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Synthesis and SAR of 2-aryl pyrido[2,3-*d*]pyrimidines as potent mGlu5 receptor antagonists

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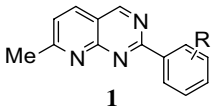
Abstract—A novel series of potent 2-aryl pyrido[2,3-*d*]pyrimidine mGlu5 receptor antagonists are described. The synthesis and pharmacological activities of these analogs are discussed.

Non-competitive metabotropic glutamate receptor 5 (mGlu5) antagonists are viewed as having promise against a number of CNS and peripheral diseases including the treatment of pain, anxiety, gastro-esophageal reflux disease (GERD), Fragile X, and Parkinson's disease.[1] The mGlu5 receptor is one of eight known metabotropic glutamate receptors which comprise a family of G-protein-coupled receptors categorized into three groups based on their sequence homology, pharmacology, and preferred signal transduction mechanism. Group I mGlu receptors (mGlu1 and mGlu5) are positively coupled to phosphatidylinositol hydrolysis/Ca^{2+} mobilization, while group II (mGlu2 and mGlu3) and group III (mGlu receptors 4, 6, 7, and 8) are both coupled in an inhibitory manner to adenylyl cyclase.[2]

MPEP (2-methyl-6-(2-phenylethynyl)pyridine) was developed as a non-competitive mGlu5 receptor antagonist as part of a Sibia–Novartis collaboration and subsequently has been reported to show positive effects in rodent models of anxiety and pain.[3] Structurally, metabotropic glutamate receptors have a large extracellular glutamate-sensing domain coupled to a 7-transmembrane region, and a small intracellular domain that couples to the G-protein. Studies have indicated that MPEP exerts its non-competitive antagonist effect by binding in the 7-transmembrane domain.[4] The non-competitive nature of MPEP is highly desirable, since it can more readily overcome the variable and sometimes high levels of endogenous glutamate relative to a competitive antagonist.

Recently, the target has attracted much attention in the search for a small non-competitive antagonist that can be useful for the treatment of various disease states.[5–17]

Our research focused on exploring analogs as potential small molecule mGlu5 receptor antagonists which are also active models of anxiety and pain. The approach described herein targets the identification of structurally novel chemical templates which do not possess the acetylene linker found in MPEP. As such, we recently revealed a class of 2-aryl quinolines which are potent mglu5 receptor antagonists.[17]



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Keywords: Pyrido[2,3-*d*]pyrimidine; Synthesis; SAR; mGlu5 receptor antagonist; Arthritis; Rat.
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Seeking diversity in the bicycloheteroaryl template portion of the 2-aryl quinoline series, we now report a structurally novel pyrido[2,3-*d*]pyrimidine containing template (**1**), which similar to the quinoline series, possesses a promising molecular modeling overlap with MPEP (illustrated in Fig. 1). The SAR exploration of this new motif was primarily focused around substitution at the 7- and 2-position of the pyrido[2,3-*d*]pyrimidine core and optimized for mGlu5 receptor potency.

The construction of this ring system was accomplished through a simple two-step synthesis from commercially available 2-amino-6-methylnicotinaldehyde (Scheme 1). Formation of the initial amide bond required robust conditions (1 equiv of DMAP at 140 °C) due to the poor nucleophilic nature of the amino group of the 2-amino pyridine **2**. Alternate peptide coupling conditions were tried to form this amide bond with poor success. Increased yields of the first step were realized when DMAP was premixed with the acid chloride (**3**) prior to the addition of pyridine **2**. Purification of intermediate (**4**) followed by aminolysis in a sealed tube at 80 °C afforded the desired 2-aryl pyrido[2,3-*d*]pyrimidine scaffold (**1**). Several 2-aryl pyrido[2,3-*d*]pyrimidines lacking a 7-methyl substituent (**20**) were additionally synthesized from 2-amino-nicotinaldehyde in a similar manner.[18]

To probe the activity of the 7-methylpyrido[2,3-*d*]pyrimidine series, compounds **5** through **22** were prepared (Table 1) and tested in an mGlu5 receptor functional (FLIPR) based assay.[19] Overall, the biological data revealed good activity with a well-defined SAR for this novel series of mGlu5 receptor antagonists. It was observed that substitution at the C3-position of the 2-aryl ring was necessary for good potency as highlighted by the 3-methyl- and 3-bromo-containing analogs (**11** and **12**). However, it appears polar groups are not well tolerated since the complete loss of activity was observed for compounds **9** and **10**. Further SAR revealed that disubstitution in the C3- and C5-aryl positions provides enhanced potency relative to monosubstitution in the

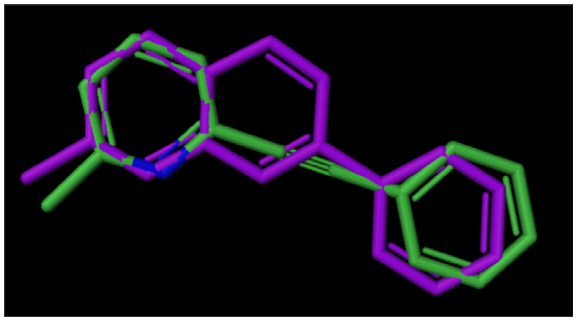
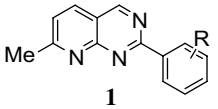


Figure 1. Overlay of MPEP (green) and compound **5** (**1** where R = H, magenta).

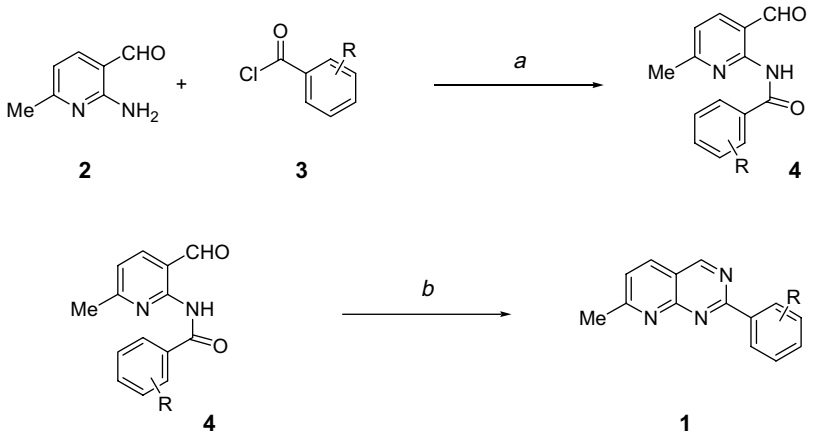
Table 1. Functional (FLIPR) activity for 7-methyl-2-aryl pyrido[2,3-*d*]pyrimidine analogs



1

Compound	R	FLIPR IC_{50}[a] (nM)
5	H	2700
6	2-Methoxy	>30,000
7	3-Methoxy	1500
8	3-Cyano	240
9	3-Methylsulfonyl	>30,000
10	3-Methanamine	>30,000
11	3-Methyl	53.0
12	3-Bromo	38.0
13	4-Methylcarboxy	>30,000
14	4-Bromo	>30,000
15	4-Cyano	>30,000
16	3,4-Dichloro	>30,000
17	2,4-Dichloro	>30,000
18	3-Cyano-5-methoxy	1.20
19	3,5-Dichloro	0.72
20	3,5-Dimethoxy	179
21	3-Methyl-5-fluoro	39.7
22	3,4,5-Trimethoxy	>30,000

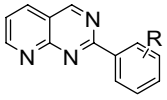
[a] Compounds were measured for potency to inhibit quisqualate stimulated calcium mobilization using FLIPR technology. The data shown were obtained using CHO cells stably expressing rat mGlu5 receptors. Values are geometric means of two or more experiments.



Scheme 1. Reagents and conditions: (a) DMAP (1 equiv), DMA, 140 °C; (b) NH_3(l), EtOH, 80 °C, sealed tube.

C3-aryl position. In particular, compound **17** (3-cyano-5-methoxy) had a 200-fold increase in potency over compound **7** (3-cyano). Additionally, compound **18** was identified as the most potent mGlu5 receptor antagonist (3,5-dichloro, IC_{50} = 0.72 nM) in this series. Interestingly, alternate di- and tri-aryl substitution patterns were not potent.

Table 2. Functional (FLIPR) activity for selected 2-aryl pyrido[2,3-*d*]pyrimidine analogs

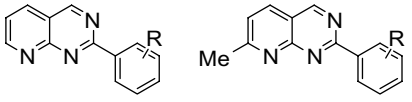


23

Compound	R	FLIPR IC_{50}[a] (nM)
24	3-Cyano	>26,100
25	3-Bromo	333
26	3-Cyano-5-methoxy	4.95
27	3,5-Dichloro	228
28	3-Methyl-5-fluoro	321

[a] Values are geometric means of two or more experiments.

Table 3. Functional (FLIPR) activity comparison between 2-aryl pyrido[2,3-*d*]pyrimidine (**23**) and the 7-methyl-2-aryl pyrido[2,3-*d*]pyrimidine (**1**)



23 **1**

Compound from series 23	Compound from series 1	R	23/1 FLIPR IC_{50} (nM)
24	**7**	3-Cyano	109X
25	**11**	3-Bromo	9X
26	**17**	3-Cyano-5-methoxy	4X
27	**18**	3,5-Dichloro	317X

Table 2 illustrates selected pyrido[2,3-*d*]pyrimidines (**24**–**28**) that were prepared and tested for mGlu5 receptor antagonist activity. These analogs in this template (**23**) lacked the 7-methyl substituent on scaffold **1**.

From this study, the 7-desmethyl pyrido[2,3-*d*]pyrimidines (Table 2) are revealed to exhibit a significant decrease in activity relative to the 7-methyl pyrido[2,3-*d*]pyrimidine series (Table 1). This implies that the 7-methyl moiety is required for optimal potency. This loss in potency is illustrated in Table 3.

The most potent compound (**18**) was evaluated in vivo (po, 10 mg/kg) in the rat monosodium iodoacetate (MIA) model of osteoarthritis (OA) pain[19,20] and the results are presented in Figure 2. In this model, MIA is injected into the right knee joint of a rat resulting in OA-like pain causing the animal to shift its weight onto the left leg. This shift, referred to as change in hind paw weight distribution, can be reversed by commonly utilized therapies for OA pain including naproxen, acetaminophen, and the COX-2 specific inhibitor, rofecoxib.[20,21] The time course results of a single dose oral administration of **18** revealed there was a significant inhibition of change in hind paw weight distribution versus vehicle at both the 2 and 4 h post-dose time points. This effect was shown to be quite similar to the oral effect of the same single dose of the COX-2 inhibitor, rofecoxib and further supports the mGlu5 receptor as a reasonable target to alleviate the signs and symptoms of arthritis.

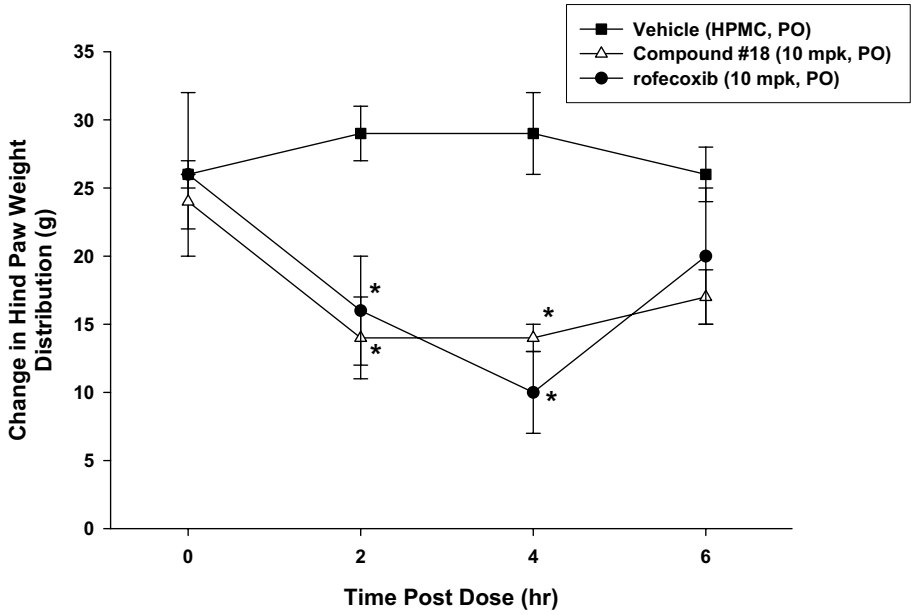


Figure 2. Data are expressed as means ± SEM. Dosing was 1 h pre-treatment (po). Statistically significantly differences were determined using a one-way analysis of covariance adjusted for multiplicity of statistical testing by a Hochberg's Procedure ($^*P < 0.05$ vs vehicle at same time point). $N = 8$ rats per group.

In summary, a series of readily accessible 2-aryl pyrido[2,3-*d*]pyrimidines were synthesized that possessed potent mGlu5 receptor antagonist activity. Of the 2-aryl pyrido[2,3-*d*]pyrimidines studied, it appears that appropriate substitution at the C3-position of the aryl ring and further substitution at the 3,5-positions of the aryl ring are preferred for mGlu5 receptor antagonist activity. In particular non-polar moieties appear to provide optimal antagonist activity. Additional substitution of 7-methyl onto the pyrido[2,3-*d*]pyrimidine ring yielded more potent analogs than the corresponding 7-desmethyl analogs. In vivo activity (po) of compound **18** was comparable in activity to the reference standard (COX-2 inhibitor, rofecoxib) in the rat MIA model. These results validate that the 2-aryl pyrido[2,3-*d*]pyrimidine is a promising new class of mGlu5 receptor antagonists and provides an additional tool beyond MPEP to investigate the important area of glutamate research.

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18. Representative synthetic example: 2-(3-fluoro-5-methylphenyl)pyrido[2,3-*d*]pyrimidine **28**. To a solution of 4-dimethylaminopyridine (0.872 g, 7.139 mmol) in dimethylacetamide (3 mL) was added 3-fluoro-5-methylbenzoyl chloride (1.540 g, 8.923 mmol) and the combined mixture was stirred at room temperature wherein a solid forms. A solution of 2-amino-nicotinaldehyde (0.871 g, 7.139 mmol) in dimethylacetamide (3 mL) was added to the solid mixture and heated to 140 °C for 18 h. The crude mixture was concentrated in vacuo and purified by silica gel chromatography eluting with ethyl acetate/hexanes to afford 0.712 g (39%) of 3-fluoro-*N*-(3-formylpyridin-2-yl)-5-methylbenzamide as an off-white solid. This amide was then dissolved in ethanol (30 mL) and ammonia (5 mL) was added and the combined mixture was heated in sealed bomb at 80 °C for 12 h. Upon cooling, the bomb was opened and the contents were concentrated in vacuo and purified by silica gel chromatography eluting with ethyl acetate/hexanes to afford 0.378 g (57%) of **28** as an off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6) *d* 2.54 (s, 3H), 7.27 (d, $J = 8.1$ Hz, 1H), 7.45 (dd, $J = 8.2$, 4.3 Hz, 1H), 8.06 (d, $J = 8.1$ Hz, 1H), 8.26 (s, 1H), 8.6 (dd, $J = 8.1$, 2.1 Hz, 1H), 9.3 (dd, $J = 4.3$, 2.1 Hz, 1H), 9.08 (s, 1H) HPLC:Phenomenex Develosil Combi RP3 4.6 × 50 mm, 4 min gradient, 50% CH_3CN/50% H_2O/0.1% HCO_2H to 98% CH_3CN/2% H_2O/0.1% HCO_2H; 0.5 min hold, 4.0 min run, 4 mL/min UV detection across 214, 254, 280 nm, 2.018 min retention time; MS (APCI): *m/z* 240.2 [M+H].
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21. All experimental procedures were conducted in an AAALAC International-accredited facility in compliance with the United States Department of Agriculture Animal Welfare Act Regulations and the Guide for the Care and Use of Laboratory Animals and were approved by the PGRD Ann Arbor Institutional Animal Care and Use Committee prior to initiation of the studies.