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Benzazoles as allosteric potentiators of metabotropic glutamate receptor 2 (mGluR2): Efficacy in an animal model for schizophrenia

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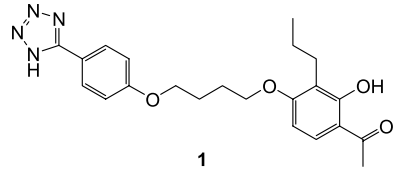
Abstract—Metabotropic glutamate receptor 2 (mGluR2) has been implicated in a variety of CNS disorders, including schizophrenia. Disclosed herein is the development of a new series of allosteric potentiators of mGluR2. Structure–activity relationship studies in conjunction with pharmacokinetic data led to the discovery of indole **5**, which is active in an animal model for schizophrenia.

Glutamate is the primary excitatory neurotransmitter in the central nervous system. It activates two distinct classes of receptors: the ionotropic glutamate receptors and the metabotropic glutamate receptors. The ionotropic glutamate receptors, which mediate fast synaptic transmission, are ligand-gated ion channels, including the NMDA, AMPA, and Kainate receptors. The metabotropic glutamate receptors (mGluRs) are G-protein-coupled receptors that modulate glutamatergic activity. To date, eight mGlu receptor subtypes have been identified and categorized into three groups based on primary structure, second messenger coupling, and pharmacology: group I (mGluRs 1 and 5); group II (mGluRs 2 and 3); and group III (mGluRs 4, 6, 7, and 8).[1]

The group II metabotropic glutamate receptors have been implicated in a variety of disease states, including anxiety and schizophrenia.[2] Moreover, non-selective group II mGluR agonists[3] have shown efficacy in animal models for schizophrenia[4] and in human clinical trials for anxiety.[5] It is believed that this functional activity comes from activation of mGluR2 because of this receptor's role in regulating glutamate release into the synapse.[6,7]

Unfortunately, agonists that are selective for mGluR2 versus mGluR3 have not been reported. This is most likely a result of the high degree of sequence homology at the glutamate-binding site among group II mGlu receptors. The development of positive modulators that do not bind at the glutamate-binding site is an alternate strategy for selectively activating mGluR2. An allosteric-binding site involved in the positive modulation of metabotropic glutamate receptor 2 has been discovered.[8] Compounds that bind at this site potentiate the activation of mGluR2 in the presence of glutamate, but these potentiators show no activity in the absence of glutamate.[9,10]



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Keywords: Metabotropic glutamate receptor; mGlu2; mGluR2; Allosteric potentiator; Schizophrenia.
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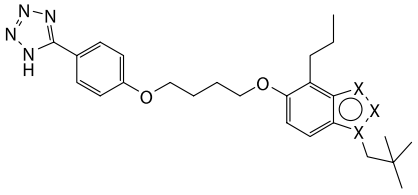
Compound **1** was identified from a screening campaign as an allosteric potentiator of mGluR2. This compound is moderately potent (EC_{50} = 348 nM) with a low level of potentiation (31%),[11] displays no activity at mGluR3,[12] has a poor pharmacokinetic profile in rats ($t_{1/2}$ = 0.2 h; Cl_p = 33 mL/min/kg; %F = 63),[13] and is not significantly brain penetrant (B/P = <0.01).[14] The data obtained for compound **1** directly translated into our goals for identifying new potentiators: (i) increase potency and potentiation, (ii) maintain selectivity versus mGluR3, (iii) increase brain penetration, and (iv) improve the pharmacokinetic profile. In this paper, we describe the elaboration of compound **1** into a new series of mGluR2-selective potentiators, and subsequent evaluation of the most-promising compound in an in vivo model for schizophrenia.

Our structure–activity relationship studies[15] began by replacing the hydroxyketone motif of compound **1**. We reasoned that the hydroxyketone is constrained by an internal hydrogen bond and therefore could be replaced with a variety of heterocycles (Table 1). These heterocycles were initially substituted with a *neo*-pentyl group because prior work related to compound **1** indicated that higher levels of potentiation can be achieved with bulkier ketone substituents.[16] Of all the heterocycles incorporated into compound **1**, the indole and benzotriazole ring systems stand out above the rest. Indole **5** (559 nM, 98% pot.), although slightly less potent than compound **1**, gives a 3-fold increase in percent potentiation. Benzotriazole **9** (188 nM, 106% pot.) is 3-fold more potent than indole **5** with a similar level of potentiation. Unfortunately, benzotriazole **9** shows activity at mGluR3 (936 nM, 115% pot.), whereas indole **5** does not.[17] For this reason, as well as synthetic ease and versatility, further SAR utilized the indole ring system.

Next, we explored the potential for varied substitution about the indole ring (Table 2). Initial work focused on the indole nitrogen substituent. Starting from a methyl group, lengthening the alkyl group

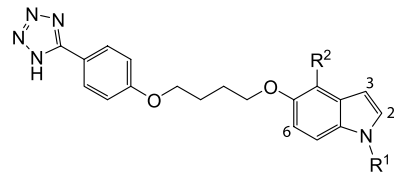
Table 1. Benzazoles as acetophenone replacements



Compound	Ring	hmGluR2: GTPγS binding[a]	
		EC_{50} (nM)	% Potentiation[b]
2		788	73
3		na	
4		na	
5		559	98
6		1172	44
7		984	29
8		6367	12
9		188	106

[a] Values are means of two or more experiments (na, not active at 10 μM).
[b] Percent of maximal glutamate response (1 mM).

Table 2. Indole substitution



Compound	R^1	R^2	Ring substituent	hmGluR2: GTPγS[a]	
				EC_{50} (nM)	% Potentiation[b]
10	Me	*n*Pr	—	na	
11	*n*Pr	*n*Pr	—	652	57
12	*n*Pent	*n*Pr	—	598	74
13	*i*Pr	*n*Pr	—	1130	23
14	*i*Bu	*n*Pr	—	765	62
15	CH_2cPr	*n*Pr	—	755	82
16	CH_2cHex	*n*Pr	—	228	101
5	CH_2CMe_3	*n*Pr	—	559	98
17	CH_2CMe_3	H	—	3591	47
18	CH_2CMe_3	Me	—	1070	39
19	CH_2CMe_3	Br	—	2263	62
20	CH_2CMe_3	*n*Pr	2-Me	559	73
21	CH_2CMe_3	*n*Pr	3-Me	na	
22	CH_2CMe_3	*n*Pr	6-OMe	na	

[a] Values are means of two or more experiments (na, not active at 10 μM).
[b] Percent of maximal glutamate response (1 mM).

(**10** → **11** → **12**) has a positive impact on both potency and potentiation. Branched alkyls are beneficial when the branching is one atom removed from the nitrogen (**13** vs. **14**). Further increase in size (**15** → **16**; **14** → **5**) reveals the optimal substituents to be large lipophilic groups such as *neo*-pentyl and methylenecyclohexane. Additional compounds were made with non-alkyl substituents (acyl, sulfonyl, benzyl, and pyridyl) on the indole nitrogen, but all were inactive. Cursory investigation of the C4 substituent (**17**–**19**) shows no improvement as compared to *n*-propyl. Additionally, placing small substituents around the indole ring slightly reduces (**20**) or completely eliminates (**21**, **22**) activity.

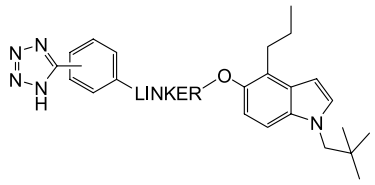
Finally, we examined the linker connecting the indole ring to the phenyl tetrazole (Table 3). Shortening or lengthening the linker (**23**–**25**) significantly reduces activity. However, the inclusion of conformational constraints within the linker provides a considerable benefit when oriented properly *vide infra* (**26**–**30**). Installation of a *trans*-olefin (**26**) slightly reduces both potency and potentiation.[18] Direct incorporation of a phenyl ring (**27**, **28**), although detrimental to the overall activity, suggests the existence of an orientational preference. Expanding on this possibility, elimination of the ether linkage between the two phenyl rings and varying the position of the tetrazole gives compounds **29** and **30** that are more potent than *n*-butyl-linked indole **5** and maintain the same level of potentiation.

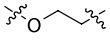
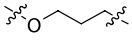
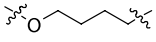
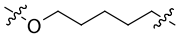
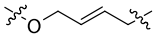
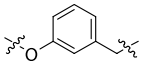
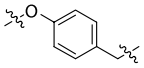
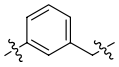
Having identified a new series of mGluR2-selective potentiators with improved potency and potentiation, we sought to demonstrate in vivo efficacy in an animal model for schizophrenia. Since mGluR2 is a CNS target, we needed to first assess the brain penetrability of the most potent compounds. Selected compounds were dosed intraperitoneal (IP) at 20 mg/kg and at 2 h, brain and plasma levels were taken (Table 4). As with our lead compound (**1**), no compound was detected in the brain with biphenyl-linked indoles **29** and **30**. Fortunately, all three *n*-butyl-linked benzazoles that we tested (**5**, **9**, and **16**) were brain penetrant. Of these, compound **5** was best with an average brain level of 600 nM and a brain/plasma ratio of 0.16. Subsequently, the pharmacokinetic profile in rats for compound **5** was obtained ($t_{1/2}$ = 2.2 h; Cl_p = 11 mL/min/kg; %F = 12).[13]

With the identification of indole **5** as the most-promising compound from this series, we examined its effect in a behavioral model for schizophrenia, the modulation of ketamine-induced hyperactivity in rats (Fig. 1).[19] As previously observed with mGluR2/3 agonists,[4] the mGluR2-selective potentiator indole **5** attenuates the effect of ketamine on activity levels.

In summary, we have disclosed the development of a new series of allosteric potentiators for mGluR2. Our studies led to the discovery of indole **5**, an mGluR2-selective potentiator that is moderately brain

Table 3. Linker modifications



Compound	Linker	Tetrazole	hmGluR2: GTPγS[a]	
			EC_{50} (nM)	% Potentiation[b]
23		*Para*	1356	39
24		*Para*	3544	40
5		*Para*	559	98
25		*Para*	1010	51
26		*Para*	779	84
27		*Para*	451	73
28		*Para*	879	66
29		*Para*	318	94
30		*Meta*	134	107

[a] Values are means of two or more experiments.
[b] Percent of maximal glutamate response (1 mM).

Table 4. Rat brain penetration data[a]

Compound	Brain level (nM)	*B*/*P*[b]
1	—	<0.01
5	600	0.16
9	75	0.01
16	200	0.02
29	—	<0.01[c]
30	—	<0.01[d]

[a] 20 mpk IP, 2 h ($n = 3$).
[b] Brain level/plasma level.
[c] Plasma level was 22 μM.
[d] Plasma level was 7 μM.

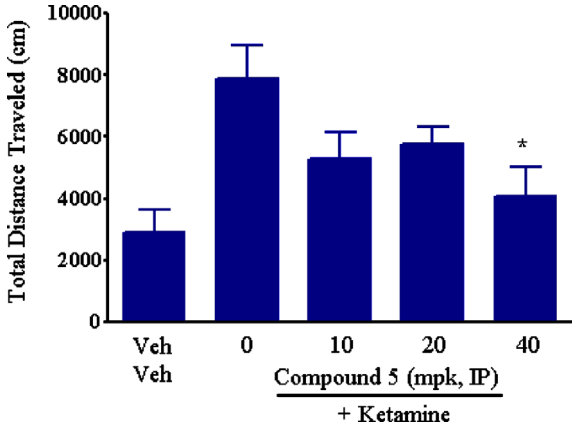


Figure 1. Schizophrenia Model: modulation of ketamine-induced hyperactivity. Rats ($n = 10$/group) were dosed with compound **5** (IP) or vehicle (IP) 60 min prior to receiving ketamine (25 mpk SC). Data are presented as the group mean (±SEM) recorded for the total duration of the 120-min test period. Data were analyzed by a one-way ANOVA followed by Dunnett's *t*-tests, $*p < 0.05$ compared to vehicle/ketamine-treated rats.

penetrant, has acceptable rat PK, and most importantly, is active in an animal model for schizophrenia. Indole **5** represents an attractive lead for the development of potent compounds for the treatment of schizophrenia.

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We thank Merryl Cramer for performing the PK and brain/plasma ratio studies.

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11. The effect of these compounds was characterized in the [^{35}S]GTPγS binding assay using a cell line expressing human mGluR2. See Ref. 8a for a detailed description of this assay. First an EC_{10} (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 EC_{50} 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 an allosteric potentiator. Non-specific binding was determined by addition of 10 μM unlabeled GTPγS. All compounds were tested to a minimum of $n = 2$. The difference in EC_{50} was generally <2-fold and the difference in percentage potentiation was generally <15% with respect to the values quoted in the tables.
12. Compound **1** was inactive up to 10 μM at mGluR3 as well as mGluRs 1, 4, 5, 7, and 8.
13. Sprague–Dawley rats; 2 mpk IV ($n = 2$); 10 mpk PO ($n = 3$).
14. B/P = ratio of brain level to plasma level.
15. All compounds were synthesized by methods previously described by us (Ref. 10). For installation of the C4 propyl group see: Moody C. J. *J. Chem. Soc., Perkin Trans. 1* **1984**, 1333. All final compounds displayed spectral data (NMR, MS) that were consistent with the assigned structure.
16. See Refs. 10a,b for general SAR; results with *neo*-pentyl are not published.
17. Active compounds were screened for activity at mGluR3 using the [^{35}S]GTPγS binding assay (see Ref. 11). All compounds except **9** were inactive up to 10 μM.
18. A compound containing the *cis*-olefin was made in a related series and showed no activity.
19. In healthy humans, ketamine induces behavioral disruptions and cognitive deficits that mimic some aspects of schizophrenia. Similar behavioral disruptions, typified by increased locomotion (hyperactivity), are seen in ketamine-treated rodents. This model assesses the effect of a compound on the locomotion of ketamine-treated animals. See Ref. 4.