

Allosteric potentiators of the metabotropic glutamate receptor 2 (mGlu2). Part 3: Identification and biological activity of indanone containing mGlu2 receptor potentiators

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Abstract—We have identified and synthesized a series of phenyl-tetrazolyl and 4-thiopyridyl indanones as allosteric potentiators of the metabotropic glutamate receptor 2. Structure activity relationship studies directed toward improving the potency and level of potentiation, as well as PK properties, led to the discovery of **28** (EC_{50} = 186 nM), which displayed activity in a rodent model for schizophrenia.

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1. Introduction

Glutamate is the major excitatory neurotransmitter in the CNS and plays an important role in many CNS functions. Glutamate receptors are classified into two main types, ionotropic (iGlu), which are glutamate mediated ion channels, and metabotropic (mGlu), which are a class of G-protein coupled receptors.^{1,2} Currently, mGlu receptors are divided into eight subtypes and three main groups (I–III). Group II (mGlu2 and -3) mGlu receptors are mainly concentrated presynaptically and generally inhibit neurotransmission. Therefore, agents targeting group II mGlu receptors may have utility in a variety of CNS disorders^{3–5} including epilepsy, anxiety, and schizophrenia.⁶ Recently, nonselective mGlu2/3 receptor agonists^{7–9} have shown activity in numerous animal models as well as human clinical trials.^{10,11} These agonists are generally rigid glutamate analogs. However, compounds selective for mGlu2 over mGlu3 have not been discovered using this approach. Therefore, another strategy for selectivity involves the discovery of allosteric modulators that do not bind at the glutamate binding

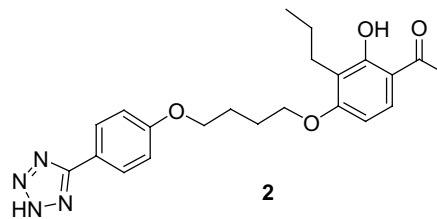
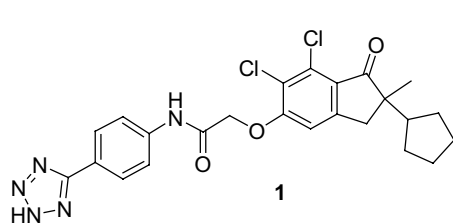
site.^{12–14} Screening of the Merck sample collection for allosteric modulators of the mGlu2 receptor identified phenyl-tetrazolyl indanone **1** (EC_{50} = 600 nM, 86% potentiation, with potentiation being defined as the response obtained using the test compound up to 10 μ M plus an EC_{10} of glutamate normalized to the maximal response obtained with glutamate alone)¹⁵ along with phenyl-tetrazolyl acetophenone **2** (EC_{50} = 348 nM, 31% potentiation), which has been disclosed previously.^{16,17} Indanone **1** displayed no activity in the absence of glutamate as well as no activity at mGlu3 in the presence or absence of glutamate, confirming it was a selective mGlu2 receptor modulator. Concurrent to our work on compound **2**, we investigated similar approaches to improve the potency, brain penetration, and biological activity of compound **1**. This paper outlines the discovery of a brain penetrant, nontetrazole containing mGlu2 receptor potentiator that shows activity in a rodent model for schizophrenia after systemic dosing.

2. SAR studies

In order to improve the potency and PK properties of compound **1**, three areas were addressed: (1) the effect of the linker between the indanone and the aryl tetrazole, (2) the effect of groups on the indanone, and (3) the replacement of the tetrazole. All compounds

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described herein were synthesized and tested as racemates unless otherwise noted. Likewise, all compounds described herein showed no activity at mGlu3 or other mGlu receptors.

We began with modification of the linker, as indanone **1** displayed poor PK parameters (vide infra) and we felt that the amide linkage was potentially responsible for this. Therefore, initial efforts focused on removing the amide. As shown in Table 1, complete removal of the amide moiety to give biphenyl compound **3** led to a total loss of potency. We surmised that there needed to be some minimal distance between the indanone and the tetrazole for potency. We then prepared a series of alkyl tetrazoles (**4–6**) to address this question. Indeed, the 3 carbon linker **4** displayed poor potency but the 4 and 5 carbon linker gave moderately potent compounds **5** and **6**, the former with an EC_{50} of 589 nM. Reincorporating the aryl group further increased the potency, as long as the tetrazole was in the *meta*- or *para*-position. As shown, *ortho*-substituted **7** was not active, whereas both the *meta*- (**8**) and *para*- (**9**) substituted compounds displayed significantly improved potency compared to the original amide lead, with the latter being slightly preferred. Interestingly, construction of an all carbon linked compound (**10**), the immediate analog of **9**, led to a 5–6-fold decrease in activity. Because of this we focused only on compounds with at least one oxygen atom in the linker. The final compounds examined (**11–13**) showed that extending the linker by 2 (**11**), 3 (**12**), or 4 (**13**) more atoms gave compounds of similar activity to **9**, indicating that there was some flexibility in the SAR concerning longer linker lengths. However, subsequent SAR utilized the simple benzylic linkage due to the observation that no obvious potency boost was obtained with the longer linkers and we desired to keep molecular weight down. We also investigated the stereochemical requirements for mGlu2 receptor potentiation in this series. Compound **9** was also resolved and only the (–) isomer was found to be active ((–) isomer EC_{50} = 122 nM, 90% potentiation; (+) isomer EC_{50} > 10 μ M).

With the result of compound **9** in hand, we next focused on modification of the group α to the ketone on the indanone. As shown in Table 2, a range of substituents are tolerated. Removal of the methyl group in **9** gave a monosubstituted compound (**14**) that displayed a 2-fold increase in potency (to 122 nM), which represents one of the most potent compound in this series. Other groups in place of the cyclopentyl also displayed potency, with isopropyl (**15**) and propyl (**16**) being appropriate surrogates. When only smaller alkyl groups are present, for example, two methyl groups (**17**), potency is diminished,

indicating that there appears to be an aliphatic binding area. Potency also decreased with a phenyl group (**18**). Interestingly when two larger groups, for example, a butyl group and a cyclopentyl group (**19**) are present, activity is 5-fold less indicating a limit to the steric bulk that can be accommodated at the binding site.

Due to issues associated with poor brain penetration for the tetrazole containing compounds, we investigated replacements for the tetrazole group as illustrated in Table 3. Simple methylation of the tetrazole led to a complete loss of activity (**20**). A sulfonamide derivative was also not potent (**21**). A number of other groups containing an acidic proton gave moderate levels of potency and potentiation. For example, a simple carboxylic acid (**22**), an imide (**23**) and an acyl sulfonamide (**24**) showed activity close to the tetrazole. Due to our previous success in a related series of compounds¹⁸ replacing the tetrazole with a thiopyridine moiety, we investigated incorporating this group. Gratifyingly, this strategy gave a compound (**25**) that was close in potency to the original lead.

To further optimize the potency of compound **25**, we reinvestigated the indanone as well as ketone substitution. Similar to our results with the tetrazole containing compounds, both smaller aliphatic (bis-methyl) and larger aliphatic (*n*-butyl/cyclopentyl) on the indanone gave less potent compounds. A phenyl group also gave diminished potency and potentiation (data not shown). Much larger improvements were seen with the incorporation of monosubstituted indanones **26–28** wherein the methyl group of **25** was not present. One of the most promising compounds in this series was **28**, displaying both good potency (186 nM) and extremely high levels of potentiation (169%), implying that this compound gave a much stronger response than even the maximal glutamate response at the mGlu2 receptor. The implications of this high level of potentiation are not understood at this time, although it should be noted that this compound also does not display activity in the absence of glutamate. Due to its potency, this compound was profiled further (vide infra). We also investigated several dimethyl substituted indanones in the place of dichloro indanones and found compounds with good, but slightly lessened potency. For example, **29** showed 3-fold less potency than **26** and **30** was slightly less potent than **28**. Both **29** and **30** also had lower levels of potentiation. It should be noted that compounds such as **30**, which had only a 48% level of potentiation, as well as other compounds with lower levels of potentiation, displayed no antagonist activity at the mGlu2 receptor, rather only partial agonist like behavior.

Table 1. Binding affinities for linker-modified indanones

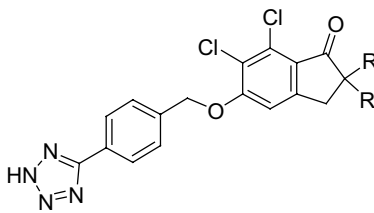
Compd	Group	hmGlu2	
		GTP γ S binding EC ₅₀ (nM) ^a	% Potentiation ^b
1		600	86
3		NA ^c	—
4		5000	30
5		589	63
6		761	91
7		NA ^c	—
8		371	79
9		223	85
10		1160	30
11		276	75
12		270	89
13		225	97

^a Value represents mean of two or more experiments.^b Result expressed as a percentage of the maximum glutamate response at 1 mM.^c NA denotes not active <10 μ M concentration.

3. PK properties and biological activity

A number of indanones were investigated for their rat pharmacokinetics (Table 5). As stated above, initial lead **1** showed extremely poor rat PK, presumably due to the amide linkage. However, tetrazole containing compounds **9** and **12** showed greatly improved PK with clearances of 35 and 19 mL/min/kg as well as bioavaila-

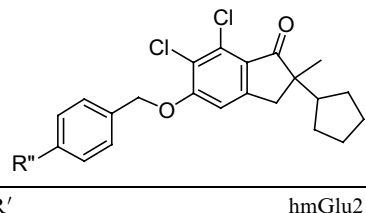
bilities of 65% and 14%, respectively. However, these compounds had very low brain penetration (<1%). As has been observed before,¹⁸ the thiopyridine replacement improved the brain penetration. For example, compound **28** has a brain/plasma ratio of 108%. Although the absolute brain levels were low, we felt that this compound would be suitable for in vivo evaluation due to this increased brain penetration. The model we

Table 2. Modifications to ketone substitution


Compd	R	R'	hmGlu2	
			GTPγS binding EC ₅₀ (nM) ^a	% Potentiation ^b
9	-CH ₃		223	85
14	-H		122	80
15	-H		195	80
16	-H		282	57
17	-CH ₃	-CH ₃	782	35
18	-CH ₃	-Ph	629	58
19	-nC ₄ H ₉		1090	24

^a Value represents mean of two or more experiments.^b Result expressed as a percentage of the maximum glutamate response at 1 mM.

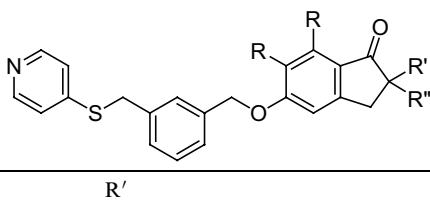
chose to investigate was the blockade of ketamine induced hyperactivity, which is a rodent model for schizo-

Table 3. Tetrazole replacements


Compd	R'	hmGlu2	
		GTPγS binding EC ₅₀ (nM) ^a	% Potentiation ^b
9		223	85
20		NA ^c	—
21	-SO ₂ NH(CH ₃)	NA ^c	—
22	-CO ₂ H	892	76
23		442	54
24		515	105
25		562	114

^a Value represents mean of two or more experiments.^b Result expressed as a percentage of the maximum glutamate response at 1 mM.^c NA denotes not active <10 μM concentration.

phrenia.¹⁹ In this assay compound **28** was found to be efficacious after a systemic dose (ip) at 40 mpk (Fig. 1).

Table 4. Activity of thiopyridines


Compd	R	R'	R'	hmGlu2	
				GTPγS binding EC ₅₀ (nM) ^a	% Potentiation ^b
25	-Cl	-CH ₃		562	114
26	-Cl	-H		165	100
27	-Cl	-H		839	106
28	-Cl	-H		186	169
29	-CH ₃	-H		596	70
30	-CH ₃	-H		291	48

^a Value represents mean of two or more experiments.^b Result expressed as a percentage of the maximum glutamate response at 1 mM.

Table 5. Selected rat pharmacokinetic parameters

Compd	Cl ^a (mL/min/kg)	% F ^a	Brain/plasma ^b	Brain level ^b (nM)
1	>100	0	ND ^c	—
9	35	65	<0.01	—
12	19	21	<0.01	—
28	ND ^c	ND ^c	1.08	240

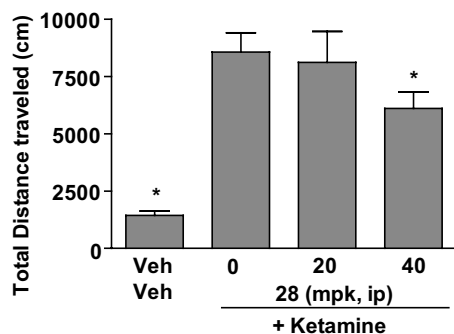
^a Dosed 2 mpk iv and 10 mpk po.^b Dosed 20 mpk ip, levels at 2 h.^c Not determined.

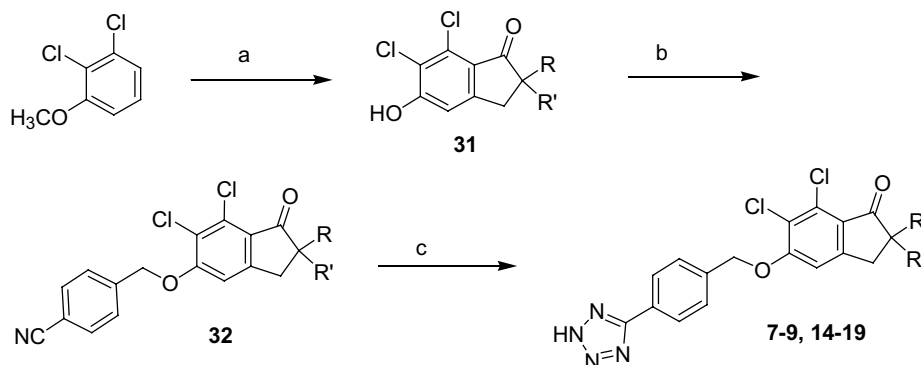
Figure 1. Modulation of ketamine hyperactivity in rats by mGlu2 potentiator **28**. Subjects were dosed with **28** (ip) or vehicle (ip) 30 min before receiving sc injections of ketamine (25 mg/kg). Activity data (total distance traveled) are presented as the group mean ($\pm$ S.E.M) recorded for the total duration of the 120 min test period. Data were analyzed by a one-way ANOVA followed by Dunnett's *t*-tests. **p* < 0.05 compared to vehicle/ketamine-treated rats.

4. Chemistry

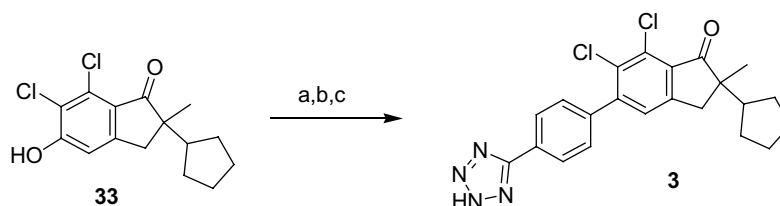
The compounds described in Tables 1 and 2 were synthesized as outlined in Schemes 1–3.²⁰ Starting from dichloroanisole, this compound was converted to the appropriate indanone (**31**) following the literature procedure in five steps in overall good yield.²¹ The appropriately substituted indanone **31** was then alkylated to give a cyano precursor **32**, which was converted to the desired tetrazole using a tin catalyzed reaction with trimethylsilyl azide. In this manner, compounds **7–9** and **14–19** were prepared. Compounds **4–6** and **11–13** were prepared in a similar fashion using the appropriate alkyl bromide/nitrile following the same steps.

Compound **3** was prepared as shown in Scheme 2. Indanone **33** was first converted into the corresponding triflate using triflic anhydride. It was then subjected to a palladium catalyzed cross-coupling with 4-cyanobromobenzene to give the desired nitrile precursor, which was transformed into tetrazole **3** as described previously. Likewise, compound **10** was accessed via a cross-coupling strategy, this time a Sonagashira coupling of the same triflate with 4-cyanoethynylbenzene. The nitrile **34** was first converted to the tetrazole, then the triple bond was fully reduced using palladium on carbon to give **10** (Scheme 3).

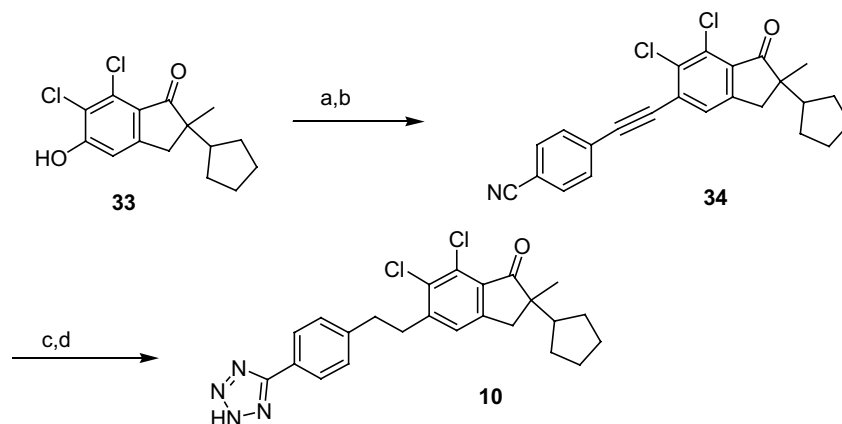
The compounds in Table 3 were prepared as outlined in Scheme 4. Indanone **33**, prepared as described above, was alkylated with methyl-4-(bromomethyl)benzoate in good yield. The ester was then hydrolyzed to give acid



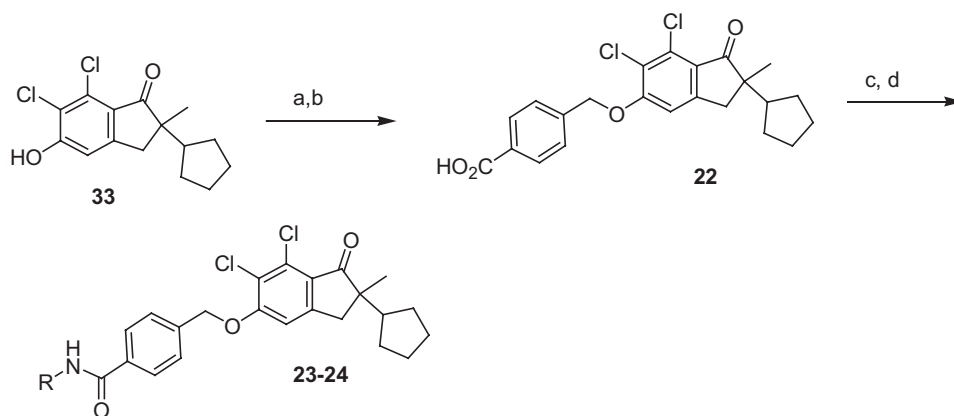
Scheme 1. Reagents and conditions: (a) five steps, see Ref. 21; (b) α -bromo-*p*-tolunitrile, K₂CO₃, acetone, 45 °C; (c) TMS-N₃, Bu₂SnO, toluene, reflux.



Scheme 2. Reagents and conditions: (a) PhNTf₂, Cs₂CO₃, CH₃CN/DMF 9/1, 88%; (b) 4-cyanophenylboronic acid, Na₂CO₃, Pd(PPh₃)₄, DME/H₂O 2.5/1, 45 °C, 17%; (c) TMS-N₃, Bu₂SnO, toluene, reflux, 60%.



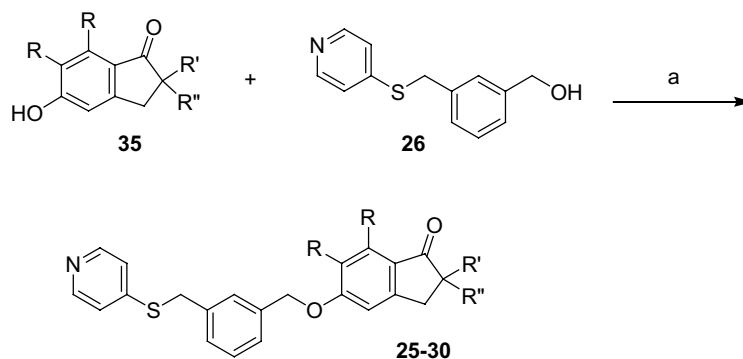
Scheme 3. Reagents and conditions: (a) PhNTf_2 , Cs_2CO_3 , $\text{CH}_3\text{CN}/\text{DMF}$ 9/1, 88%; (b) 4-cyanoethynylbenzene, $\text{PdCl}_2(\text{PPh}_3)_2$, CuI , Et_3N , 65°C , 41%; (c) TMS-N_3 , Bu_2SnO , toluene, reflux, 63%; (d) H_2 , 10% Pd/C , MeOH , quant.



Scheme 4. Reagents and conditions: (a) methyl-4-(bromomethyl)benzoate, K_2CO_3 , acetone, 45°C , 88%; (b) LiOH , THF/water 1/1, 99%; (c) $(\text{COCl})_2$, DMF , CH_2Cl_2 ; (d) RNH_2 , NaH , THF , 0°C .

22. This was then converted into compounds **23–24** by first converting the acid into the acid chloride then reacting the acid chloride with the appropriate anion prepared via the amide or sulfonamide and sodium hydride. Compound **21** was prepared via an alkylation in a similar manner as with **22**, using the appropriate sulfonamide benzyl bromide. Compound **20** was accessed via alkylation of compound **9** with methyl iodide.

Lastly, compound **25** and the compounds in Table 4 were synthesized under Mitsunobu type conditions with an appropriately substituted indanone (**35**) and benzylic alcohol **26**, which was made as described previously. Reaction of these two precursors with di-*tert*-butylazodicarboxylate and triphenylphosphine in THF gave the desired compounds. In this fashion **25–30** were obtained in generally good yields (Scheme 5).



Scheme 5. Reagents: (a) DTAD , PPh_3 , THF .

5. Conclusion

In conclusion, a new class of indanone mGlu2 receptor potentiators has been described. Optimization of the series has led to compounds such as **28**, which shows activity after systemic dosing in rodent models with relevance to schizophrenia. This result helps validate the potential application of mGlu2 receptor potentiators in a variety of CNS disorders. Further optimization and application of this series of compounds will be reported in due course.

Acknowledgments

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References and notes

- Pin, J.-P.; Acher, F. *Curr. Drug Targets: CNS Neurol. Disord.* **2002**, *1*, 297; Conn, P. J.; Pin, J. P. *Ann. Rev. Pharmacol. Toxicol.* **1997**, *37*, 205.
- Schoepp, D. D.; Jane, D. E.; Monn, J. A. *Neuropharmacology* **1999**, *38*, 1431.
- Lam, A. G.; Soriano, M. A.; Monn, J. A.; Schoepp, D. D.; Lodge, D.; McCulloch, J. *Neurosci. Lett.* **1998**, *254*, 121.
- Kingston, A. E.; O'Neill, M. J.; Lam, A.; Bales, K. R.; Monn, J. A.; Schoepp, D. D. *Eur. J. Pharmacol.* **1999**, *377*, 155.
- Helton, D. R.; Tizzano, J. P.; Monn, J. A.; Schoepp, D. D.; Kallman, M. J. *J. Pharmacol. Exp. Ther.* **1998**, *284*, 651.
- Chavez-Noriega, L. E.; Schaffhauser, H.; Campbell, U. C. *Curr. Drug Targets: CNS Neurol. Disord.* **2002**, *1*, 261.
- Monn, J. A.; Valli, M. J.; Massey, S. M.; Wright, R. A.; Salhoff, C. R.; Johnson, B. G.; Howe, T.; Alt, C. A.; Rhodes, G. A.; Robey, R. L.; Griffey, K. R.; Tizzano, J. P.; Kallman, M. J.; Helton, D. R.; Schoepp, D. D. *J. Med. Chem.* **1997**, *40*, 528.
- Monn, J. A.; Valli, M. J.; Massey, S. M.; Hansen, M. M.; Kress, T. J.; Wepsiec, J. P.; Harkness, A. R.; Grutsch, J. L., Jr.; Wright, R. A.; Johnson, B. G.; Andis, S. L.; Kingston, A.; Tomlinson, R.; Lewis, R.; Griffey, K. R.; Tizzano, J. P.; Schoepp, D. D. *J. Med. Chem.* **1999**, *42*, 1027.
- Nakazato, A.; Kumagai, T.; Sakagami, K.; Yoshikawa, R.; Suzuki, Y.; Chaki, S.; Ito, H.; Taguchi, T.; Nakanishi, S.; Okuyama, S. *J. Med. Chem.* **2000**, *43*, 4893.
- Levine, L.; Gaydos, B.; Sheehan, D.; Goddard, A. W.; Feighner, J.; Potter, W. Z.; Schoepp, D. D. *Neuropharmacology* **2002**, *43*, 294.
- Grillon, C.; Cordova, J.; Levine, L. R.; Morgan, C. A., III. *Psychopharmacology* **2003**, *168*, 446; Schoepp, D. D.; Wright, R. A.; Levine, L. R.; Gaydos, B.; Potter, W. Z. *Stress* **2003**, *6*, 189.
- Knoflach, F.; Mutel, V.; Jolidon, S.; Kew, J. N. C.; Malherbe, P.; Viera, E.; Wichmann, J.; Kemp, J. A. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 13402.
- (a) Johnson, M. P.; Baez, M.; Jagdmann, G. E., Jr.; Britton, T. C.; Large, T. H.; Callagaro, D. O.; Tizzano, J. P.; Monn, J. A.; Schoepp, D. D. *J. Med. Chem.* **2003**, *46*, 3189; (b) Barda, D. A.; Wang, Z.; Britton, T. C.; Henry, S. S.; Jagdmann, G. E.; Coleman, D. S.; Johnson, M. P.; Andis, S. L.; Schoepp, D. D. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3099; (c) Hu, E.; Chua, P. C.; Tehrani, L.; Nagasawa, J. Y.; Pinkerton, A. B.; Rowe, B. A.; Vernier, J.-M.; Hutchinson, J. H.; Cosford, N. D. P. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 5071.
- Schaffhauser, H.; Rowe, B. A.; Morales, S.; Chavez-Noriega, L. E.; Yin, R.; Jachec, C.; Rao, S. P.; Bain, G.; Pinkerton, A. B.; Vernier, J.-M.; Bristow, L. J.; Varney, M. A.; Daggett, L. P. *Mol. Pharmacol.* **2003**, *64*, 798.
- The effect of these compounds was characterized in the [³⁵S]-GTPγS binding assay using a cell line expressing human mGlu2 receptor. See Ref. **14** for a detailed description of this assay. First, an EC₁₀ (1 μM) of glutamate was added to the cell line followed immediately by the test compound at varying concentrations. The response was then compared to a response using a saturating amount of glutamate (1 mM) to give both an EC₅₀ and a percent potentiation (the response normalized to the maximum response of glutamate alone). The same experiment was carried out in the absence of glutamate to test if the compound was truly a positive allosteric modulator. Nonspecific binding was determined by addition of 10 μM unlabeled GTPγS.
- Pinkerton, A. B.; Vernier, J.-M.; Schaffhauser, H.; Rowe, B. A.; Campbell, U. C.; Rodriguez, D. E.; Lorrain, D. S.; Baccei, C. S.; Daggett, L. P.; Bristow, L. J. *J. Med. Chem.* **2004**, *47*, 4595.
- Pinkerton, A. B.; Cube, R. V.; Hutchinson, J. H.; Rowe, B. A.; Schaffhauser, H.; Zhao, X.; Daggett, L. P.; Vernier, J.-M. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 5329.
- Pinkerton, A. B.; Cube, R. V.; Hutchinson, J. H.; James, J. K.; Gardner, M. F.; Rowe, B. A.; Schaffhauser, H.; Daggett, L. P.; Vernier, J.-M. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 5867.
- Lorrain, D. S.; Schaffhauser, H.; Campbell, U. C.; Baccei, C. S.; Correa, L. D.; Rowe, B.; Rodriguez, D. E.; Anderson, J. J.; Varney, M. A.; Pinkerton, A. B.; Vernier, J.-M.; Bristow, L. J. *Neuropsychopharmacology* **2003**, *28*, 1622.
- All final compounds displayed spectral data (NMR, MS) that was consistent with the assigned structure.
- Woltersdorf, O. W., Jr.; deSolms, S. J.; Schultz, E. M.; Cragoe, E. J., Jr. *J. Med. Chem.* **1977**, *20*, 1400.