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Discovery and in vitro and in vivo profiles of 4-fluoro-*N*-[4-[6-(isopropylamino) pyrimidin-4-yl]-1,3-thiazol-2-yl]-*N*-methylbenzamide as novel class of an orally active metabotropic glutamate receptor 1 (mGluR1) antagonist

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ABSTRACT

We identified 4-fluoro-*N*-[4-[6-(isopropylamino)pyrimidin-4-yl]-1,3-thiazol-2-yl]-*N*-methylbenzamide **27** as a potent mGluR1 antagonist. The compound possessed excellent subtype selectivity and good PK profile in rats. It also demonstrated relatively potent antipsychotic-like effects in several animal models. Suitable for development as a PET tracer, compound **27** would have great potential for elucidation of mGluR1 functions in human.

Glutamate is one of the major excitatory neurotransmitters in the central nervous system (CNS) and acts on ionotropic glutamate receptors including NMDA and non-NMDA receptors and on G-protein coupled metabotropic glutamate receptors (mGluRs). mGluRs are classified into eight subtypes (three groups) based on sequence homology, coupling mechanisms to G-protein and pharmacological properties. mGluRs are considered to be drug targets for the modulating glutamate transmission in the treatment of various neurological and psychiatric diseases including pain, epilepsy, Parkinson's disease, cognitive disorders, drug abuse, anxiety and schizophrenia.[1–4]

Since CPCCOEt[5,6] was identified as the first noncompetitive mGluR1 antagonist with moderate affinity, a number of rather potent and selective ligands have been developed. In general, noncompetitive mGluR1 antagonists act at the seven transmembrane domain, not at the glutamate binding N-terminal of the extracellular domain, and their structural features are very different from glutamate. For example, thiazolo[3,2,*a*]benzimidazole amide derivatives,[7] 2,4-dicarboxypyrrole esters,[8] quinoline derivatives[9] and triazafluorenones[10] were reported to be low nano-molar mGluR1 antagonists. These structurally diverse agents have facilitated the elucidation of action mechanisms of noncompetitive mGluR1 antagonists in vitro and revealed their therapeutic potential by using established animal models. In order to conduct clinical trail to identify therapeutic utility of an mGluR1 antagonist, noncompetitive mGluR1 antagonists need to improve their oral bioavailability. Previous attempts to improve oral bioavailability without impairing potency have met with little success.

We have reported three kinds of potent and selective mGluR1 antagonist, such as FTIDC **1**,[11–13] isoindolinone analog **2**,[14] and quinoline analog **3**[15] (Fig. 1). Our antagonists are structurally diverse but have an aryl-triazole group in common. Consequently completely structurally distinct classes were still needed to strengthen our therapeutic concept. In order to obtain structurally novel hit compounds suitable for development into an orally available mGluR1 antagonist, we primarily screened Merck Research Laboratories (MRL) chemical library using CHO cells expressing human mGluR1a, with antagonistic activity measured by FLIPR assay. Among the hit compounds, biaryl amide compound **4** possessing no triazole group showed moderate antagonistic activity on human mGluR1 receptors with IC_{50} value of 210 nM and good mGluR5/mGluR1 selectivity (Fig. 2).

In this Letter we describe the structure–activity relationship of hit compound **4** and identify a potent, selective and orally active mGluR1 antagonist in which potency and physicochemical properties are appropriately balanced.

Various biarylamide derivatives **4**–**17** and **23** were synthesized as shown in Scheme 1. Aryl-methylkenone or aryl bromoketone were cyclized with thiourea to afford 2-amino-5-aryl-thiazole. Condensation of 2-aminothiazole with various acid chlorides under basic condition provided compounds **4**–**17** and **23**.

The terminal 2- or 5-pyrimidine derivatives were prepared from commercially available 2,4-dibromothiazole as shown in Scheme

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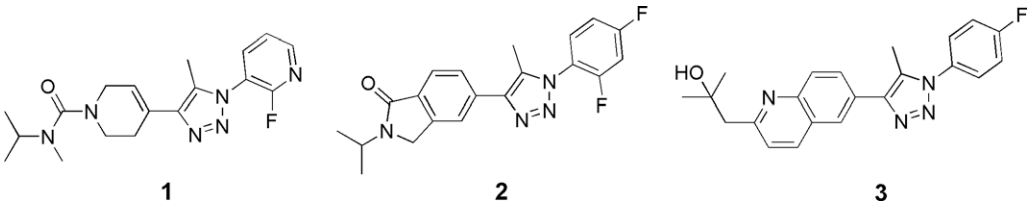


Figure 1. Structure of reported mGluR1 antagonists.

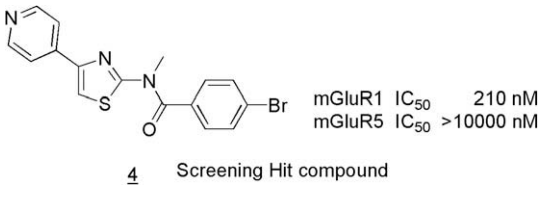
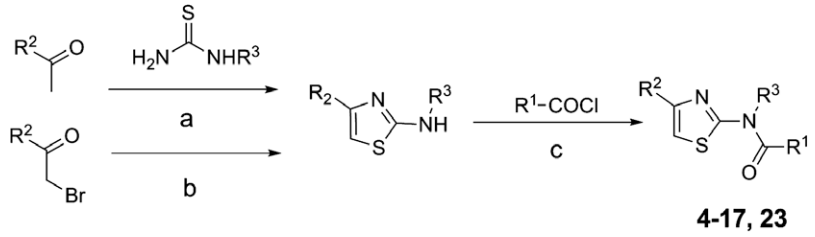


Figure 2. Hit compound derived from HTS screening.

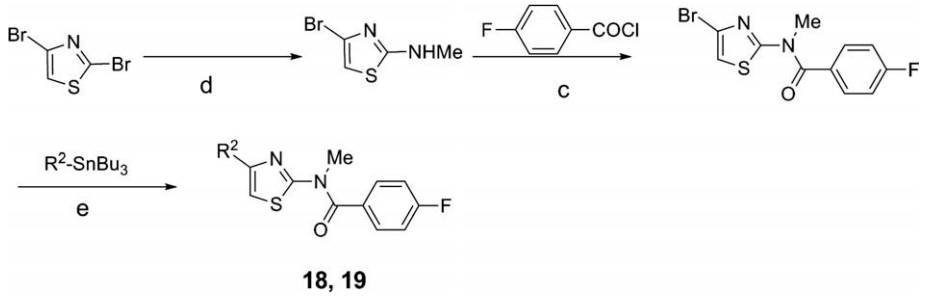
2. Dibromothiazole reacted with methylamine to give 5-bromo-2-methylaminothiazole, followed by condensation with 4-fluorobenzoyl chloride to provide 2-benzoylamide-4-bromothiazole. Finally, Stille coupling with appropriate tri-butyltin compounds in the presence of tetrakis (triphenylphosphine) palladium afforded pyrimidine derivatives **18** and **19.**

N-Alkylamide derivatives **20**–**22** were synthesized as shown in Scheme 3. Non-alkylated amide **23** was prepared from non-substituted thiourea as shown in Scheme 1. Compound **23** was treated with NaH, followed by appropriate alkyl iodide to yield *N*-alkylamide derivatives **20**–**22.**

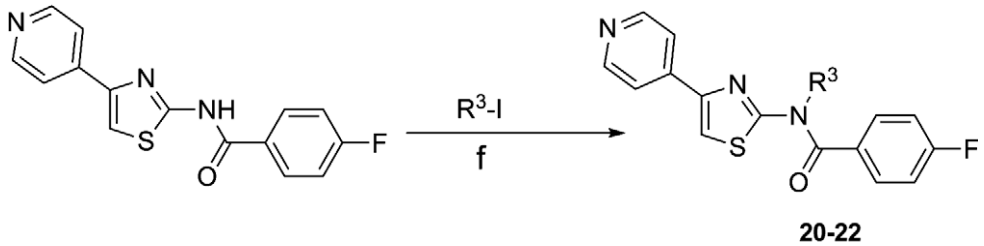
6-Substituted pyrimidine derivatives **24–28** were synthesized from 4,6-dichloropyrimidine as shown in Scheme 4. 4,6-Dichloropyrimidine was reacted with 1-ethoxyvinyl-tri-*N*-butyltin under Stille coupling condition to give mono-ethoxyvinylpyrimidine. Ethoxyvinyl derivative was reacted with *N*-bromosuccinimide, followed by treatment with *N*-methylthiourea to provide 4-pyrimidinyl-2-methylaminothiazole. Aminothiazole derivative was condensed with 4-fluorobenzoyl chloride, followed by substitution of pyrimidine 6-position with several amines to give 6-substituted pyrimidine derivatives **24**–**28**.



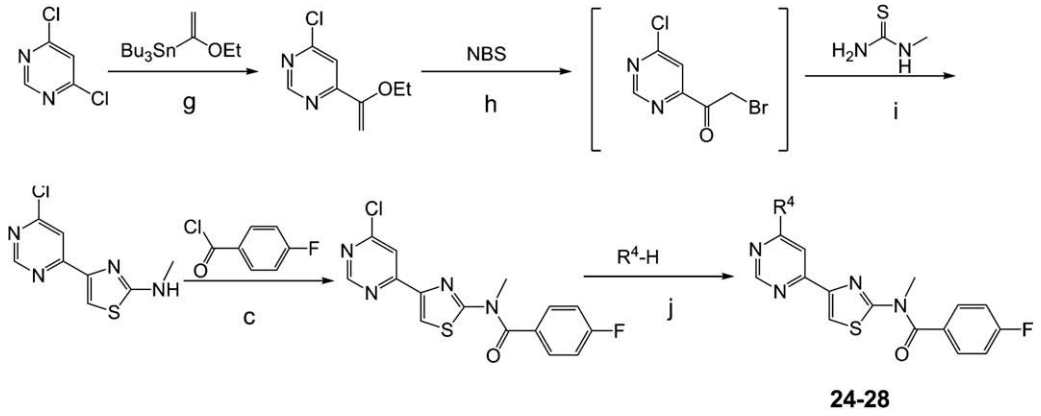
Scheme 1. Synthesis of biarylamide derivatives **4**–**17** and **23**. Reagents and conditions: (a) I_2, 100 °C, 3–60%; (b) Et_3N, EtOH, reflux, 70% for R_2: 4-pyridyl R_3: Me, 95% for R_2: phenyl R_3: Me; (c) Et_3N, toluene, reflux, 8–77%.



Scheme 2. Synthesis of pyrimidine derivatives **18** and **19**. Reagents and conditions: (d) $MeNH_2$, MeOH, rt, 15%; (c) Et_3N, toluene, reflux, 73%; (e) $Pd(Ph_3P)_4$, toluene, 100 °C, 8% for R_2: 4-pyrimidyl, 15% for R_2: 2-pyrimidyl.



Scheme 3. Synthesis of *N*-alkyl derivatives **20**–**22**. Reagents and conditions: (f) NaH, DMF, rt, 62% for R_3: ethyl, 51% for R_2: *n*-propyl, 5% for R_2: *iso*-propyl.

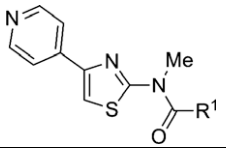


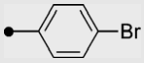
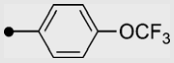
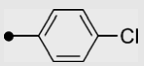
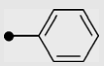
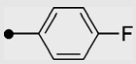
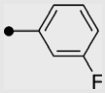
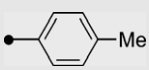
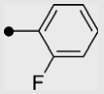
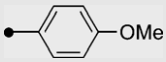
Scheme 4. Synthesis of 6-substituted pyrimidine derivatives **24–28**. Reagents and conditions: (g) $Pd(Ph_3P)_4$, DMF, 120 °C, 75%; (h) THF–H_2O, rt; (i) EtOH, rt, 73% for two steps; (c) Et_3N, toluene, reflux, 90%; (j) K_2CO_3, THF, 90 °C, 20–74%.

Our goals were to investigate the structure–activity relationship of this lead class and to identify structurally diverse mGluR1 antagonists which have comparable profiles to our previously developed antagonists **2** and **3**.

Modification of R^1 moiety was initially performed since bromobenzene is not a preferable group for drug design, and derivatization of R^1 is relatively simple. Various kinds of carboxylic acids were introduced and revealed that an aromatic ring is essential to show mGluR1 antagonistic activity (data not shown). Therefore, substituted benzene analogues were tested as a bromobenzene replacement, Table 1 highlights the SAR of the R^1 substitutent. Fluoro analogue **6** was the best among halide analogues **2–4**. We considered that an electron-withdrawing group might be necessary. However electron-donating methyl derivative **7** also showed potent antagonistic activity. Relatively large substituents **8** and **9** were not tolerated in this lead class. Based on these results, only small substituents were accepted in terms of mGluR1 antagonistic activity. Among fluorobenzene analogues **6**, **11** and **12**, it became clear that substitution at 4- (*para*) position was preferable.

Table 1
SAR of right-hand substituent

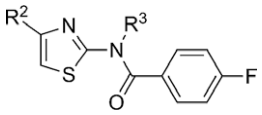


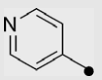
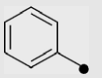
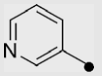
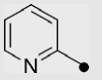
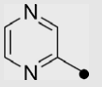
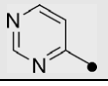
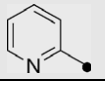
No	R^1	IC_{50}[a] ± SEM (nM) $hmGluR_1$	No	R^1	IC_{50}[a] ± SEM (nM) $hmGluR_1$
4		210 ± 68	9		>10,000
5		62 ± 17	10		80 ± 9.7
6		49 ± 16	11		100 ± 10
7		83 ± 15	12		130 ± 15
8		1500 ± 15			

[a] The IC_{50} value is the mean of multiple results (at least three independent determinations performed in duplicate) with standard error of the means.

Next, we investigated the SAR of R^2 portion in order to remove potentially toxic 4-pyridine structure of the left-hand substituent which may cause CYP inhibition.[16] Replacement of the 4-pyridine group with various kinds of functional groups was tested as shown in Table 2. Alkyl groups demonstrated poor antagonistic activity (data not shown). Phenyl analogue **13** also did not show mGluR1 antagonistic activity. Nitrogen containing aromatic groups were essential to show potent antagonistic activity. Various kinds of hetero-aromatic groups were examined. Among six-membered hetero aryl analogues **14–19**, 2-pyridyl analogue **15** and 4-pyrimidyl analogue **17** showed potent antagonistic activity. The effects of *N*-alkyl group substitution at R^3 were checked using 2-pyridyl analogue **15** as a template (**15** and **20–23**). Only small alky groups such as

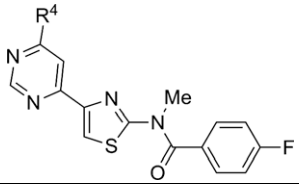
Table 2
SAR of left-hand substituent



No	R^2	R^3	IC_{50}[a] ± SEM (nM) $hmGluR_1$	No	R^2	R^3	IC_{50}[a] ± SEM (nM) $hmGluR_1$
6		Me	49 ± 16	18		Me	1500 ± 470
13		Me	>10,000	19		Me	5700 ± 440
14		Me	230 ± 130	20		Et	18 ± 2.4
15		Me	6.0 ± 1.0	21		^{n}Pr	81 ± 24
16		Me	49 ± 17	22		^{i}Pr	2000 ± 340
17		Me	10 ± 2.9	23		H	980 ± 120

[a] The IC_{50} value is the mean of multiple results (at least three independent determinations performed in duplicate) with standard error of the means.

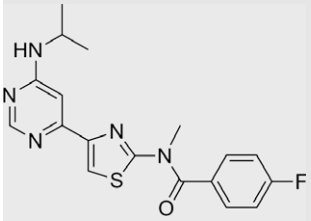
Table 3
SAR of 5-substituted pyrimidine derivatives



No	R^4	IC_{50}[a] ± SEM (nM)		Metabolic stability in human liver microsomes[b] (remaining%)	Solubility in pH7.4 potassium phosphate buffer[c] (μM)
		$hmGluR^1$	$hmGluR_5$		
18		10 ± 2.9	6600 ± 1400	37	11.5
24		2.4 ± 0.32	>10,000	64	1.6
25		1.8 ± 0.25	7900 ± 2100	20	4.5
26		3.7 ± 0.74	9200 ± 770	35	1.3
27		5.1 ± 2.0	7000 ± 1900	60	45
28		77[d]	>10,000[d]		

[a] The IC_{50} value is the mean of multiple results (at least three independent determinations performed in duplicate) with standard error of the means.
[b] 1 μM of compound was incubated in NADPH fortified liver microsomes (0.25 mg-protein/mL) for 30 min at 37 °C.
[c] Method is described in detail in Ref. 17.
[d] The values are the means of two experiments.

Table 4
Profiles of **27**



27

In vitro profiles
Group 1
$hmGluR1$ (IC_{50})[a] 5.1 nM
$hmGluR5$ (IC_{50})[a] 7000 nM
Group 2
$hmGluR2$ (IC_{50})[a] >10,000 nM
Group 3
$hmGluR8$ (IC_{50})[a] >10,000 nM
Quisqualic acid binding site (IC_{50}) >10,000 nM
In vivo profiles
Brain penetrability[b]
Mouse brain/plasma concn 0.17 nmol/g/0.19 μM
Pharmacokinetics[c]
Rat *F*: 53%, $T_{1/2}$: 2.3 h, , CLp: 28 mL/min/kg
In vivo efficacy[d]
Rat PPI disruption model MED 1.0 mg/kg, PO
Mouse hyperlocomotion model MED 0.3 mg/kg, PO

[a] The IC_{50} value is the mean of multiple results (at least three independent determinations performed in duplicate) with standard error of the means.
[b] Plasma and brains of mice were collected at 0.5 h after drug administration (3 mg/kg, po) and drug concentrations were measured ($n = 3$ mice/group).
[c] Pharmacokinetic study was conducted in fasted Sprague-Dawley rats ($n = 3$) dosed at 1 mg/kg for intravenous dosing and dosed at 3 mg/kg for oral dosing as a solution in water.
[d] Method is described in detail in Refs. 12,20.

methyl **15** and ethyl **20** were accepted and amide NH proton should be blocked (**23**) in terms of mGluR1 antagonistic activity.

Since the pyridine at the end of the molecule still had little concern for CYP inhibition, pyrimidine analogue **17** was used as a template for further modification.[16]

Finally, the effects of substituents at pyrimidine ring on intrinsic potency and solubility were investigated. Introduction of methyl group at 2-, 5- or 6-position of the pyrimidine ring were tested and revealed that position 6 was the most effective in terms of improvement of intrinsic potency (data not shown). Characterization of pyrimidine analogues revealed that they showed insufficient microsomal stability and poor solubility in aqueous media.[17] Therefore, in order to improve metabolic stability and water solubility, introduction of hydrophilic substituents was examined. Among them, amine analogue **24** had good microsomal stability as well as good antagonistic activity. However, mGluR1 antagonists require good brain penetration showing in vivo efficacy; therefore alkyl groups should be introduced on the nitrogen at 5-position since H-bond donors are known to impair brain penetration.[18,19]

The primary amine of **24** was replaced with alkylamines. Unfortunately, metabolic stability and water solubility of 6-amino pyrimidine derivatives **25** and **26** decreased compared with non-amino analogue **18**. Only isopropylaminopyrimidine analogue **27** showed good microsomal stability and excellent water solubility in addition to good mGluR1 antagonistic activity (see Table 3).

Detailed profiles of isopropylaminopyrimidine analogue **27** are shown in Table 4.

Compound **27** had potent antagonistic activity against human mGluR1 receptor with an IC_{50} value of 5.1 nM. Excellent selectivity over other subtypes was exhibited; IC_{50} values were 7000 nM (human mGluR5), >10,000 nM (human mGluR2) and >10,000 nM (human mGluR8).

Modes of action of **27** were examined by [^{3}H]quisqualate (a potent Group I mGluR receptor agonist known to bind at the glutamate binding site) binding with human mGluR1 receptors. No displacement of [^{3}H]quisqualate (>10,000 nM) binding indicated that compound **27** bound at a site different from the glutamate binding site. Therefore, compound **27** is a potent and noncompetitive mGluR1 antagonist (allosteric antagonist) with excellent subtype selectivity.

Compound **27** also demonstrated antipsychotic-like activities in several kinds of in vivo animal models. In mice, **27** antagonized methamphetamine-induced hyperlocomotion at 0.3 mg/kg po. Furthermore, **27** (1.0 mg/kg po) reversed disruption of prepulse inhibition (PPI) caused by methamphetamine or ketamine in rats.[12,20] As a final point, **27** had acceptable DMPK profiles, improved microsomal stabilities, and good oral bioavailability ($F = 53\%$) in rats as well as good brain penetrability.

In summary, compound **27**[21] showed quite potent mGluR1 antagonistic activity, excellent subtype selectivity and also good PK profile in rats. Moreover it also demonstrated antipsychotic-like effects in several animal models. Furthermore development of **27** with an *N*-methyl amide moiety in which the methyl group can be introduced in the molecule in the last step of preparation would be suitable for development of a PET tracer to examine in vivo pharmacodynamics of mGluR1 antagonist. Therefore, compound **27** has great potential for elucidating the functions of mGluR1 in humans.

Acknowledgments

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21. Analytical data of **27** as a HCl salt: ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.23 (6H, d, J = 6.3 Hz), 3.64 (3H, s), 4.25–4.33 (1H, m), 7.27 (1H, s), 7.40 (2H, t, J = 8.8 Hz), 7.79 (2H, dd, J = 8.8, 4.6 Hz), 8.45 (1H, s), 8.73 (1H, s), 9.29–9.37 (1H, m). MS(ESI+): m/z 372.2 $[M+H]^+$.