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Allosteric potentiators of the metabotropic glutamate receptor 2 (mGlu2). Part 2: 4-Thiopyridyl acetophenones as non-tetrazole containing mGlu2 receptor potentiators

Anthony B. Pinkerton,* Rowena V. Cube, John H. Hutchinson, Joyce K. James, Michael F. Gardner, Hervé Schaffhauser, Blake A. Rowe, Lorrie P. Daggett and Jean-Michel Vernier

Merck Research Laboratories, MRLSDB2, 3535 General Atomics Court, San Diego, CA 92121, USA

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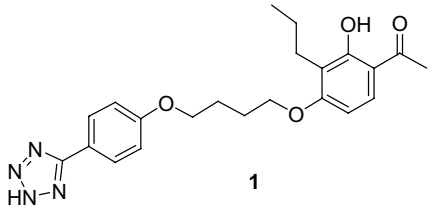
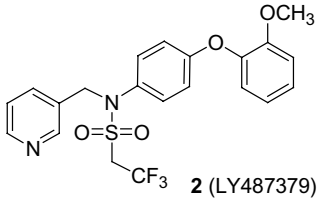
Abstract—We have identified and synthesized a series of 4-thiopyridyl acetophenones as positive allosteric potentiators of the metabotropic glutamate receptor 2. Structure–activity relationship studies directed toward replacement of the tetrazole in the initial lead led to the discovery of **16** (EC_{50} = 340 nM), which showed improved brain penetration over the initial lead.

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 identified a selective mGlu2 receptor potentiator, phenyl-tetrazolyl acetophenone **1** (EC_{50} = 348 nM, 31% potentiation, with potentiation being defined as the response obtained using the test compound up to 10 μM plus an EC_{10} of glutamate normalized to the maximal response obtained with glutamate alone).[15–17] However, this compound, while displaying in vivo activity, needed to be dosed icv due to poor brain penetration. Working on the hypothesis that the tetrazole, or other acidic functionality, was



1



2 (LY487379)

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* Corresponding author. Tel.: +1 858 202 5379; fax: +1 858 202 5752; e-mail: anthony_pinkerton@merck.com

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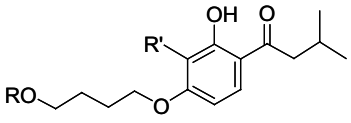
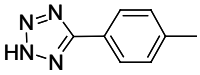
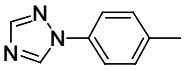
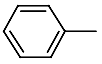
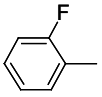
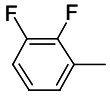
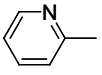
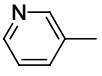
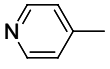
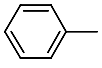
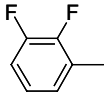
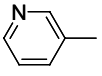
responsible for the poor brain penetration, we investigated the replacement of the tetrazole with a variety of groups that would maintain potency. Concurrent to this work, researchers at Lilly recently disclosed a structurally distinct class of mGlu2 receptor potentiators (e.g., **2**, $EC_{50} = 1700\,nM$, 52% potentiation), which, unlike compound **1**, contains no acidic protons.[13] This paper outlines the discovery of a new class of brain penetrant, nontetrazole containing mGlu2 receptor potentiators from the initial tetrazole containing lead **1**.

2. SAR studies

Our starting point for the SAR, as outlined in Table 1, was the previously described lead (**3**), which displayed both good activity (229 nM) as well as level of potentiation (89%).[16] Attempts at replacing the tetrazole with other 5 membered heterocycles gave mostly inactive or very weakly active compounds. For example, *N*-linked triazole **4** had a 20-fold drop in potency from compound **3**. Removing the tetrazole group altogether (**5**) also led

Table 1. Binding affinities for tetrazole replacements



Compd	R	R′	hmGlu2	
			GTPγS binding EC_{50} (nM)[a]	% Potentiation[b]
1	—	—	348	31
2	—	—	1700	52
3		–CH_3	229	89
4		–CH_3	4237	18
5		–CH_3	4550	24
6		–CH_3	1987	30
7		–CH_3	384	18
8		–CH_3	3273	33
9		–CH_3	1717	46
10		–CH_3	3855	40
11		–Br	1500	53
12		–Br	558	88
13		–Br	575	67

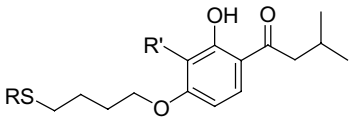
[a] Value represents mean of two or more experiments.
[b] Result expressed as a percentage of the maximum glutamate response at 1 mM.

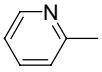
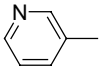
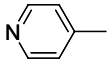
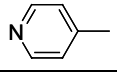
to a 20-fold drop in potency. This result led us to investigate a large number of simple substitutions on the aromatic ring (halogen, methyl, methoxy), which gave compounds that were either inactive or similar in activity to phenyl derivative **5** (data not shown). The most potent compounds to arise from these permutations were the mono fluorophenyl derivative **6** and the 2,3-difluorophenyl derivative **7**. In the case of compound **7** good potency was observed (384 vs 229 nM for the tetrazole compound **3**) albeit with low potentiation. The disclosure from researchers at Lilly of pyridine derivatives such as compound **2** led us to investigate the incorporation of pyridine in hope of accessing an additional binding site. Both 2- and 4-pyridyl derivatives **8** and **10** did not show improved potency. However, 3- and derivative **9** showed a 2-fold increase in potency and also a modest (46%) level of potentiation compared to the simple phenyl derivative **5**. Previous work[16] showed that replacement of the methyl substituent on the phenyl ring with a bromine led to an increase in potency and potentiation. Gratifyingly, when an acetophenone moiety containing a bromine in the place of a methyl was examined, even better potencies and levels of potentiation were obtained. This was the case both for the simple phenyl derivative **11**, and, more importantly, the 3-pyridyl compound **13**, which displayed potency and potentiation approaching that of the tetrazole containing lead **3**. Likewise, 2,3-difluorophenyl derivative **12** showed good potency and high levels of potentiation. However, compound **12**, along with compound **7** were not pursued further due to poor rat PK properties (see Table 4).

Due to relatively poor aqueous solubility for compound **13**, we turned our attention to examining sulfur linked pyridines in place of oxygen linked pyridines (Table 2). Interestingly, with a sulfur linkage, the potency of 2-, 3-, and 4-pyridyl derivatives were increased for **14**–**16** although the level of potentiation was lower. For example, 3-pyridyl *O*-linked potentiator **9** displayed potency of 1717 versus 1093 nM for **15**, but the relative potentiation was 26% for **9** and 46% for compound **15**. The implications of this disparity are not understood at the present time. Importantly, 4-thiopyridyl derivative **16** was found to have potency of 340 nM, which is similar potency to the more active tetrazole containing leads. It was also soluble in water as the HCl salt. Compound **16**, therefore, became a compound suitable for further investigation (vide infra). Contrary to previous results, incorporating the bromine into the acetophenone moiety for this compound gave a compound (**17**) that was less potent than **16**, albeit with considerably higher potentiation (82% vs 33%). Oxidation of the sulfur in compound **16** to the sulfoxide and sulfone gave compounds that displayed much diminished potency. Likewise the *N*-oxide of compound **16** had decreased potency (data not shown).

With the result of compound **16** in hand, we then sought to further optimize the activity of the 4-thiopyridine compounds through modification of the linker (Table 3). When the linker was either shortened (**18**) or lengthened (**19**) by one carbon, inactive compounds were obtained, which initially seemed to indicate a very tight SAR. However, we were subsequently pleased to find that a number of aryl linkers could be incorporated to give active compounds. For example, bis-benzylic linked compounds **20** and **21** showed both good potency and potentiation. In particular, compound **20**, with activity of 819 nM and 68% potentiation was investigated further for PK in rats. Likewise, mono-benzylic compounds **22**–**24** showed similar levels of potency. Incorporating a *t*-butyl group α to the ketone in place of the isopropyl group gave a compound, **25**, that

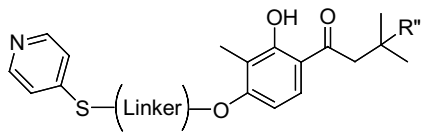
Table 2. Activity of thiopyridines

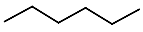
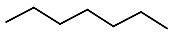
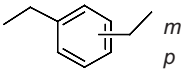
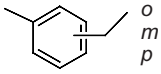
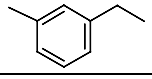


Compd	R	R′	huGlu2	
			GTPγS binding EC_{50} (nM)[a]	% Potentiation[b]
14		$-CH_3$	1532	21
15		$-CH_3$	1093	26
16		$-CH_3$	340	33
17		–Br	676	82

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

Table 3. Effect of different linkers



Compd	Linker	R″	huGlu2	
			GTPγS binding EC_{50} (nM)[a]	% Potentiation[b]
16		–H	340	33
18		–H	NA[c]	—
19		–H	NA[c]	—
20	 *m*	–H	819	68
21	*p*	–H	770	78
22	 *o*	–H	677	38
23	*m*	–H	890	58
24	*p*	–H	1060	38
25		$–CH_3$	578	79

[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.

displayed improved potency and level of potentiation (79%). This compound was also examined for its rat PK properties.

3. PK properties

With a number of potent thiopyridine compounds in hand, we then examined the brain/plasma ratio in rats for several of these compounds. For reference, the brain/plasma ratios for tetrazole compounds **1** and **3** are included. As shown in Table 4 the brain to plasma ratios for tetrazoles **1** and **3** were extremely low (<5%). LY487379 (**2**) displayed modest brain/plasma ratios, although considerably better than tetrazole leads **1** and **3**. In contrast, thiopyridine **16** displayed an excellent brain/plasma ratio after sc dosing. Gratifyingly, the brain/plasma ratios for compounds **20** and **25** were also much improved, giving brain levels close to or exceeding the EC_{50} at 2 h post dosing.

Table 4. Selected rat PK parameters and brain/plasma ratios

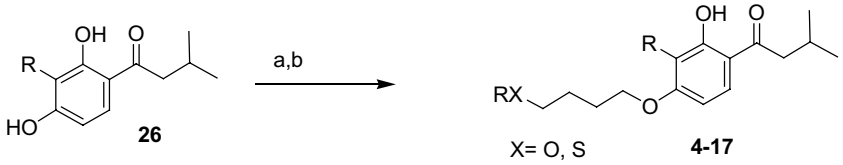
Compd	Cl[a] (mL/min/kg)	%F[a]	Brain/plasma[b]	Brain level[b] (nM)
1	33	63	0.01	—
2	70	<1	0.15	10
3	15	59	0.03	—
12	75	0	ND[c]	ND[c]
16	33	3	1.10	400
20	ND[c]	ND[c]	1.20	330
25	ND[c]	ND[c]	0.21	340

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

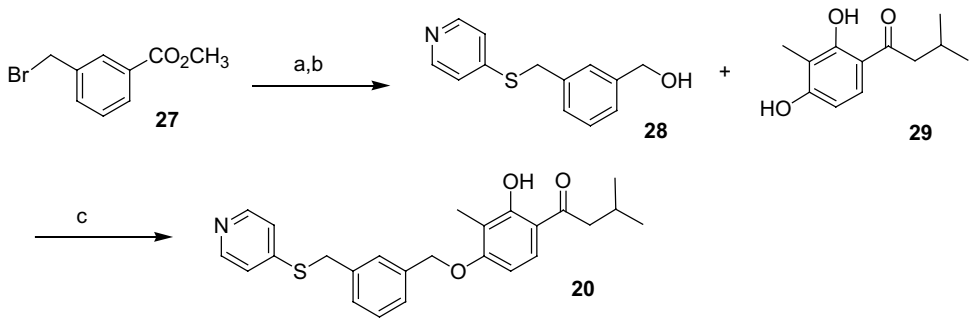
4. Chemistry

The compounds described in Tables 1 and 2 were synthesized as described below (Scheme 1). The synthesis began with acetophenone derivatives **26**,[15,16] which were first alkylated selectively with 1,4-dibromobutane. Compounds **4**–**17** were then made via alkylation of the appropriate phenol or thiophenol in good yields.[18]

The compounds in Table 3 were synthesized in a manner exemplified by the example of compound **20** (Scheme 2). Beginning from commercially available 3-(bromo-



Scheme 1. Reagents and conditions: (a) 1,4-dibromobutane, K_2CO_3, acetone, 45 °C; (b) RXH, K_2CO_3, acetone, 45 °C.



Scheme 2. Reagents and conditions: (a) 4-mercaptopyridine, K_2CO_3, acetone, 45 °C, 85%; (b) $LiAlH_4$, THF, 0 °C, 80%; (c) DTAD, PPh_3, THF, 43%.

methyl) methyl benzoate (**27**), 4-mercaptophenol was added to give the thioether. The ester was then reduced using lithium aluminum hydride in tetrahydrofuran. The resulting benzylic alcohol **28** was then reacted with acetophenone **29** under Mitsunobu conditions[19] using di*tert* butylazodicarboxylate and triphenylphosphine to give the desired product in moderate yield. The other compounds in Table 3 were synthesized from 4-(bromomethyl) methyl benzoate (for **21**), or *ortho*-, *meta*-, and *para*-(pyridin-4-ylthio)phenyl]methanol (for **22**, **23**, and **24**), respectively. Compounds **18** and **19** were synthesized from 1,3-dibromobutane or 1,5-dibromobutane as illustrated in Scheme 1.

5. Conclusion

In summary we have described herein the discovery of a new class of allosteric modulators of the mGlu2 receptor, in which the phenyl tetrazolyl group of the lead structure has been replaced by a 4-thiopyridine group. This advancement has increased our understanding of the structural variations allowed for mGlu2 receptor activity and has led to a brain penetrant class of potentiators. The lead compounds from this series, **16** and **20** showed brain/plasma levels > 1 and had potencies of 340 and 819 nM with potentiation levels of 33% and 68%, respectively. The compounds, therefore, had potency comparable to the original tetrazole containing lead as well as greater potency compared to **2** (LY487379, EC_{50} = 1700 nM, 52% potentiation). These compounds, along with the other compounds outlined herein, were selective for mGlu2 over mGlu3 as well as the other mGlu receptors. Further work will focus on continued optimization as well as use of these potentiators in vivo models.

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17. The effect of these compounds was characterized in the [^{35}S]-GTPγS binding assay using a cell line expressing human mGlu2 receptor. See Ref. 14 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 a positive allosteric modulator. Nonspecific binding was determined by the addition of 10 μM unlabeled GTPγS.
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