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Rapid hit to lead evaluation of pyrazolo[3,4-*d*]pyrimidin-4-one as selective and orally bioavailable mGluR1 antagonists

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Abstract—Our HTS effort yielded a preferential mGluR1 pyrimidinone antagonist **1** with lead-like characteristics. Rapid hit to lead (HTL) study identified compounds with improved functional activity and selectivity such as **1b** with little improvements in ADME properties. Addition of an aminosulfonyl group on the *N*-1 aromatic ring led to **2f**, a compound with similar in vitro biochemical profiles as those of **1b** but drastically improved in vitro ADME properties. These improvements were paralleled by rat PK study characterized by low clearance and quantitative bioavailability. Compound **2f** represented a true lead-like molecule that is amenable for further lead optimization (LO) evaluation.

Hit to lead (HTL) is playing an increasingly important role in drug discovery.[1–3] At the onset of such processes, biologically active molecules (hits) are first identified, most often by high-throughput screening (HTS) of company or commercial libraries, and evaluated. When selecting the hits for HTL study, special attention is given to those so-called 'lead-like' molecules, namely compounds with low molecular weight (MW < 450), relatively low hydrophobicity ($-4 < \mathrm{clog}\,P < 4.5$), and synthetic accessibility.[4,5] Through rapid structure–activity relationship (SAR) study of the hit series, the goal of HTL is to not only improve the in vitro profile including biochemical potency and sub-type selectivity, but also to evaluate and address the absorption, distribution, metabolism, and excretion (ADME) properties. Early profiling of the ADME properties will enable the project team to assess the likelihood of developing an orally bioavailable molecule in the ensuing lead optimization (LO) stage, the goal of which is to generate potential drug candidates. HTL will either provide high quality lead to facilitate the LO or help disqualify a series of compounds due to insurmountable liabilities, which will reduce unnecessary efforts and potential attrition in late discovery phases.

Glutamic acid (Glu) is the major excitatory amino acid (EAA) neurotransmitter in the central nervous system (CNS) and is implicated in pathological processes leading to a number of neurological and psychiatric diseases.[6–8] The transmitter function of Glu is modulated by two types of receptors: the ionotropic glutamate receptors (iGluRs) and metabotropic glutamate receptors (mGluRs).[9–11] mGluRs belong to the type 3 superfamily of G-protein coupled receptors (GPCRs) with seven transmembrane (TM) domains and comprise eight members that can be subdivided into three groups according to sequence homology, signal transduction mechanism, and pharmacological profile[12,13]: group I ($\mathrm{mGluRs}_{1/5}$), group II ($\mathrm{mGluRs}_{2/3}$), and group III ($\mathrm{mGluRs}_{4/6/7/8}$). A specific role for group I mGluRs in nociceptive processing has been demonstrated by pharmacological, immunohistochemical, and in situ hybridization.[14–21] A role of dorsal horn group I mGluRs, particularly the mGluR1 receptor, in acute nociception has been described in behavioral[22–24] and electrophysiological studies in vitro[25] or in vivo,[26] suggesting that selective mGluR1 antagonists may serve as novel analgesics. Recently, non-amino acid, selective mGluR1 antagonists have been reviewed.[27] Some of them, includ-

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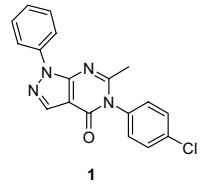
ing CPCCOEt (7-(hydroxyimino)cyclopropa[*b*]chromen-1a-carboxylate ethyl ester),[28] BAY36-7620,[29] R-214127[30], 16259585[31], and dicarboxypyrroles[32], have been shown to inhibit receptor activity in a non-competitive manner by interacting with the transmembrane domain VII, a location remote from the glutamate binding site. Some of the most recently reported potent, non-competitive mGluR1 antagonists include quinoline derivatives,[33] β-carbolines,[34] thiazolo [3,2-*a*]benzimidazole-2-carboxamide (YM-298198) analogs[35], and triazfluorenone analogs, which had been shown to be effective in different pain models.[36]

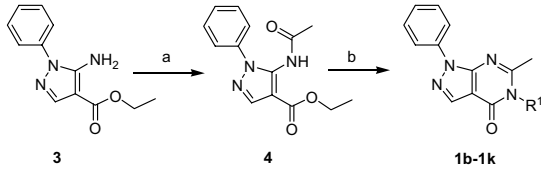
Despite the structure diversity of known mGluR1 antagonists, only poor to modest oral bioavailabilities were achieved.[32,33,36] To identify additional novel, potentially orally bioavailable small-molecule mGluR1-selective antagonists, a high-throughput screening (HTS) effort on Abbott compound collection was carried out. A unique pyrazolopyrimidinone analog **1** (Fig. 1) was shown to be a preferred mGluR1 non-competitive antagonist (IC_{50} = 242 nM) exhibiting much weaker potency against mGluR5 (IC_{50} = 9600 nM). Our HTL study on compounds **1** to improve in vitro potency, subtype selectivity, and ADME properties is presented herein.



1

hmGluR1 FLIPR EC_{50} = 242 nM
hmGluR5 FLIPR EC_{50} = 9600 nM

Figure 1.



3 **4** **1b-1k**

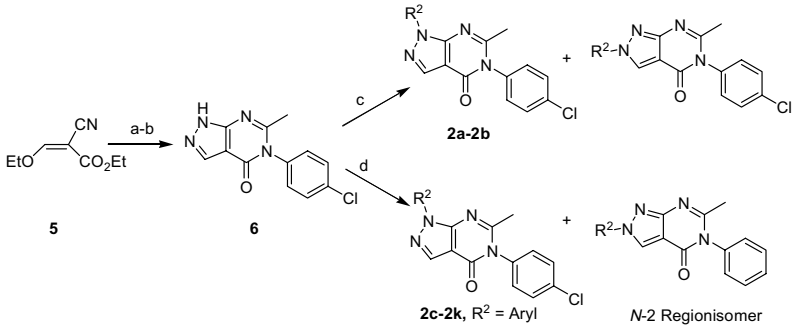
Scheme 1. Reagents and conditions: (a) i—Ac_2O, reflux, overnight; ii—NH_4OH, MeOH, 2 h; (b) R^1NH_2, $POCl_3$, 165 °C, 2 h.

HTS hit **1** is a small lead-like molecule (MW = 336, clog *P* = 4.2)[4,5] with a rigid structure scaffold and potential multiple points to introduce diversity and therefore represents a valid starting point for HTL study. Our strategy was to keep the pyrazolopyrimidine pharmacophore intact and modulate two cyclic appendage groups (R^1 and R^2) sequentially. Analogs with different R^1 groups (**1a**–**1i**), shown in Scheme 1, were prepared under solvent-free conditions in moderate to good yields using $POCl_3$[37] to assist the condensation and cyclization from **4** and the corresponding amines. Analogs with alkyl R^2 (**2a** and **2b**) were generated using a modified Mitsunobu procedure[38] shown in Scheme 2. Compounds with aryl R^2 groups (**2c**–**2l**) were prepared by copper-catalyzed C–N bond formation[39] under microwave conditions. Despite moderate yield (20–40%), this synthesis allowed rapid access to these analogs that otherwise would require multi-step synthesis. In these reactions K_3PO_4 was a superior base to K_2CO_3. For both alkylation procedures, *N*-2 regioisomers were produced in about 1:3 ratio with respect to the desired *N*-1 isomers and they were isolated by HPLC.

These compounds were evaluated in a calcium influx Fluorometric Imaging Plate Reader (FLIPR) assay employing 1321N1 cells transfected with human mGluR1 or human mGluR5 receptors (co-expressing rat GLAST).[40] The antagonist potency, expressed as IC_{50}, is the concentration at which 50% of the Ca^{2+} flux induced by 10 μM glutamate is blocked.

Concurrent with the antagonist activity characterization, we decide to start evaluating the ADME properties of the project compounds to identify possible orally bioavailable compounds. Aqueous solubility, in vitro stability in liver microsomes, and passive membrane permeability[41] were measured on the most potent analogs to monitor such progress.

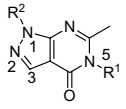
Different R^1 groups were first investigated as shown in Table 1. During the HTS, a closely related analog **1a**, a *meta*-chloro isomer of **1**, was found to be inactive. Subsequent modifications of R^1 groups were focused



5 **6** **2a-2b** **2c-2k**, R^2 = Aryl *N*-2 Regionisomer

Scheme 2. Reagents and conditions: (a) NH_2NH_2, EtOH, 75 °C, 2 h; (b) i—Ac_2O, reflux, overnight; ii—step b in Scheme 1; (c) R^2OH, DBAD, P-PPh_3, THF, DMF; (d) R^2X, CuI, ligand, K_3PO_4, THF, DMSO, 180 °C, microwave, 30 min.

Table 1. Antagonist potencies of 5-substituted pyrazolo[3,4-*d*]pyrimidin-4-ones



1a-i, 2a-l

Compound	R^1	R^2	FLIPR IC_{50} ± SEM[a] (nM)		c log *P*[c]	Solubility (μM)	Microsomal clearance[d] (L/h/kg)	Permeability ($\times 10^{-6}$ cm/s)
			hmGluR1	hmGluR5				
1	4-Cl–C_6H_4	Ph	242 ± 60	9600 ± 1200	4.20	18	7.0	29.6
1a	3-Cl–C_6H_4	Ph	>100,000	ND[b]				
1b	4-Br–C_6H_4	Ph	78 ± 7	>100,000	4.35	14	6.9	19.4
1c	4-OH–C_6H_4	Ph	2400 ± 160	>100,000				
1d	4-CF_3O–C_6H_4	Ph	>100,000	>100,000				
1e	4-NH_2SO_2–C_6H_4	Ph	>100,000	>100,000				
1f	3-F-4-Cl–C_6H_4	Ph	800 ± 160	>100,000	4.34	18	11	ND[b]
1g	Cycloheptyl	Ph	760 ± 160	ND[b]				
1h	Piperidin-1-yl	Ph	91 ± 20	2800 ± 800	2.55	19	108	5.49
1i	Azepan-1-yl	Ph	240 ± 50	3600 ± 370				
2a	4-Cl–C_6H_4	Cycloheptyl	520 ± 130	>100,000				
2b	4-Cl–C_6H_4	Cyclohexylmethyl	>100,000	>100,000				
2c	4-Cl–C_6H_4	2-CH_3–C_6H_4	350 ± 70	9500 ± 2200				
2d	4-Cl–C_6H_4	3-CH_3–C_6H_4	180 ± 3	>100,000	4.69	30	24	13.4
2e	4-Cl–C_6H_4	3-Cl–C_6H_4	220 ± 30	>100,000	4.91	11	11	5.6
2f	4-Cl–C_6H_4	3-NH_2SO_2–C_6H_4	127 ± 6	>100,000	2.70	42	< 2.5	17.6
2g	4-Cl–C_6H_4	4-NH_2SO_2–C_6H_4	1412 ± 200	>100,000				
2h	4-Cl–C_6H_4	4-Cl–C_6H_4	1100 ± 250	>100,000				
2i	4-Cl–C_6H_4	Pyridin-2-yl	>100,000	>100,000				
2j	4-Cl–C_6H_4	Pyridin-3-yl	230 ± 16	>100,000	2.79	73	4.0	21.4
2k	4-Cl–C_6H_4	Pyrimidin-5-yl	9800 ± 1730	>100,000				
2l	4-Cl–C_6H_4	Pyrazin-2-yl	>100,000	>100,000				

[a] Mean value for antagonist IC_{50} calculated from at least three determinations ± SEM in FLIPR assay using 1321N1 cells expressing either human mGluR1 or mGluR5 receptors.
[b] ND, not determined.
[c] Calculated log *P*.
[d] Rat microsomal scaled intrinsic clearance.

on *para*-substituted analogs accordingly. The *para*-chloro group of R^1 could be replaced by a bromo to give **1b**, which is more potent and selective. Other substituents at this position such as hydroxy (**1c**), trifluoromethoxy (**1d**), and aminosulfonyl (**1e**), however, either decreased or abolished the mGluR1 antagonist activity, although they were not active against mGluR5 either. Additional substitution on the phenyl ring did not improve the antagonist activity (**1f**). Saturated cycloalkyl groups and their variants were also prepared and tested. Those compounds with ring sizes of 6 and above were active (**1g**–**1i**), indicating a putative large hydrophobic pocket on the receptor that can accommodate these non-polar cycloalkyl R^1 rings. Despite being more potent than **1**, **1h** and **1i** were weakly active against mGluR5, therefore less selective.

The initial hit **1** had relatively low solubility and high microsomal clearance but good membrane permeability. More active and selective analog **1b** showed no significant improvement in solubility and microsomal stability. Compound **1h** exhibited much higher microsomal clearance and poorer permeability comparing to **1**, indicating faster metabolism of the piperidine ring. Addition of a fluoro group (**1f**) did not slow down the microsomal clearance, suggesting that modification of R^2 might be necessary to improve both antagonist activity and metabolism profile. Based on the above observations our focus was switched to R^2 modifications, keeping the *para*-chloro on the R^1 phenyl ring. One postulation was to introduce electron-withdrawing groups at R^2 to slow down the metabolism while maintaining or improving the in vitro profile.

In vitro testing indicated that all the *N*-2 regioisomers were inactive (data not shown), highlighting that right spatial alignment of different fragments is critical to generating favorable interactions with the receptor. Like R^1, large hydrophobic groups such as cycloheptyl were favored over smaller rings. The bioisosteric cyclohexylmethyl R^2 (**2b**) was inactive. In contrast to the *para*-substitution preference of the R^1 phenyl ring, compounds with *meta*-substituted R^2 groups were more active (**2d**–**2f**) with no detectable mGluR5 antagonism, with **2f** being the most potent. Similar substitution at the *para*-position led to weaker compounds (**2g** vs **2f**, **2h** vs **2e**) than their *meta*-cogeners. Substitution at the *ortho*-position (**2c**) provided a weaker and less selective mGluR1 antagonist. Meanwhile, different nitrogen-containing heterocycles were also surveyed. While 2-pyridyl R^2 (**2i**) was inactive, the 3-pyridyl analog (**2j**) showed comparable mGluR1 antagonism as **1**, consistent with the previous observation that *meta*-substituents were beneficial to the antagonist activity. Two *meta*-embed-

Table 2. Mean pharmacokinetic parameters in Sprague–Dawley rats (iv, ip, po 1 mg/kg)

Compound		AUC[b]	V_β[c]	CL[d]	C_{max}[e]	t_{max} (h)	$t_{1/2}$ (h)	F (%)
1b	iv[a]	1285	3.1	0.8			2.5	
2f		6150	0.4	0.2			1.5	
1b	ip[a]	389			0.17	0.25	0.25	30
2f		7790			1.54	1.7	1.7	Quant[f]
1b	po[a]	100			0.03	1.3	1.3	8
2f		6240			2.1	1.2	1.2	Quant[f]

[a] $n = 9$ animal per study.
[b] ng h/mL.
[c] L/kg.
[d] ng/mL.
[e] μg/mL.
[f] Quantitative.

ded nitrogens (**2k**) resulted in much weaker activity, and compound with *ortho*- and *meta*-embedded nitrogens (**2l**) was inactive.

Among these active analogs, **2d** had better solubility but faster clearance. The electron-withdrawing *meta*-chloro analog (**2e**) had higher clog*P*, similar solubility and clearance as **1** but much lower permeability. Incorporation of aminosulfonyl (**2f**), a polar, electron-withdrawing group, however, decreased clog*P*, improved solubility and stability in microsomes significantly while maintained relative good permeability. The *meta*-embedded pyridyl (**2j**) exhibited similar ADME profile.

The overall favorable in vitro characteristics of **2f** prompted us to evaluate its pharmacokinetic (PK) profile in rat (Table 2). Comparing to **1b**, intravenous (iv) administration of **2f** in rat revealed a much lower clearance (0.2 L/h/kg vs 0.8 L/h/kg), agreeing in rank order with their in vitro microsomal metabolism rate. The calculated volume of distribution of **2f** was much less than that of **1b**, which contributes to its lower iv half-life (1.5 h vs 2.5 h). Despite this, compound **2f** has substantially improved intraperitoneal (ip) and oral (po) plasma exposure and peak concentrations of those of **1b**. The bioavailabilities of **2f** are quantitative in both cases.

In conclusion, efficient HTL study, characterized by the parallel evaluations of both the biochemical and in vitro ADME profiles, was carried out on the HTS hit **1** as novel mGluR1 antagonists. The distinctive preference of *para*-substituents at R^1 and *meta*-groups at R^2 is noteworthy. R^1 modifications offered compounds with better potency and subtype selectivity but similar ADME properties. Nevertheless, careful choosing and incorporation of electron-withdrawing group at R^2 led to significant improvements of ADME properties. Within this structure class the in vitro ADME data were predictive of what was observed in in vivo PK study in terms of clearance and bioavailability. This HTL effort quickly turned a 'lead-like' HTS hit **1** into a true lead molecule **2f** with high in vitro potency and superb physicochemical properties. The identification of orally bioavailable mGluR1 selective antagonists will aid the understanding of mGluR1 related pharmacology and potentially benefit the discovery of therapies from selective mGluR1 modulation.

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Supplementary data

Experimental procedures, compound characterization data, and ADME assay methods associated with this article can be found, in the online version. Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.bmcl.2007.05.028.

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