ACS Medicinal Chemistry Letters

pubs.acs.org/acsmedchemlett Letter

Discovery of MK-8768, a Potent and Selective mGluR2 Negative Allosteric Modulator

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Cite This: *ACS Med. Chem. Lett.* 2023, 14, 1088−1094 Read Online

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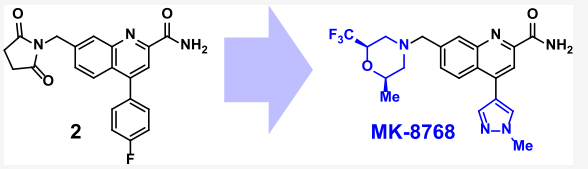
ABSTRACT: Glutamate plays a key role in cognition and mood, and it has been shown that inhibiting ionotropic glutamate receptors disrupts cognition, while enhancing ionotropic receptor activity is pro-cognitive. One approach to elevating glutamatergic tone has been to antagonize presynaptic metabotropic glutamate receptor 2 (mGluR2). A desire for selectivity over the largely homologous mGluR3 motivated a strategy to achieve selectivity through the identification of mGluR2 negative allosteric modulators (NAMs). Extensive screening and optimization efforts led to the identification of a novel series of 4-arylquinoline-2-carboxamides. This series was optimized for mGluR2 NAM potency, clean off-target activity, and desirable physical properties, which resulted in the identification of improved C4 and C7 substituents. The initial lead compound from this series was Ames-positive in a single strain with metabolic activation, indicating that a reactive metabolite was likely responsible for the genetic toxicity. Metabolic profiling and Ames assessment across multiple analogs identified key structure−activity relationships associated with Ames positivity. Further optimization led to the Ames-negative mGluR2 negative allosteric modulator MK-8768.



KEYWORDS: *mGluR2, glutamate, Ames, cognition*

The role of glutamate as a major excitatory neurotransmitter in the CNS[1] is well-known, and the effects of glutamate are mediated primarily through ionotrophic and metabotropic glutamate receptors (mGluRs).[2−4] Inhibiting the ionotropic glutamate receptors (NMDA and AMPA receptors) can disrupt cognition,[5] and activation of these receptors as a mechanism to enhance cognition[6−8] is associated with a number of challenges including desensitization and adverse events. The challenge of avoiding these unwanted effects while still elevating glutamatergic tone is potentially ameliorated through antagonism of presynaptic glutamate receptors.[9] Group II mGluRs (mGluR2, mGluR3) are presynaptic[10] and are autoinhibitory GPCRs such that inhibition of mGluR2 has been shown to increase synaptic glutamate, increase postsynaptic activity and plasticity, and potentially improve learning and memory.[11,12] Promisingly, antagonists[13] of mGluR2/3 show efficacy in preclinical cognition assays,[14,15] but mGluR3-driven hyperlocomotion and increased wake[16] have motivated the desire for mGluR2 selective ligands. Unfortunately, obtaining selective orthosteric antagonists has been elusive, presumably due to the high level of homology between mGluR2 and mGluR3. Therefore, selective inhibition of mGluR2 may be more achievable with allosteric ligands. The potential impact of developing a safe mGluR2 negative allosteric modulator (NAM) is significant, as a drug with this mechanism of action could have positive effects on a range of CNS disorders including Alzheimer's disease cognition[17] and depression[18−20]—both of which have significant unmet medical need.

In addition to a number of publications from other institutions on their efforts[21−24] toward identifying selective mGluR2 inhibitors, our company recently published[25] an approach to identification of selective mGluR2 NAMs, which led to the discovery of a 4-arylquinoline-2-carboxamide series. A high throughput screen identified compound **1** (Figure 1); initial SAR studies demonstrated that C2-carboxamide was

Received: May 17, 2023
Accepted: June 29, 2023
Published: July 12, 2023

https://doi.org/10.1021/acsmedchemlett.3c00210
ACS Med. Chem. Lett. 2023, 14, 1088−1094

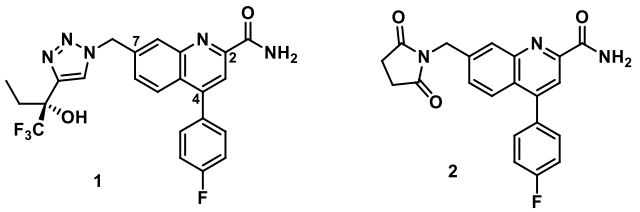


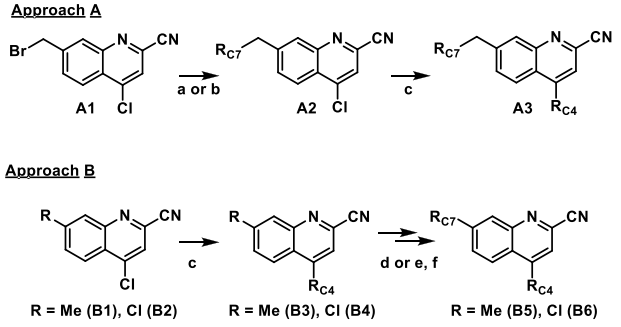
Figure 1. Screening hit **1** and tool compound **2**.

required for potency, the C4 vector preferred lipophilic groups, and C7 tolerated polar groups. Compound **2** was identified following these early studies, and it demonstrated the properties commensurate with an in vivo tool compound.

Compound **2** has good mGluR2 inhibition potency (FLIPR IC_{50} = 9 nM), full selectivity against mGluR3 (FLIPR $IC_{50} \geq$ 10 000 nM), and excellent rat oral pharmacokinetics (100%F). Compound **2** also was used to demonstrate that a selective mGluR2 NAM could show desirable activity in a mouse delayed nonmatch to position (DNMTP)[26] cognition assay. While **2** proved to be a good tool compound, it did have several properties that required optimization. The major areas we looked to improve were brain penetration, solubility, and off-target selectivity. **2** is a substrate for P-gp active transport[27] (rat P-gp BA:AB = 7.0), and thus the goal to advance an improved analog with optimum brain penetration was to eliminate this liability. The off-target challenges with **2** were primarily ion channels (hERG-binding[28] IC_{50} = 27 μM) and pregnane X receptor (PXR) agonism[29] (EC_{50} = 0.9 μM, 158% max agonism), which can lead to CYP induction and significant drug−drug interactions.

The initial strategy to identify an improved mGluR2 NAM was to further expand the C4 and C7 SAR to balance the overall properties with a focus on four identified key issues (P-gp, solubility, PXR, hERG). The synthetic tractability of the carboxamide series was a key feature that we were able to exploit to broadly explore both C4 and C7 substitutions with chemical libraries which enabled many analogs to be prepared quickly and a more rapid advancement of the series (Scheme 1).[30] In order to explore C4 diversity, approach A was used for many analogs—in this sequence, the C7 benzyl halide of intermediate **A1** was initially functionalized (S_N2, Suzuki, etc.), and the resulting intermediate **A2** was then subsequently reacted at C4 with Suzuki or similar cross-couplings. C7-SAR could be expanded using approach B, which simply flips the order of operations—starting from **B1**/**B2**, the initial Suzuki reaction to give **B3**/**B4** is followed by halogenation and functionalization of the C7-methyl group (R = Me) or direct functionalization if R = Cl. Both approaches pivoted on a late-stage conversion of 2-cyanoquinolines **A3** and **B5**/**B6** to the desired primary carboxamides.

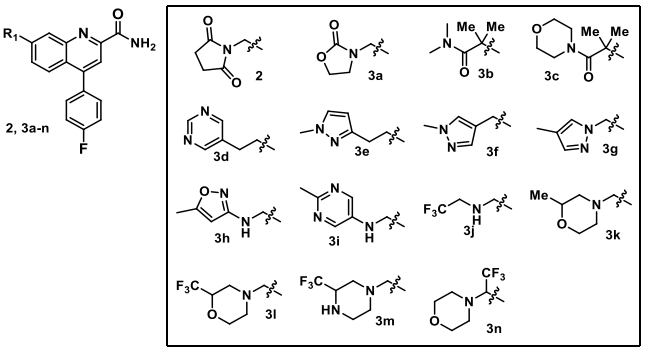
Scheme 1. General Approach to C4 and C7 Libraries



[a]R_{C7} amine or heterocycle, DIEA or K_2CO_3, DMF or CH_3CN. [b]$Pd(dppf)Cl_2$, R_{C7}-$B(OH)_2$, K_3PO_4, dioxane/water, 100 °C. [c]R_{C4}-$B(OH)_2$, $Pd(PPh_3)_4$, Na_2CO_3, dioxane/water, 100 °C. [d]B5—NBS, AIBN, CCl_4, then R_{C7} amine or heterocycle, DIEA or K_2CO_3, DMF or CH_3CN. [e]B6−potassium vinyltrifluoroborate, $Pd(OAc)_2$, RuPhos, Cs_2CO_3, dioxane, 60 °C. [f]9-BBN, THF, 60 °C, then $Pd_2(dba)_3$, cataCXium, K_2CO_3, water.

Examination of the C7 vector with multiple libraries and targeted singletons further validated the previously published[25] early SAR with a wide variety of polar functionality being tolerated including amides, carbamates, heterocycles, and amines (Table 1). Reducing the number of carbonyl groups and removing the amide of **2** did lead to improved solubility and reduced P-gp efflux but led to a large reduction in mGluR2 FLIPR potency and no improvement in other properties (**3a**). Acyclic amides were broadly tolerated with a ∼10-fold loss in potency and either no change in solubility (**3b**) or improved solubility with a large increase in P-gp efflux (**3c**). Complete removal of carbonyl functionality to give either homobenzylic (**3d**, **3e**) or benzylic (**3f**, **3g**) C- or N-linked aromatic heterocycles maintained much of the potency of **2** and generally provided analogs with no P-gp-transport liability, but analogs were poorly soluble and had low micromolar inhibition in the hERG binding assay. In an attempt to improve solubility, amino heterocycles were examined (**3h**, **3i**) and were generally

Table 1. C7 SAR



#	mGluR2 IC_{50} (nM)	pH 7 soly (μM)	PXR (EC_{50} μM,% max)	Rat P-gp (BA:AB)/Papp (10^{-6}cm/s)[b]	hERG[c] IC_{50} (μM)
2	4	4	0.9, 158%	7.0/33	27
3a	123	127	9.7, 95%	3.3/36	14[32]
3b	37	<2	3.5, 110%	5.0/38	40
3c	40	143	0.3, 122%	45/32	20.6
3d	7	<1	>30, 47%	1.0/31	>60
3e	15	<1	9.2, 97%	0.6/25	2.9
3f	13	4	>30, 21%	0.7/31	3.5
3g	27	<2	16.7, 53%	0.9/27	1.9
3h	15	3	>30, 49%	1.5/30	3.3
3i	19	7	>30, 1%	13/31	6.2
3j	22	6	>30, 50%	0.2/24	10.2
3k[a]	43	103	>30, 26%	NT	5.5
3l[a]	24	2	16.8, 73%	0.6/24	5.5
3m[a]	12	105	7.1, 99%	0.3/27	11.2
3n[a]	41	<2	4.9, 184%	1.4/28	11.2

[a]Mixture of enantiomers. [b]P-gp transport ratio BA/AB determined using LLC-MDR1 cells. [c]hERG-[^{35}S]-MK-499 binding assay[28]

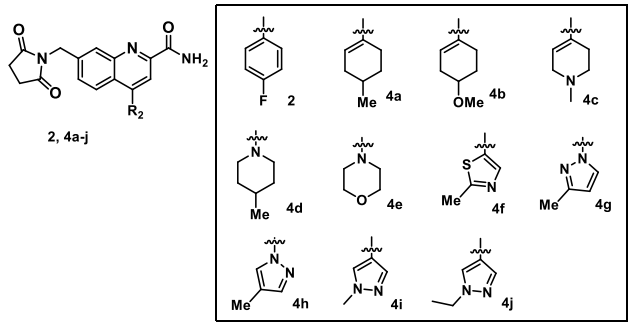
https://doi.org/10.1021/acsmedchemlett.3c00210
ACS Med. Chem. Lett. 2023, 14, 1088−1094

similar to the related homobenzylic heterocycles but did not lead to significant improvements in solubility or in other parameters, with the exception of **3i**, which had greatly reduced PXR activity. Reduction of aromatic content is a well-established strategy[31] to improve solubility and other properties, and while the simple primary benzyl amine was not potent (data not shown), a variety of alkyl and, in particular, fluoroalkyl substituted amines (**3j**−**n**) maintained reasonable potency. Fluoroalkyl amines were not included to address any specific liability but in order to increase library diversity. The methyl-substituted morpholine **3k** improved solubility and PXR activity significantly, and the corresponding trifluoromethyl-piperazine **3m** improved potency and was a P-gp nonsubstrate.

Overall, the extensive exploration of C7 with a C4-fluorophenyl led to diverse effects on properties, but it was challenging to balance multiple properties in parallel.

Examining the impact on properties in the same way for the C4 vector again was enabled with a library-focused approach (Table 2). As was communicated in the initial report,[25]

Table 2. C4 SAR



#	mGluR2 IC_{50} (nM)	pH 7 soly (μM)	PXR (EC_{50} μM,% max)	Rat P-gp (BA:AB)/Papp (10^{-6}cm/s)	hERG[c] IC_{50} (μM)
2	4	4	0.9, 158%	7.0/33	27
4a[a]	8	<1	0.6, 115%	1.4/32	>60
4b[a]	68	125	NT	19/38	NT
4c	4030	203	NT	NT	NT
4d	18	<2	1.0, 152%	11/35	17
4e	3170	157	2.8, 87%	NT	>60
4f	41	64	6.0, 137%	18/30	>60
4g	>5000	46	NT	NT	NT
4h	74	99	>30, 54%	17/30	NT
4i	67	162	20, 70%	22/24	27
4j	107	150	30, 30%	NT	NT

[a]Mixture of enantiomers. [b]P-gp transport ratio BA/AB determined using LLC-MDR1 cells. [c]hERG-[^{35}S]-MK-499 binding assay

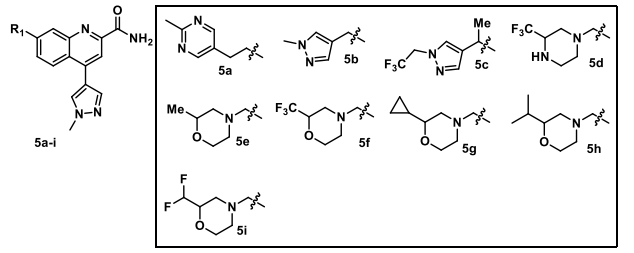
lipophilic groups were preferred at this position, and while substitution of the fluorophenyl ring was tolerated, the impact on other properties was generally minimal. With the primary goal of improving solubility, the sp^3 content of the molecules was increased with the introduction of saturated cycloalkyl and heterocycles (**4a**−**e**). 4-Methylcyclohexene derivative **4a** displayed excellent mGluR2 NAM potency and was a non-P-gp substrate, but the solubility was not improved. Attempts to introduce heteroatoms into targeted positions either negatively impacted P-gp (**4b**, **4d**) or significantly reduced potency (**4c**, **4e**). This strategy did improve solubility, but the improvement was only modest for the analogs that maintained some potency (**4b**, **4d**).

A wide range of heteroaromatic groups was then explored. Methylthiazole analogue **4f** maintained reasonable potency (41 nM) and pH 7 solubility (41 μM) but did not positively impact PXR or P-gp.

3-Methylpyrazole **4g** lost all potency, but isomeric **4h** was much more active (74 nM) and also showed some improvement in pH 7 solubility (99 μM) and PXR (>30 μM, 54%). *N*-methyl analogue **4i** also improved PXR and solubility while maintaining similar potency (67 nM). Further extending the *N*-alkyl group led to a loss in potency, but **4j** did maintain the positive impact on the other key optimization parameters.

Given the benefits of multiple parameters and the demonstrated (Table 1) ability to reduce P-gp efflux with C7 substituents, *N*-methylpyrazole was selected to explore further, with the primary goals being to increase potency and decrease P-gp efflux while maintaining or improving the positive impacts on solubility, PXR, and hERG binding. As before, libraries were used to expand on the C7 SAR with the C4 *N*-methylpyrazole-containing core (Table 3). With the C4,

Table 3. C4 *N*-Methylpyrazole SAR



#	mGluR2 IC_{50} (nM)	pH 7 soly (μM)	PXR (EC_{50} μM,% max)	rat P-gp (BA:AB)/Papp (10^{-6}cm/s)	hERG[c] IC_{50} (μM)
5a	56	141	>30, 8%	9.6/30	>60
5b	53	105	>30, 2%	6.6/36	>60
5c[a]	11	107	19, 75%	18/29	10
5d[a]	122	175	>30, 5%	4.2/29	>20
5e[a]	166	175	>30, 1%	NT	>60
5f[a]	12	132	>30, 24%	1.3/30	19
(*R*)-**5f**	11	142	>30, 9%	1.7/29	>60
5g[a]	32	175	1.7, 100%	3.4/29	51
5h[a]	23	142	29, 51%	1.3/34	23
5i[a]	67	193	NT	NT	NT

[a]Mixture of enantiomers. [b]P-gp transport ratio BA/AB determined using LLC-MDR1 cells. [c]hERG-[^{35}S]-MK-499 binding assay

fluorophenyl, homobenzylic, and benzylic heterocycles were highly potent. However, when combined with the C4 *N*-methylpyrazole (**5a**,**b**), no significant increase in potency compared to **4i** was observed, and moderate (53−56 nM) potency was maintained. Promisingly, the key off-targets (PXR and hERG) were significantly improved, and the P-gp transport was also reduced compared to **4i**. Potency could be improved with additional substitution (**5c**), but activity on PXR and hERG was significantly increased, and the P-gp-transport also increased. Interestingly, while the C4-fluorophenyl containing C7-trifluoromethylpiperazine (**3m**) was one of the more potent early analogs, the corresponding C4-*N*-methylpyrazole (**5d**) lost significant potency. In contrast, the related morpholines maintained (**5e**) or improved (**5f**) upon

https://doi.org/10.1021/acsmedchemlett.3c00210
ACS Med. Chem. Lett. 2023, 14, 1088−1094

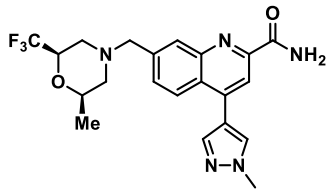
the potency present with the C4-fluorophenyl, and both analogs also had promising profiles with low PXR activity and excellent solubility. **5f** was not a substrate for P-gp. Additional 3-substituted morpholine analogs were then explored, but the SAR was quite sensitive, with small alkyl groups (**5g**,**h**) eroding the PXR selectivity and other small structural modifications (**5i**) leading to a loss in potency. While both enantiomers of the racemic trifluoromethyl-containing analogue (**5f**) maintained good potency (mGluR2 NAM IC_{50}: S-isomer = 31 nM/R-isomer = 11 nM), the more potent R-isomer ((*R*)-**5f**) was selected for further profiling. (*R*)-**5f** maintained the excellent selectivity (mGluR3 NAM IC_{50} = 7800 nM) of **2** and showed significant improvement in solubility (pH7 = 142 μM), PXR ($EC_{50} \geq 30$ μM, 9% max), and P-gp ((BA:AB; rat/human) = 1.7/0.8; P_{app} = 29). (*R*)-**5f** also possessed an excellent ion channel profile (hERG/$CaV_{1.2}$/$NaV_{1.5}$ (IC50) $\geq$ 60/22/>30 μM) as well as a high level of general off-target selectivity (no hits in Panlabs[33] panel of >120 targets tested at 30 μM). (*R*)-**5f** was also orally bioavailable in rodents (rat = 100%F @ 20 mpk) and demonstrated activity in the mouse DMNTP assay (data not shown). While (*R*)-**5f** appeared to have a very attractive profile, it was discovered in an exploratory 5-strain Ames assessment[34] that in the presence of metabolic activation (rat S9 fraction) in a single strain (TA97a), (*R*)-**5f** was Ames-positive (>2-fold increase in revertants at $\geq$300 μg/plate), thus indicating a risk of mutagenicity. This result prevented (*R*)-**5f** from advancing further, but since the Ames-positive result was only in the presence of metabolic activation, we explored the sites of metabolism with hopes to identify a way to mitigate the problematic pathway.

In human, dog, and rat hepatocytes as well as in rat and dog in vivo, the metabolism of (*R*)-**5f** features several C7-trifluoromethyl morpholine oxidation products as well as demethylation of the C4-*N*-methylpyrazole (Supporting Information S2). We then assessed the single strain Ames profile of analogs with changes in the C4 and C7 region using a screening paradigm in TA97a with rat S9 only. The results from this effort (Supporting Information S3) revealed that changes in the C4 vector did not lead to changes in Ames-positivity in TA97a with rat S9, but minor or major changes in the C7 substituent result in single-strain Ames-negative analogs, indicating the likely cause of the Ames positivity in (*R*)-**5f** is an unidentified/unconfirmed reactive metabolite or intermediate on the C7-trifluoromethylmorpholine. While all di- or trisubstituted C7-trifluoromethylmorpholines (S2−7 to S2−13) were Ames negative in TA97a with rat S9, the (2*R*,6*R*)-2-methyl-6-(trifluoromethyl) morpholine isomer possessed the best combination of potency, P-gp, solubility, and hERG inhibition and advanced to become MK-8768.

A more complete profile of MK-8768 is shown in Table 4. MK-8768 is a 9.6 nM inhibitor of mGluR2 with complete selectivity against mGluR1,3,4,5,6,8 (IC_{50} > 10 000 nM). MK-8768 also demonstrated an excellent overall selectivity profile with weak to no activity on the hERG, IK_s, and $Na_{v1.5}$ ion channels and no confirmed activities < 10 μM in a Panlabs screen of >120 off-targets. MK-8768 is a nonsubstrate for rat, human, and monkey P-gp; has high passive permeability; and demonstrated good brain penetration in rats ($Kp_{u,u}$ > 1; CSF: $[plasma]_u$ = 1). A lack of significant activity at PXR or inhibition (reversible and time-dependent) of cytochrome P450s indicates that MK-8768 has a low risk for being a perpetrator of drug−drug interactions. In human hepatocytes, the major routes of metabolism were hydroxylation of the morpholine ring region and dehydrogenation of the morpholine region. *N*-Demethylation of the pyrazole was also observed in human hepatocytes but was a more significant pathway in rats and monkeys. Interestingly, similar to (*R*)-**5f**, metabolism on the C7-morpholine was the major metabolic pathway (Supporting Information S4), but MK-8768 was Ames negative in a full five-strain Ames assay. In rats, dogs, and monkeys, MK-8768 exhibited moderate clearance, and the effective half-life ranged from 1.7 h in monkeys to 3.3 h in rats. The oral bioavailability was 32% and 34% in rats and dogs, respectively. The in vivo efficacy of MK-8768 was then assessed in a rhesus monkey object retrieval detour (ORD) task,[35,36] which is an assessment of executive function and attention. In the ORD assay, performance on difficult, but not easy, trials is disrupted by scopolamine, ketamine, and PFC lesions. MK-8768 was coadministered (Figure 2) with a dose of scopolamine titrated to produce the targeted level of impairment (~90% correct difficult trials reduced to ~60% correct difficult trials), and a significant reversal of the scopolamine deficit was achieved by intramuscular doses from 0.03 to 1 mg/kg (see Supporting Information S1 for details). The efficacious doses produced unbound plasma concentrations from 3−65 nM, which represents 0.3−6.5-fold the in vitro mGluR2 IC_{50}. Higher doses of MK-8768 (3 mg/kg) were not effective in improving difficult trial performance, indicative of an inverted U-shaped dose−effect curve that oftentimes accompanies pro-cognitive mechanisms in preclinical studies.

MK-8768 can be prepared as a single enantiomer starting from (*R*)-3,3,3-trifluoropropylene oxide (TFPO) and (*S*)-2-chloropropanoic acid in the longest linear sequence of eight steps in 31% overall yield (Scheme 2).

Table 4. Profile of MK-8768



mGluR2 FLIPR IC_{50}	9.6 nM
mGluR1,3,4,5,6,8 FLIPR IC_{50}	>10000 nM
P-GP[a] (BA:AB; rat/human/monkey); Papp	2.1/1.5/1.4; 32
PXR EC_{50}	>30 μM, 12% @ 30 μM
hERG/IK_s/$NaV_{1.5}IC_{50}$[b]	22/>30/>30 μM
five strain Ames	negative
CYP IC_{50} (1A2, 2B6, 2C8, 2C9, 2C19, 2D6, 3A4)	>26 μM, no time dependent inhibition
tosylate salt xtal soly (SGF/FaSSIF/FeSSIF)	4.1/0.44/2.1 mg/mL
plasma fraction unbound (human/rat/dog/monkey)	2.5%/4%/4.5%/4.3%
rat PK (CL; Vd; $T_{1/2,eff}$; bioavailability)[c]	36 mL/min/kg; 5.7 L/kg; 1.8 h; 32% F
dog PK (CL; Vd; $T_{1/2,eff}$; bioavailability)[d]	24 mL/min/kg; 7.3 L/kg; 3.3 h; 34% F
monkey PK (CL; Vd; $T_{1/2,eff}$)[e]	22 mL/min/kg; 3.2 L/kg; 1.7 h

[a]P-gp transport ratio BA/AB determined using LLC-MDR1 cells. [b]Determined using PatchXpress automated patch-clamp system. [c]Rat: 1 mg/kg IV, 2 mg/kg PO. [d]Dog: 0.25 mg/kg IV, 0.5 mg/kg PO. [e]Monkey: 0.5 mg/kg IV.

https://doi.org/10.1021/acsmedchemlett.3c00210
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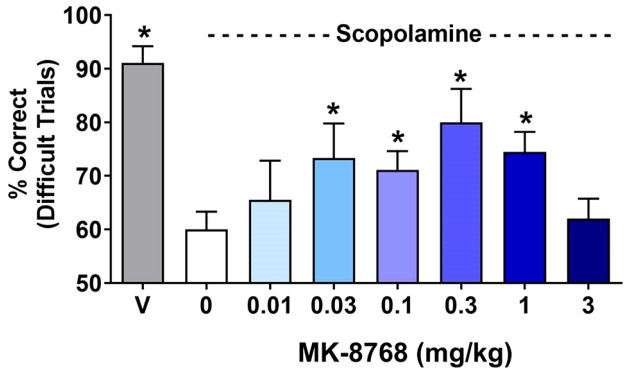
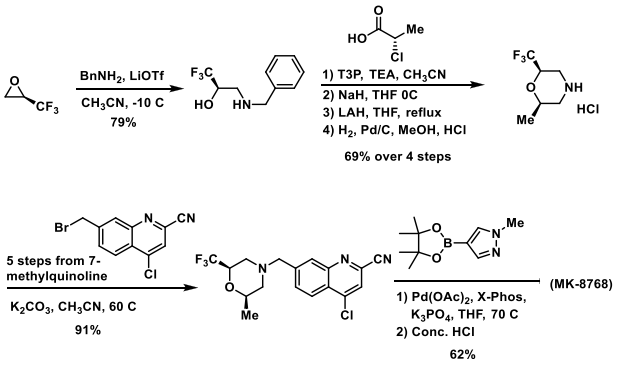


Figure 2. MK-8768 efficacy in the rhesus ORD task. MK-8768 improved object retrieval in rhesus monkeys following scopolamine impairment. *Indicates significantly different than animals given vehicle prior to scopolamine (white bar).

Scheme 2. Synthesis of MK-8768



In summary, initial screening for mGluR2 inhibition identified a series of mGluR2-selective quinoline carboxamides that were optimized for potency, brain penetration, solubility, and off-target profile. This led to the identification of (*R*)-**5f**, which has an excellent all-around profile but was Ames-positive in a single strain with metabolic activation. SAR studies established the problematic functionality as the monosubstituted C7-trifluoromethylmorpholine, and Ames-negative disubstituted analogue MK-8768 was subsequently identified as the optimum analog. MK-8768 is a potent, selective, highly soluble mGluR2 NAM with good oral pharmacokinetics and demonstrated efficacy in a rhesus monkey model of executive function and attention. Further investigations of MK-8768 in additional preclinical or clinical settings will be published at a later date.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsmedchemlett.3c00210.

Experimental details and HRMS data of the synthesis of MK-8768; HRMS data for examples 1−5i and S3−1 to S3−13; in vitro metabolism profile for (R)-5f and MK-8768; Ames TA97a (with rat S9) SAR (PDF)

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The design, synthesis, and characterization of compounds was conducted by M.T.R., P.J.M., B.H., Z.M., Y.S., P.d.L., J.L.F., Y.H., J.M.-C.W., Z.-Q.Y., J.J.P., D.M.H., J.J.M., H.Z., C.J.B., and A.C. The compounds were analyzed and further characterized by M.L.C., I.H., L.M., J.O., V.N.U., J.B.S., D.C.D., V.P., D.M.E., J.D.V., and J.M.U. The DMPK characterization of compounds was conducted by KLF, BL, YL. The work was managed by R.E.D., J.T.K., J.M.U., and R.S.M. M.T.R. wrote the manuscript with help in review from all authors.

Funding

These studies were funded by Merck Sharp and Dohme LLC, a subsidiary of Merck and Co., Inc., Rahway, NJ, USA.

Notes

The authors declare the following competing financial interest(s): All authors were employees of Merck Sharp & Dohme LLC, a subsidiary of Merck & Co., Inc., Rahway, NJ, USA at the time of their contribution to this work.

■ ACKNOWLEDGMENTS

We would like to thank all mGluR2 NAM project members who contributed to this work.

■ ABBREVIATIONS

CNS, central nervous system; $mGluR_2$, metabotropic glutamate receptor 2; NAM, negative allosteric modulator; P-gp, P-glcoprotein; HPLC, high-performance liquid chromatography; DIEA, diisopropylethyl amine; RuPhos, 2-dicyclohexylphosphino-2′,6′-diisopropoxybiphenyl; cataCXium, Di(1-adamantyl)-*n*-butylphosphine; NMDA, *N*-methyl-D-aspartate; AMPA, *α*-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; FLIPR, fluorescence imaging plate reader; DNMTP, delayed nonmatch to position; hERG, human ether-a-go-go related gene; PXR, pregnane X receptor; SAR, structure−activity relationship; $CaV_{1.2}$, calcium channel, voltage-dependent, L type, alpha 1C subunit; $NaV_{1.5}$, sodium channel protein type 5 subunit alpha; CYP, cytochrome P450; PK, pharmacokinetics; CL, clearance; Vd, volume of distribution; ORD, object retrieval detour

■ REFERENCES

(1) Fonnum, F. Glutamate: a neurotransmitter in mammalian brain. *J. Neurochem.* **1984**, *42* (1), 1−11.
(2) Kew, J. N. C.; Kemp, J. A. Ionotropic and metabotropic glutamate receptor structure and pharmacology. *Psychopharmacology (Berlin, Ger.)* **2005**, *179* (1), 4−29.
(3) Reiner, A.; Levitz, J. Glutamatergic Signaling in the Central Nervous System: Ionotropic and Metabotropic Receptors in Concert. *Neuron* **2018**, *98* (6), 1080−1098.
(4) Niswender, C. M.; Conn, P. J. Metabotropic glutamate receptors: physiology, pharmacology, and disease. *Annu. Rev. Pharmacol. Toxicol.* **2010**, *50*, 295−322.
(5) Keifer, J.; Zheng, Z. AMPA receptor trafficking and learning. *Eur. J. Neurosci* **2010**, *32* (2), 269−277.
(6) Lynch, G. Memory enhancement: the search for mechanism-based drugs. *Nat. Neurosci.* **2002**, *5* (Suppl), 1035−1038.
(7) Tang, Y.-P.; Shimizu, E.; Dube, G. R.; Rampon, C.; Kerchner, G.; Zhuo, M.; Liu, G.; Tsien, J. Z. Genetic enhancement of learning and memory in mice. *Nature (London)* **1999**, *401* (6748), 63−69.
(8) Morrow, J. A.; Maclean, J. K. F.; Jamieson, C. Recent advances in positive allosteric modulators of the AMPA receptor. *Curr. Opin. Drug Discovery Dev.* **2006**, *9* (5), 571−579.
(9) Conn, P. J.; Christopoulos, A.; Lindsley, C. W. Allosteric modulators of GPCRs: a novel approach for the treatment of CNS disorders. *Nat. Rev. Drug Discovery* **2009**, *8* (1), 41−54.
(10) Shigemoto, R.; Kinoshita, A.; Wada, E.; Nomura, S.; Ohishi, H.; Takada, M.; Flor, P. J.; Neki, A.; Abe, T.; Nakanishi, S.; Mizuno, N. Differential presynaptic localization of metabotropic glutamate receptor subtypes in the rat hippocampus. *J. Neurosci.* **1997**, *17* (19), 7503−7522.
(11) Xi, Z.-X.; Baker, D. A.; Shen, H.; Carson, D. S.; Kalivas, P. W. Group II metabotropic glutamate receptors modulate extracellular glutamate in the nucleus accumbens. *J. Pharmacol. Exp. Ther.* **2002**, *300* (1), 162−171.
(12) Kim, S. H.; Steele, J. W.; Lee, S. W.; Clemenson, G. D.; Carter, T. A.; Treuner, K.; Gadient, R.; Wedel, P.; Glabe, C.; Barlow, C.; Ehrlich, M. E.; Gage, F. H.; Gandy, S. Proneurogenic Group II mGluR antagonist improves learning and reduces anxiety in Alzheimer A*β* oligomer mouse. *Mol. Psychiatry* **2014**, *19* (11), 1235−1242.
(13) Qunies, A. a. M.; Emmitte, K. A. Negative allosteric modulators of group II metabotropic glutamate receptors: A patent review (2015 - present). *Expert Opin. Ther. Pat.* **2021**, *31* (8), 687−708.
(14) Shimazaki, T.; Kaku, A.; Chaki, S. Blockade of the metabotropic glutamate 2/3 receptors enhances social memory via the AMPA receptor in rats. *Eur. J. Pharmacol.* **2007**, *575* (1−3), 94−97.
(15) Woltering, T. J.; Wichmann, J.; Goetschi, E.; Knoflach, F.; Ballard, T. M.; Huwyler, J.; Gatti, S. Synthesis and characterization of 1,3-dihydro-benzo[b][1, 4]diazepin-2-one derivatives: Part 4. In vivo active potent and selective non-competitive metabotropic glutamate receptor 2/3 antagonists. *Bioorg. Med. Chem. Lett.* **2010**, *20* (23), 6969−6974.
(16) Wood, C. M.; Wafford, K. A.; McCarthy, A. P.; Hewes, N.; Shanks, E.; Lodge, D.; Robinson, E. S. J. Investigating the role of mGluR2 versus mGluR3 in antipsychotic-like effects, sleep-wake architecture and network oscillatory activity using novel Han Wistar rats lacking mGluR2 expression. *Neuropharmacology* **2018**, *140*, 246−259.
(17) Francis, P. T. Glutamatergic Approaches to the Treatment of Cognitive and Behavioural Symptoms of Alzheimer's Disease. *Neurodegener. Dis.* **2008**, *5* (3−4), 241−243.
(18) Campo, B.; Kalinichev, M.; Lambeng, N.; El Yacoubi, M.; Royer-Urios, I.; Schneider, M.; Legrand, C.; Parron, D.; Girard, F.; Bessif, A.; Poli, S.; Vaugeois, J.-M.; Le Poul, E.; Celanire, S.

Characterization of an mGluR2/3 Negative Allosteric Modulator in Rodent Models of Depression. *J. Neurogenet.* **2011**, *25* (4), 152−166.
(19) Krystal, J. H.; Mathew, S. J.; D'Souza, D. C.; Garakani, A.; Gunduz-Bruce, H.; Charney, D. S. Potential psychiatric applications of metabotropic glutamate receptor agonists and antagonists. *CNS Drugs* **2010**, *24* (8), 669−693.
(20) Sanacora, G.; Treccani, G.; Popoli, M. Towards a glutamate hypothesis of depression. *Neuropharmacology* **2012**, *62* (1), 63−77.
(21) Felts, A. S.; Rodriguez, A. L.; Smith, K. A.; Engers, J. L.; Morrison, R. D.; Byers, F. W.; Blobaum, A. L.; Locuson, C. W.; Chang, S.; Venable, D. F.; Niswender, C. M.; Daniels, J. S.; Conn, P. J.; Lindsley, C. W.; Emmitte, K. A. Design of 4-Oxo-1-aryl-1,4-dihydroquinoline-3-carboxamides as Selective Negative Allosteric Modulators of Metabotropic Glutamate Receptor Subtype 2. *J. Med. Chem.* **2015**, *58* (22), 9027−9040.
(22) Zhang, X.; Xiao, Z.; Kumata, K.; Yamasaki, T.; Josephson, L.; Zhang, M.-R.; Wang, L.; Liang, S. H. Positron Emission Tomography (PET) Imaging of Metabotropic Glutamate Receptor Subtype 2 (mGlu2) Based on a Negative Allosteric Modulator Radioligand. *Neuromethods* **2021**, *164*, 23−37.
(23) Childress, E. S.; Wieting, J. M.; Felts, A. S.; Breiner, M. M.; Long, M. F.; Luscombe, V. B.; Rodriguez, A. L.; Cho, H. P.; Blobaum, A. L.; Niswender, C. M.; Emmitte, K. A.; Conn, P. J.; Lindsley, C. W. Discovery of Novel Central Nervous System Penetrant Metabotropic Glutamate Receptor Subtype 2 (mGlu2) Negative Allosteric Modulators (NAMs) Based on Functionalized Pyrazolo[1, 5-a]pyrimidine-5-carboxamide and Thieno[3, 2-b]pyridine-5-carboxamide Cores. *J. Med. Chem.* **2019**, *62* (1), 378−384.
(24) Bollinger, K. A.; Felts, A. S.; Brassard, C. J.; Engers, J. L.; Rodriguez, A. L.; Weiner, R. L.; Cho, H. P.; Chang, S.; Bubser, M.; Jones, C. K.; Blobaum, A. L.; Niswender, C. M.; Conn, P. J.; Emmitte, K. A.; Lindsley, C. W. Design and Synthesis of mGlu2 NAMs with Improved Potency and CNS Penetration Based on a Truncated Picolinamide Core. *ACS Med. Chem. Lett.* **2017**, *8* (9), 919−924.
(25) Shu, Y.; Diamond, T. L.; Hershey, J. C.; Huang, S.; Magliaro, B. C.; O'Brien, J. A.; Schlegel, K.-A. S.; Puri, V.; Uebele, V. N.; Uslaner, J. M.; Wang, C.; Converso, A. Discovery of 4-arylquinoline-2-carboxamides, highly potent and selective class of mGluR2 negative allosteric modulators: From HTS to activity in animal models. *Bioorg. Med. Chem. Lett.* **2020**, *30* (9), 127066.
(26) Dunnett, S. B.; Evenden, J. L.; Iversen, S. D. Delay-dependent short-term memory deficits in aged rats. *Psychopharmacology (Berlin)* **1988**, *96* (2), 174.
(27) Doan, K. M. M.; Humphreys, J. E.; Webster, L. O.; Wring, S. A.; Shampine, L. J.; Serabjit-Singh, C. J.; Adkison, K. K.; Polli, J. W. Passive permeability and P-glycoprotein-mediated efflux differentiate central nervous system (CNS) and non-CNS marketed drugs. *J. Pharmacol. Exp. Ther.* **2002**, *303* (3), 1029−1037.
(28) Priest, B. T.; Bell, I. M.; Garcia, M. L. Role of hERG potassium channel assays in drug development. *Channels (Austin)* **2008**, *2* (2), 87−93.
(29) Sinz, M. W. Evaluation of pregnane X receptor (PXR)-mediated CYP3A4 drug-drug interactions in drug development. *Drug Metab. Rev.* **2013**, *45* (1), 3−14.
(30) Bungard, C. J.; Converso, A.; De Leon, P.; Hanney, B.; Hartingh, T. J.; Manikowski, J. J.; Manley, P. J.; Meissner, R.; Meng, Z.; Perkins, J. J.; Rudd, M. T.; Shu, Y. Preparation of quinoline carboxamide and quinoline carbonitrile derivatives as mGluR2-negative allosteric modulators. WO2013066736, 2013.
(31) Lovering, F.; Bikker, J.; Humblet, C. Escape from Flatland: Increasing Saturation as an Approach to Improving Clinical Success. *J. Med. Chem.* **2009**, *52* (21), 6752−6756.
(32) Zhang, X.; Zhang, Y.; Chen, Z.; Shao, T.; Van, R.; Kumata, K.; Deng, X.; Fu, H.; Yamasaki, T.; Rong, J.; Hu, K.; Hatori, A.; Xie, L.; Yu, Q.; Ye, W.; Xu, H.; Sheffler, D. J.; Cosford, N. D. P.; Shao, Y.; Tang, P.; Wang, L.; Zhang, M.-R.; Liang, S. H. Synthesis and preliminary studies of 11C-labeled tetrahydro-1,7-naphthyridine-2-carboxamides for PET imaging of metabotropic glutamate receptor 2. *Theranostics* **2020**, *10* (24), 11178−11196.
(33) Eurofins Discovery - Your Novel Molecule Can Change the World.https://www.eurofinsdiscovery.com/.
(34) Escobar, P. A.; Kemper, R. A.; Tarca, J.; Nicolette, J.; Kenyon, M.; Glowienke, S.; Sawant, S. G.; Christensen, J.; Johnson, T. E.; McKnight, C.; Ward, G.; Galloway, S. M.; Custer, L.; Gocke, E.; O'Donovan, M. R.; Braun, K.; Snyder, R. D.; Mahadevan, B. Bacterial mutagenicity screening in the pharmaceutical industry. *Mutat. Res., Rev. Mutat. Res.* **2013**, *752* (2), 99−118.
(35) Diamond, A.; Zola-Morgan, S.; Squire, L. R. Successful performance by monkeys with lesions of the hippocampal formation on AB and object retrieval, two tasks that mark developmental changes in human infants. *Behav Neurosci* **1989**, *103* (3), 526−537.
(36) Taylor, J. R.; Elsworth, J. D.; Roth, R. H.; Sladek, J. R., Jr.; Redmond, D. E. Jr., Cognitive and motor deficits in the acquisition of an object retrieval/detour task in MPTP-treated monkeys. *Brain* **1990**, *113* (3), 617−637.

https://doi.org/10.1021/acsmedchemlett.3c00210
ACS Med. Chem. Lett. 2023, 14, 1088−1094