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Bioorganic & Medicinal Chemistry Letters 14 (2004) 5329–5332
Bioorganic & Medicinal Chemistry Letters

Allosteric potentiators of the metabotropic glutamate receptor 2 (mGlu2). Part 1: Identification and synthesis of phenyl-tetrazolyl acetophenones

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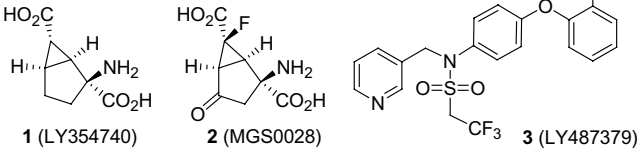
Received 25 June 2004; revised 7 August 2004; accepted 9 August 2004
Available online 3 September 2004

Abstract—We have identified and synthesized a series of aryl-tetrazoyl acetophenones as positive allosteric potentiators of the metabotropic glutamate receptor 2. Structure activity relationship studies directed toward improving the potency and level of potentiation led to the discovery of **22** (EC_{50} = 93 nM, 128% potentiation).

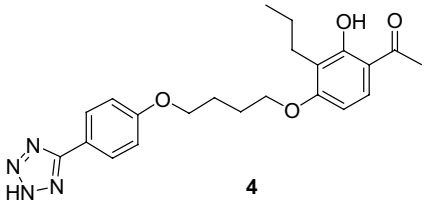
1. Introduction

Glutamate is the transmitter of the large majority of fast excitatory synapses in the CNS and plays an important role in a wide variety of CNS functions. The actions of glutamate are mediated by the activation of both ionotropic glutamate receptors (iGlu), which are glutamate-gated ion channels, as well as the metabotropic glutamate receptors (mGlu), which are a class of G-protein coupled receptors. Eight subtypes of the mGlu receptors have been identified, which fall into three main groups.[1,2] Group I consists of mGlu1 and -5, which are primarily stimulatory. Groups II (mGlu2 and -3) and III (mGlu4, -6, -7, -8), however, are presynaptic and generally inhibit neurotransmission. Therefore, agents targeting mGlu receptors may have utility in a variety of clinical conditions[3–5] including epilepsy, anxiety, and schizophrenia.[6] The physiological importance of group II mGlu receptors has been shown by the efficacy of rigid glutamate analogs such as (1*S*,2*S*,5*R*,6*S*)-2-aminobicyclo[3.1.0]hexane 2,6-dicarboxylic acid (**1**, LY354740)[7,8] and (1*R*,2*S*,5*S*,6*S*)-2-amino-6-fluoro-4-oxobicyclo[3.1.0]hexane 2,6-dicarboxylic acid (**2**, MGS0028)[9] in both animal models as well as human clinical trials.[10,11] Both compounds are non-selective mGlu2/3 receptor agonists.



1 (LY354740) **2** (MGS0028) **3** (LY487379)

Agonists selective for mGlu2 over mGlu3 have not, as yet, been discovered, presumably due to the high degree of sequence homology between group II mGlu receptors. Therefore, another strategy for selectivity involves the discovery of allosteric modulators that do not bind at the glutamate binding site.[12] Researchers at Lilly have recently disclosed 3-pyridyl sulfonamides such as **3** (LY487379) as allosteric modulators of the mGlu2 receptor, which do not bind at the glutamate binding site.[13,14] This paper details the discovery and SAR of a new class of mGlu2 receptor selective potentiators.



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Keywords: Metabotropic glutamate receptors; GPCRs.
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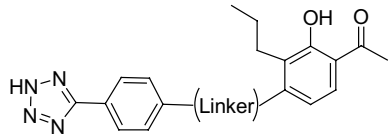
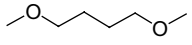
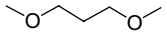
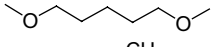
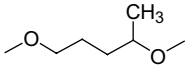
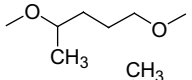
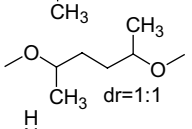
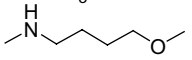
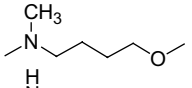
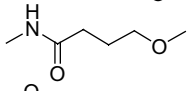
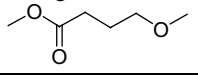
doi:10.1016/j.bmcl.2004.08.020

Screening of the Merck sample collection led to the discovery of compound **4**,[15] which displayed moderate activity as an mGlu2 receptor potentiator, with an EC_{50} of 348 nM and a 31% level of potentiation. Percent potentiation is defined as the response obtained using the test compound plus an EC_{10} of glutamate normalized to the maximal response obtained with glutamate alone.[17] In the absence of glutamate, **4** showed no activity at the mGlu2 receptor, confirming that the compound is a positive allosteric potentiator. Compound **4** displayed a similar profile to the Lilly potentiator **3**, which in our assays had an EC_{50} of 1.7 μM with 52% potentiation. Compound **4**, as well as all of the other compounds described below showed no activity at the human mGlu3 receptor in the presence or absence of glutamate, as well as at the other mGlu receptors (-1, -4, -5, -7, -8) (data not shown). Therefore, our initial goal was to improve both the potency and especially the level of potentiation. Three strategies were employed: (1) investigation of different linkers between the acetophenone and the phenyl tetrazole, (2) modification of the acetophenone moiety, and (3) examination of various tetrazole replacements.

Table 1. Effect of different linkers



Compd	Linker	hmGlu2	
		GTPγS binding EC_{50} (nM)[a]	% Potentiation[b]
3	—	1700	52
4		348	31
5		1418	41
6		3313	75
7		918	76
8		495	67
9		241	52
10		939	35
11		2013	98
12		NA[c]	
13		7517	30

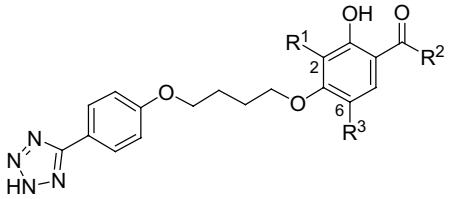
[a] Value represents mean of two or more experiments.
[b] Result expressed as a percentage of the maximal glutamate response at 1 mM.
[c] NA = denotes not active @ 10 μM concentration.

We initially examined the modification of the linker between the aryl tetrazole and the acetophenone to in an attempt to ascertain the requirements for activity and potentiation. These efforts are summarized in Table 1 in which a range of different linkers were explored starting from the initial lead **4**. When the linker was either shortened (**5**) or lengthened (**6**) by one atom, potency decreased dramatically, which initially indicated a very tight SAR. Interestingly, additions of methyl groups either adjacent to the acetophenone (**7**) tetrazole (**8**) or both (**9**) gave a modest increase in the level of potentiation, and in the case of compound **9** a small potency boost also compared to compound **4**. It should be noted that compound **9** is a racemic mixture of diastereomers. This indicates that relatively small modifications of the linker could improve activity. However, other simple modifications of the linker were not beneficial. The inclusion of nitrogen (**10** and **11**) as well as incorporation of an amide (**12**) or ester (**13**) gave significantly lower activity. Whether this is due to an electronic effect or due to conformation is unclear.

The effects of the modification of the acetophenone moiety are detailed in Table 2. From the initial lead **4**, it was found that removing the propyl group at position 2 (**14**) led to loss of activity. Incorporation of a methyl group in the same position resulted in a 10-fold loss of potency (**15**). As with smaller groups such as methyl, larger groups were also not tolerated at this position. For example, a pentyl group (**16**) led to loss of activity. Switching to a bromo substituent (**17**) regained some of the lost potency and also dramatically increased the potentiation (31–98%). The loss in potency shown with a methyl group in position 2 could be compensated for by substituting the ketone with a larger lipophilic group (R^2). Indeed, potency increased from methyl (**15**) to ethyl (**18**) to propyl (**19**) to isobutyryl (**20**). Interestingly, with two larger lipophilic groups (**21**) potency dropped off, indicating that too much steric hindrance around the acetophenone was not optimal. Drawing on the result for compound **17**, wherein a bromine on the acetophenone increases the level of potentiation, a bromo substituent was reincorporated on the aryl ring with the isobutyryl group. We were pleased to find this effect additive, with compound **22** showing an EC_{50} of 93 nM with greater than 100% potentiation. This represents one of the most potent compounds in this series. Further examination of the acetophenone moiety indicated that in contrast to position 2, substitution at position 6 on the phenyl ring (**23**) led to a loss of activity. Likewise, incorporation of cycloalkyl groups on the ketone (R^2) led to compounds that were considerably less potent that compound **22** (data not shown).

Lastly, we investigated potential replacements for the tetrazole in compound **4**. As shown in Table 3, a number of other acidic groups could substitute for the tetrazole. In general, acidic groups with a similar pK_a to the tetrazole were active, albeit with diminished potency. For example, switching to a carboxylic acid (**24**) led to a fivefold drop in potency. Likewise, an acyl sulfonamide (**25**) showed lowered activity compared to compound **4**. Attempts to improve activity by adding

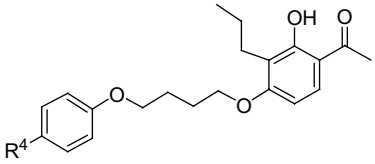
Table 2. Binding affinities for acetophenone modifications

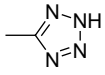
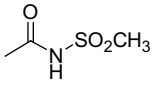


Compd	R^1	R^2	R^3	hmGlu2	
				GTPγS binding EC_{50} (nM)[a]	% Potentiation[b]
4	$-CH_2CH_2CH_3$	$-CH_3$	–H	348	31
14	–H	$-CH_3$	–H	NA[c]	
15	$-CH_3$	$-CH_3$	–H	3365	52
16	$-CH_2(CH_2)_3CH_3$	$-CH_3$	–H	NA[c]	
17	–Br	$-CH_3$	–H	878	99
18	$-CH_3$	$-CH_2CH_3$	–H	380	60
19	$-CH_3$	$-CH_2CH_2CH_3$	–H	304	100
20	$-CH_3$	$-CH_2CH(CH_3)_2$	–H	229	89
21	$-CH_2CH_2CH_3$	$-CH_2CH(CH_3)_2$	–H	421	74
22	–Br	$-CH_2CH(CH_3)_2$	–H	93	128
23	–H	$-CH_3$	$-CH_2CH_2CH_3$	NA[c]	

[a] Value represents mean of two or more experiments.
[b] Result expressed as a percentage of the maximal glutamate response at 1 mM.
[c] NA = denotes not active @ 10 μM concentration.

Table 3. Tetrazole replacements



Compd	R^4	hmGlu2	
		GTPγS binding EC_{50} (nM)[a]	% Potentiation[b]
4		348	31
24	$-CO_2H$	1823	54
25		1041	29
26	$-SO_2NH_2$	NA[c]	—
27	$-CONH_2$	NA[c]	—

[a] Value represents mean of two or more experiments.
[b] Result expressed as a percentage of the maximal glutamate response at 1 mM.
[c] NA = denotes not active @ 10 μM concentration.

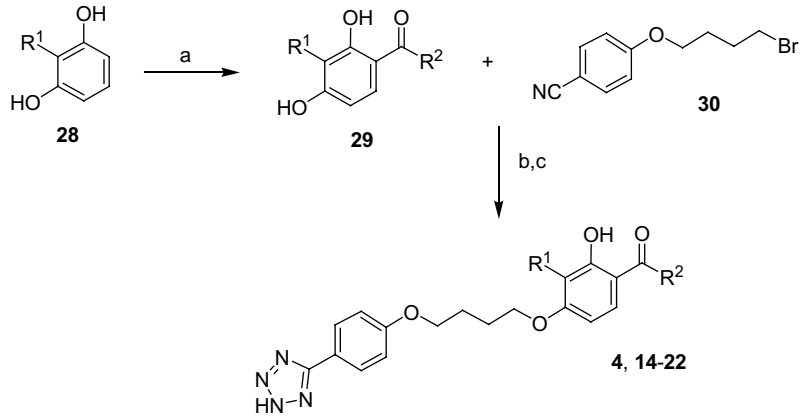
various substituents on the phenyl tetrazole that would modulate the pK_a were unsuccessful (data not shown). Also, the *meta*-substituted phenyl tetrazole gave similar activity, although the *ortho* tetrazole was less active (data not shown). Lastly, compounds without acidic protons were generally not active. For example, sulfonamides (**26**) and simple amides (**27**) had no activity compared to the parent tetrazole. Further work examining tetrazole replacements will be discussed in a subsequent paper.

2. Chemistry

The compounds described in Tables 1 and 2 and were synthesized in a straightforward manner with the representative method shown below (Scheme 1). The synthesis began with 2-substituted resorcinols (**28**), which were reacted with an acid chloride in the presence of aluminum trichloride overnight to give acetophenone derivatives **29**.[18] These were then alkylated selectively using alkyl bromide **30** with potassium carbonate in acetone to give the cyano precursor to the desired compounds. The final products[19] were accessed via a tin catalyzed tetrazole formation using trimethylsilyl azide in refluxing toluene in generally good yields. Compounds **24**–**27** were prepared using a similar alkylation strategy, with a hydrolysis of an ester to give the acid **24** instead of tetrazole formation. Compounds **5**–**13** were synthesized in a similar fashion as outlined in Scheme 1 using the appropriate alkyl bromide equivalent of compound **30**.

3. Conclusion

In summary we have described herein the discovery of phenyl-tetrazolyl acetophenones as potent positive allosteric modulators of the mGlu2 receptor, which have no intrinsic activity in the absence of glutamate. These studies provided us with some understanding of the structural requirements necessary for mGlu2 receptor potentiator activity, and led to potent compounds with high levels of potentiation, for example, compound **22** (EC_{50} = 93 nM, 128% potentiation). This represents a significant improvement over the initial lead **4** as well as compound **3**, which, when tested in house in our



Scheme 1. Reagents and conditions: (a) R^2COCl, $AlCl_3$, CH_2Cl_2; (b) K_2CO_3, acetone, 45 °C; (c) TMS-N_3, Bu_2SnO, toluene, reflux.

assays, displayed a potency of 1.7 μM with 52% potentiation.[14] Further exploration of this series of compounds is in progress and will be disclosed in due course.

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15. Screening of compounds was carried out using a Ca^{2+} flux functional (FLIPR384) assay using a stable cell line co-expressing the human mGlu2 receptors coupled to a promiscuous G-protein (Gα16). Receptor activity was detected by changes in [Ca^{2+}], measured using the fluorescent, Ca^{2+} sensitive dye fura-2. For further information, see: Varney, M. A.; Cosford, N. D.; Jachec, C.; Rao, S. P.; Sacaan, A.; Lin, F. F.; Bleicher, L.; Santori, E. M.; Flor, P. J.; Allgeier, H.; Gasparini, F.; Kuhn, R.; Hess, S. D.; Velicelebi, G.; Johnson, E. C. *J. Pharmacol. Exp. Ther.* **1999**, *290*, 170–181.
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17. For the GTPγS assay, 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 a positive allosteric modulator. Non-specific binding was determined by addition of 10 μM unlabeled GTPγS.
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19. All final compounds displayed spectral data (NMR, MS) that was consistent with the assigned structure.