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Synthesis, SAR and Unanticipated Pharmacological Profiles of Analogues of the mGluR5 Ago-potentiator ADX-47273

Darren W. Engers^{a,†}, Alice L. Rodriguez^{a,†}, Richard Williams^a, Alexis A. Hammond^b, Daryl Venable^a, Oluwatomi Oluwatola^c, Gary A. Sulikowski^c, P. Jeffrey Conn^a, and Craig W. Lindsley^{a,c}

Craig W. Lindsley: craig.lindsley@vanderbilt.edu

^a Department of Pharmacology, Vanderbilt Program in Drug Discovery, Vanderbilt University Medical Center, MRBIV (Langford)-12415D, Nashville, TN 37232-6600 (USA), Fax: (+1) 615-343-6532

^b Program in Neuroscience, Meharry Medical College, 1005 Dr. D.B. Todd, Jr. Blvd., Nashville, TN 37028 (USA)

^c Department of Chemistry, Vanderbilt University, Nashville, TN 37232 (USA)

Abstract

An iterative analogue library synthesis strategy rapidly developed comprehensive SAR for the mGluR5 ago-potentiator ADX-47273. This effort identified key substituents in the 3-position of oxadiazole that engendered either mGluR5 ago-potential or pure mGluR5 positive allosteric modulation. The mGluR5 positive allosteric modulators identified possessed the largest fold shifts (up to 27.9-fold) of the glutamate CRC reported to date as well as providing improved physicochemical properties.

Keywords

mGluR5; ago-potentiator; positive allosteric modulator (PAM); negative allosteric modulator (NAM); schizophrenia; glutamate

Correspondence to: Craig W. Lindsley, craig.lindsley@vanderbilt.edu.

[†]These authors contributed equally to this work.

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With an onset in late adolescence, schizophrenia, a complex psychiatric disorder characterized by a combination of negative (social withdrawal, blunting of emotional responses, anhedonia) and positive (hallucinations, delusions, paranoia, disorganized behavior) symptoms along with significant cognitive dysfunction is a debilitating disease that requires lifelong, daily maintenance therapy at a cost to society of \$65 billion a year.[1] The prevailing dogma by which schizophrenia has been managed for decades states that excessive dopaminergic transmission in the forebrain underlies the disease – the so-called “dopamine hypothesis” or “dopamine hyperfunction hypothesis”. [2] The rationale for this hypothesis is based on the fact that all clinically relevant antipsychotic agents, both typical (haloperidol) and atypical (clozapine, olanzapine), possess significant antagonist activity at the dopamine D2 receptor; however, these agents have a slow onset of action and mainly treat the positive symptoms of schizophrenia, with limited to no effect on the negative and cognitive symptoms, thereby representing a substantial unmet medical need.[3] Moreover, all of these agents bind to a number of neurotransmitter receptors such as dopamine (D1–D4), serotonin (5-HT1A, 1D, 2A, 2C, 6 and 7), adrenergic (α 1, α 2), histamine (H1) and muscarinic (M1); therefore, the observed efficacy can more accurately be ascribed to polypharmacology.[4–10]

The *N*-methyl-D-aspartate (NMDA) receptor antagonist phencyclidine (PCP) has been shown to induce the positive, negative and cognitive symptoms of schizophrenia in healthy patients and elicit a resurgence of symptoms in stable schizophrenics.[10] In the clinic, the observation that administration of the NMDA receptor co-agonist glycine provides a modest improvement in schizophrenic patients suggests that increasing NMDA receptor activation may provide a therapeutic benefit.[11] These observations led to the NMDA receptor hypofunction hypothesis as an alternative theory for the underlying cause of schizophrenia.[12] According to this hypothesis, any agent that can potentiate NMDA receptor currents, either directly by action on modulatory sites on the NMDA receptor (i.e., the glycine co-agonist binding site) or indirectly by activation of GPCRs known to potentiate NMDA receptor function (i.e., mGluR5), has the potential to ameliorate the symptoms of schizophrenia.[13]

Glutamate is the major excitatory transmitter in the central nervous system, exerting its effects through either ionotropic or metabotropic glutamate receptors. The metabotropic glutamate receptors (mGluRs) are members of the GPCR family C, characterized by a large extracellular amino-terminal binding domain. To date, eight mGluRs have been cloned, sequenced and assigned to three groups (Group I: mGluR1 and mGluR5; Group II: mGluR2 and mGluR3; Group III: mGluRs 4,6,7,8) based on their structure, coupling to effector mechanisms and pharmacology.[14] Achieving mGluR subtype selectivity has been difficult with orthosteric agonists, which are typically analogs of glutamate, quisqualate or phenyl glycine. Recently, our laboratories have reported on the discovery of positive allosteric modulators (PAMs) of mGluR5, compounds which alone have no effect on mGluR5 function, but potentiate mGluR5 receptor response in the presence of sub-threshold levels of the native agonist glutamate. We have also identified compounds that display allosteric agonist activity at higher concentrations and are more accurately termed mGluR5 ago-potentiators. By virtue of binding at an allosteric site on the receptor, these ligands afford high mGluR5 sub-type selectivity (>100-fold selectivity versus mGluR1,2,3,4,7,8).[15] We have identified (Figure 1) three chemotypes of mGluR5 PAMs, represented by DFB (**1**),[16] CPPHA (**2**),[17] and the MPEP-like **3**. [18] In addition, we discovered the ago-potentiator CDPPB (**4**).[19] Interestingly, **1**, **3**, and **4** have been shown to bind at the MPEP binding site, while **2** binds at a yet undefined second allosteric site on mGluR5.[16–19] CDPPB (**4**) was the first centrally active mGluR5 PAM/ago-potentiator, which allowed us to validate *in vivo* that allosteric activation of mGluR5 possessed an antipsychotic profile in rat behavioral models.[19] However, CDPPB (**4**) displayed poor solubility in most vehicles, limiting its utility for further *in vivo* studies, and a disparity between binding and functional potency. Moreover, due to the intrinsic allosteric agonist activity, though weak, we were unable to validate pure mGluR5 potentiation as the mechanism for the

in vivo activity. Lead optimization efforts of the CDPPB (**4**) scaffold were unable to address these issues.[19,20]

In 2005, Addex disclosed the structurally distinct ago-potentiator ADX-47273 (**5**) and subsequently produced patents.[21,22] Researchers at both Addex[21,22] and Wyeth[23] have recently reported on the *in vivo* efficacy of ADX-47273 (**5**) in a number of preclinical antipsychotic and cognition models, further validating selective mGluR5 activation as a potential new mechanism to address the complex symptom clusters (positive, negative and cognitive symptoms) of schizophrenia. However, ADX-47273 (Figure 2) is a more potent ago-potentiator (mGluR5 PAM EC₅₀ = 168 nM, 107% Glu Max, 9-fold shift @ 1 μM; Allosteric agonist EC₅₀ = 9.5 μM, 65% Glu Max) than CDPPB (**4**), but still suffers from poor physiochemical properties due to a lack of solubilizing moieties.[18–22]

In order to further validate potentiation of mGluR5 as a therapeutic approach for the treatment of the positive, negative and cognitive symptom clusters of schizophrenia and advance the science concerning mGluR5, pure mGluR5 PAMs and mGluR5 ago-potentiators with improved pharmacological and physiochemical properties are required. Little has been disclosed concerning the SAR and pharmacological profiles of ADX-47273 and its analogues, [21,22] so our lab initiated a campaign to explore the ADX47273 scaffold in an effort to improve the physiochemical properties of ADX-47273, determine if a pure PAM could be identified in this series and address three questions: 1) are alternative aryl/heteroaryl rings tolerated at the 3-position of the oxadiazole, 2) are alternative amides tolerated and 3) is the (*S*) stereochemistry required for mGluR5 PAM activity (Figure 3)?

We elected to pursue a focused library approach to explore the SAR of ADX-47273. First, we prepared a small library of analogues **6** evaluating known phenyl isosteres to replace the 4-FPh moiety of the 3-position of the oxadiazole within ADX-47273 in an attempt to incorporate solubilizing and/or polar groups while maintaining the 4-FPh amide and the (*S*)-stereochemistry (Table 1). Pyridine isosteres afforded intriguing SAR. Analogue **6a**, a pyridyl congener of the 4-FPh moiety of ADX-47273, lost ~8-fold in potency (EC₅₀ = 1,460 nM), while the 2-pyridyl analogue **6b** lost only 2-fold in potency, but maintained efficacy (EC₅₀ = 348 nM, 109% Glu Max). The 4-pyridyl isomer **6d** was comparable to **6a**, while the 3-pyridyl **6c** lost significant potency (EC₅₀ = 5,000 nM). The 2-thienyl congener **6e** (EC₅₀ = 170 nM) was equipotent to ADX-47273, and the 2-pyrimidinyl analogue **6f** lost all PAM activity.

These data then led to design of second generation library wherein the optimal 3-position groups with submicromolar EC₅₀s (4-FPh (**5**), 2-pyridyl (**6b**) and 2-thienyl (**6e**) from Table 1) were maintained while evaluating a diverse set of twelve acylating agents. In this second generation library (Figure 4) the (*S*)-stereochemistry of the 3-piperidine carboxylic acid was again maintained to provide analogues **10a-l**, **11a-l** and **12a-l**.

Ultimately, we followed two synthetic routes to access ADX-47273 analogs **10–12** (Scheme 1).[24] In route I, three (*Z*)-*N'*-hydroxyimides **7** (R = 4-FPh, 2-thienyl, 2-pyridyl) were coupled under standard EDCI/HOBt conditions with (*S*)-(tert-butoxycarbonyl)piperidine-3-carboxylic acid, followed by refluxing in 1,4-dioxane to afford oxadiazoles **8**. The Boc group was removed with 4 N HCl/dioxane to provide **9**, followed by typical acylation chemistry with 12 diverse acid chlorides to provide ADX-47273 analogs **10** (R = 4-FPh), **11** (R = 2-thienyl) and **12** (R = 2-pyridyl). Alternatively, analogs **10–12** could be accessed according to route II, wherein the oxadiazole is installed in the final step. In this scenario, (*S*)-piperidine-3-carboxylic acid **13** is converted to the corresponding methyl ester **14**, followed by typical acylation chemistry to deliver analogs **15** in good yields. Saponification with LiOH provides the corresponding acids which were then converted into the corresponding analogs **10** (R = 4-FPh), **11** (R = 2-thienyl) and **12** (R = 2-pyridyl). We employed route I for the initial library generation

of 36 ADX-47273 analogs **10–12**, and generally relied on route II for scale-up of interesting compounds.[24]

Robust SAR was observed for ADX-47273 analogs **10–12**, but the most striking finding was that a subtle change in the nature of the substituent in the 3-position of the oxadiazole afforded either potent mGluR5 ago-potentiators (**10** and **11**) or pure mGluR5 positive allosteric modulators (**12**), with little or no detectable agonist activity. As shown in Table 1, analogs **10a–10f** and **10j** were potent with submicromolar EC₅₀s (133–714 nM) and efficacious (92–107% glutamate max) mGluR5 ago-potentiators when the amide moiety was either a fluorinated or cyano-containing benzamide or a 2-thienyl amide. Other amides (heterocyclic, aryl and cycloalkyl) possessed EC₅₀s > 1 μM, and were therefore not useful as potential *in vivo* candidates.

A striking similar trend of activity was noted with amide analogues in the 2-thienyl series **11** (Table 3). Both potency and efficacy paralleled the 4-FPh series **10** for analogues **11a–g** (EC₅₀s 144–1,170 nM) and efficacious (90–109% glutamate max). In this instance as well, other amides (heterocyclic, aryl and cycloalkyl) possessed EC₅₀s > 1 μM, and were therefore not useful as potential *in vivo* candidates.

All of the analogs **10a–10f**, **10j** and **11a–11g** possessed significant mGluR5 allosteric agonist activity as well as positive allosteric modulator activity, and are therefore more accurately described as ago-potentiators, like the parent ADX-47273 (**5/10a**). With many of these analogues, the agonist activity was so strong, that it precluded fold-shift data from being calculated, or forced these experiments to be conducted at very low doses of compound (i.e., fold shifts at 370 nM versus the typical 1–10 μM). Figure 5 highlights a prototypical ago-potentiator from this series. The raw calcium fluorescence trace clearly illustrates the intrinsic mGluR5 agonist activity as well as the potentiation of an EC₂₀ concentration of glutamate. Full CRCs confirm an EC₅₀ for potentiation of 133 nM and an agonist EC₅₀ of 5 μM for **10c**. Like most of the analogues **10a–10f**, **10j** and **11a–11g**, the intrinsic agonist activity required that fold shift experiments be conducted at compound concentrations of only 370 nM; however, 4- to 5-fold shifts of the glutamate CRC were still observed. Moreover, all of these analogues were selective for mGluR5 (no activity at mGluRs 1, 2, 3, 4, 7 or 8). As with the parent, ADX-47273 (**5**), none of these analogues offered an improvement in solubility across pharmaceutically acceptable vehicles (clogPs were >4.5) and overall physiochemical properties were poor. Still, this effort provided potent and efficacious ago-potentiators for further studies.

Analogues **12a–12d**, **12f** and **12g**, which contained a 2-pyridyl moiety in the 3-position of the oxadiazole, afforded an unanticipated pharmacological profile. The potency and efficacy of these analogues **12** were comparable to, or slightly less potent (EC₅₀s 244–757 nM) and efficacious (89–109% glutamate max) than **10a–10f**, **10j** and **11a–11g** (Table 4). In addition to providing a basic nitrogen atom capable of salt formation, analogues **12a–12d**, **12f** and **12g** demonstrated an unexpected profile—pure mGluR5 positive allosteric modulation. Indeed, all of these analogues either displayed no mGluR5 agonism, or only a trace (<10% at 30 μM) at high compound concentrations.

Figure 6 highlights a prototypical mGluR5 positive allosteric modulator, (**12b**) from this series. The raw calcium fluorescence trace clearly illustrates a complete lack of intrinsic mGluR5 agonism, but a robust potentiation of an EC₂₀ concentration of glutamate. Full CRCs confirm an EC₅₀ for potentiation of 244 nM with no agonism by compound alone up to 30 μM, a finding in sharp contrast to **10a–10f**, **10j** and **11a–11g**. As there was no agonist activity, fold shift experiments could be conducted at standard concentrations, and (**12b**) afforded a strong 14-fold shift of the glutamate CRC at 1 μM. Other analogs in this series provided similar fold

shifts, but **(12c)** elicited an unprecedented 27.9-fold shift of the glutamate CRC at 1 μM – the largest fold-shift reported to date for an mGluR5 PAM. Moreover, all of these analogues were selective for mGluR5 (no activity at mGluRs 1, 2, 3, 4, 7 or 8). Unlike the parent, ADX-47273 (**5**) and **10a–10f**, **10j** and **11a–11g**, the corresponding HCl salts of **12a–12d**, **12f** and **12g** offered an improvement in solubility across pharmaceutically acceptable vehicles ($\text{clogP} < 3.6$ – a full log improvement) and overall physiochemical properties were improved, such that homogeneous dosing solutions could be obtained in tox-friendly vehicles (saline (5–25 mg/mL), β -cyclodextrin (10–20 mg/mL) as opposed to the **10** and **11** series which only afforded homogeneous solutions/microsuspensions in toxic PEG/DMSO vehicles ($\sim 5\text{mg/mL}$).

Surprisingly, one analogue **12k**, with a cyclobutyl amide, demonstrated a ‘switch’ in mode of pharmacology and was found to be an mGluR5 negative allosteric modulator (NAM). While weak ($\text{IC}_{50} = 8.7\text{ }\mu\text{M}$, 23% Glu Max), this is the first time we have observed this ‘switch’ of modes of pharmacology in a non-MPEP chemotype (Figure 7).[16,18]

Finally, we wanted to evaluate the stereochemistry at the C3 position of the piperidine-3-carboxylic acid **13**, as ADX-47273 (**5**) was the first reported mGluR5 PAM possessing a chiral center, and we wanted to determine if there was enantioselective potentiation. Following the synthetic routes in Scheme 1 and substituting (*R*)-piperidine-3-carboxylic acid for the previously employed (*S*)-congener **13**, allowed us to synthesize and evaluate the corresponding (*R*)-enantiomers of representative analogs from the **10–12** series and address this issue. As shown in Table 5, the (*R*)-enantiomers are uniformly 9- to 10-fold less potent than the corresponding (*S*)-enantiomers, but equally efficacious. This constitutes the first reported example of enantioselective potentiation of mGluR5, and while Addex has only reported on the (*S*)-enantiomers, this work quantifies the importance of the (*S*)-stereochemistry for activation of mGluR5.

In summary, we explored the chemical space surrounding the mGluR5 ago-potentiator ADX-47273 (**5**) employing an iterative library design comprised of three small libraries (~ 60 compounds). This effort identified potent mGluR5 ago-potentiators **10a–10f**, **10j** and **11a–11g** which possessed either a 4-FPh or 2-thienyl moiety in the 3-position of the oxadiazole core. Quite unexpectedly, when the 3-position was substituted with a 2-pyridyl moiety **12a–12d**, **12f** and **12g**, a new series of potent mGluR5 positive allosteric modulators resulted, which lacked the intrinsic agonist activity of **10a–10f**, **10j** and **11a–g**, and afforded 14-to 27.9-fold shifts – the largest ever observed for mGluR5. Moreover, analogues **12a–12d**, **12f** and **12g** could form HCl salts which displayed improved solubility and physiochemical properties. Quite unexpectedly, we identified one ADX-47273 analogue **12k**, demonstrated a ‘switch’ in mode of pharmacology to a negative allosteric modulator – an observation previously reserved to MPEP-like scaffolds. Finally, we discovered that the (*S*)-enantiomer for analogues **10–12** is required for mGluR5 activation, and represents the first example of enantioselective potentiation. With these new tools, we are poised to evaluate, *in vivo*, the effects of pure mGluR5 potentiation versus ago-potentiation of mGluR5 in preclinical antipsychotic and cognition models. These experiments are in progress and will be reported in due course.

Experimental Section

Full experimental details for representative mGluR5 PAMs, general details for analog synthesis and full experimental details for the *in vitro* assays are available in the Experimental Section.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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24. For full synthetic and pharmacology details, please see the Experimental Section.

Figure 1.
Representative Chemotypes of mGluR5 Positive Allosteric Modulators **1**, **2** and **3** and the mGluR5 Ago-potentiator **4**.

Figure 2.

In vitro profile of the mGluR5 ago-potentiator ADX-47273 (5), highlighting the intrinsic allosteric agonist activity (5 alone) to activate mGluR5 in the absence of an EC₂₀ of glutamate. In the presence of an EC₂₀ of glutamate, ADX-47273 (5) is a potent mGluR5 positive allosteric modulator with an EC₅₀ for potentiation of 168 nM and a 9-fold shift of the glutamate response curve at 1 μ M.

Figure 3.
Three key areas to explore in the lead optimization of ADX-47273 (**5**).

Figure 4.
Second generation (3 × 12) library design for analogues **10–12**.

Figure 5.

In vitro pharmacological profile of **10c**. A) Raw calcium fluorescence trace showing mGluR5 receptor activation (agonism) in the presence of **10c** alone added at $t=4s$, followed by potentiation of an EC_{20} concentration of glutamate added 140s later, indicating **10c** is also an mGluR5 PAM; B) Concentration-response-curves for both potentiation ($EC_{50} = 133$ nM) and agonism ($EC_{50} = 5$ μ M); C) Fold-shift assay employing 370 nM concentration of **10c** elicits a 5-fold leftward shift of the glutamate CRC.

Figure 6.

In vitro pharmacological profile of **12b**. A) Raw calcium fluorescence trace showing no mGluR5 receptor activation (agonism) in the presence of **12b** alone added at t=4s, followed solely by potentiation of an EC₂₀ concentration of glutamate added 140s later, indicating **12b** is an mGluR5 PAM; B) Concentration-response-curve for potentiation (EC₅₀ = 244 nM) and again showing no mGluR5 activation by **12b**/vehicle alone; C) Fold-shift assay employing 1 μM concentration of **12b** elicits a 14-fold leftward shift of the glutamate CRC.

12k

Negative Allosteric Modulator (NAM)

$IC_{50} = 8.7 \mu M$, 23% Glu Max

Figure 7.
Identification of an mGluR5 NAM within the ADX PAM Chemotype.

Scheme 1.

Synthesis of analogs **10–12** of ADX-47273 (**5**). Reagents and conditions: Route (I): a) (*S*)-1-(*tert*-butoxycarbonyl)piperidine-3-carboxylic acid, EDCI, HOBT, 1,4-dioxane, reflux (R = 4-FPh, 51%; R = 2-thienyl, 66%; R = 2-pyridyl, 44%); b) 4 N HCl/dioxane, DCM (82–99%); c) R₁COCl, DIEA, DCM (74–95%); Route (II): d) SOCl₂, MeOH (99%); e) (i) R₁COCl, DIEA, DCM; (ii) LiOH, THF/MeOH (88–92%); f) (*Z*)-*N'*-hydroxyimide, EDCI, HOBT, 1,4-dioxane, reflux (R = 4-FPh, 56%; R = 2-thienyl, 68%; R = 2-pyridyl, 57%).

Table 1Structures and activities of ADX-47273 analogs **6**.

6		
Compd	R ₁	Potentiato
ADX-47273 (5)		16

Compd	R ₁	Potentiato
6a	<p>1,4</p>

Compd	R ₁	Potentiator
6b		34
6c		5,00

Compd	R ₁	Potentiato
6d		1,41
6e		17
6f		>1

^aEC₅₀ values are the average of at least three determinations at an EC₂₀ concentration of glutamate

^b% Glu Max is the maximum response of compounds relative to the maximal glutamate response – at least three determinations.

Table 2Structures and activities of ADX-47273 analogs **10**.

10		
Compd	R ₁	Potentiator
5/10a ADX-47273		163

Compd	R ₁	Potentiator
10b		219
10c		133

Compd	R ₁	Potentiator
10d		16d

Compd	R ₁	Potentiator
10e		714
10f		520

Compd	R ₁	Potentiator
10j		435

^aEC₅₀ values are the average of at least three determinations at an EC₂₀ concentration of glutamate

^b% Glu Max is the maximum response of compounds relative to the maximal glutamate response – at least three determinations.

Table 3

Structures and activities of ADX-47273 analogs **11**.

Compd	R ₁	Potentiator EC ₅₀
6e/11a		170±20

Compd	R ₁	Potentiator EC ₅₀
11b		212±2

Compd	R ₁	Potentiator EC ₅₀
11c		144±2

Compd	R ₁	Potentiator EC ₅₀
11d		183±20

Compd	R ₁	Potentiator EC ₅₀
11e		1,150±1

Compd	R ₁	Potentiator EC ₅₀
11f		1,090±1

Compd	R ₁	Potentiator EC ₅₀
11g		1,170±2

^aEC₅₀ values are the average of at least three determinations at an EC₂₀ concentration of glutamate

^b% Glu Max is the maximum response of compounds relative to the maximal glutamate response – at least three determinations.

Table 4

Structures and activities of ADX-4723 analogs **12**.

Compd	R ₁	Potentiator EC ₅₀
6b/12a		348±70

12

Compd	R ₁	Potentiator EC ₅₀
12b	<p>Chemical structure of the R₁ substituent for compound 12b, which is a 2,6-difluorophenyl group.</p>	244±2

12

Compd	R ₁	Potentiator EC ₅₀
12c		304±3

Compd	R ₁	Potentiator EC ₅₀
12d		594±8
12f		566±9

12

Compd	R ₁	Potentiator EC ₅₀
12g	<p>Chemical structure of R₁ for compound 12g, showing a 4-(trifluoromethyl)phenyl group.</p>	757±20

^aEC₅₀ values are the average of at least three determinations an EC₂₀ concentration of glutamate

^b% Glu Max is the maximum response of compounds relative to the maximal glutamate response – at least three determinations.

Table 5Comparison of activities of (*R*)- and (*S*)-enantiomeric analogs of ADX-47273.

<div style="display: flex; justify-content: space-around; align-items: center;"> <div style="text-align: center;"> <p>10-12</p> </div> <div style="text-align: center;"> <p>16-18</p> </div> </div>			
Compd	R	Potentiator EC ₅₀ (nM) ^a	%Glu Max ^b
5/10a (<i>S</i>), ADX-47273 16a (<i>R</i>)	4-FPh	168±28 1680±362	107±5 108±6
6e/11a (<i>S</i>) 17a (<i>R</i>)	2-thienyl	170±28 2170±189	98±4 101±5
6b/12a (<i>S</i>) 18a (<i>R</i>)	2-pyridyl	348±70 2440±487	109±2 92±2

^aEC₅₀ values are the average of at least three determinations an EC₂₀ concentration of glutamate^b% Glu Max is the maximum response of compounds relative to the maximal glutamate response – at least three determinations.