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3-Cyano-5-fluoro-*N*-arylbenzamides as negative allosteric modulators of $mGlu_5$: Identification of easily prepared tool compounds with CNS exposure in rats

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ABSTRACT

Development of SAR in a 3-cyano-5-fluoro-*N*-arylbenzamide series of non-competitive antagonists of $mGlu_5$ using a functional cell-based assay is described in this Letter. Further characterization of selected potent compounds in in vitro assays designed to measure their metabolic stability and protein binding is also presented. Subsequent evaluation of two new compounds in pharmacokinetic studies using intraperitoneal dosing in rats demonstrated good exposure in both plasma and brain samples.

Glutamate is the major excitatory transmitter in the mammalian CNS, exerting its effects through both ionotropic and metabotropic glutamate receptors. The metabotropic glutamate receptors (mGlus) belong to family C of the G-protein-coupled receptors (GPCRs). The eight mGlus discovered to date have been further divided according to their structure, preferred signal transduction mechanisms and pharmacology (Group I: $mGlu_1$ and $mGlu_5$; Group II: $mGlu_2$ and $mGlu_3$; Group III: $mGlu_4$, $mGlu_6$, $mGlu_7$, and $mGlu_8$).[1] Selectively targeting a specific mGlu through the use of an orthosteric ligand can be difficult due to the highly conserved nature of that binding site. A strategy that has proven effective for achieving selectivity has been the design and use of allosteric modulators of the target.[2]

The non-competitive $mGlu_5$ antagonists, also known as negative allosteric modulators (NAMs), 2-methyl-6-(phenylethynyl) pyridine (MPEP)[3] and 3-[(2-methyl-1,3-thiazol-4-yl)ethynyl]pyridine (MTEP)[4] are important tool compounds and have demonstrated efficacy in numerous preclinical models of disease, including pain,[5] anxiety,[6] gastroesophageal reflux disease (GERD),[7] and fragile X syndrome.[8] In addition, recent extensive work with these compounds has established their utility in numerous animal models of drug addiction. Attenuation of various cocaine seeking behaviors in mice,[9] rats,[10] and squirrel monkeys[11] has been reported with these compounds. Further work established their efficacy in animal models with other drugs of abuse, including nicotine,[10f] morphine,[12] methamphetamine,[13] and alcohol.[14]

Recent years have seen growing clinical evidence of the potential utility for antagonists of $mGlu_5$. Addex Pharmaceuticals has disclosed positive data from phase II clinical studies with the $mGlu_5$ NAM ADX10059 in GERD[15] and acute migraine.[16] FRAXA Research Foundation and Neuropharm have been exploring the potential of fenobam in treating fragile X syndrome and early results from these studies in patients have been positive.[17] Novartis has reported on efforts with their $mGlu_5$ antagonist AFQ056 directed toward identification of the first approved treatment for Parkinson's disease levodopa-induced dyskinesia (PD-LID), which is a complication that arises following dopamine-replacement

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therapy.[18] The link between $mGlu_5$ antagonism and PD-LID was further bolstered by Addex's recent communication describing the efficacy of both ADX10059 as well as their second generation $mGlu_5$ antagonist ADX48621 in a non-human primate model of PD-LID.[19,20]

Such preclinical and now clinical validation of $mGlu_5$ antagonists makes it an attractive area for further research. We have been interested in the identification of new chemotypes for the design of $mGlu_5$ non-competitive antagonists and have recently reported some of the results from this effort.[21] This previously described work was based on the development of hits identified using a functional cell-based high-throughput screen. We have also focused a portion of our $mGlu_5$ NAM effort on rational design and scaffold hopping approaches. One such area centered on the development of SAR in a 3-cyano-5-fluoro-*N*-arylbenzamide series and is the subject of this Letter.

Numerous research groups have devoted significant effort toward the discovery of new and improved $mGlu_5$ NAMs in recent years.[22] An examination of some of the primary literature describing the SAR of various $mGlu_5$ NAM chemotypes revealed some common structural features. One such feature was the presence of a 3-cyano-5-fluorophenyl ring in several of the most potent analogs across multiple chemical series (Table 1). We developed a chemical plan in order to build around this common structural motif. Significant effort has been detailed by the aforementioned research groups around the phenyl portion of their respective templates. Our plan was to fix this phenyl ring in the form of a 3-cyano-5-fluorobenzamide in order to expand the SAR around the amine portion of such a template. One of the advantages of such an approach was that analogs could be prepared in a single step through the coupling of commercially available 3-cyano-5-fluorobenzoic acid with amines under standard conditions. Using such an approach with a high-throughput preparative LC/MS system[27] allowed for the rapid generation of libraries.[28]

Our initial focus was the evaluation of new heteroaryl amines (Table 2). Compounds were evaluated in our functional assay, which measures the ability of the compound to block the mobilization of calcium by glutamate in HEK293A cells expressing rat $mGlu_5$.[29] A survey of pyridyl amines (**1**–**3**) revealed that only 2-aminopyridine derivative **1** was moderately potent. Thiazole **4** and pyrazole **5** were weak antagonists, while isoxazole **6** was inactive. Appendage of a 6-methyl group (**7**) onto **1** yielded a near 50-fold improvement in potency.[30] A similar modification resulted in an even more dramatic improvement in the case of 4-methylthiazole **8**, which is more than 150 times potent than **4**. Such extreme shifts in potency due to relatively minor structural modifications are quite common when working with allosteric modulators of GPCRs.

Table 1
$mGlu_5$ NAMs with a common 3-cyano-5-fluorophenyl ring

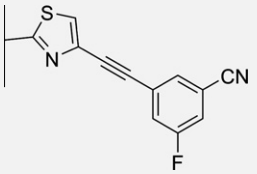
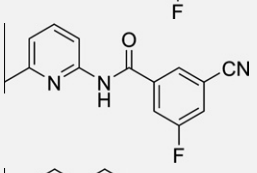
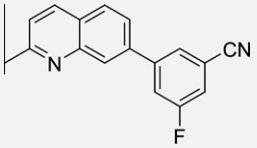
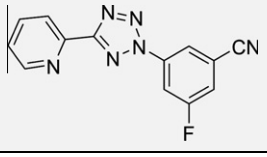
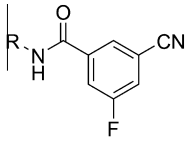
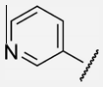
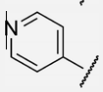
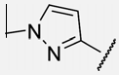
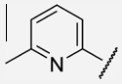
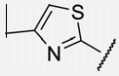
Organization	Structure	Reference
Merck & Co.		23
National Institute on Drug Abuse		24
Pfizer		25
Merck & Co.		26

Table 2
Heteroaryl amides



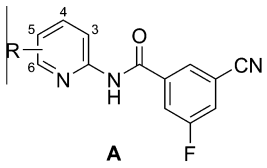
Entry	R	$mGlu_5$ IC_{50}[a] (nM)	% Glu max[a,b]
1		3010 ± 510	5.4 ± 0.6
2		>30,000	–
3		>30,000	–
4		>10,000[c]	37.4 ± 0.8
5		>10,000[c]	46.7 ± 1.4
6		>30,000	–
7		65 ± 23	1.2 ± 0.7
8		59 ± 14	1.0 ± 0.3

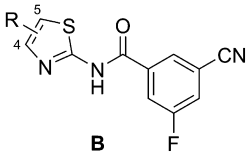
[a] Calcium mobilization $mGlu_5$ assay; values are average of $n \geqslant 3$.
[b] Amplitude of response in the presence of 30 μM test compound as a percentage of maximal response (100 μM glutamate); average of $n \geqslant 3$.
[c] CRC does not plateau.

Having observed the dramatic impact of substitution on potency, we decided to explore this further in the context of pyridine **1** and thiazole **4** (Table 3). Methyl substitution at the 3-position (**9**) of the pyridine ring produced an inactive compound, while the 4-methyl analog **10** was a weak antagonist. Moreover, 4,6-dimethyl analog **11** also lacked potency, indicating that addition of a 6-methyl group to **10** failed to rescue activity. Choosing to investigate alternative substituents at the 6-position, we found that 6-chloropyridine **12** and 6-ethylpyridine **15** were only slightly less potent than **7**. While 6-trifluoromethylpyridine **13** was only a weak antagonist, 6-methoxypyridine **14** was a moderate to weak partial antagonist. In the case of such compounds, the CRC clearly plateaus at approximately 20% of the glutamate maximum. A similar phenomenon was observed in the case of 5-methylthiazole **16**. Such partial antagonists have been noted and characterized in other $mGlu_5$ NAM chemotypes.[31] 4,5-Dimethylthiazole **17** was a full antagonist, albeit 10-fold less potent than **8**. 4-Cyclopropylthiazole **18** demonstrated a 15-fold reduction in potency compared to **8**; conversely, the loss of potency was even more severe in the case of 4-*tert*-butylthiazole **19**.

We were also interested in understanding the tolerance for amides without heteroaryl groups and turned our attention to that

Table 3
Substituted heteroaryl amides



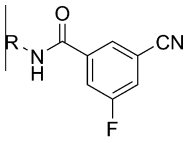


Entry	Series	R	$mGlu_5$ IC_{50}[a] (nM)	% Glu max[a,b]
7	A	6-Me	65 ± 23	1.2 ± 0.7
9	A	3-Me	>30,000	—
10	A	4-Me	>10,000[c]	38.6 ± 3.1
11	A	4,6-di-Me	>30,000	—
12	A	6-Cl	250 ± 40	2.2 ± 0.2
13	A	6-CF_3	>10,000[c]	19.6 ± 2.8
14	A	6-OMe	1510 ± 215	17.3 ± 0.4
15	A	6-Et	505 ± 145	1.7 ± 0.5
8	B	4-Me	59 ± 14	1.0 ± 0.3
16	B	5-Me	863 ± 149	17.5 ± 6.8
17	B	4,5-Di-Me	594 ± 102	2.4 ± 0.4
18	B	4-*cyc*-Pr	900 ± 269	1.5 ± 0.5
19	B	4-*tert*-Bu	>10,000[c]	29.7 ± 9.3

[a] Calcium mobilization $mGlu_5$ assay; values are average of $n \geqslant 3$.
[b] Amplitude of response in the presence of 30 μM test compound as a percentage of maximal response (100 μM glutamate); average of $n \geqslant 3$.
[c] CRC does not plateau.

area next (Table 4). A survey of several simple cylcoalkyl amides yielded only inactive compounds such as **20**. Only adamantyl amide **21** was a weak antagonist. More interesting was phenyl amide **22**, which was also a relatively weak antagonist. Still, given the aforementioned impact observed with substitution in the case of the heteroaryl amides, the thorough evaluation of substituted phenyl amides was a logical next step.

Table 4
Alkyl and phenyl amide SAR



Entry	R	$mGlu_5$ IC_{50}[a] (nM)	% Glu max[a,b]
20	Cyclohexyl	>30,000	—
21	Adamantyl	>10,000[c]	34.7 ± 6.2
22	Phenyl	5440 ± 1480	12.5 ± 4.7
23	2-Fluorophenyl	>30,000	—
24	3-Fluorophenyl	2160 ± 35	22.1 ± 1.1
25	4-Fluorophenyl	>10,000[c]	69.2 ± 2.2
26	2-Chlorophenyl	>30,000	—
27	3-Chlorophenyl	45 ± 21	0.6 ± 0.2
28	4-Chlorophenyl	>30,000	—
29	2-Methylphenyl	>30,000	—
30	3-Methylphenyl	122 ± 29	2.1 ± 0.2
31	4-Methylphenyl	>30,000	—
32	2-Methoxyphenyl	>30,000	—
33	3-Methoxyphenyl	>10,000[c]	35.5 ± 5.6
34	4-Methoxyphenyl	>30,000	—
35	3-(Trifluoromethyl)phenyl	543 ± 60	0.9 ± 0.3
36	3-Cyanophenyl	489 ± 70	1.4 ± 0.3
37	3-Ethynylphenyl	331 ± 70	2.3 ± 0.3
38	3-Ethylphenyl	4940 ± 1250	2.4 ± 0.2
39	3-*tert*-Butylphenyl	>30,000	—
40	3-(Methylsulfonyl)phenyl	>30,000	—
41	3-Chloro-2-fluorophenyl	347 ± 80	2.5 ± 0.5
42	3-Chloro-4-fluorophenyl	377 ± 43	43.0 ± 8.0
43	3-Chloro-5-fluorophenyl	1830 ± 446	38.5 ± 5.2
44	5-Chloro-2-fluorophenyl	3780 ± 488	29.8 ± 6.6

[a] Calcium mobilization $mGlu_5$ assay; values are average of $n \geqslant 3$.
[b] Amplitude of response in the presence of 30 μM test compound as a percentage of maximal response (100 μM glutamate); average of $n \geqslant 3$.
[c] CRC does not plateau.

An iterative examination of all positions of the phenyl ring with small substituents of varying electronic character quickly established some clear SAR trends (Table 4). First, substitution at the 2- and 4-positions of the phenyl ring led to reductions in potency relative to **22**. Second, substitution at the 3-position with a chloro (**27**) or methyl (**30**) group improved potency by more than 40-fold. 3-Fluoro (**24**) substitution yielded a moderately potent partial antagonist, while 3-methoxy (**33**) substitution was disfavored. Having established a preference for substitution at the 3-position, we focused on that location for additional SAR development. Both trifluoromethyl (**35**) and cyano (**36**) analogs were potent antagonists, although less effective than **27** and **30**. While a terminal alkyne (**37**) group gave a compound similar in potency to **35** and **36**, larger alkyl groups such as ethyl (**38**) and *tert*-butyl (**39**) were not well tolerated. Introduction of a polar methylsulfone (**40**) group was also not tolerated. In order to understand the potential options that would be available for future optimization of the metabolic stability of compounds within this series, we decided to fix the 3-chloro substituent and examine the effects of fluorination at all other positions on the phenyl ring. Fluorination at the 2-position (**41**) decreased potency over sevenfold relative to **27**. Interestingly, 4-fluoro regioisomer **42** demonstrated near identical potency to **41**, but was a clear partial antagonist. The remaining analogs **43** and **44** were also partial antagonists; however, their potency was reduced relative to **42**.

Having some compounds with excellent potency in our functional assay, we decided to further evaluate them in a radioligand binding affinity assay measuring the ability of compound to displace [^{3}H]3-methoxy-5-(pyridin-2-ylethynyl)pyridine, a close structural analog of MPEP (Table 5).[32] The binding affinity K_i values for these compounds were generally in good agreement (within fivefold) with their potency in the functional assay. The affinity data was also consistent with the allosteric nature of these ligands and confirms their interaction with the MPEP binding site. The same four compounds were also tested for their activity in $mGlu_{1-4}$ and $mGlu_{7-8}$ assays and were found to be inactive when tested at 10 μM against those receptors.

In addition to the binding assays, the same four compounds were evaluated for their metabolic stability[33] and propensity to bind plasma proteins[34] (Table 6). Only thiazole **8** demonstrated good stability in both rat and human liver microsomes. The remaining compounds were notably less stable in human liver

Table 5
Binding affinity of selected compounds

Compound	$mGlu_5$ IC_{50}[a] (nM)	$mGlu_5$ K_i[b] (nM)
7	65	157
8	59	102
27	45	206
30	122	100

[a] IC_{50} values are an average of $n \geqslant 3$.
[b] K_i values are an average of $n = 2$.

Table 6
In vitro DMPK

Compound	Liver microsomes[a]		Plasma protein binding[b]	
	Rat	Human	Rat	Human
7	46.5	7.0	84.0	89.9
8	89.9	70.3	92.4	98.4
27	96.8	18.0	93.7	97.1
30	69.7	19.5	95.3	98.3

[a] Liver microsomes expressed as percent parent remaining at $t = 15$ min.
[b] Plasma protein binding expressed as percent bound.

Table 7
PK of **8** and **27** following IP dosing in rats (10 mg/kg)

	8		27	
	Plasma	Brain	Plasma	Brain
C_{max} (μg/mL or μg/g)	7.65	28.77	0.97	1.09
T_{max} (h)	0.25	0.25	0.25	0.25
AUC (μg h/mL or μg h/g)[a]	17.82	72.96	1.20	1.71
Brain to plasma ratio	4.1:1		1.4:1	

[a] AUC measured from 0 to 4 h.

microsomes than in rat liver microsomes. Still, the stability of **8** and **27** in rat liver microsomes was supportive of their further study. Free fraction was greatest with pyridine **7**, although free fraction in rat plasma proteins was considerable with the other three compounds. All compounds were more highly bound to human plasma proteins than to rat plasma proteins.

Evaluation of the in vitro DMPK data indicated that **8** and **27** would both be potentially interesting compounds for evaluation in vivo. As such, both compounds were studied in rat PK experiments using intraperitoneal dosing (Table 7).[35] Exposure of **27** was good in both plasma and brain with a brain to plasma ratio greater than 1 to 1. Impressive exposure was observed with **8**, which showed excellent levels in both plasma and particularly brain. In fact, the brain to plasma ratio for **8** was greater than 4 to 1.

In summary, we have discovered and characterized two new $mGlu_5$ NAM in vivo tool compounds using a rational drug design approach based on common features of known antagonists. Compounds **8** and **27** potently inhibited the mobilization of calcium by an EC_{80} concentration of glutamate in HEK293A cells expressing rat $mGlu_5$. Their interaction with the known allosteric binding site was confirmed with a radioligand binding assay, and selectivity over other mGlus was established. Both compounds can be prepared in a single, simple synthetic step from inexpensive, readily available starting materials. Furthermore, these compounds are distinct from the 1,2-diarylalkyne chemotype that has been employed in the bulk of published preclinical in vivo studies to date. Our current plans include evaluation of these compounds in various rat models of diseases relevant to $mGlu_5$ and will be the subject of future communications.

Acknowledgments

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28. Prior to biological testing, all compounds were analyzed by LCMS and determined to be ⩾95% pure, and selected compounds were further characterized by proton NMR. For a large scale synthesis, compounds can also be purified via flash chromatography on silica gel. For example, synthesis and characterization of **8** was as follows: 2-amino-4-methylthiazole (5.00 g, 44 mmol), 3-cyano-5-fluorobenzoic acid (7.23 g, 44 mmol), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (8.4 g, 44 mmol) and 4-dimethylaminopyridine (0.535 g, 4.4 mmol) were dissolved in CH_2Cl_2 (100 ml) and stirred at rt overnight. Water was added and the layers were separated. The organic layer was dried ($MgSO_4$), filtered, and concentrated in

vacuo. Purification by flash chromatography on silica gel afforded 10.18 g (89%) of **8** as a white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.36 (s, 1H), 8.20 (d, J = 9.3 Hz, 1H), 8.12 (d, J = 8.12 Hz, 1H), 6.84 (s, 1H), 2.30 (s, 3H); HRMS calcd for $C_{12}H_8FN_3OS$ (M+H$^+$), found 262.0450. Synthesis and characterization of **27** was as follows: 3-chloroaniline (5.00 g, 39 mmol), 3-cyano-5-fluorobenzoic acid (7.23 g, 41 mmol), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (7.89 g, 41 mmol) and 4-dimethylaminopyridine (503 mg, 4.1 mmol) were dissolved in CH_2Cl_2 (300 ml) and stirred at rt overnight. Water was added and the layers were separated. The organic layer was dried ($MgSO_4$), filtered, and concentrated in vacuo. Purification by flash chromatography on silica gel afforded 9.80 g (91%) of the **27** as a white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.58 (s, 1H), 8.27 (t, J = 1.2 Hz, 1H), 8.14–8.08 (m, 2H), 7.92 (t, J = 2.0 Hz, 1H), 7.68–7.66 (m, 1H), 7.42 (t, J = 8.1 Hz, 1H), 7.21–7.19 (m, 1H); HRMS calcd for $C_{14}H_8ClFN_2O$ (M+H$^+$), found 275.0385.

29. HEK293A cells expressing rat mGlu$_5$ were cultured and plated as previously described. The cells were loaded with a Ca^{2+}-sensitive fluorescent dye and the plates were washed and placed in the Functional Drug Screening System (Hamamatsu). Test compound was applied to cells 3 s after baseline readings were taken. Cells were incubated with the test compounds for 140 s and then stimulated with an EC_{20} concentration of glutamate; 60 s later an EC_{80} concentration of agonist was added and readings taken for an additional 40 s. Allosteric modulation by the compounds was measured by comparing the amplitude of the responses at the time of glutamate addition plus and minus test compound. For a more detailed description of the assay, see Ref. 31a.
30. Compound **7** has also been disclosed by the group at NIDA (Ref. 24). Functional activity in our assay is in good agreement with their published data.
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33. The test compounds (1 μM) were incubated for 15 min at 37 °C with shaking, in medium containing human/rat liver microsomes, phosphate buffer, and the cofactor NADPH.
34. A 96-well rapid equilibrium dialysis (RED) apparatus (Thermo Scientific) was used to determine the free fraction in rat and human plasma for compound.
35. Compounds were dosed at 10 mg/kg intraperitoneally as microsuspensions in 10% tween 80 in male Sprague Dawley rats. Brain and plasma samples were collected at 0.25, 0.5, 1, 2, and 4 h post dose. IP dosing was chosen as a convenient route to help maximize exposure.