 20 nM). An examination of the pharmacokinetic parameters of this series unfortunately showed them to be unstable in a microsomal incubation assay, limiting their use as in vivo tool molecules. Although optimization in this scaffold was possible, the tight SAR descriptive of mGlu4 ligands is highlighted by the chloro-regioisomer **25**. The transposition of this moiety's chlorine atom on the central aryl ring resulted in a derivative with complete loss of activity.

Although significant progress had been made up to this point on improving in vitro potency and overall physicochemical properties of various ligands, an orally active CNS penetrant mGlu4 PAM had yet to be realized. To that end, East and co-workers reported in 2010 the identification of a modestly potent lead **26** (EC_{50} ~5 μM), found via an HTS campaign and shown in Scheme 6.[60] This compound, with obvious structural similarities to several well-known mGlu5 NAMs/weak mGlu4 PAMs (**27** MPEP, **28** SIB 1893),[61−63] served as the starting point to investigate the potential SAR. In the course of resynthesis, they found that the precursor amine **29** was responsible for the potency at the mGlu4 receptor, with approximately 1 μM affinity. The ensuing SAR optimization afforded minimal improvements to overall affinity, as noted earlier a well precedented result, and the

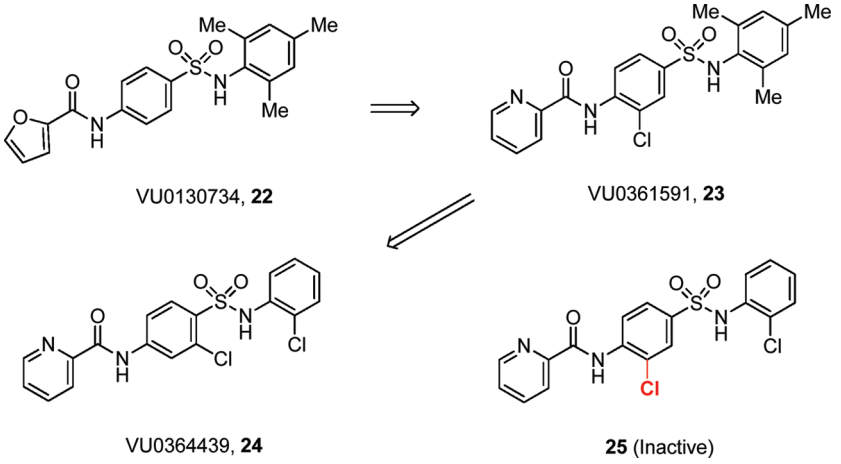
Scheme 4. Evolution of Vanderbilt University Lead



Scheme 6. Discovery of an Orally Active mGlu4 PAM

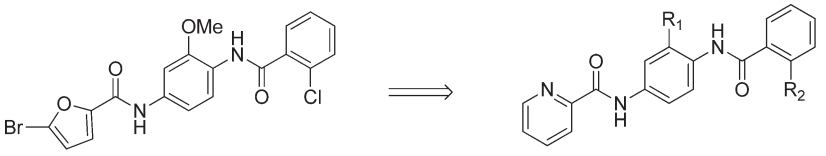


Scheme 5. Optimization of VU0130734



dx.doi.org/10.1021/cn200043e |ACS Chem. Neurosci. 2011, 2, 433–449

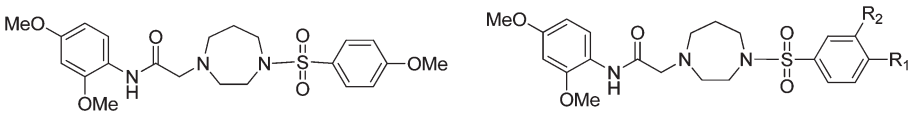
Scheme 7. Additional Vanderbilt mGlu4 PAMs



CID 1314024, **30**

R^1 = OMe, R^2 = Cl; VU0415374, **31**
R^1 = Cl, R^2 = Cl; VU0366037, **32**
R^1 = Cl, R^2 = Me; VU0366038, **33**

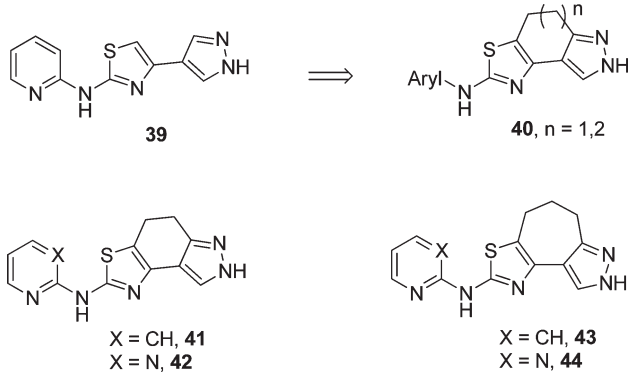
Scheme 8. First mGlu4 PAMs containing a basic moiety



VU0105737 **34**

R^1 = CF_3, R^2 = H (EC_{50} 2.5 μM) **35**
R^1 = H, R^2 = H (EC_{50} 3.5 μM), **36**
R^1 = Me, R^2 = H (EC_{50} 3.3 μM), **37**
R^1 = Me, R^2 =Me (EC_{50} 1.3 μM), **38**

Scheme 9. Tricyclic mGlu4 Ligands



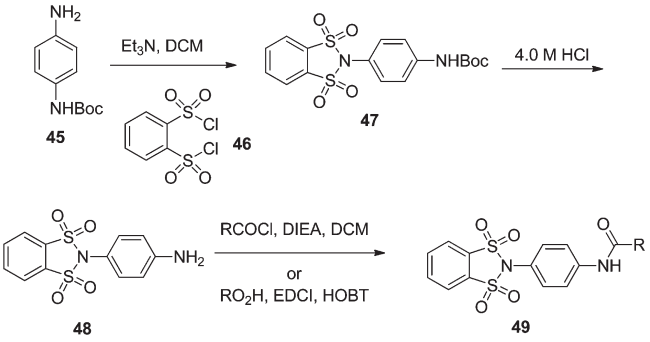
39

40, n = 1,2

X = CH, **41**
X = N, **42**

X = CH, **43**
X = N, **44**

Scheme 10. Synthesis of Novel 1,1,3,3-Tetraoxidobenzo-[*d*][1,3,2]dithiazole Derivatives as mGlu4 PAMs



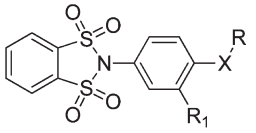
45 **46** **47** **48** **49**

researchers focused further examination on the in vivo properties of derivative **29**. The pharmacokinetic profiling showed **29** to be a high clearance, moderate volume of distribution compound with ~10% unbound in plasma, 51% oral bioavailability, and a B/P ratio of ~3. When dosed orally at 1, 10, and 30 mg/kg in the haloperidol induced catalepsy model, **29** showed a dose dependent reduction in catalepsy at all doses, with an ED_{50} estimated at ~1 mg/kg.

Hopkins, Niswender, and co-workers recently reported the discovery of a series of phenylpicolinamides, represented by **30** (Chempub CID 1314024) (Scheme 7), adding to the growing list of mGlu4 PAMs identified through the Vanderbilt HTS campaign.[64] The commercially available compound was moderately active (EC_{50} 1.4 μM) and bore a noteworthy similarity to the earlier identified lead **22** VU0130734. Utilizing the knowledge gained in the SAR around the earlier sulfonamide (series **22**−**25**), the group modified the furyl group as before to the 2-pyridyl moiety **31** (VU0415374), resulting in a 10-fold affinity improvement and the highest reported fold-shift to date (EC_{50} 100 nM, FS 72). Through SAR optimization, it was noted that the functional potency and fold-shift data did not track and again the optimization was hampered by extremely tight SAR. Derivatives **32** (VU0366037, EC_{50} 517 nM, FS 18.3) and **33** (VU0366038, EC_{50} 958 nM, FS 9.6) were chosen in addition to the methoxy derivative **31** in order to investigate their potential as tool compounds possessing CNS penetrant qualities. Examination of the PK properties of this series showed acceptable in vitro clearance and good plasma and brain exposure (**33**: B/P 1) among a number of examples, when dosed subcutaneously (sc) at 10 mg/kg in aqueous 10% Tween-80. The ability to administer these via sc dosing in aqueous formulations is a clear advantage among many of the previously identified mGlu4 PAM ligands, which required icv dosing, and highlights their contribution to the tools being identified.

As work continued in an effort to expand the structural diversity while improving the physicochemical properties, the Vanderbilt group reported a novel series of mGlu4 PAMs containing for the first time a basic amine and a less than planar

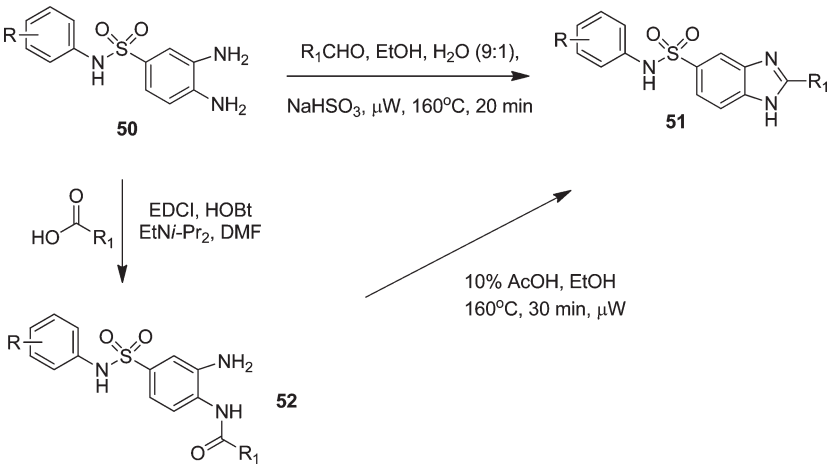
Table 2. Novel 1,1,3,3-Tetraoxidobenzo[*d*][1,3,2]dithiazole Derivatives as mGlu4 PAMs



49a-n

compd	X	R	R_1	hmGlu$_4$ EC_{50} (nM)	GluMax (%)
49a	NHCO	phenyl	H	203	146
49b	NHCO	2-furyl	H	50	284
49c	NHCO	cyclohexyl	H	128	96
49d	NHCO	2-pyridyl	H	38	254
49e	NHCO	6-fluoro-2-pyridyl	H	179	198
49f	NHCO	2-pyrimidine	H	286	247
49g	NHCO	2-thiazol	H	142	216
49h	NHCO	3-fluoro-2-pyridyl	H	50	69
49i	NHCO	3,5-difluoro-2-pyridyl	H	1650	195
49j	NHCO	2-furyl	F	9130	125
49k	NHCO	2-pyridyl	F	739	62
49l	CONH	2-pyridyl	H	113	39
49m	$NHCH_2$	2-pyridyl	H	2140	161
49n	$NHC(CH_2)_2$	2-pyridyl	H	134	165

Scheme 11. Synthesis of Novel Benzoimidazolesulfonamide as mGlu4 PAMs

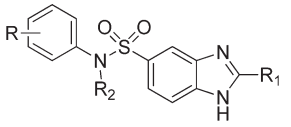


aromatic scaffold.[65] The identification of **34** (VU0105737), shown in Scheme 8, a moderately potent derivative (EC_{50} 7.5 μM), containing a homopiperazinyl group, served as the starting point for the ensuing SAR investigation. Unfortunately, after preparation of a variety of derivatives examining the various regions, the SAR again showed limited scope and depth. There were a number of analogues with affinity improvements on the order of 3−6 fold, **35**−**38**; however, these were far outnumbered by the analogues with significant loss of potency, even with very minor changes to the substitution patterns. Furthermore, it was noted that the pharmacokinetic properties were less than optimal. Nonetheless, the additional chemical diversity contribution to the growing list of mGlu4 ligands serves to expand the possibilities for future novel ligand design.

As the quantity and quality of mGlu4 PAM tools has progressed and the utility in various in vivo assays has shown the potential utility of this target, interest in mGlu4 has risen as is evidenced by the increase in patent application disclosures. The patent work of the past decade will be detailed in the following section; however, the specific potencies and SAR are often difficult, or impossible, to ascertain from these disclosures. Despite the paucity of pertinent data in patent applications, the supply of recent disclosures has afforded a number of starting points to allow for expansion of the chemical space in this area. Doller and co-workers, from Lundbeck Research, have recently reported on a series of compounds with excellent potency and pharmacokinetic properties.[66] Initiated as an exploration of the Addex polyheterocyclic scaffold,[67] the group undertook an investigation of the rational design of alternate novel scaffolds with the aid of molecular modeling. Resynthesis of one of the noted examples **39** (Scheme 9), confirmed the parent compound to be a moderately potent mGlu4 PAM. A subsequent SAR

examination of various substituted analogues of the thiazole and pyrazole rings and an examination of the key low-energy conformers led the group to propose and then synthesize the ring-constrained analogues represented by **40** (n = 1, 2). It must be noted that Addex disclosed structures of this type in a subsequent patent application, although they were unknown at the time this work was undertaken. The 6-membered ring derivatives, when properly outfitted with the 2-pyridyl **41** or 2,6-pyrimidyl ring **42**, showed excellent potency with EC_{50}'s of 220 nM and 65 nM, respectively, at the mGlu4 PAM site. The modeling exercise supported this very well and furthermore suggested the 7-membered congeners to be a more suitable fit.

Table 3. Novel Benzoimidazolesulfonamide as mGlu4 PAMs

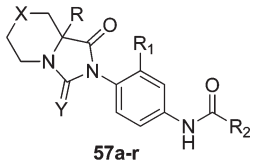


51a-u

compd	R	R_1	R_2	$hmGlu_4$ EC_{50} (nM)	GluMax (%)
51a	2-Br	phenyl	H	499	60
51b	2-Cl	phenyl	H	353	77
51c	2-F	phenyl	H	320	87
51d	2,4-diF	phenyl	H	296	73
51e	2,6-diF	phenyl	H	318	38
51f	2-Cl	2-pyridyl	H	72.3	226
51g	2-F	2-pyridyl	H	186	246
51h	2-Cl	2-pyridyl	Me	3530	166
51i	2-F	2-pyridyl	Me	1110	61
51j	2-Cl	2-thiazol	H	770	194
51k	2-F	2-thiazol	H	756	222
51l	2-Cl	3-thiophenyl	H	361	111
51m	2-F	3-thiophenyl	H	369	42
51n	2-Cl	3-pyridyl	H	5250	84
51o	2-Cl	cyclohexyl	H	1760	141
51p	2-Cl	6-chloro-2-pyridyl	H	116	98
51q	2-Cl	6-fluoro-2-pyridyl	H	111	145
51r	2-Cl	3-fluoro-2-pyridyl	H	747	192
51s	2-Cl	3,5-difluoro-2-pyridyl	H	473	123
51t	2-Cl	5-methyl-2-furanyl	H	76	52
51u	2-Cl	5-chloro-2-furanyl	H	199	76

To that end, these analogues were prepared and demonstrated improved potencies of 9 and 7 nM, respectively, for the corresponding derivatives **43** and **44**. The SAR of the pyridyl/pyrimidyl moiety was then expanded, but with unrealized gain, not unlike all previous studies described. In vitro pharmacokinetic studies showed the pyridyl derivatives (**41** and **43**) to be far superior to the pyrimidyl examples (**42** and **44**), and these were chosen for further profiling. Functional studies showed the pyridyl derivatives to be selective (>10 μM) versus the mGlu1, 2, 3, 5, and 7 receptors. In addition, in vivo pharmacokinetic studies in Sprague−Dawley rats showed them to be orally bioavailable, with B/P of 0.6−0.8 (1 h time point) when dosed at 10 mg/kg. The potential utility of these compounds continues to be explored, and results in efficacy models will be reported in due course.

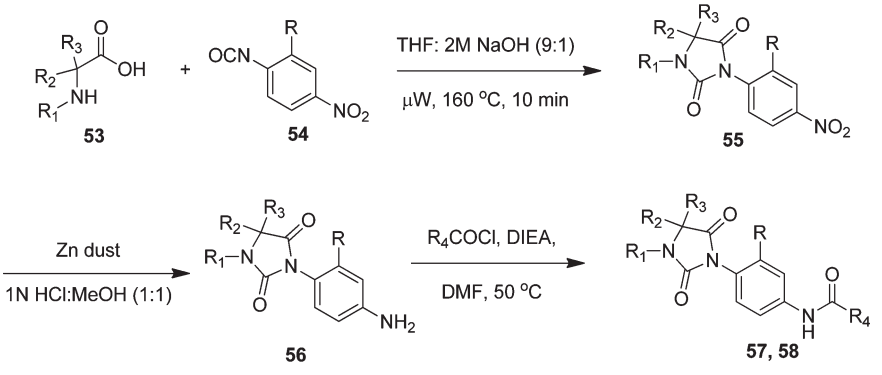
Table 4. Novel 2,5-Dioxoimidazolidin-1-yl)phenyl)-acetamide Derived mGlu4 PAMs



57a-r

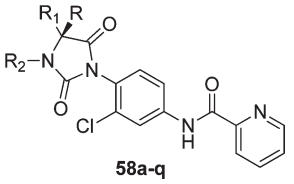
compd	X	Y	R	R_1	R_2	$hmGlu_4$ EC_{50} (nM)
57a	CH_2	O	H	OMe	2-pyridyl	2700
57b	CH_2	O	H	H	2-pyridyl	3120
57c	CH_2	O	H	Cl	2-pyridyl	814
57d	CH_2	S	H	Cl	2-pyridyl	367
57e	CH_2	O	*S*-H	Cl	2-pyridyl	393
57f	CH_2	O	*R*-H	Cl	2-pyridyl	1820
57g	CH_2	O	H	Cl	6-F-2-pyridyl	1670
57h	CH_2	O	H	Cl	4-thiazole	1980
57i	CH_2	O	H	Cl	4-pyrimidine	2580
57j	CH_2	O	H	Cl	3-F-2-pyridyl	3310
57k	O	O	H	Cl	2-pyridyl	2180
57l	O	S	H	Cl	2-pyridyl	1470
57m	S	O	H	Cl	2-pyridyl	403
57n	SO_2	O	H	Cl	2-pyridyl	3030
57o	NH	O	H	Cl	2-pyridyl	4200
57p	NMe	O	H	Cl	2-pyridyl	Inactive
57q	CH_2	O	Me	Cl	2-pyridyl	434
57r	CH_2	S	Me	Cl	2-pyridyl	185

Scheme 12. Synthesis of 2,5-Dioxoimidazolidin-1-yl)phenyl)acetamides as an mGlu4 PAM



dx.doi.org/10.1021/cn200043e |*ACS Chem. Neurosci.* 2011, 2, 433–449

Table 5. Novel 2,5-Dioxoimidazolidin-1-yl)phenyl)-acetamide Derived mGlu4 PAMs



58a-q

compd	R	R_1	R_2	$hmGlu_4$ EC_{50} (nM)
58a	H	H	Me	7060
58b	Me	Me	H	4210
58c	$(CH_2)_3$		H	1280
58d	$(CH_2)_4$		H	795
58e	$(CH_2)_5$		H	229
58f	$(CH_2)_6$		H	222
58g	$(CH_2)_5$		Me	178
58h	bicyclo[2.2.1]heptane		H	217
58i	bicyclo[2.2.1]heptane		Me	325
58j	phenyl	H	H	380
58k	$C(CH_3)_3$	H	H	200
58l	$C(CH_3)_3$	H	$CH(CH_3)_2$	200
58m	benzyl	Me	H	604
58n	benzyl	Me	Me	197
58o	benzyl	Me	Ethyl	236
58p	4-F-benzyl	Me	H	335
58q	4-F-benzyl	Me	Me	217

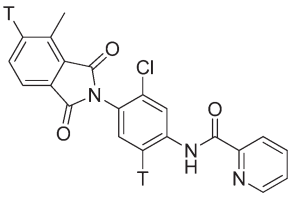


Figure 3. Radiolabeled phthalimide derivative as mGlu4 PAMs.

It is apparent from the myriad of past reports, and these new revelations, that the SAR has been disappointedly narrow in scope as well as depth for all series investigated. This theme is unfortunately one that has been noted in the investigation of positive allosteric modulation for other GPCR receptors as well, and has hampered the progress in several disease areas.[68,69] A greater understanding of the structural nature of Class C GPCRs would undoubtedly be a benefit to this as well as other areas. Although, to date, there is not a single report of a crystal structure for a Class C GPCR; there are several groups in academia and industry focused on this important objective.[70−72] It can only be surmised that the future of the design of allosteric modulators for this class of GPCRs will benefit from the realization of these powerful structure-based tools.

4. RECENT PATENT DISCLOSURES OF MGLU4 RECEPTOR LIGANDS

The following section highlights the recent patent activity in the $mGlu_4$ PAM area. In the period 2009−2011, there have been

Scheme 13. Synthesis of Novel Phthalimide Derivatives as mGlu4 PAMs

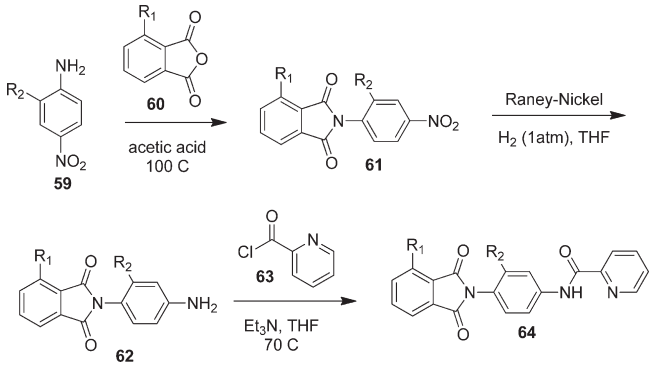
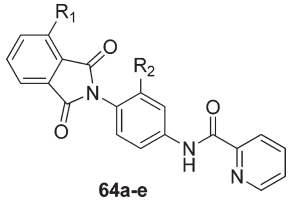


Table 6. Novel Phthalimide Derivatives as mGlu4 PAMs



64a-e

compd	R_1	R_2	$hmGlu_4$ EC_{50} (nM)
64a	H	H	712
64b	H	Me	1724
64c	F	Cl	36
64d	Me	Cl	11
64e	Me	Br	12

a total of 10 patent applications published from three separate groups: Vanderbilt University, Merck Research Laboratories, and Addex Pharmaceuticals. In 2007, Merck & Co., Inc. and Addex Pharmaceuticals had reportedly partnered to develop novel PAMs targeting $mGlu_4$ for the treatment of PD and potentially other undisclosed indications.[73]

4.1. Vanderbilt University Patent Disclosures. *4.1a. 1,1,3,3-Tetraoxidobenzo[d][1,3,2]dithiazaoles.* In 2010, the Vanderbilt University team published a patent application (WO2010/088406) pertaining to highly polar 1,1,3,3-tetraoxidobenzo[*d*][1,3,2]dithiazaoles (Scheme 10).[74] The application exemplified 18 compounds, 14 of which are shown in Table 2. The synthesis began with the bis-sulfonamide formation from 1,2-benzenedisulfonyl dichloride, **46**, and *N*-Boc-*p*-phenylenediamine, **45**. After deprotection of the Boc group in compound **47** under acidic conditions to give **48**, the appropriate amide was obtained from either the acid chloride or the carboxylic acid. Compounds **49a**−**n** were synthesized in a similar fashion.

The SAR for the 1,1,3,3-tetraoxidobenzo[*d*][1,3,2]dithiazole derivatives is shown in Table 2. The initial SAR analysis involved modification of the amide portion (X−R) with aryl, heteroaryl, and cycloalkyl groups. The most potent compounds in this series involved heteroaryl amide substituents (2-pyridyl, **49d**, 38 nM, 254% GluMax; 2-furyl, **49b**, 50 nM, 284% GluMax). Substituted heteroaryl groups are also well tolerated (3-fluoro-2-pyridyl, **49h**, 50 nM, 69% GluMax) although with diminished GluMax

dx.doi.org/10.1021/cn200043e |*ACS Chem. Neurosci.* 2011, 2, 433–449

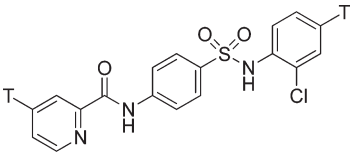
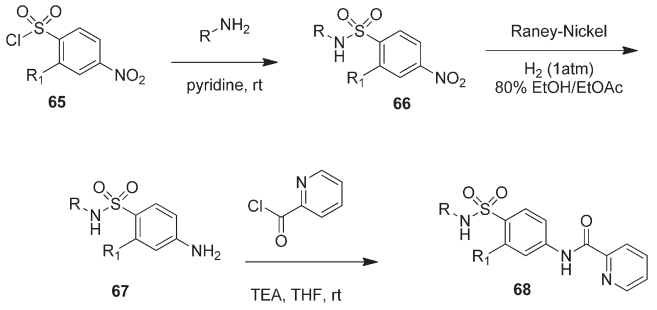


Figure 4. Radiolabeled sulfonamide derivative as an mGlu4 PAM.

Scheme 14. Synthesis of Novel Sulfonamide Derivatives as mGlu4 PAMs



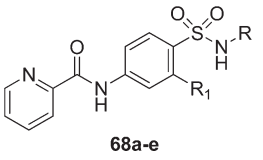
response. Reversal of the amide (**49l**) or removal (**49m** and **49n**) led to compounds of reduced potency.

In addition to activity at the mGlu4 receptor, the Vanderbilt application also shows in vivo PK parameters for two of the compounds after ip administration (**49b** and **49c**). The data shows a brain/plasma ratio of ~0.6−1 for both compounds with concentrations well above the EC_{50}. Although no further in vivo data is reported, the PK data suggests these compounds may show efficacy in preclinical animal models or PD.

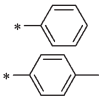
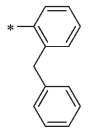
4.1b. Benzoimidazolesulfonamide and Indolesulfonamides. A patent application from Vanderbilt University was recently published (WO2011/011722) describing various benzoimidazolesulfonamides and indolesulfonamides.[75] The application contains over 53 exemplified compounds with EC_{50} and GluMax (%) provided. The series were prepared by one of three synthetic routes shown in Scheme 11. First, standard coupling of commercially available 2-phenyl-1*H*-benzo[*d*]imidazole-5-sulfonyl chloride with various anilines provided sulfonamides **51** in high yield (not shown). An alternative route involved the formation of the benzimidazole in a one pot procedure from 3,4-diamino-*N*-(2-chlorophenyl)benzenesulfonamide and pyridine carboxaldehyde under oxidative conditions affording desired product **51**. The third synthetic route, a stepwise approach also shown in Scheme 11, consisted of an amide coupling of the previously mentioned diamine, followed by dehydration under microwave conditions to provide benzoimidazolesulfonamide **51**.

A number of benzimidazole compounds are exemplified in the patent and a brief SAR analysis is provided in Table 3. The Vanderbilt group assayed halogen substituents for the R group on the aryl sulfonamide with all groups (F, Cl, or Br) being of modest potency (300−500 nM) and modest GluMax (60−87%). Moving to the eastern portion (2-benzimidazole substituent), the group utilized aryl and heteroaryl groups at R_1. Moving from the phenyl (**51b**) to 2-pyridyl (**51f**), there was a 5-fold increase in potency (353 nM, **51b** vs 72 nM, **51f**) and a significant increase in GluMax (77% vs 226%). Other heteroaryl groups were evaluated; however, they were much less potent than the 2-pyridyl group (2-thiazol, **51j**, 770 nM; 3-thiophene,

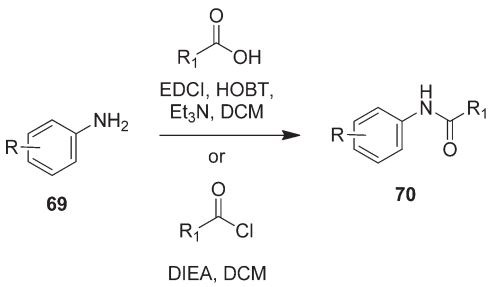
Table 7. Novel Sulfonamide Derivatives as $mGlu_4$ PAMs



68a-e

compd	R	R_1	$hmGlu_4$ EC_{50} (nM)
68a		H	33
68b		OMe	6076
68c		H	1104
68d		H	141
68e		H	182

Scheme 15. Synthesis of Novel Amide Derivatives as mGlu4 PAMs



51l, 361 nM; 3-pyridyl, **51n**, 5250 nM). However, substituted furan derivative (5-methyl-2-furanyl, **51t**, 76 nM) was observed to be of equal potency with the 2-pyridyl derivative.

4.1c. Imidazolidine-2,4-dione Scaffold. In 2011, a patent application (WO2011/029104) pertaining to imidazolidine-2,4-dione from the Vanderbilt group was published.[76] The application exemplified 100 compounds with full experimental procedures for 47 specifically exemplified compounds. A representative synthesis, shown in Scheme 12, began with the cyclization of an appropriate amino acid, **53**, and 2-substituted-4-nitrophenylisocyanate, **54**, under basic conditions to afford the desired dione **55**. The reduction of the nitro group with activated zinc provided aniline **56** which is then coupled with an acid chloride to provide the desired imidazolidine-2,4-dione, **57** (tetrahydroimidazo[1,5-*a*]pyridine-1,3(2*H*,5*H*)-dione) or **58** (imidazolidine-2,4-dione).

As mentioned, a number of 2,5-dioxoimidazolidin-1-yl)phenyl)acetamides compounds are exemplified in the patent application, and a brief SAR analysis is provided in Tables 4 and 5. The first set of compounds contains a [4.3.0]bicyclo ring system (Table 4), and a variety of carbocycles and heterocycles are presented. As with the previous compounds, the 2-pyridyl amide

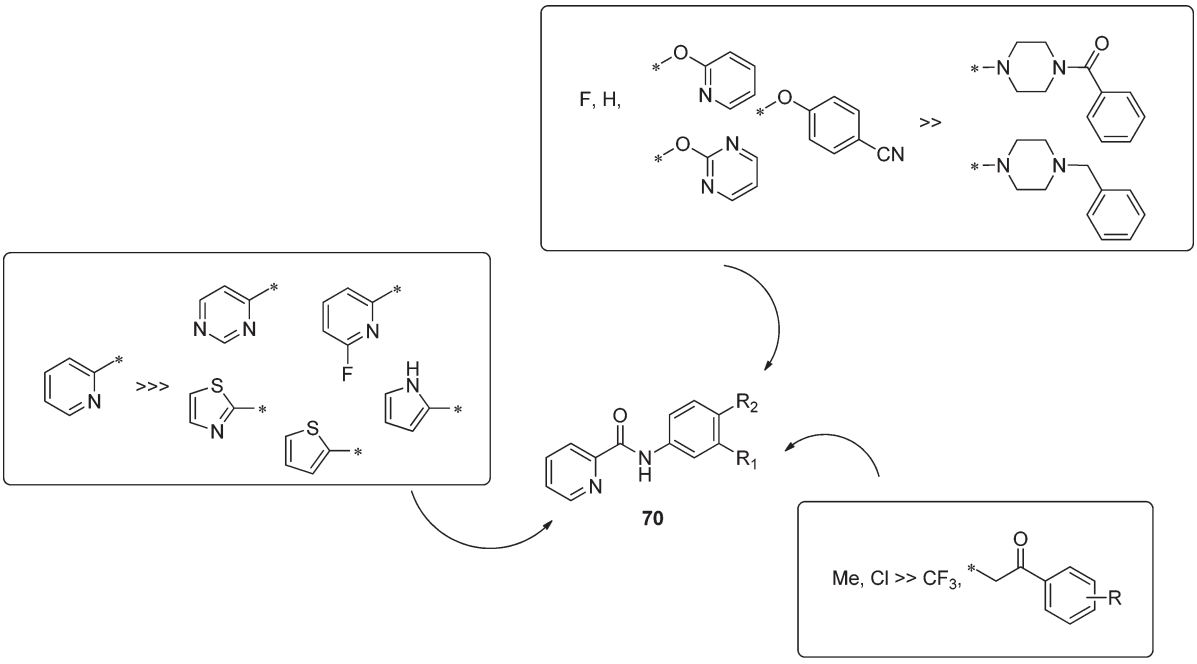
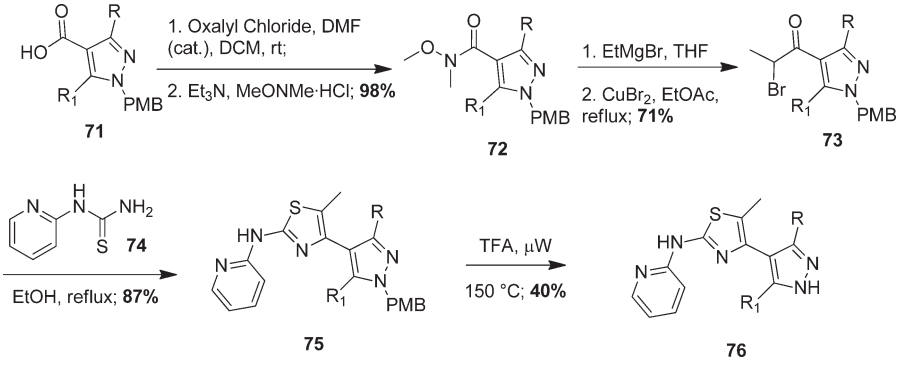


Figure 5. SAR overview of heterobiarylamides mGlu4 PAMs.

Scheme 16. Synthesis of Novel Heteroaromtic Derivatives as mGlu4 PAMs



(R_2) displays the best potency. The Vanderbilt team investigated a number of substituents at the R_1 group (methoxy, hydrogen, chlorine) and the Cl substitution reportedly provided the most potent compound (EC_{50} = 814 nM). Moving to the 2,5-dioxoimidazolidine moiety, a thiocarbonyl substitution led to more potent compounds (**57d**, 367 nM vs **57c**, 814 nM; **57l**, 1470 nM vs **57k**, 2180 nM; **57r**, 185 nM vs **57q**, 434 nM). Methyl substitution at R also led to a 2-fold increase in potency (**57q**, 434 nM vs **57c**, 814 nM). The compound also displayed an enantiopreference as the (*S*)-enantiomer was 5-fold more potent than the (*R*)-enantiomer (**57e**, 393 nM vs **57f**, 1820 nM).

The non-bicyclo compounds are summarized in Table 5. It can be easily seen that large groups led to increased activity at the $mGlu_4$ receptor. The unsubstituted (R,R_1) was weakly active (**58a**, 7060 nM) compared to the *gem*-dimethyl compound (**58b**, 4210 nM), and increasing ring size led to sub-micromolar compounds with the cyclohexyl (**58e**, 229 nM) and cycloheptyl (**58f**, 222 nM) being the most potent. *N*-Methylation was tolerated as well (**58g**, 178 nM). Bulkier groups did not lead to an increase in potency compared to the cycloalkyl derivatives (cyclohexyl, **58e**, 229 nM vs bicyclo[2.2.1]heptane, **58h**, 217 nM). Noncyclized substitutions were also well tolerated with the *tert*-butyl (**58k**, 200 nM), benzyl (**58m**, 604 nM), and 4-fluorobenzyl (**58p**, 335 nM) groups all showing nanomolar activity. In addition to *N*-methylation being tolerated, larger groups were also accommodated (ethyl, **58o**, 236 nM; isopropyl, **58l**, 200 nM).

4.2. Recent Merck Research Laboratories Patent Disclosures. *4.2a. Phthalimide Derivatives.* In 2010, a patent application from Merck Research Laboratories (WO2010/033349) was published describing a series of phthalimide derivatives.[77] The application exemplified 43 compounds including radiolabeled $mGlu_4$ phthalimide that provides a valuable option for ligand binding site displacement studies (Figure 3). A representative synthesis, shown in Scheme 13, was achieved by heating phthalic anhydride, **60**, with 4-nitroaniline, **59**, in acetic acid to afford **61**. In the presence of Raney-Nickel and a hydrogen atmosphere, the nitro group in **61** was reduced to the aniline, **62**, and the

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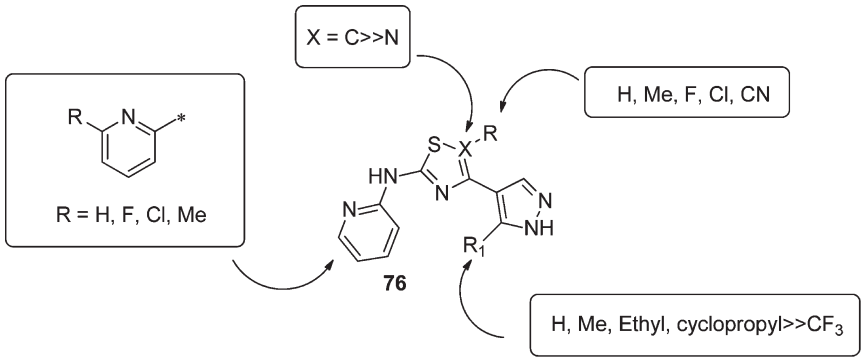
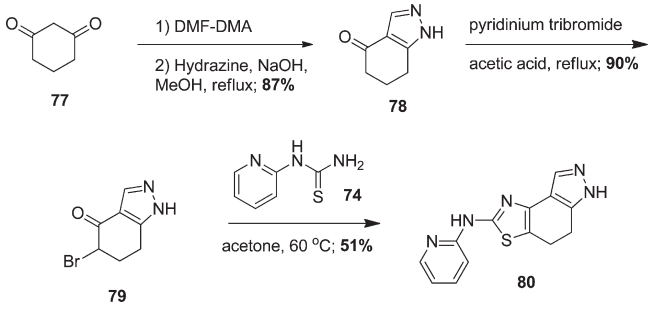


Figure 6. SAR overview of Addex's 2-aminothiazole mGlu4 PAMs.

Scheme 17. Synthesis of Novel Heterotricyclic Derivatives as mGlu4 PAMs



picolinamide **64** was formed under acid chloride coupling conditions.

Of the 43 exemplified compounds, only 5 compounds had published potencies (EC_{50}), shown in Table 6. While it is difficult to formulate an SAR perspective from the limited data set, a combination of substitution, specifically chlorine and bromine, on the internal aromatic ring (R_2 position) and substitution on the phthalimide (R_1 position), appeared to improve potency.

4.2b. Sulfonamide Derivatives. In 2010, a patent application from Merck Research Laboratories (WO2010/033350) was published describing a series of sulfonamide derivatives.[78] The application exemplified 50 compounds including radiolabeled $mGlu_4$ sulfonamide that provides a valuable option for ligand binding site displacement studies (Figure 4). The synthesis of compound **68**, shown in Scheme 14, was achieved by coupling the aniline with the substituted 4-nitrophenyl sulfonyl chloride, **65**. After the nitro group reduction with Raney-Nickel provided the desired aniline **67**, standard acid chloride coupling with pyridine-2-carbonyl chloride afforded picolinamide **68**. Other exemplified compounds were synthesized following this procedure.

Again, much like Merck's phthalimide patent, very limited SAR data was presented. The Merck group only reports 2-picolinamide amides while varying the internal phenyl ring, and the sulfonamide portion of the molecule (Table 7). The internal phenyl substituents (R_1) reveal that hydrogen is more potent than OMe, although a clear SAR picture is difficult to ascertain with these limited examples.

4.3. Recent Addex Pharmaceuticals Patent Disclosures. *4.3a. Amide Derivatives.* In 2009, a patent application from Addex Pharmaceuticals (WO2009/010454) was published describing a series of amide derivatives.[79] The application exemplified 44 compounds with full experimental procedures for 8 compounds. A general synthesis of compounds of type **70** is shown in Scheme 15 utilizing standard amide formation conditions.

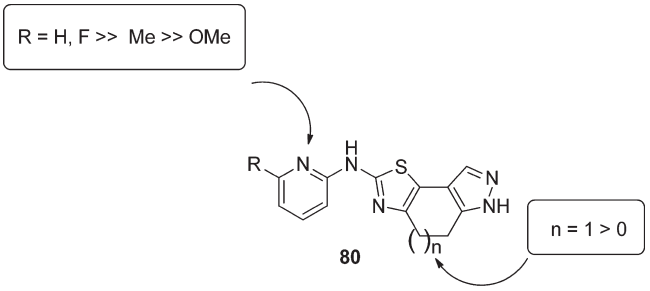
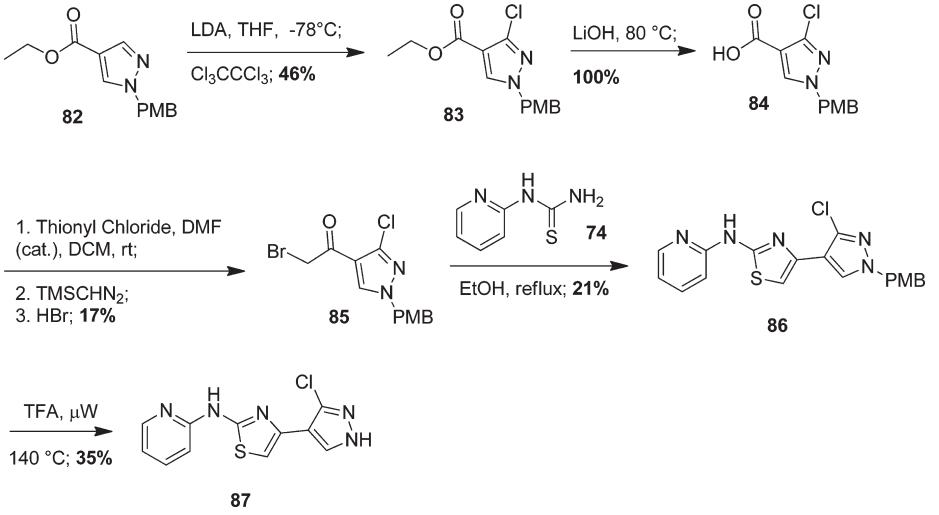


Figure 7. SAR overview of Addex's tricyclic mGlu4 PAMs.

The Addex patent does not reveal any EC_{50} potency values, but rather a range of activity. Thus, a true SAR evaluation cannot be performed (potency ranges are $EC_{50} < 1\ \mu M$ and $1\ \mu M < EC_{50} < 10\ \mu M$); however, general trends can be determined from this application (Figure 5). Much like the Vanderbilt and Merck applications, the Addex group has shown that 2-picolinamides ($<1\ \mu M$) are more potent than other heterocyclic amide compounds ($>1\ \mu M$). Small substituents in the 3-position of the phenyl group (R_1) are more active than larger groups. The 4-position of the phenyl group (R_2) is much more tolerant of substitution as aryl ethers are as active as halogen substituents.

4.3b. Heteroaromatic Derivatives. In 2009, a patent application from Addex Pharmaceuticals was published (WO2009/010455) describing a series of heteroaromatic derivatives.[67,80] The application exemplified 63 compounds with full experimental procedures for 19 compounds. It appears from the details that an attempt to expand the SAR of this heteroaromatic scaffold required a tremendous synthetic effort utilizing a total of 13 different synthetic routes. A representative synthesis is shown in Scheme 16. The protected 4-pyrazole carboxylic acid, **71**, was treated with oxalyl chloride and catalytic DMF followed by the addition of *N,N*-dimethylhydroxylamine hydrochloride to provide the desired amide, **72**, in good yield. Addition of ethyl magnesium bromide to Weinreb amide **72** afforded ethyl ketone, which upon treatment with $CuBr_2$ under refluxing conditions gave α-bromo ketone **73**. The combination of pyridinyl thiourea, **74**, and α-bromo ketone **73** in refluxing ethanol afforded thiazole **75** in good yield. Subsequent deprotection of the para-methoxy

Scheme 18. Synthesis of Novel 2-Aminothiazole Derivatives as mGlu4 PAMs



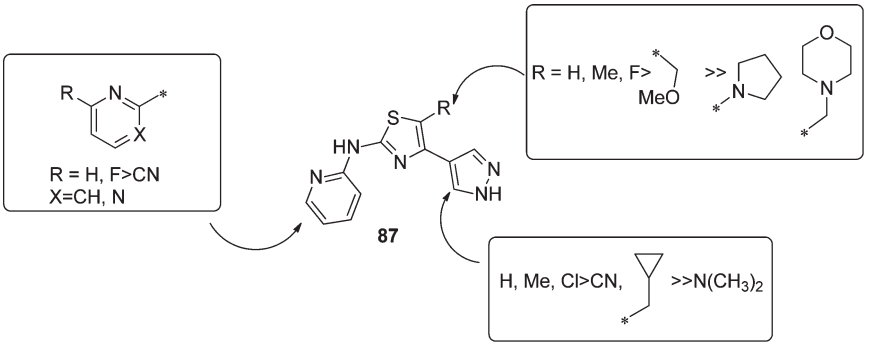
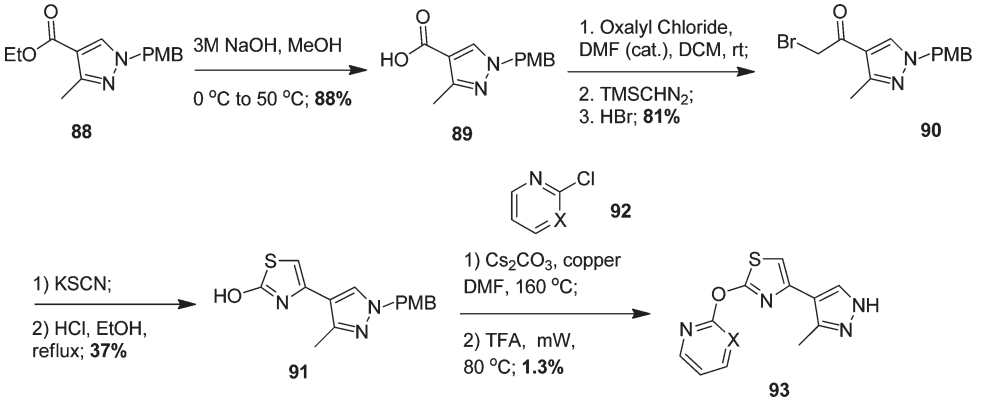


Figure 8. SAR overview of Addex's 2-aminothiazole mGlu4 PAMs.

Scheme 19. Synthesis of Novel Heteroaromatic Ether Derivatives as mGlu4 PAMs



benzyl group under acidic conditions gave the desired heteroaromatic compound **76**.

Again, much like the previous patent application from Addex Pharmaceuticals, no EC_{50} values are given; just ranges of potencies. However, in contrast to the previous amide patent application, this new application shows more potent compounds with potency ranges (EC_{50} < 500 nM; 500 nM < EC_{50} < 1 μM) portrayed in Figure 6. A number of compounds showed potency of <500 nM with substituted pyridines being the desired left-hand group. The Addex group looked at thiazole and thiadiazole internal ring moieties (X = C, N) with the substituted thiazole (R ≠ H) as the desired group. Lastly, substituted NH-pyrazole groups were the only right-hand groups evaluated with a number of R_1 groups being tolerated.

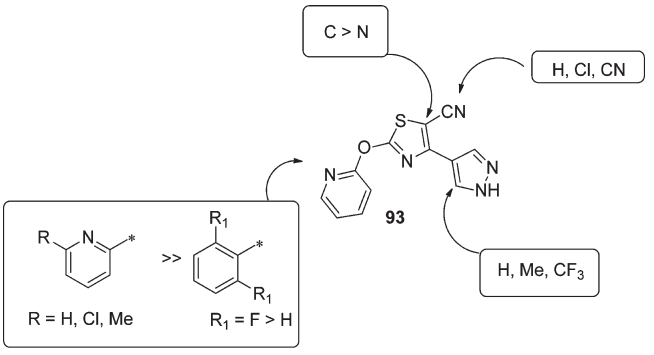


Figure 9. SAR overview of 2-oxythiazole mGlu4 PAMs.

4.3c. Heterotricyclic Derivatives. In 2010, a patent application from Addex Pharmaceuticals (WO2010/079238) was published describing a series of heterotricyclic derivatives.[81] These compounds are very similar to the work described earlier by the Lundbeck group (vide supra). The application exemplified 34 compounds with full experimental procedures for 6 compounds. This series was an extension of earlier work (WO2009/010455) by cyclizing the thiazole and pyrazole ring systems. A representative synthesis, shown in Scheme 17, begins with the condensation of 1,3-cyclohexadione, **77**, and DMF·DMA followed by addition of hydrazine under basic conditions to afford the desired pyrrazole **78** in excellent yield. After bromination with pyridinium tribromide in refluxing acetic acid, the α-bromo ketone **79** was subjected to pyridinyl thiourea, **74**, to provide the desired heterotricyclic adduct **80** in good yield.

By cyclizing the thiazole and pyrazole moieties into a tricyclic system, the Addex group reports compounds of type **80** with EC_{50}'s less than 100 nM (their most potent compounds to date), portrayed in Figure 7. Very limited examples were presented, with again the 2-pyridyl substituents being the most potent. In addition, both 5- and 6-membered fused ring systems showed potency of <100 nM.

4.3d. 2-Aminothiazole Derivatives. In 2010, a patent application from Addex Pharmaceuticals (WO 2010/079239) was published thoroughly describing a series of thiazole derivatives similar to their 2009 patent application.[82] The application exemplified 83 compounds with full experimental procedures for 25 compounds. A representative synthesis is shown in Scheme 18 which shows significant similarity to a previously described synthetic route (Scheme 16). The protected ethyl 4-pyrazole carboxylate, **82**, was deprotonated with LDA solution at −78 °C, and subsequent addition of hexachloroethane resulted in the chloropyrazole **83**. After saponification of the ethyl ester, the acid **84** was treated with thionyl chloride affording the acid chloride. Trimethylsilyldiazomethane addition to the acid chloride followed by HBr addition provided the α-bromoketone **85**. Compound **85** was refluxed with pyridyl thiourea, **74**, to afford the PMB protected amino thiazole **86** in 21% yield. Subsequent deprotection with TFA under microwave conditions provided the desired compound, **87**.

The SAR described in this patent application is nearly identical to those previously exemplified; however, the Addex groups converge on the thiazole as the central aryl group (in preference to the thiadiazole), portrayed by compounds of type **87** in Figure 8. In addition, the Addex group further exemplifies numerous more examples than previously disclosed

4.3e. Heteroaromatic Ether Derivatives. In 2011, a patent application from Addex was published describing a series of heteroaromatic ether derivatives.[83] The application exemplified 21 compounds with full experimental procedures for 7 compounds. This is the first disclosure of aromatic and heteroaromatic ethers providing excellent potency in Addex's thiazole/pyrazole scaffold. A representative synthesis, shown in Scheme 19, begins with saponification of the ethyl ester, **88**, with NaOH and MeOH. The resulting acid, **89**, was converted to the α-bromoketone, **90**, following a three-step protocol in 81% overall yield. The α-bromoketone, **90**, is converted to the 2-hydroxythiazole, **91**, via reaction with KSCN followed by HCl in EtOH at reflux (37% yield). Finally, the desired ether compounds **93** were synthesized after S_NAr displacement of the heteroaryl chloride followed by PMB deprotection.

As with the previous patent application (WO2009/010455), pyridyl derivatives were the most potent examples (<100 nM), with substituted thiazoles and NH-pyrazole moieties representing preferred compounds. The general substitution is portrayed in Figure 9.

With the exception of the first patent application from Addex Pharmaceuticals (WO2009/010454), all of their subsequent patents have centered around a common thiazole central aryl ring system. The thiazole system has been adorned with either a pyridyl or pyrimidyl amine or ether in the 2-position of the thiazole with a substituted NH-pyrazole in the 4-position. As the patents from Addex have converged to this common structural class, it will remain to be seen if future publications in the public domain will be forthcoming describing perhaps the full SAR and in vivo efficacy details.

5. SUMMARY

There has been significant interest in the area of the metabotropic glutamate receptors in the past decade. The therapeutic potential of the various receptors holds great promise for several areas, in particular the neuroscience field. The mGlu4 subtype receptor has shown enormous potential as a target for the treatment of PD with several groups building the foundations of a strong preclinical case. The search for selective ligands has led to several key advances, notably in allosteric modulation, and reports of detailed SAR as well as an increase in patent applications are beginning to highlight the interest. Although the discovery of a greater set of diverse ligands has enabled an expanded understanding of the receptor pharmacology and efficacy, further medicinal chemistry optimization will be needed to fully enable the potential of mGlu4 therapeutics.

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Author Contributions

Albert Robichaud, Corey Hopkins, Craig Lindsley and Darren Engers each contributed to this manuscript by writing segments, creating schemes and tables, editing the document and preparing the final forms. Albert Robichaud and Corey Hopkins were the key contacts at Lundbeck and Vanderbilt University, respectively, and had the main responsibility of the structure, content and flow of the manuscript.

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