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3-(2-Ethoxy-4-{4-[3-hydroxy-2-methyl-4-(3-methylbutanoyl)-phenoxy]butoxy}phenyl)propanoic acid: a brain penetrant allosteric potentiator at the metabotropic glutamate receptor 2 (mGluR2)

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Abstract—We have identified and synthesized a brain penetrant propanoic acid as an allosteric potentiator of the metabotropic glutamate receptor 2. Structure–activity relationship studies directed toward improving the potency, level of potentiation and brain penetration led to the discovery of **8** (EC_{50} = 1200 nM, 77% potentiation, 119% brain/plasma in rat, 20 mpk ip, brain level of 5700 nM).

1. Introduction

The neurotransmitter, glutamate, plays an important role in a wide variety of CNS functions, exerting its effect on both the ionotropic glutamate receptors, which are glutamate-gated ion channels, as well as the metabotropic glutamate receptors (mGlu), which are in the class of G-protein coupled receptors. Eight subtypes of the mGlu receptors fall into three main groups.[1,2] Group I consists of mGlu1 and −5, which have mainly been shown to be stimulatory. Groups II (mGlu2 and −3) and III (mGlu 4, −6, −7, −8), however, are often concentrated presynaptically and generally inhibit neurotransmission. Therefore, agents targeting the mGlu receptors may have utility in a variety of diseases[3–5] including epilepsy, anxiety, and schizophrenia.[6] The physiological importance of group II mGlu receptors has been shown by the efficacy of a rigid glutamate analogs such as (1*S*,2*S*,5*R*,6*S*)-2-aminobicyclo[3.1.0]hexane 2,6-dicarboxylic acid[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[9] in both animal models as well as human clinical trials.[10,11] Both compounds are non-selective mGlu2/3 receptor agonists. Due to the high degree of sequence homology between group II mGlu receptors, selective agonists for mGlu2 over mGlu3 have not, as yet, been discovered. Therefore, another strategy for selectivity involves the discovery of allosteric modulators that do not bind at the glutamate binding site.[12–14] This paper details the discovery and SAR of a class of selective mGlu2 receptor potentiators.

Screening[15–17] of the Merck sample collection led to the discovery of tetrazole **1**, which displayed moderate activity as an mGluR2 potentiator. Utilizing tetrazole **1** as the lead compound, three strategies were employed to improve the potency, level of potentiation and brain penetration. The first strategy focused on the modification of the acetophenone moiety, leading to the discovery of tetrazole **2**.[18] The second strategy investigated different linkers between the acetophenone and the phenyl tetrazole functional groups.[18] The third strategy focused on the replacement of the tetrazole functionality with the goal of increasing brain penetration and hence improving the pK profile of the compounds. A variety of tetrazole replacements were examined, which led to

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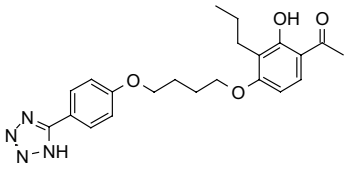
the discovery of 4-thiopyridyl acetophenone (**3**) as a potent mGluR2 potentiator.[19] During these efforts, 7-hydroxycoumarin (**4**) was also found to be a suitable tetrazole replacement. (EC_{50} 1400 nM, 45% potentiation, with potentiation being defined as the response obtained using the test compound up to 10 μM plus an EC_{10} of glutamate normalized to the maximal response obtained with glutamate alone.) Compound **4** and the other analogs described below only showed activity at mGluR2 with no activity observed for mGluR3 as well as the other mGluRs. Herein we describe the detailed efforts on the replacement of the tetrazole with 7-hydroxycoumarin (**4**), chromanone, and propanoic acid functionalities.



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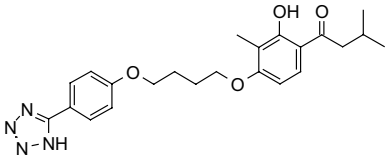

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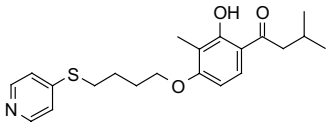

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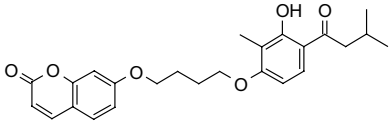

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2. Biology

Table 1 illustrates the SAR modifications of lead compound **4**. Reduction of the 7-hydroxy-coumarin moiety to lactone **5** gave a favorable result of enhancing both the binding affinity (406 nM) and level of potentiation of 67% as compared to **4**. Reduction and hydrolysis of the 7- hydroxycoumarin moiety to the carboxylic acid–phenol **6** also led to an improvement in potency when compared to lead **4** (1400–801 nM), but unfortunately no substantial increase in level of potentiation (45–66%) was observed. The same result was again seen for methyl ester **7** with an increase in binding affinity of 478 nM, but no substantial increase in level of potentiation at 56%. Ethers **8** and **9** did not give more potent compounds than the lead **4** (1180 and 2200 nM, respectively), but their high levels of potentiation (77% and 173%, respectively), justified further study. Replacement of the methyl group with a bromide at the acetophenone portion of the molecule as in compound **10** gave a threefold increase in binding (1400–360 nM) and an almost twofold increase in level of potentiation (45–73%) as compared to lead **4**. These positive results encouraged us to further investigate the modifications of the 7-hydroxycoumarin moiety.

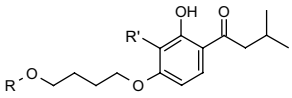
To single out which specific groups in the 7-hydroxycoumarin functionality were required for potency, a subsequent SAR study focused on two subseries: (1) the simple propionic acid analogs and (2) the fused six-membered ring ethers. The first subseries studied how the four atom chain affected the activity without the phenol or ether moiety present. The second subseries focused on cyclic ethers and simple phenol analogs.

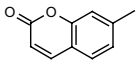
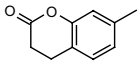
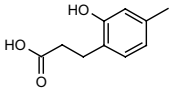
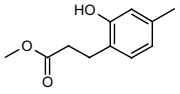
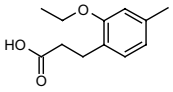
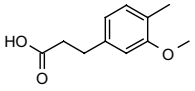
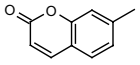
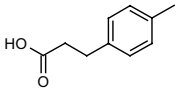
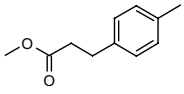
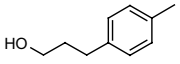
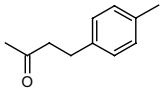
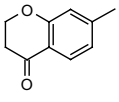
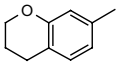
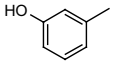
Removal of the phenol moiety as in acid **11** when compared to the phenol–acid **6** gave mixed results: a loss in potency (801–1450 nM), but a slight increase in potentiation level (66–82%). Comparison of the methyl esters **12** and **7** showed a decrease in potency (478–3619 nM) and no change in potentiation (56–54%). These results indicate the roles of the phenol and the carboxylic acid/ester are additive for binding affinity, but not necessarily additive for level of potentiation. Minimal activity in both potency and level of potentiation were observed in the propanol analog **13** and the 2-butanone compound **14**. Likewise, modification to the cyclic ethers **15** and **16** gave weakly potent compounds. Surprisingly, the simple phenol **17**, although showing a weak binding affinity of 1580 nM, gave a decent level of potentiation of 69%. From these results, an acidic proton either from a phenol or carboxylic acid is required to maintain a moderate level of potentiation (>50%).

Subsequent testing of all compounds listed in Table 1 for mGluR3 potency showed no activity (>10,000 nM), further providing evidence that potentiators can be used to enhance selectivity for mGluR2 over mGluR3.

Pharmacokinetic data of the tetrazole lead **2** and selected coumarin analogs (**6**, **8**, and **10**) are shown in Table 2. Both tetrazole **2** and phenol/carboxylic acid **6** showed a brain to plasma ratio of less than 3%. Capping of the phenol as in ethyl ether **8**[20] gratifyingly increased the brain to plasma ratio to 1.19 with a brain level of 5700 nM, the highest level of brain penetration of the series. At this point it is unclear what the causes for the improved brain penetration for compound **8** are. None of the compounds tested (**2**, **6**, **8**, **10**) were found

Table 1. Binding affinities for tetrazole replacements



Compounds	R	R′	GTPγS binding EC_{50} (nM)[a]	% Potentiation[b]
2	—	—	229	89
3	—	—	340	33
4		–CH_3	1400	45
5		–CH_3	406	67
6		–CH_3	801	66
7		–CH_3	478	56
8		–CH_3	1180	77
9		–CH_3	2200	173
10		–Br	360	73
11		–CH_3	1450	82
12		–CH_3	3619	54
13		–CH_3	3200	39
14		–CH_3	1100	28
15		–CH_3	869	34
16		–CH_3	1090	29
17		–CH_3	1580	69

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

to be Pgp substrates. It should be noted that the aqueous solubility for compound **8** was considerably higher than for phenol/acid **6** and tetrazole **2**; thus a higher plasma level was obtained for compound **8** compared to compound **6**. Interestingly, this was not the case for tetrazole **2**, which had high plasma levels but essentially no brain

Table 2. Rat PK profile comparisons between tetrazole **2** and coumarin analogs **6**, **8** and **10**

Compound	Cl[a] (mL/min/kg)	Vd (L/kg)	$t_{1/2}$ (h)	%F[a]	Plasma[b] (nM)	Brain[b] (nM)	Brain/plasma[b]
2	15	0.5	1.5	63	8200	<LOQ[d]	<0.03
6	ND[c]	ND[c]	ND[c]	ND[c]	1380	<LOQ[d]	<0.03
8	ND[c]	ND[c]	ND[c]	ND[c]	4789	5700	1.19
10	18	1.81	2.4	0	66	<LOQ[d]	<0.03

[a] Dosed 2 mpk iv ($n = 2$) and 10 mpk po ($n = 3$) in Sprague-Dawley rats.
[b] Dosed 20 mpk ip ($n = 3$) in Sprague-Dawley rats, animals sacrificed at 2 h and brain and plasma levels analyzed.
[c] Not determined.
[d] Limit of quantification.

penetration. This effect was observed for the other tetrazole containing compounds.[18,19] One potential explanation for the high brain levels for **8** is an unidentified transporter. The most potent coumarin analog, bromide **10**, unfortunately showed poor pK characteristics presumably due to its very poor aqueous solubility.

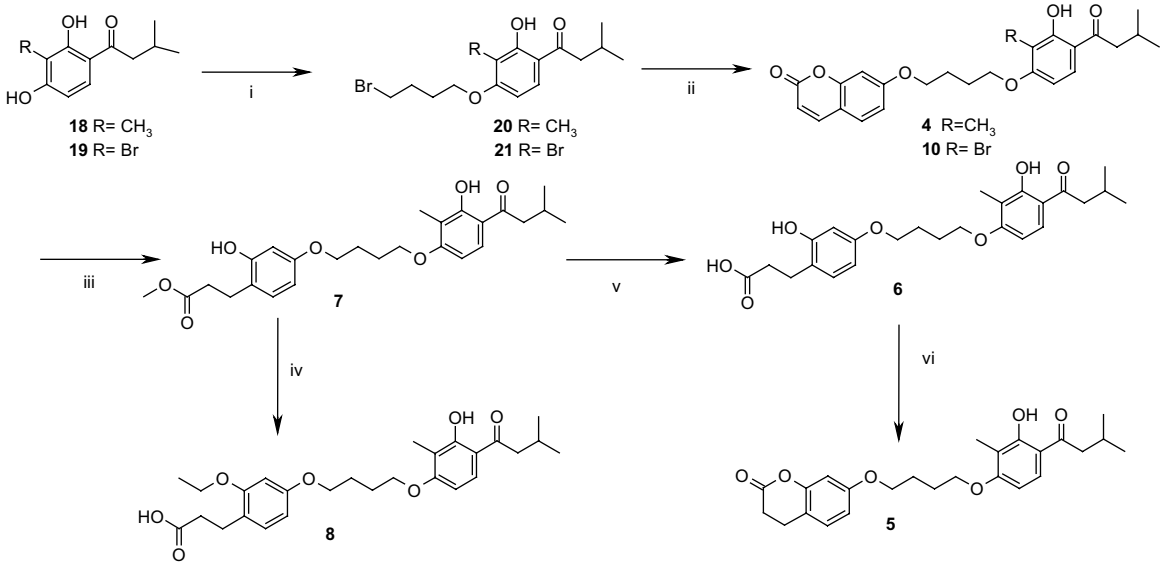
3. Chemistry

The synthesis for the 7-hydroxycoumarin compound **4** and its analogs described herein is outlined in Schemes 1 and 2.[21] The acetophenone derivatives **18** and **19**, were alkylated with 1,4-dibromobutane, using potassium carbonate in acetone to afford the precursors **20** and **21**, respectively. 7-Hydroxycoumarin was alkylated by these precursors to give compounds **4** and **10**. Compound **4** was further hydrogenated in methanol to afford the methyl ester **7**. Hydrolysis of **7** with lithium hydroxide produced the carboxylic acid **6**, which was then lactonized using *p*-toluenesulfonic acid in refluxing benzene to give the reduced coumarin analog **5**. The alkylation of phenol **7** with iodoethane and subsequent ester hydrolysis afforded compound **8**.[20] To synthesize compounds **9**, **11**–**14**, and **18**, intermediate **20** was alkylated with the appropriate phenols utilizing conditions similar to those outlined in Scheme 1.

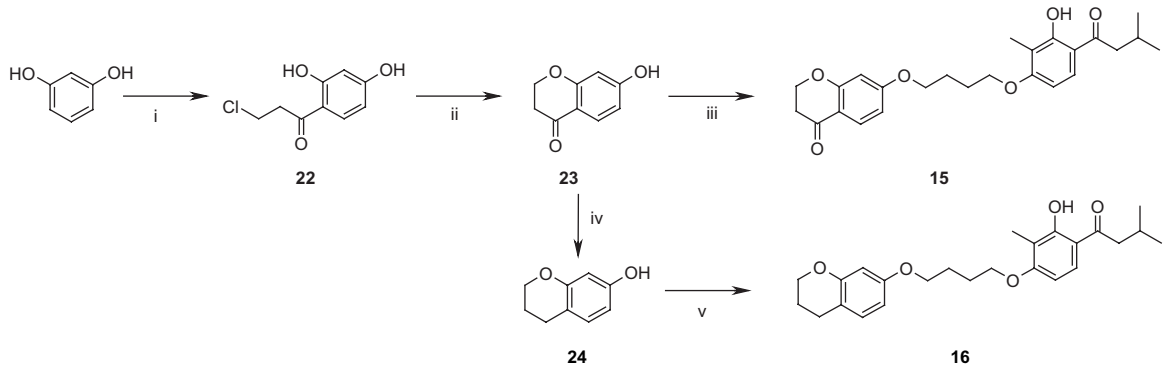
The synthesis of compounds **15** and **16** began with a reaction of resorcinol with 3-chloropropionic acid to give the chloride **22** (Scheme 2).[22] Intramolecular cyclization of chloride **22** afforded ketone **23**, which was then alkylated with bromide **20** to afford ketone **15**. Hydrogenation of intermediate **23** produced the cyclic ether **24** as the only product. Compound **24** was then further alkylated with bromide **20** to give compound **16**.

4. Conclusion

In summary, we have disclosed a new series of allosteric potentiators at the mGlu receptor 2 by replacing the tetrazole moiety of the lead compound **2** with 7-hydroxycoumarin type analogs. SAR strategies led to compounds with moderate potency, high levels of potentiation and good brain penetration. Although the lead tetrazole **2** showed good potency of 229 nM and a high level of potentiation of 89%, this compound was not brain penetrant. From this SAR, we have found a highly brain penetrant compound in ether **8** with a brain to plasma ratio of 1.19 with brain levels at 5700 nM. Ether **8**, although less potent at 1180 nM, shows a high level of potentiation of 77%. This led us to further analyze **8** and other coumarin type analogs for full pK and in vivo profiles.



Scheme 1. Reagents and conditions: (i) dibromobutane, K_2CO_3, acetone, 40 °C, 75%; (ii) 7-hydroxycoumarin, K_2CO_3, acetone 40°C, 75%; (iii) 10% Pd/C, MeOH, H_2, 1 atm, 8 h, 97%; (iv) (a) iodoethane, K_2CO_3, acetone, 40°C, 42%, (b) 2.5 N lithium hydroxide, dioxane/water, 4 h, 96%; (v) 2.5 N lithium hydroxide, dioxane, rt, 4 h, 98%; (vi) *p*-TsOH, benzene, reflux, 2 h, 65%.



Scheme 2. Reagents and conditions: (i) 3-chloropropionic acid, triflic acid, 80 °C, 60%; (ii) 2.0 N sodium hydroxide, 87%; (iii) **20**, K_2CO_3, acetone, 40 °C, 42%; (iv) 10% Pd/C, ethyl acetate, H_2, 1 atm, 12 h, 100%; (v) **20**, K_2CO_3, acetone, 40 °C, 46%.

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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.
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17. The effect of these compounds were confirmed and further characterized in the [^{35}S]-GTPγS binding assay. First, an EC_{10} (1.0 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. See Ref. 14 for a detailed description of this assay.
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20. Proton NMR of compound **8** is consistent with proposed structure. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 13.03 (s, 1H), 7.63–7.61 (d, 1H), 7.07–7.05 (d, 1H), 6.46–6.40 (m, 3H), 4.16–4.12 (m, 2H), 4.06–4.00 (m, 4H), 2.92–2.89 (m, 2H), 2.79–2.78 (d, 2H), 2.68–2.64 (m, 2H), 2.30–2.28 (m, 1H), 2.13 (s, 3H), 2.07–2.00 (m, 4H), 1.44–1.41 (t, 3H), 1.03–1.02 (d, 6H). HRMS 473.2546 (M^+), 495.2337 (M^++Na). HPLC–MS showed greater than 95% purity for compound **8**.
Strong hydrogen bonding between the phenol proton and the ketone greatly downshifts the phenol proton singlet to 13 ppm, a diagnostic signal in the proton NMR for all of the analogs described herein. This singlet is observed in compound **8**. Therefore, we conclude that ethylation occurs regioselectively as specified.
21. All final compounds displayed spectral data (^{1}H NMR, HPLC–MS) consistent with the assigned structure (purity >95%).
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