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Letter

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Synthesis and Evaluation of Metabotropic Glutamate Receptor Subtype 5 Antagonists Based on Fenobam

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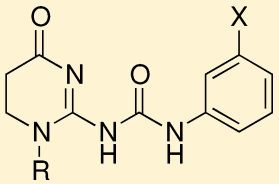
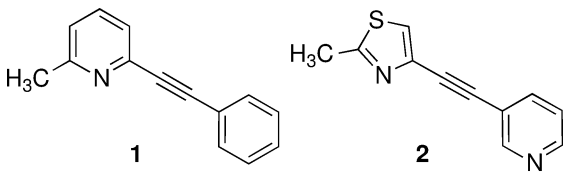
Supporting Information

ABSTRACT: In an effort to discover potent and selective metabotropic glutamate receptor subtype 5 (mGluR5) antagonists, 15 tetrahydropyrimidinone analogues of 1-(3-chlorophenyl)-3-(1-methyl-4-oxo-4,5-dihydro-1*H*-imidazol-2-yl)-urea (fenobam) were synthesized. These compounds were evaluated for antagonism of glutamate-mediated mobilization of internal calcium in an mGluR5 in vitro efficacy assay. The IC_{50} value for 1-(3-chlorophenyl)-3-(1-methyl-4-oxo-1,4,5,6-tetrahydropyridine)urea (**4g**) was essentially identical to that of fenobam.



KEYWORDS: *Fenobam, metabotropic glutamate receptors, drug addiction, mGluR5 antagonist*

Metabotropic glutamate receptor subtype 5 has been linked to various central nervous system disorders including addiction, pain, fragile X syndrome, depression, and anxiety.[1−5] The discovery of 3-methyl-6-(phenylethynyl)-pyridine (MPEP; **1**) and (2-methyl-1,3-thiazol-4-yl)-ethynylpyridine (MTEP; **2**) as potent and selective metabotropic glutamate receptor 5 (mGluR5) antagonists has led to



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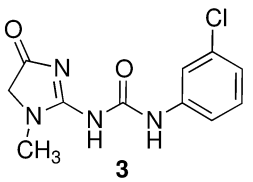

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intensive investigations focused on developing new analogues.[6−9]

During the 1970s and 1980s, McNeil Laboratories developed fenobam (**3**) as a potential anxiolytic but with a then unknown molecular target.[10−12] Apparently because of mixed results from clinical studies, fenobam's further development was discontinued.[10−13] In 2005, fenobam (**3**) was shown to be a potent and selective mGluR5 antagonist prompting structure−activity relationship studies using fenobam as the lead compound.[14−17] Despite considerable progress in the search for mGluR5 antagonists, identification of potent mGluR5 antagonists with better drug-like properties remains a challenge.

In this paper, we describe the synthesis of a novel class of compounds (**4a−m**) containing a tetrahydropyrimidinone moiety as a replacement of the imidazolinone moiety in fenobam (**3**). This class of compounds has been proposed to possess anxiolytic properties in patent literature,[18] but neither chemical properties nor their activity as mGluR5 antagonists has ever been explored.

The tetrahydropyrimidinone series of compounds **4a−m** was synthesized as outlined in Scheme 1. First, **5a−c** were prepared as described in literature procedures.[19] Condensation of commercially available phenyl isocyanates **6a−f** with **5a−c** in chloroform provided target compounds **4a−m** in moderate to good yields after recrystallization from ethanol. (See Table 1 for % yields.)

Alteration of the urea linkage in **4g** with thiourea linkage was achieved by reaction of **5b** with 3-chlorophenylisothiocyanate **7** to provide **8** (Scheme 2). The reaction of **9** with 3-chlorophenyl isocyanate **6a** provided **10** in 45% yield.

The 15 tetrahydropyrimidinone analogues of fenobam **4a−m**, **8**, and **10** were evaluated for antagonism of glutamate-mediated mobilization of internal calcium in an mGluR5 in vitro efficacy assay. Compounds were tested for in vitro efficacy using Chinese hamster ovary (CHO-K1) cells stably transfected with the human mGluR5 complementary DNA (cDNA). Ca^{2+} was measured using a FlexStation3 (Molecular Devices Corp.). IC_{50} values were calculated from a four-parameter logistic equation fit to the calcium flux concentration−response data using Prism (GraphPad Software, San Diego, CA). All compounds fully antagonized the action of glutamate and did not display agonist activity at 10 μM. The IC_{50} values for **4a−m**, **8**, and **10** along with IC_{50} value for the standard mGluR5 antagonist fenobam are listed in Table 1. The tetrahydropyrimidinone analogues **4a−f**, which do not have an *N*-methyl, had

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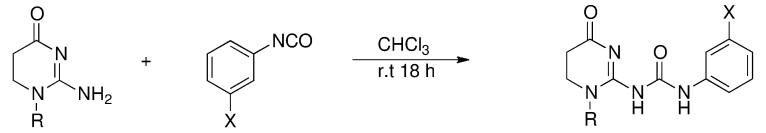
Scheme 1. Synthesis of Tetrahydropyrimidinone Series of Compounds 4a−m



5a; R = H
5b; R = CH_3
5c; R = CH_2CH_3

6a: X = Cl
6b: X = Br
6c; X = F
6d; X = CH_3
6e; X = CF_3
6f; X = CN

4a; R = H; X = Cl
4b; R = H; X = Br
4c; R = H; X = F
4d; R = H; X = CH_3
4e; R = H; X = CF_3
4f; R = H; X = CN
4g; R = CH_3; X = Cl
4h; R = CH_3; X = Br
4i; R = CH_3; X = F
4j; R = CH_3; X = CH_3
4k; R = CH_3; X = CF_3
4l; R = CH_3; X = CN
4m; R = CH_2CH_3; X = Cl

Table 1. Inhibition of Human mGluR5-Mediated Intracellular Calcium Mobilization Values for Compounds 3, 4a−m, 8, and 10

compd	yield (%)[a]	IC_{50} (nM)[b]
3		43.0 ± 5.20
4a	78	8700 ± 3600
4b	75	9300 ± 1300
4c	71	9200 ± 480
4d	25	3900 ± 2100
4e	70	6000 ± 1400
4f	79	4000 ± 1700
4g	24	50.0 ± 10.0
4h	52	96.0 ± 21.0
4i	38	2400 ± 310
4j	35	870 ± 360
4k	24	1200 ± 280
4l	27	100 ± 30.0
4m	65	250 ± 35.0
8	40	570 ± 100
10	45	850 ± 81.0

[a]Yield for syntheses **4a−m** from reaction of **5a−c** with **6a−f**, synthesis of **8** from reaction of **5b** with **7**, and synthesis of **10** from reaction of **9** with **6a**. [b]The data represent the means ± SEs from at least three independent experiments except **4d** and **8** where $n = 2$.

weak antagonist efficacy at the mGluR5 receptor. The most potent analogue was the 3-methylphenyl substituted analogue **4d**, which had an IC_{50} = 3900 nM. N-Methylation of the tetrahydropyrimidinone ring in **4a−f** to give compounds **4g−l**, respectively, resulted in increased antagonist efficacy. The 3-chlorophenyl *N*-methyltetrahydropyrimidinone fenobam analogue **4g** with an IC_{50} = 50 nM had an antagonist efficacy similar to that found for fenobam (IC_{50} = 43.0 nM). However, changing the chloro group in **4g** to other substituents did not improve the potency, although the bromo and cyano analogues (**4h** and **4l**) were in the same potency range (96.0 and 100 nM, respectively). Replacing the *N*-methyl group in **4g** with an *N*-ethyl group to obtain **4m** and alteration of the urea linkage with thiourea to obtain **8** led to a significant reduction in antagonist efficacy. Even adding a double bond to the tetrahydropyrimidinone ring of **4g** to give **10** resulted in significant decrease in potency. Because pharmacokinetic (PK) studies in humans have indicated variable plasma levels for fenobam,[20] even small changes in the structure of fenobam (**3**) could lead to a compound with better drug-like properties. While beyond the scope of this letter, future PK and metabolism studies will help reveal the potential of **4g** as a useful mGluR5 antagonist.

In summary, 15 tetrahydropyrimidinone fenobam analogues were synthesized and tested for antagonism at mGluR5. The meta-substituted 3-chloro-, 3-bromo-, and 3-cyano-analogues **4g**, **4h**, and **4l** possessed low nanomolar IC_{50} values in an mGluR5 efficacy assay. Thus, these compounds, particularly **4g**, represent new lead structures for the development of pharmacotherapies for treating addiction and other central nervous system disorders.

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures for the synthesis and elemental analysis data for **4a−m**, **8**, and **10**. This material is available free of charge via the Internet at http://pubs.acs.org.

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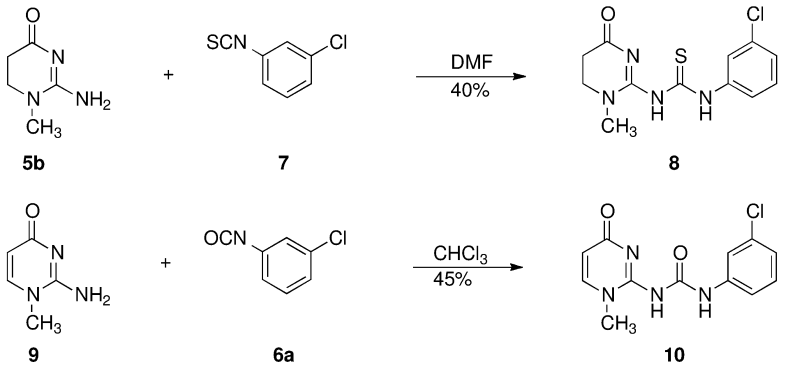
Scheme 2. Synthesis of Fenobam Analogues 8 and 10



5b **7** **8**

9 **6a** **10**

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Notes

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■ ABBREVIATIONS

CHO-K1, Chinese hamster ovary; mGluR5, metabotropic glutamate receptor 5; MPEP, 3-methyl-6-(phenylethynyl)-pyridine; MTEP, (2-methyl-1,3-thiazol-4-yl)ethynylpyridine; PK, pharmacokinetic; tPSA, total polar surface area; cDNA, complementary DNA

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