N-Acyl-*N′*-arylpiperazines as negative allosteric modulators of mGlu$_1$: Identification of VU0469650, a potent and selective tool compound with CNS exposure in rats

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Abstract

Development of SAR in an *N*-acyl-*N′*-arylpiperazine series of negative allosteric modulators of mGlu$_1$ using a functional cell-based assay is described in this Letter. Characterization of selected compounds in protein binding assays was used to aid in selecting VU0469650 for further profiling in ancillary pharmacology assays and pharmacokinetic studies. VU0469650 demonstrated an excellent selectivity profile and good exposure in both plasma and brain samples following intraperitoneal dosing in rats.

Keywords

Glutamate; GPCR; mGlu$_1$; Allosteric modulator; CNS; Piperazine

ʟ-Glutamic acid (glutamate) is the major excitatory neurotransmitter in the mammalian central nervous system (CNS). Glutamate produces its effects through binding to both ionotropic and metabotropic glutamate receptors. The metabotropic glutamate receptors (mGlus) belong to family C of the G protein-coupled receptors (GPCRs). Further classification of the mGlus discovered to date has been 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] Many of these receptors have attracted significant attention as promising targets for the treatment of a variety of CNS related disorders. The design of drug-like compounds that selectively target a specific mGlu has been complicated by the fact that the orthosteric binding site of the mGlus is highly conserved. An alternative strategy that has proven successful for overcoming such selectivity hurdles has been the optimization of compounds that modulate the function of the receptor through interaction with an allosteric binding site.[2]

An area within the mGlu allosteric modulator field that has garnered substantial interest has been the design and application of selective small molecule negative allosteric modulators (NAMs) of mGlu$_1$.[3] Several tool compounds have been discovered during recent years

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further establishing the link between mGlu$_1$ antagonism and the potential treatment of CNS related disorders such as addiction,[4] anxiety,[5] epilepsy,[6] pain,[5a,7] and psychotic disorders. [5a,8] Compounds **1–6** (Fig. 1) are representative of the mGlu$_1$ NAM chemotypes that have been discovered to date and examples of molecules with demonstrated efficacy in vivo. For example, JNJ16259685 (**1**) was efficacious in a rat model of anxiety[9] as well as rat[10] and squirrel monkey[11] models of addiction. A-841720 (**2**) was established as an analgesic in rat models of neuropathic pain[12] and post-operative pain.[13] Inhibition of nociceptive pain was observed with **3** in rats[14] and with YM298198 (**4**) in mice.[15] Antipsychotic activity as measured by acoustic prepulse inhibition models was noted with **5**[16] and **6**[17] in rats and with **1**[8] and **4**[8] in mice. Also of interest, recent research has identified a potential role for mGlu$_1$ inhibition in the treatment melanoma[18] and certain types of breast cancer.[19]

We have recently become interested in the discovery and development of structurally novel mGlu$_1$ NAMs with properties suitable for evaluation in rodent models of a variety of CNS disorders. A potential starting point for a hit to lead optimization program focused on mGlu$_1$ NAMs was identified through cross screening during the course of our recent work in the mGlu$_5$ NAM field (Fig. 2).[20] The adamantyl carboxamides **7** and **8** were mGlu$_5$ NAMs that were also found to behave as mGlu$_1$ NAMs in a functional cell based assay.[21] Our mGlu$_1$ assay measures the ability of the compound to block the mobilization of calcium by an EC$_{80}$ concentration of glutamate in cells expressing human mGlu$_1$.

One attractive feature of this scaffold was the fact that robust chemistry would allow to us to rapidly vary three distinct portions of the chemotype and evaluate considerable synthetic diversity (Scheme 1). For instance, commercially available and enantiopure piperazines **9** ($R^1 = CH_3$) were useful for evaluating substitution of the piperazine core. For the purpose of evaluating SAR about the aryl portion of the chemotype (R^2), substituted piperazines **9** were reacted with adamantoyl chloride to afford amide intermediates **10**. Removal of the carbamate protecting group under acidic conditions gave amine intermediates **11**. Target compounds were accessible from amines **11** through nucleophilic aromatic substitution reactions with aryl fluorides or Buchwald–Hartwig[22] amination reactions with suitable aryl halides.[23] For evaluation of SAR around the amide portion of the scaffold, a simple reorganization of the synthetic transformations was required. Reaction of amines **9** with either 2-fluoropyridine or 2-bromopyridine under appropriate conditions provided intermediate **12**. Following cleavage of the protecting group, amines **13** were readily converted to the target amide compounds using well established methods.[24]

Evaluation of methyl substituted piperazines was one of our first areas of investigation (Table 1). New compounds were prepared with the 2-pyridyl group found in hit **7** (**14–17**). Evaluation of these enantiopure analogs yielded some interesting results. A preference with regard to the mGlu$_1$ NAM activity was observed for one enantiomer versus the other in the case of both the 2-methyl and 3-methyl analogs. With the 2-methyl analogs a 15- fold preference was observed for the *R*-enantiomer (**15** vs **14**), while with the 3-methyl analogs a sixfold preference was observed for the *R*-enantiomer (**17** vs **16**). Furthermore, both **15** and **17** were approximately fourfold more potent than the unsubstituted comparator **7**. Moreover, an equally substantial discovery was noted upon testing of **15** and **17** in our functional mGlu$_5$ assay.[25] Both compounds were only weak antagonists in this assay (IC$_{50}$ >10 ¼M***). In short order, we had moved from a hit compound with little to no group I mGlu selectivity to two compounds with more than 60-fold selectivity for mGlu$_1$ versus mGlu$_5$.

We next chose to evaluate SAR around the amide portion of the chemotype in the context of both the (*R*)-2-methyl piperazine (series I) and the (*R*)-3-methyl piperazine (series II) cores (Table 2). A number of analogs (**18–35**) were prepared in an attempt to identify

replacements for the 1-adamantyl group. This was not generally successful; however, there were some notable exceptions. In the context of the (R)-2-methyl piperazine but not the (R)-3-methyl piperazine, the cyclooctyl (**26**) and the *trans*-(*tert*-butyl)cyclohexyl (**30**) groups demonstrated weak to moderate NAM activity against mGlu$_1$. Additionally, the bicyclo[3.3.1]nonan-3-yl (**32**) group was essentially equipotent to **15**, though this was again not effective in the case of the (R)-3-methyl piperazine (**33** vs **17**). It has been reported that the cubyl group can function as an effective and less lipophilic isosteric replacement for the adamantyl group.[26] Unfortunately, such a modification was not effective in our chemotype (**34** and **35**).

Our attention next moved from the amide to the aryl portion of the template (Table 3). Using an approach similar to that previously described, we again observed only weak antagonists (**37**, **41**, **43**, **45**) and a compound (**39**) with no activity at the top concentration tested (30 μM) in the context of the (R)-3-methyl piperazine. Fortunately, the same was not observed with the (R)-2- methyl piperazine analogs. Though 4-pyridyl analog **38** demonstrated only weak mGlu$_1$ NAM activity, 3-pyridyl analog **36** exhibited good potency. Good to moderate potency was also noted with pyrimidines **40** and **44** and pyrazine **42**. Finally, the 2-cyanophenyl analog **46** had potency within twofold of that observed with **15** and represented an improvement over the unsubstituted comparator piperazine **8** (Fig. 2).

Having seen little progress in identifying improved analogs with the (R)-3-methyl piperazine core, we chose to focus our remaining efforts on attempting to improve upon the results observed with (R)-2-methyl piperazine analogs **15** and **46** (Table 4). Knowing that very subtle structural modifications can dramatically affect activity with allosteric modulators, we prepared fluorinated analogs of **15** (**48**–**50**). Though all three compounds exhibited good potency, 6-fluoropyridine **48** was the most potent and only slightly less potent than **15**.We also prepared heteroaryl analogs (**51**–**54**) of **46** as a means to reduce lipophilicity. Most of these derivatives demonstrated good (**51** and **53**) to moderate (**52**) potency; however, analog **54** represented a nearly threefold improvement relative to **46** and the most potent compound from within the chemotype identified to date.

Calculation of the ligand-lipophilicity efficiency (LLE) values for our most potent analogs identified analog **54** as the compound with the best balance of potency and lipophilicity, two factors linked to drug-likeness (Table 5).[27] We also decided to examine these same compounds for their propensity to non-specifically bind to rat plasma proteins.[28] Binding to proteins can limit the amount of drug available to interact with the target, and thus impact efficacy in an in vivo setting. Since we are primarily interested in CNS applications of an mGlu$_1$ NAM, we also chose to further profile interesting compounds by measuring their binding to rat brain homogenates. Not surprisingly, the measured fraction unbound tracked well with the calculated log$P$ values. In the instances where brain homogenate binding was measured, there was reduced fraction unbound in brain homogenates relative to plasma. Our experience is that such a relationship between the unbound fraction in brain homogenates versus plasma is often but not always the case. Gratifyingly, the most potent analog **54** also demonstrated an increased fraction unbound compared to most of the other compounds tested.

Compound **54** (VU0469650) was also examined in cell based functional assays for its selectivity against the other members of the mGlu family and was determined to be inactive against mGlu$_{2–8}$ at 10 μM.[29] Additionally, we tested the compound in a functional cell based assay against rat mGlu$_1$ and found little difference across species.[30] With over 100-fold selectivity against the other mGlus, we also wanted to assess the selectivity of the compound against a wider variety of targets. As such, we submitted the compound to a commercially available radioligand binding assay panel of 68 clinically relevant GPCRs, ion

channels, kinases, and transporters,[31] and no significant responses were found at a concentration of 10 μM.[32]

Given the results summarized herein, we chose to evaluate VU0469650 in a rat pharmacokinetic study (Table 6).[33] In order to understand the rate of metabolism in vivo, a study was conducted using intravenous (IV) dosing. VU0469650 exhibited moderate to high clearance, a moderate volume of distribution, and a half-life of approximately 1 hour. We reasoned that such a clearance profile was supportive of further studies and proceeded to evaluate the compound in a plasma-brain level experiment using intraperitoneal (IP) dosing. Given the moderate to high clearance observed in the IV experiment, the IP dosing route was chosen in an attempt to maximize exposure. The 30 min post-dose time point was selected as it would be a relevant time point for many behavioral experiments of interest. We were pleased to see that the CNS penetration for VU0469650 was excellent and that the absolute levels in the brain were quite good. Taking into account the aforementioned brain homogenate binding data, we estimate the unbound fraction in the brain to be 32 nM. If that is indeed the case, a threefold increase in dose (30 mg/kg) should provide unbound drug levels near the functional IC_{50} for $mGlu_1$.

In conclusion, we have identified a new $mGlu_1$ NAM tool compound from within a series of *N*-acyl-*N*′-arylpiperazines using a hit to lead optimization plan initiated from a cross screening hit. VU0469650 can be prepared using a simple three step procedure and readily available starting materials. The compound is also potent and selective with excellent CNS penetration in rats. Future plans include evaluation of the compound in rodent behavioral assays associated with CNS disorders linked to $mGlu_1$ signaling. Results and observations from such studies will be the subject of future communications.

Acknowledgments

References and notes

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21. HEK 293A TREx cells stably expressing human mGlu$_1$ were plated in black-walled, clear-bottomed, poly-D-lysine coated 384-well plates in 20 μL of assay medium (DMEM containing 10% dialyzed FBS, 20 mM HEPES, and 1 mM sodium pyruvate) at a density of 20 K cells/well. The cells were induced with 50 ng/mL tetracycline and grown overnight at 37 °C in the presence of 5% CO$_2$. The next day, medium was exchanged to assay buffer (Hank's balanced salt solution, 20 mM HEPES, and 2.5 mM probenecid) using an ELX405 microplate washer, leaving 20 μL/ well, followed by addition of 20 μL/well 2× Fluo-4 AM (2.3 μM final) indicator dye (prepared as a DMSO stock and mixed in a 1:1 ratio with pluronic acid F-127) in assay buffer, and incubation for 45 m at 37 °C. The dye was then exchanged to assay buffer using an ELX405, leaving 20 μL/well. Ca^{2+} flux was measured using the Functional Drug Screening System (FDSS7000, Hamamatsu, Japan). Compounds were serially diluted 1:3 into 10 point concentration response curves and transferred to daughter plates using the Echo acoustic plate reformatter. Compounds were diluted into assay buffer to a 2× stock using a Thermo Fisher Combi which was applied to cells at t = 3 s. Cells were incubated with the test compounds for 2.3 min and then stimulated with an EC$_{20}$ concentration of glutamate; 1.9 min later an EC$_{80}$ concentration of glutamate was added and readings taken for an additional 1.7 min. Data were collected at 1 Hz. Concentration response

curves were generated using a four point logistical equation with XLfit curve fitting software for Excel.

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23. Synthesis of **54** (VU0469650) is representative of route I. (*R*)-4-*N*-Boc-2-methyl piperazine (665 mg, 3.32 mmol), 1-adamantoyl chloride (990 mg, 4.98 mmol), and DIEA (1.15 mL, 6.64 mmol) were dissolved in 2.0 mL of dichloromethane, and the reaction was monitored by LCMS. Upon completion, 5.0 mL of MeOH was added, and the mixture was concentrated in vacuo. The residue was purified by flash chromatography. The purified product was immediately dissolved in 1.0 mL of MeOH and excess 4 N HCl in dioxanes (4.0 mL, 16 mmol). Once the protecting group had cleaved as judged by LCMS, an aqueous solution of saturated sodium bicarbonate was added until the pH was basic. The mixture was extracted with dichloromethane, and the organic layers were concentrated in vacuo to afford 814 mg (94% yield) of adamantan-1-yl((*R*)-2-methylpiperazin-1-yl)methanone as a white solid. Adamantan-1-yl((*R*)-2-methylpiperazin-1-yl)methanone (355 mg, 1.35 mmol) and 2-cyano-3-fluoropyridine (1.42 mL, 5.41 mmol) were dissolved in 4.0 mL of NMP in a microwave reaction vial and heated in the microwave for 10 min at 250 °C. The reaction mixture was purified via reverse phase chromatography (0.1% TFA in H_2O/MeCN). The fractions containing the product were combined and neutralized by the addition of aqueous saturated sodium bicarbonate. The mixture was extracted with dichloromethane, and the organic layers were combined and concentrated in vacuo. The residue was dissolved in 1.0 mL of MeOH and 4 N HCl in dioxanes (3.0 mL, 12 mmol) and stirred for 2 h. The mixture was concentrated in vacuo to afford 212 mgs (39% yield) of the HCl salt as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.30 (dd, *J* = 4.3, 1.4 Hz, 1H), 7.67 (dd, *J* = 8.6, 1.3 Hz, 1H), 7.62 (dd, *J* = 8.6, 4.4 Hz, 1H), 4.74 (m, 1H), 4.35 (d, *J* = 13.7 Hz, 1H), 3.44 (t, *J* = 12.1 Hz, 2H), 3.27 (m, 1H), 2.93 (dd, *J* = 12.1, 3.5 Hz, 1H), 2.84 (td, *J* = 12.8, 2.8 Hz, 1H), 2.02–1.83 (m, 9H), 1.75–1.60 (m, 6H), 1.32 (d, *J* = 6.7 Hz, 3H). HRMS (TOF, ES[+]) $C_{22}H_{28}N_4O$ [M+H][+] calculated 365.2341; found 365.2344. $[\alpha]_D^{25}$ −45.7° (*c* = 6.0, MeOH).

24. Synthesis of **17** is representative of route II. (*R*)-4-*N*-Boc-2-methyl piperazine (490 mg, 2.44 mmol), 2-bromopyridine (257 μL, 2.69 mmol), Xantphos (212 mg, 0.366 mmol), Pd$_2$(dba)$_3$ (112 mg, 0.112 mmol), and NaO*t*Bu (705 mg, 7.34 mmol) were added to 5.0 mL of dioxanes in a microwave reaction vial. The reaction was heated for 10 min in the microwave at 120 °C and monitored by LCMS. Upon completion, the mixture was cooled and filtered through a pad of celite and concentrated in vacuo. The crude material was purified by flash chromatography and immediately dissolved in 1.0 mL of MeOH with stirring. 4 N HCl in dioxanes (4.0 mL, 16 mmol) was added. Once the protecting group had cleaved as judged by LCMS, an aqueous solution of saturated sodium bicarbonate was added until the pH was basic. The mixture was extracted with dichloromethane, and the organic layers were concentrated in vacuo to afford 260 mg (60%) of (*R*)-2-methyl-1-(pyridin-2-yl)piperazine as a colorless liquid. (*R*)-2-Methyl-1-(pyridin-2-yl)piperazine (260 mg, 1.47 mmol), 1-adamantoyl chloride (437 mg, 2.20 mmol), and DIEA (508 μL, 2.93 mmol) were dissolved in 1.0 mL of dichloromethane and monitored by LCMS. Upon completion, 5.0 mL of MeOH was added, and the mixture was concentrated in vacuo. The crude product was purified via reverse phase chromatography (0.1% TFA in H_2O/MeCN). The fractions containing the product were combined and neutralized by the addition of aqueous saturated sodium bicarbonate. The mixture was extracted with dichloromethane, and the organic layers were combined and concentrated in vacuo to afford 200 mg (40% yield) of the product as a white solid. ^{1}H NMR ^{1}H NMR (400 MHz, DMSO*d*$_6$) δ 8.12–8.07 (m, 1H), 7.52 (t, *J* = 8.0 Hz, 1H), 6.79 (Rotamer 1, d, *J* = 8.6, 0.65 H) 6.73 (Rotamer 2, d, *J* = 8.6 Hz, 0.35 H), 6.64–6.59 (m, 1H), 4.64 (Rotamer 1, m, 0.65 H), 4.49 (Rotamer 2, m, 0.35 H), 4.4–4.22 (m, 1H), 4.16–3.95 (m, 2H), 3.27 (m, 2.35 H), 2.80–2.69 (m, 0.65H), 2.02–1.85 (m, 9H), 1.75–1.62 (m, 6H), 1.12 (Rotamer 1, d, *J* = 6.6 Hz, 2H), 0.95 (Rotamer 2, d, *J* = 6.6 Hz, 1H); HRMS (TOF, ES[+]) $C_{21}H_{30}N_3O$ [M+H][+] calculated 340.2389; found 340.2389. $[\alpha]_D^{25}$ −20.2° (*c* = 6.0, MeOH).

25. HEK293A cells expressing rat mGlu$_5$ were cultured and plated. 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 Sharma S, Rodriguez AL, Conn PJ, Lindsley CW. Bioorg. Med. Chem. Lett. 2008; 18:4098. [PubMed: 18550372]

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28. Binding to rat plasma proteins and brain homogenates were measured using equilibrium dialysis according to methods similar to those described in Kalvass JC, Maurer TS. Biopharm. Drug Dispos. 2002; 23:327. [PubMed: 12415573]

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30. Analogous to the method described in Ref.21 with the exception that HEK 293A TREx cells stably expressing rat mGlu$_1$ were used.

31. LeadProfilingScreen®, Eurofins Panlabs, Inc. http://www.eurofinspanlabs.com.

32. Significant responses are defined as those that inhibited more than 50% of radioligand binding. In the case of VU0469650, no inhibition greater than 41% (adenosine A$_1$ and sigma σ$_1$) was observed.

33. Male Sprague–Dawley rats weighing between 250 and 300 g were purchased from Harlan Laboratories (Indianapolis, IN). VU0469650 was used as its mono-HCl salt for these studies. For the IV study, cannulated animals with catheters surgically implanted in the carotid artery and jugular vein were used. Blood collections via the carotid artery were performed at 0.033, 0.117, 0.25, 0.5, 1, 2, 4, 7, and 24 h after administration via the jugular vein catheter. For the IP study, rats were euthanized and decapitated 30 min after compound administration, and both blood and brain samples were collected. Following protein precipitation, the supernatants of all plasma and brain homogenate samples were analyzed by means of LC–MS/MS. All animal studies were approved by the Vanderbilt University Medical Center Institutional Animal Care and Use Committee.
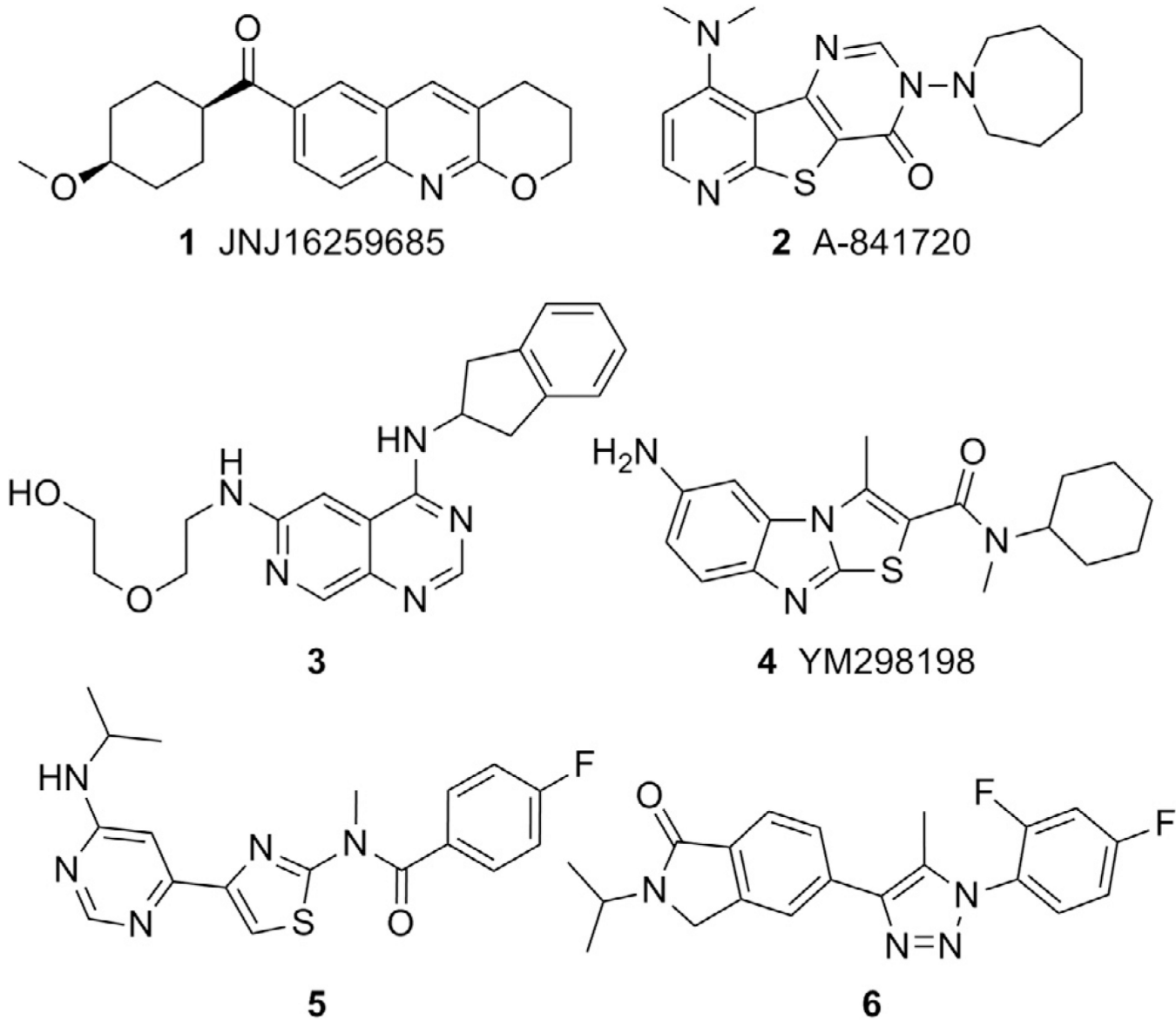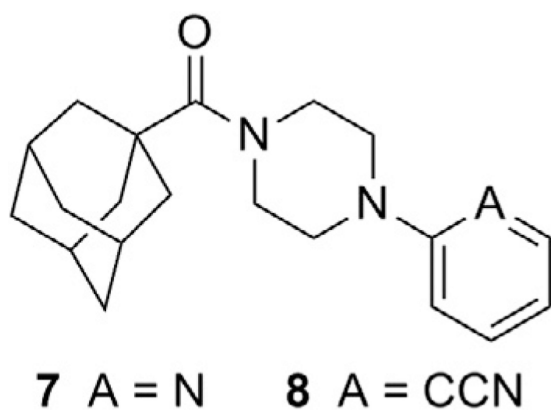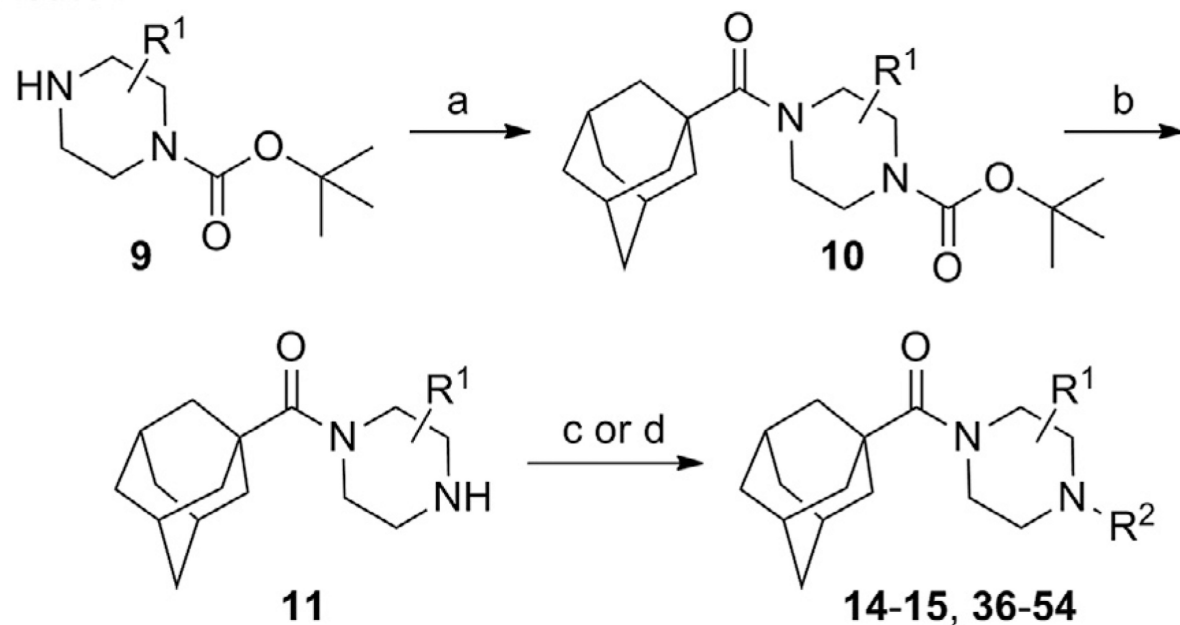
Figure 1.
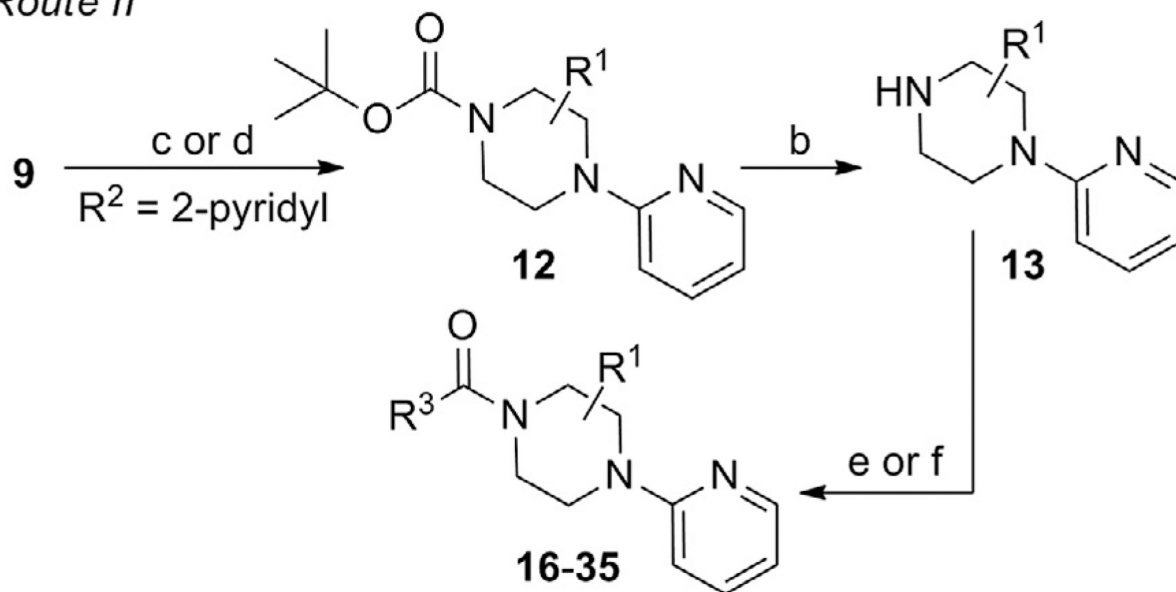mGlu$_1$ NAM compounds with in vivo efficacy.

	human mGlu$_1$ IC$_{50}$	rat mGlu$_5$ IC$_{50}$
7	630 nM	990 nM
8	649 nM	2350 nM

7 A = N   **8** A = CCN

Figure 2.
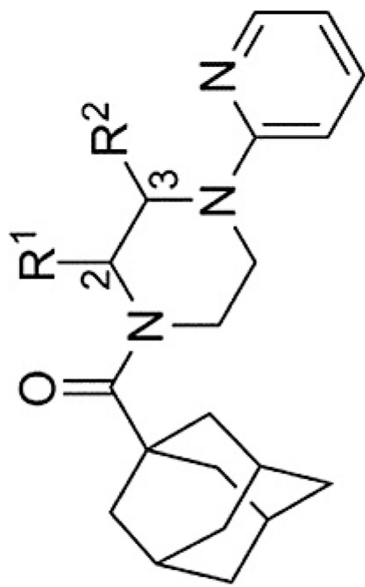mGlu$_1$ NAM starting points for hit to lead optimization.

Scheme 1.
Reagents and conditions: (a) 1-adamantoyl chloride, DIEA, CH_2Cl_2; (b) HCl, MeOH, dioxanes; (c) R^2F, NMP, μ wave, 250 °C, 10 min; (d) R^2X (X = Cl, Br, or I), $Pd_2(dba)_3$ or $Pd(OAc)_2$, Xantphos, NaO^tBu or Cs_2CO_3, dioxanes, μ wave, 120 °C, 10 min or 100 °C, 18 h; (e) R^3COCl, DIEA, CH_2Cl_2; (f) R^3CO_2H, HATU, DIEA, CH_2Cl_2.

Table 1

Chiral piperazine analogs

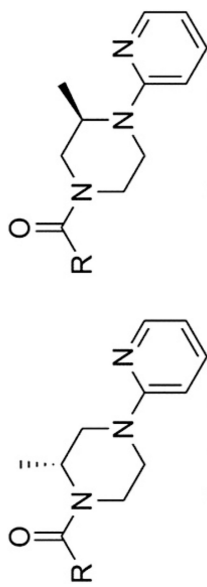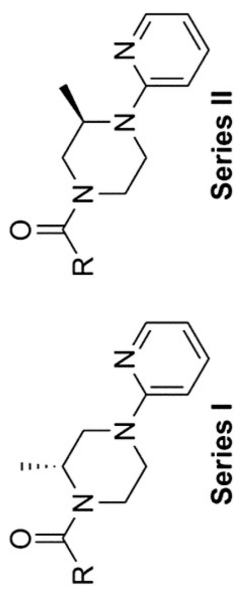| Compd	R¹	R²	mGlu₁ pIC₅₀[a] (±SEM)	mGlu₁ IC₅₀ (nM)	% Glu max[a,b] (±SEM)
7	H	H	6.20 ± 0.21	630	1.1 ± 0.8
14	(S)-CH₃	H	5.62 ± 0.18	2420	-1.4 ± 2.5
15	(R)-CH₃	H	6.79 ± 0.15	161	2.5 ± 0.5
16	H	(S)-CH₃	6.01 ± 0.13	988	1.8 ± 1.0
17	H	(R)-CH₃	6.78 ± 0.14	166	2.0 ± 0.4

[a] Calcium mobilization mGlu₁ assay; values are average of n 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 3.

Table 2

Amide SAR

Series I   Series II

Compd	Series	R	$mGlu_1$ $pIC_{50}{}^a$ (±SEM)	$mGlu_1$ IC_{50} (nM)	% Glu max^{a,b} (±SEM)
15	I		6.79 ± 0.15	161	2.5 ± 0.5
17	II		6.78 ± 0.14	166	2.0 ± 0.4
18	I		<4.5	>30,000	—
19	II		<4.5	>30,000	—
20	I		<4.5	>30,000	—
21	II		<4.5	>30,000	—
22	I		<5.0^c	>10,000	51.8 ± 3.3
23	II		<4.5	>30,000	—
24	I		<5.0^c	>10,000	29.9 ± 2.1
25	II		<5.0^c	>10,000	62.6 ± 3.3
26	I		5.18 ± 0.06	6630	0.0 ± 8.2
27	II		<5.0^c	>10,000	52.9 ± 3.3
28	I		<5.0^c	>10,000	32.1 ± 3.1
29	II		<4.5	>30,000	—
30	I		5.44 ± 0.08	3610	0.3 ± 0.4

Series I   Series II

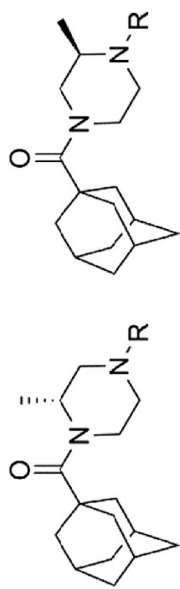| Compd	Series	R	mGlu$_1$ pIC$_{50}$^{a} (±SEM)	mGlu$_1$ IC$_{50}$ (nM)	% Glu max^{a,b} (±SEM)
31	II		<5.0^c	>10,000	44.1 ± 3.7
32	I		6.78 ± 0.11	167	1.3 ± 0.2
33	II		<5.0^c	>10,000	12.2 ± 10.6
34	I		<5.0^c	>10,000	62.7 ± 9.6
35	II		<4.5	>30,000	—

^{a}Calcium mobilization mGlu$_1$ assay; values are average of n 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 3.

^{c}CRC does not plateau.

Table 3

First generation aryl SAR

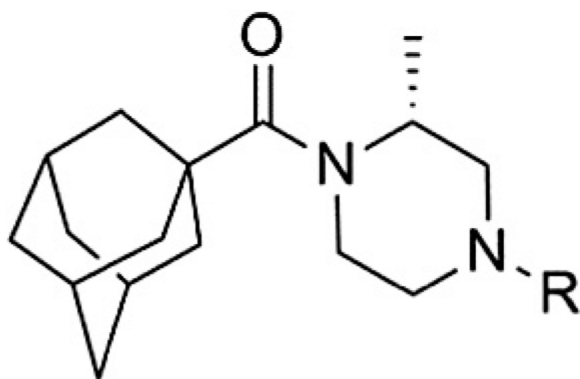| Compd	Series	R	mGlu$_1$ pIC$_{50}$[a] (±SEM)	mGlu$_1$ IC$_{50}$ (nM)	% Glu max[a,b] (±SEM)
15	I		6.79 ± 0.15	161	2.5 ± 0.5
17	II		6.78 ± 0.14	166	2.0 ± 0.4
36	I		6.22 ± 0.06	596	1.8 ± 0.2
37	II		<5.0[c]	>10,000	32.3 ± 5.2
38	I		<5.0[c]	>10,000	49.2 ± 4.5
39	II		<4.5	>30,000	—
40	I		6.15 ± 0.07	714	0.9 ± 0.4
41	II		<5.0	>10,000	17.0 ± 5.7
42	I		5.93 ± 0.08	1190	−0.3 ± 0.8
43	II		<5.0	>10,000	34.0 ± 5.1
44	I		5.88 ± 0.07	1330	−1.0 ± 2.2
45	II		<5.0	>10,000	34.7 ± 7.2
46	I		6.58 ± 0.06	264	2.1 ± 0.3
47	II		5.61 ± 0.09	2460	5.3 ± 1.7

[a] Calcium mobilization mGlu$_1$ assay; values are average of $n \geq 3$.

[b] Amplitude of response in the presence of 30 μM test compound as a percentage of maximal response (100 μM glutamate); average of n 3.

[c] CRC does not plateau.

Table 4

Second generation aryl SAR

Compd	R	mGlu$_1$ pIC$_{50}$[a] (±SEM)	mGlu$_1$ IC$_{50}$ (nM)	% Glu max[a,b] (±SEM)
15		6.79 ± 0.15	161	2.5 ± 0.5
48		6.59 ± 0.11	255	2.0 ± 0.4
49		6.38 ± 0.07	414	1.3 ± 0.9
50		6.15 ± 0.05	712	2.4 ± 0.7
46		6.58 ± 0.06	264	2.1 ± 0.3
51		6.46 ± 0.14	344	3.3 ± 1.0
52		5.86 ± 0.04	1400	−0.8 ± 3.3
53		6.40 ± 0.06	398	0.3 ± 2.2
54		7.01 ± 0.03	99	2.8 ± 0.3

[a]Calcium mobilization mGlu$_1$ assay; values are average of n 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 3.

Table 5

Protein binding results

Compd	cLogP^a	mGlu$_1$ IC$_{50}$ (nM)	LLE^b	Rat PPB^c (F_u)	Rat BHB^c (F_u)
15	4.49	161	2.30	0.016	0.011
17	4.49	166	2.29	0.017	0.008
46	4.76	264	1.82	0.003	—
48	4.74	255	1.85	0.006	—
49	4.65	414	1.73	0.012	—
51	4.22	344	2.24	0.016	—
53	3.51	398	2.89	0.081	—
54	3.60	99	3.41	0.071	0.016

[a] Calculated using ADRIANA. *Code* (www.molecular-networks.com).

[b] LLE (ligand-lipophilicity efficiency) = pIC50—cLog*P*.

[c] F_u = fraction unbound.

Table 6

Rat PK results for 54 (VU0469650)
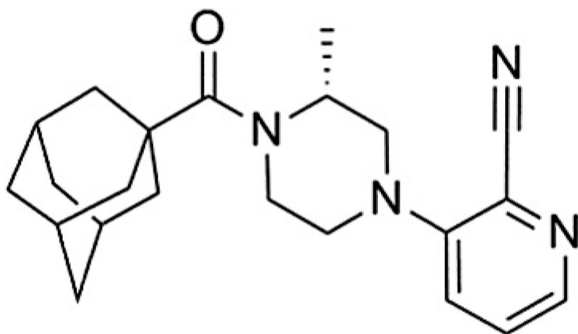
IV PK[a,c]		IP PK[b,c]	
Dose	0.2 mg/kg	Dose	10 mg/kg
Half-life	55 min	Time point	30 min
MRT	43 min	Plasma concd	509 ng/mL
CL	52 mL/min/kg	Brain concd	727 ng/mL
V_{SS}	2.2 L/kg	B/P ratio	1.4

[a] Formulation = 10% EtOH, 40% PEG400, 50% DMSO.

[b] Formulation = 10% Tween80 in water.

[c] Male Sprague–Dawley rats ($n = 2$).