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Discovery of 4-arylquinoline-2-carboxamides, highly potent and selective class of mGluR2 negative allosteric modulators: From HTS to activity in animal models

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ABSTRACT

Antagonism of the mGluR2 receptor has the potential to provide therapeutic benefit to cognitive disorders by elevating synaptic glutamate, the primary excitatory neurotransmitter in the brain. Selective antagonism of the mGluR2 receptor, however, has so far been elusive, given the very high homology of this receptor with mGluR3, particularly at the orthosteric binding site. Given that inhibition of mGluR3 has been implicated in undesired effects, we sought to identify selective mGluR2 negative allosteric modulators. Herein we describe the discovery of the highly potent and selective class of mGluR2 negative allosteric modulators, 4-arylquinoline-2-carboxamides, following a successful HTS campaign and medicinal chemistry optimization, showing potent *in vivo* efficacy in rodent.

The role of glutamate in cognitive processes including memory acquisition, consolidation and retention has been established both in animal studies and in the clinic. Inhibiting ionotropic glutamate receptors, such as NMDA and AMPA receptors, disrupts cognition,[1] whereas enhancing ionotropic receptor activity is pro-cognitive.[2,3] However, there are clear challenges associated with over-activating NMDA and AMPA receptors including the potential for inducing seizures. Alternative approaches to elevating glutamatergic tone while keeping desensitization processes intact have focused on antagonizing the presynaptic metabotropic glutamate receptors. Indeed, antagonists of mGluR2/3 show AMPA-dependent efficacy in preclinical cognition assays.[4,5] However, it has not been clear whether antagonism at one or both receptors is necessary or sufficient for efficacy. Dual mGluR2/3 inhibitors existed in the literature.[6] however, modulation of mGluR3 receptors has been implicated in undesired pharmacodynamic effects including hyperlocomotion and increased wake.[7,8] We therefore set out to identify mGluR2-selective compounds with the potential to differentiate from other glutamatergic mechanisms based on improved safety margins. Due to the high homology between the mGluR2 and mGluR3 receptors, the team's strategy to achieve high selectivity was to focus efforts on the identification of mGluR2 negative allosteric modulators rather than target the orthosteric site. We describe herein the discovery of the most potent and selective class of mGluR2 negative allosteric modulators known to date, the quinoline carboxamides. Since its discovery,[9] there has been significant activity in the field and several other promising selective mGluR2 negative allosteric modulators from the same class have been reported.[10–13]

We undertook a FLIPR-based high throughput screen which provided a number of high-quality classes (see Supporting information for assay details). One interesting chemotype is represented by compound **1** (Fig. 1), belonging to the 5-lipooxygenase inhibitors class[14] previously discovered in our laboratories. While the *in vitro* profile of **1** is very promising, we observed low brain penetration in rat PK studies, likely due to high plasma protein binding, very low solubility and activity in the PGP assay in both human and rat.[15]

To alleviate these liabilities, we proceeded to optimize **1** by first addressing the primary amide, whose polar nature we hypothesized might be at the root of poor brain permeability. However, we quickly realized that the amide is key to potency as shown in Table 1. Removal of the amide (**8**) as well as its *mono*- (**2**) and *bis*- (**3**) methylation

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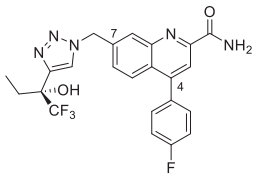
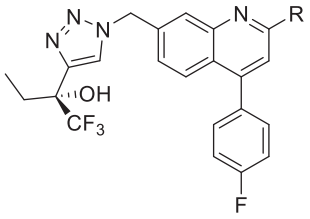
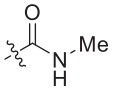
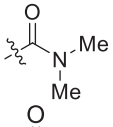
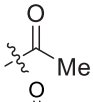
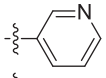


Compound **1**	
mGluR2 FLIPR IC_{50} (h)	13 nM
mGluR3 FLIPR IC_{50} (h)	>10,000 nM
PGP (h, r)	2.2, 10.9
Solubility (pH 2, 7)	<0.8 μM; <0.8 μM

Fig. 1. *In vitro* profile of compound **1**.

Table 1
SAR for the replacement of primary amide in **1**.



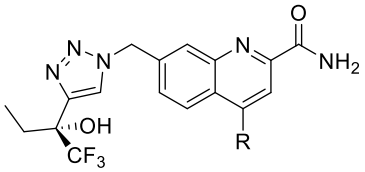
R	Cmpd	mGluR2 antag FLIPRI IC_{50}
	**1**	13 nM
	**2**	> 10,000 nM
	**3**	> 10,000 nM
	**4**	238 nM
	**5**	> 10,000 nM
	**6**	> 10,000 nM
	**7**	409 nM
	**8**	> 10,000 nM

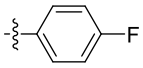
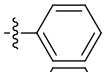
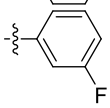
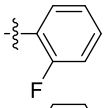
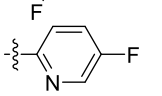
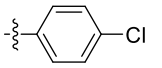
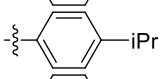
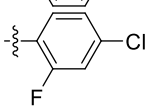
resulted in complete loss of activity. Interestingly, ketone **4** and nitrile **7** retain moderate levels of potency, while sulfone **5** and pyridine **6** are not tolerated.

We then proceeded to the exploration of the C-4 fluorophenyl group on the quinoline (Table 2). Small substituents are tolerated at all positions on the aryl (**9**–**11**), with *o*- and *p*-substitution being preferred. At the *p*-position, F and Cl are essentially equipotent (**13**), while introduction of a larger substituent (^{i}Pr, **14**) causes a drastic potency loss. *Bis*-substitution is tolerated as in compound **15**. While conversion of the aryl to a 2-pyridine retains potency, it also decreases brain penetration potential as measured by PGP (11.5 and 27.8 in human and rat, respectively). This is a general trend with this position, where we saw comparable PGP values among the various aryl substituted quinolines but significant erosion in PGP with most heterocyclic substituents we tested.

The C-7 vector was the next area to examine (Table 3) and we realized that the benzylic triazole has the highest impact on properties as activity, PGP, and PPB can all be modulated by appropriate modification of that moiety. Different 5-membered heterocycles are tolerated at C7 (*e.g.* **17**–**19**). Substitution at the α-methyl is also tolerated as in **20**-(+) and **20**-(−), but comes with drastic increase in rat PGP for

Table 2
SAR for the replacement of C4 fluorophenyl in **1**.

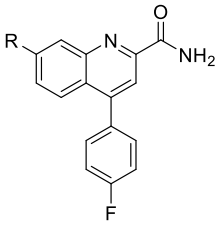


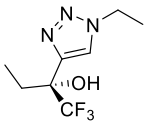
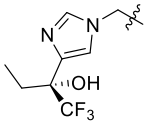
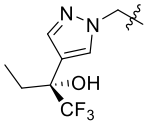
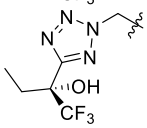
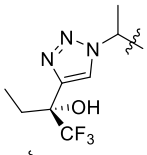
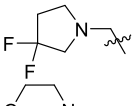
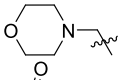
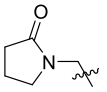
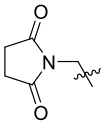
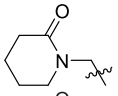
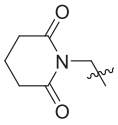
R	Cmpd	PGP h, r	mGluR2 antag FLIPRI IC_{50}
	**1**	2,2, 10.9	13 nM
	**9**		68 nM
	**10**		144 nM
	**11**		36 nM
	**12**	9.1, 4.1	36 nM
	**13**	2.1, 1.0	25 nM
	**14**		2700 nM
	**15**	2.1, 1.0	19 nM
	**16**		> 10,000 nM

both enantiomers. A significant breakthrough was the realization that removing the C7 substituent altogether provides a clear improvement in brain penetration in both human and rat (**21**) with only 10-fold loss in potency. We therefore elected to rebuild the C7 vector and explore non-heteroaryl substituents to improve the overall profile of the class, in the hopes of regaining activity while retaining the excellent properties in **21**. We indeed found that secondary amines such as **22** and **23** possess good potency and physicochemical properties while retaining good PGP values. More strikingly, we also observed exquisite and unique selectivity against mGluR3. We then proceeded to modify this vector and to lower the basicity of the amine, we synthesized corresponding amide analogue **24**, which maintained activity. Ultimately, the introduction of a second carbonyl group as in succinimide-substituted compound **25** resulted in a 20-fold potency improvement. Similar effects were observed with the corresponding 6-membered analogues containing piperidine-2,6-dione **27** and piperidin-2-one **26**.

The good activity seen with C7-unsubstituted quinoline **21** gave us an opportunity to assess the core and test the hypothesis of whether an internal hydrogen bond between the primary amide and the quinoline nitrogen favors the bioactive conformation (Table 4). This hypothesis was suggested by the observation that the addition of a second *ortho*-nitrogen to the amide as in quinazoline **28** results in complete loss of potency. To understand whether the loss was due to an unfavorable interaction of the 3-nitrogen with the binding pocket or a competing internal hydrogen bonding of the 3-nitrogen to the carboxamide, we prepared compounds **29** and **30**. In **29**, the carboxamide group is internally locked by cyclization. While we did lose activity with this modification, **29** still presents sub micromolar activity. However,

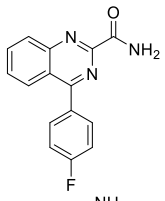
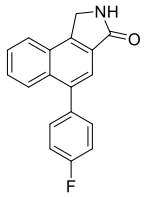
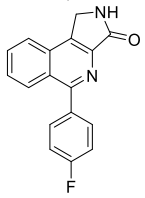
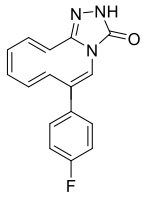
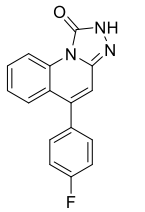
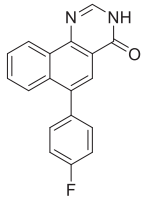
Table 3
SAR for the replacement of C7 triazole in **1** and selectivity against mGluR3.



R	Cmpd	PGP h, r	mGluR2 antag FLIPRI IC_{50}	mGluR3 antag FLIPRI IC_{50}
	**1**	2.2, 10.9	13 nm	3900 nM
	**17**	1.7, 21	16 nm	3500 nM
	**18**	1, 5.2	10 nm	> 10,000 nM
	**19**		16 nm	> 10,000 nM
	**20(+)** **20(−)**	6.3, 28.3 37.23.5	12 nm 19 nm	> 10,000 nM > 10,000 nM
	**21**	0.4, 0.4	149 nm	> 10,000 nM
	**22**	0.4, 0.5	61 nm	> 10,000 nM
	**23**	0.5, 0.9	68 nm	> 10,000 nM
	**24**		91 nm	> 10,000 nM
	**25**	1.5, 7.0	9 nm	> 10,000 nM
	**26**		58 nm	> 10,000 nM
	**27**	1.1, 3.5	21 nm	> 10,000 nM

reintroduction of the *ortho*-nitrogen in **30** caused a complete loss of potency, suggesting that this substitution is not tolerated and is the likely cause of the loss in activity in going from **21** to **28**. To interrogate the effect of the amide HBD-HBA pair position, we then designed matched pair **31** and **32**, where we observed a slight preference for a more extended HBA as in **31**. Six-membered cyclic analogue **33** also

Table 4
Quinoline carboxamide core SAR.

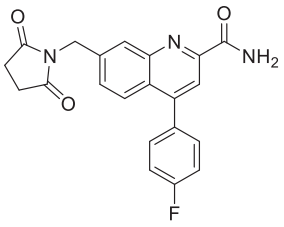
R	Cmpd	mGluR2 antag FLIPRI IC_{50}
	28	> 10,000 nM
	29	404 nM
	30	> 10,000 nM
	31	367 nM
	32	1378 nM
	33	> 10,000 nM

showed complete loss of activity. Ultimately, none of the constrained analogues designed showed comparable levels of potency to the unsubstituted primary amide, which was again revealed to be impervious to substitution.

The potency and selectivity of **25**, coupled with its excellent pharmacokinetic properties (see Fig. 2), fit the profile for a desirable *in vivo* tool compound to leverage towards achieving preclinical *in vivo* validation for this mechanism in relevant animal models.

Our first goal was to demonstrate *in vivo* target modulation with **25**. To this end, we tested the ability of **25** to reverse the effect of LY379268[16], a known mGluR2/3 orthosteric agonist. LY379268 inhibits amphetamine-induced hyperactivity and compound **25** is able to reverse the effect of LY379268 in mouse at a dose of 10 mpk (Fig. 3).

The mouse delayed non-match to position assay was selected as primary efficacy assay for the program, as it has been shown to be responsive to non-selective mGluR2/3 antagonists.[6] This assay assesses working memory. Scopolamine, a muscarinic acetylcholine receptor antagonist, was used to disrupt performance on this task given that



Compound 25

MW = 377;	LogD = 2.1
Solubility (pH7)	4.5 mM
mGluR2 FLIPR IC_{50}	8.9 nM (r)
mGluR2 GTPγS IC_{50}	20, 54 nM (h, r)
mGluR 1, 3, 4, 7, 8 FLIPR IC_{50}	>10000 nM
mGluR6 FLIPR IC_{50}	9220 nM
PPB	97.5, 97.2% (h, r)
PGP	1.5, 7.0, 9.8 (h, r, m)
Papp	29-33 cm-6/sec
MK499 IC_{50}	25 mM
CYP3A4 IC_{50}	>50 mM
PXR EC_{50}	1.1 mM

PK:	CL	$t_{1/2}$	Vd	%F
Rat	4.3	2.5	1.0	>99
Mouse	1.7	2.5	0.3	
Rhesus	7.1	2.7	1.7	>99

Fig. 2. Overall profile of compound **25**. For details of GTPγS assay, see Supplementary materials.

cholinergic deficits accompany Alzheimer's disease and that the scopolamine impairment is sensitive to non-selective antagonists. Compound **25** shows clear efficacy in this model, by affording significant dose-dependent reversal of scopolamine impairment. Plasma exposure were collected in separate animals dosed in the same manner and correlate with compound exposures (see Fig. 4).

In conclusion, *via* high throughput screening and subsequent SAR optimization, we discovered quinoline carboxamides as a highly potent and selective class of mGluR2 antagonists.[11] Compound **25** is an orally bioavailable mGluR2 negative allosteric modulator with excellent brain permeability. *In vivo*, **25** reverses the effect of mGluR2 agonist LY379268 in amphetamine-induced hyperlocomotion and shows good efficacy in the mouse delayed non-match to position assay at 10mpk. Since the discovery of this class,[8] several related carboxamides with similar mGluR2 selectivity profiles have been reported by others[9–12] and are in the clinical and preclinical space as the field continues to advance.

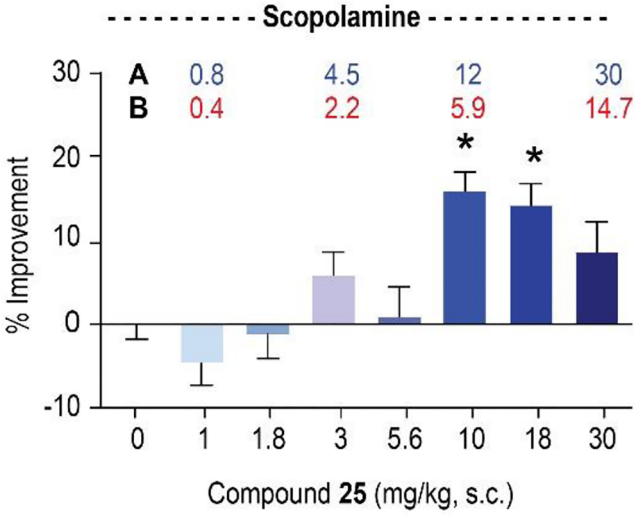


Fig. 4. Dose-responsive effects of **25** in delayed non-match to position in scopolamine-impaired mice. Values are % change from baseline. * indicates significant difference ($p < 0.05$) from 0.[17]

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

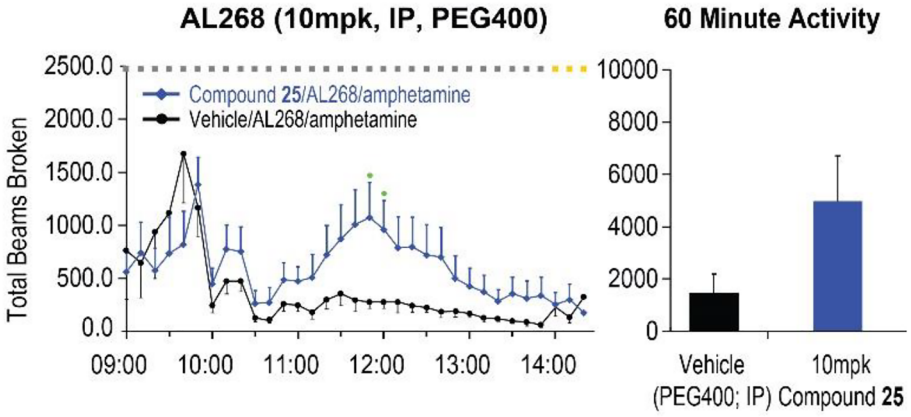


Fig. 3. Compound **25** displays *in vivo* mGluR2/3 target engagement by reversing LY379268 inhibition of Amphetamine-induced hyperactivity.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2020.127066.

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