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New positive allosteric modulators of the metabotropic glutamate receptor 2 (mGluR2): Identification and synthesis of *N*-propyl-8-chloro-6-substituted isoquinolones

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ABSTRACT

A series of *N*-propyl-8-chloro-6-substituted isoquinolones was identified as positive allosteric modulators of metabotropic glutamate receptor 2 (mGluR2 PAM) via high throughput screening (HTS). The subsequent synthesis and initial SAR exploration that led to the identification of compound **28** is described.

The metabotropic glutamate type 2 receptor (mGluR2) belongs to the group II mGlu receptors that together with the group III are negatively coupled to adenylate cyclase activation.[1] Group II mGlu receptors reduce transmission at glutamatergic synapses in brain regions where excessive glutamatergic transmission may be implicated in the pathophysiology of anxiety and schizophrenia, such as the prefrontal cortex and hippocampus. It is therefore hypothesized that activation of group II mGlu receptors may provide anxiolytic and/or antipsychotic effects.[2] Interestingly, mGlu2 receptors are located presynaptically but outside of the active zone of glutamate release (in the periphery of the synapse) where they function to reduce excitatory glutamate neurotransmission in an activity-dependent manner.[3] There are recent clinical data that strengthen the rationale for group II receptor intervention as a treatment for schizophrenia. Thus LY2140023, the orally available prodrug of the mixed mGluR2/mGluR3 orthosteric agonist LY404039, showed improvement of both positive and negative symptoms in a double-blind placebo controlled study in schizophrenic patients. Moreover, treatment with LY2140023 was safe and well tolerated and in contrast to many of the current D_2 receptor antagonist antipsychotics LY2140023 did not affect prolactin levels nor induced extrapyramidal symptoms or weight gain.[4] While both LY2140023 (tested at single dose with optimal therapeutic dose still to be determined) and olanzapine significantly separated from placebo, there was no significant difference between the two treatment groups.[5] A second phase 2 study with LY2140023 failed to show clinical improvement in schizophrenic patients but was considered inconclusive due to a greater than expected placebo response (positive control olanzapine also did not separate from placebo).[6] On the other hand, there is evidence from studies in knock-out mice that the mGlu2 receptor and not the mGlu3 receptor mediates the preclinical antipsychotic effects of the mixed mGluR2/mGluR3 agonists.[7,8]

There is growing interest in identifying positive allosteric modulators (PAMs) of the mGluR2 which bind at an alternative site to the orthosteric endogenous agonist. This approach may offer several advantages, such as increasing mGluR2 signaling with greater selectivity compared to agonists, maintaining activity based on transient and dynamic release of glutamate without inducing over-activation or desensitization, as well as increasing the likelihood to identify

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highly brain penetrant compounds as they would not be amino acid analogues.[9,10] We have recently reported a series of imidazopyridines **1**, identified by scaffold hopping with the help of computational shape and electrostatic field similarity, as a new mGluR2 PAMs chemotype (Fig. 1).[11] One of the reported and claimed mGluR2 PAMs that we used to build the model for our scaffold hopping was the isoindolone **2**, which was selected from a series of very broadly explored substituted isoindolones,[10] of which several of the most potent were the 7-chloro substituted derivatives of general formula **3**.[12–14] In this letter we report the discovery of a new series of 6,8-disubstituted isoquinolin-1-ones, **4** that shows mGluR2 PAM activity, whose general structure resembles that of reported isoindolones **3**.

High throughput screening of the Addex Pharmaceuticals compound collection in an mGluR2 PAM FLIPR (fluorometric imaging plate reader) assay resulted in the discovery of isoquinolone **5** which possessed an interesting functional activity, FLIPR pEC_{50} = 5.6. The potentiating activity of this compound was confirmed via a subsequent mGluR2 [^{35}S]-GTPγS assay (Fig. 2). Two different read outs were obtained from the GTPγS assay: (a) pEC_{50} for potentiation of the glutamate signal, and (b) % E_{MAX}[15] as the maximal response obtained using the test compound plus an EC_{20} of glutamate normalized to the maximal response obtained with glutamate alone. Compound **5** showed a pEC_{50} of 5.3 and a 104% increase in maximal glutamate effect was observed confirming the positive modulator activity of this hit. Compound **5** was tested for single point microsomal stability in human liver microsomes (HLM) showing moderate metabolic stability. Thus, compound **5** was 53% metabolized after 15 min incubation with HLM. We considered **5** as a potentially attractive starting point for an initial CNS focused exploration,[16] so we decided to start SAR exploration around positions C-6 and C-8 of the *N*-propyl-isoquinolone hit **5**.

The initial set of compounds **6**–**28** covered mainly variations of the R^2 group at positions C-6 of the isoquinolone ring while maintaining the R^1 substituent in compound **4** constant and equal to chloro (R^1 = Cl). Some examples (**29**–**31**) where $R^1 \neq$ Cl were also designed to study the similarity of this series with the reported isoindolone derivatives **3**. The target compounds **5**–**34** were prepared following synthetic strategies shown in Schemes 1–4.

The majority of final compounds were synthesized from a common intermediate **35**. The synthesis route for the preparation of **35** is shown in Scheme 1. Firstly, 4-bromo-2-methylbenzoic acid **32** was regioselectively chlorinated by treatment with *N*-chlorosuccinimide (NCS) in the presence of $Pd(OAc)_2$ to yield compound **33**.[17] Reaction of carboxylic acid **33** with *n*-propylamine under standard amide formation conditions led to benzamide **34** in excellent yield. Direct benzylic lithiation of **34** with LDA in the presence of DMF afforded 6-bromo-8-chloro-*N*-propylisoquinolone **35** in moderate yield.[18]

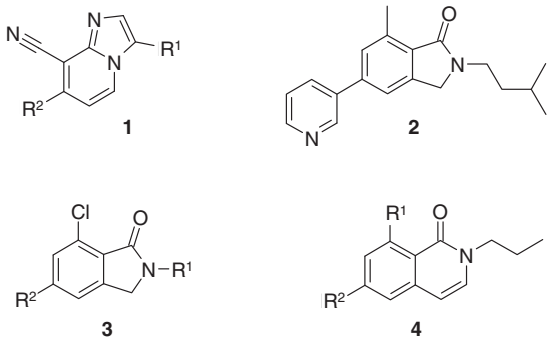


Figure 1. Structures of mGluR2 PAM reported molecules (**1**–**3**) and novel Addex/J&J chemotype (**4**).

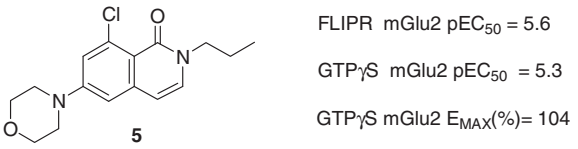
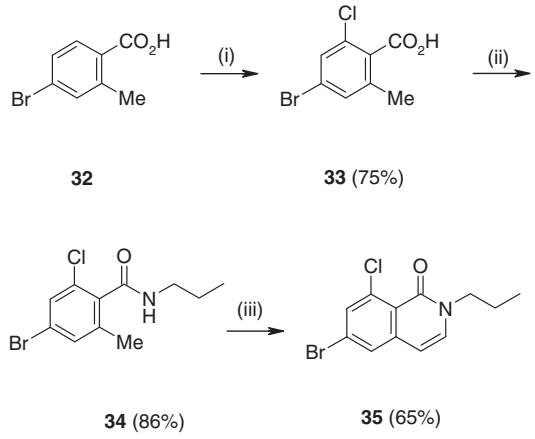
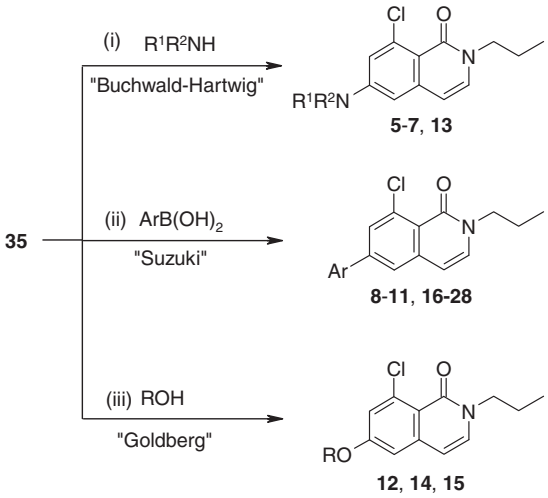


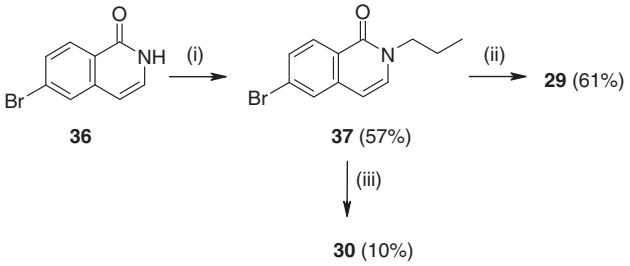
Figure 2. Isoquinolone hit **5** identified from a FLIPR mGluR2 PAM high throughput screen.



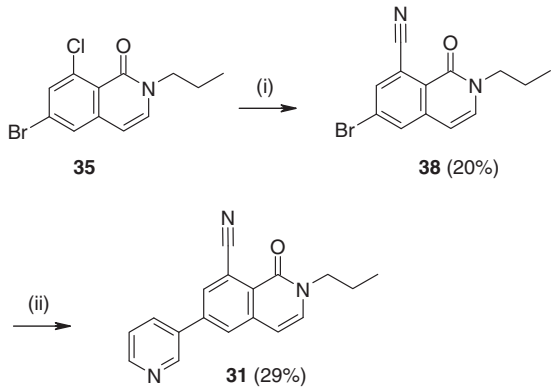
Scheme 1. Reagents and conditions: (i) NCS, $Pd(OAc)_2$, DMF, 110 °C, μW, 15 min; (ii) *n*-propylamine, EDCI, HOBt, DCM, 50 °C, 8 h; (iii) LDA, DMF, −78 °C to −10 °C, 12 h.



Scheme 2. Reagents and conditions: (i) *t*-BuONa, 10 mol % BINAP, 10 mol % $Pd_2(dba)_3$, toluene, 110 °C, 3 h, 23–71%; (ii) $Pd(PPh_3)_4$, Na_2CO_3, 1,4-dioxane, 90 °C, 2 h, 15–93%; (iii) $Pd(OAc)_2$, Cs_2CO_3, toluene, 100 °C, 3 h, 27–70%.



Scheme 3. Reagents and conditions: (i) 1-bromopropane, K_2CO_3, CH_3CN, 180 °C @ μW, 20 min; (ii) morpholine, *t*-BuONa, 10 mol % BINAP, 10 mol % $Pd_2(dba)_3$, toluene, 110 °C, 3 h; (iii) 3-pyridylboronic acid, $Pd(PPh_3)_4$, Na_2CO_3, 1,4-dioxane, 90 °C, 2 h.



Scheme 4. Reagents and conditions: (i) CuCN, NMP, 150 °C @ μW, 30 min; (ii) 3-pyridylboronic acid, $Pd(PPh_3)_4$, $NaHCO_3$, 1,4-dioxane, 90 °C, 1 h.

Intermediate **35** was converted into the targeted compounds following the synthetic approaches shown in Scheme 2. Thus, microwave assisted Buchwald–Hartwig type coupling of the 6-bromo-8-chloroisoquinolone **35** with several amines afforded the corresponding final compounds **5**–**7**, and **13**. Compounds **8**–**11** and **16**–**28** were prepared by Suzuki cross coupling between **35** and the corresponding arylboronic acids. 6-Alkoxyisoquinolones **12**, **14** and **15** were synthesized by reaction of the isoquinolone **35** with the appropriate alcohols under Goldberg type coupling reaction conditions (Scheme 2).

Compounds **29** and **30**, where R^1 = H, were prepared as it is shown in Scheme 3. Commercially available 6-bromoisoquinolone **36** was N-alkylated with 1-bromopropane using K_2CO_3 as base under microwave irradiation conditions to yield **37** in moderate yield. Compound **37** was coupled under standard Buchwald–Hartwig or Suzuki cross coupling reactions with either morpholine of 3-pyridyl boronic acid to yield the corresponding final products **29** and **30**.

Finally the 8-cyanoisoquinolone derivative **31** was prepared in two-steps from the intermediate **35**. Thus, reaction of the 6-bromo-8-chloroisoquinolone **35** with copper cyanide led to the intermediate isoquinolone **38** which was subsequently coupled with 3-pyridylboronic acid under standard Suzuki-type cross coupling reaction conditions to give the target compound **31** in 29% yield.

The variations around the R^1 and R^2 groups and the functional activity and metabolic stability data in human liver microsomes (HLM) of *N*-propylisoquinolones **5**–**31** are listed in Table 1.

To further explore the potential binding interaction of the morpholine oxygen, analogues **6** and **7** were prepared. The 4-piperidinol derivative **6** retained the potency of hit **5**, whereas the piperazine analogue **6** was only marginally active. This finding suggests that a strong basic center is not permitted in this area of the molecule. Better activity was found when the aliphatic R^2 substituent was replaced by the aromatic heterocycle pyridine (**8** and **9**) with its partial basic character.[19] The observed increase in potency was remarkable with the 3-pyridyl substituent **9** (pEC_{50} = 6.2, E_{MAX} = 83%); furthermore this was accompanied by a comparable metabolic stability (**9**: 57% metabolized vs **5**: 53% metabolized). The 5-pyrimidinyl derivative **10** did not retain the potency found with the 3- and 4-pyridyl analogues **8** and **9** and showed a remarkable drop on E_{MAX} (28%). The 4-pyrazolyl derivative **11**, which contains a somewhat acidic proton, was also well tolerated in terms of activity. In view of the good activity found with the 3-pyridyl derivative **8**, some additional 3-pyridyl analogues were prepared (**12**–**22**). Introduction of a $–CH_2O–$ or $–CH_2NH–$ linker between the 3-pyridyl ring and the isoquinolone core turned out to be detrimental for activity, with compounds **12** and **13** having pEC_{50} values of 5.6 and 5.5, respectively, with good E_{MAX} retention. This loss of potency was attenuated when longer spacers were introduced, as illustrated with compounds **14** (pEC_{50} = 6.1, E_{MAX} = 47%) and **15** (pEC_{50} = 6.2, E_{MAX} = 75%). Since none of the elongated analogues **12**–**15** proved to be superior to the directly linked pyridyl analogue **9**, we focused our exploration on evaluating the influence of substituents at position C-4 of the 3-pyridyl substituent in **9**. Overall, a wide variety of diverse substituents (**17**–**22**) were well tolerated for activity, with the exception of the dimethylaminopyridyl group in compound **16**, which led of a decrease of one log unit in activity (pEC_{50} = 5.3, E_{MAX} = 85%) and worsened metabolism (83% metabolized). The morpholine analogue **17** was found to be equipotent to compound **9** while showing an increased metabolic stability (29%). Encouraging increases on activity were achieved with compounds bearing ether substituents such as **18**–**22**. For example, a simple methoxy substituent (**18**) was tolerated for potency (pEC_{50} = 6.1) and showed a remarkable increase on the E_{MAX} (121%). Unfortunately the turnover in HLM was high (70% metabolized). Higher activity was found with the combination methoxy-fluoro derivative **19** (pEC_{50} = 6.4, E_{MAX} = 75%), but again the compound was extensively metabolized (82% metabolized). More lipophilic ethers, ending with a distal aromatic ring were also beneficial for activity, and thus compounds **20** and **21** had comparable activity to that of **18**. This may suggest the presence of a large pocket in the allosteric site that can be reached from the C-6 position of the isoquinolone core. Compound **22**, where a distal 3-pyridyl ring is part of the ether chain showed good activity (pEC_{50} = 6.5, E_{MAX} = 120%) but reduced metabolic stability (73% metabolized).[20]

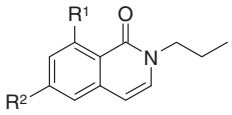
The encouraging activity results obtained with the pyridyl ethers **18**–**22** prompted us to further investigate the effect of the 3-pyridyl ring in compound **18**. Thus direct comparison between compounds **23** and **18** revealed that the pyridyl nitrogen of **18** may be optional for activity, and thus compound **23** was found to be more potent than **18**. Guided by this result, a small set of 6-phenyl-substituted isoquinolones were prepared (**24–28**). A drop in activity was observed with the phenyl ether **24** and **26** when compared to their direct pyridyl pairs **18** and **22**. A substantial improvement in potency was achieved with the more liphophilic compounds **25**, **27** and **28**.[21] Thus the 4-pyridyl ether **25** had a pEC_{50} of 6.8 and an E_{MAX} of 90%, being 11-fold more potent than the corresponding phenyl ether analogue **24**. The introduction of electron withdrawing groups in adjacent position of the distal pyridine nitrogen was beneficial for activity (**27** and **28** vs **26**). This increase was more important in compound **28** which presented a more balanced profile: pEC_{50} = 6.6, E_{MAX} = 170%, moderate metabolic stability (45% metabolized).

Finally, the role of the R^1 substituent at position C-8 was also investigated with compounds **29**–**31**. Comparison between the des-chloro analogues **29** and **30** and their chloro-substituted pairs **5** and **9** clearly revealed that a chloro-substituent at position C-8 is beneficial for potency. Replacement of the chloro atom by a cyano group (**31**) resulted in complete loss of activity.

Compound **28** was selected from this initial SAR exploration and was tested for its ability to potentiate the in vitro concentration response curve (CRC) of glutamate on cloned human mGluR2. A profile indicative of positive allosteric modulation was observed. As shown in Figure 3, the CRC of glutamate shifts to the left and upwards with increasing concentrations of compound **28**. A 5.6-fold shift in the glutamate EC_{50} was seen in the presence of 3 μM of **28** (glutamate pEC_{50} of 4.9 and 5.6 in the absence or presence of compound **28**, respectively).

Targeting the allosteric site of mGluR2 is expected to improve the chance of identifying selective mGluR2 ligands. Possible agonist or antagonist effects of compound **28** against mGluR1, 3–8 were evaluated.[22] Compound **28** was inactive in all assays, thus proving to be a selective mGluR2 PAM.

Table 1
Functional activity of representative mGluR2 PAMs **5–31**[a]



5-31

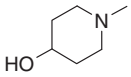
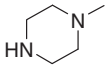
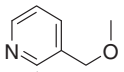
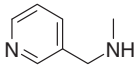
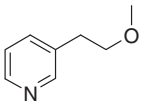
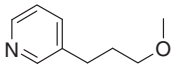
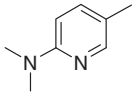
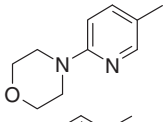
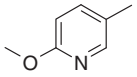
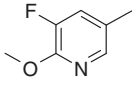
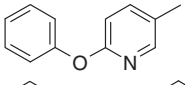
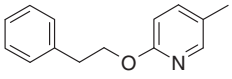
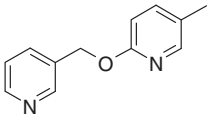
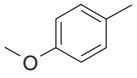
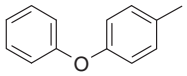
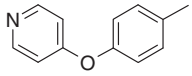
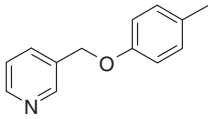
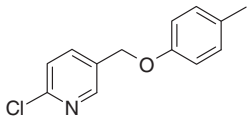
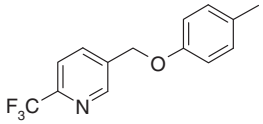
Compound	R^1	R^2	mGlu2 pEC_{50}[a]	mGlu2 E_{MAX}[a] (%)	HLM[b] (%)
5	Cl		5.3	104	54
6	Cl		5.3	106	
7	Cl		<5	47	
8	Cl		5.9	73	81
9	Cl		6.2	83	57
10	Cl		5.4	28	
11	Cl		5.8	65	
12	Cl		5.6	126	
13	Cl		5.5	82	
14	Cl		6.1	47	
15	Cl		6.2	75	
16	Cl		5.3	85	83
17	Cl		6.1	84	29
18	Cl		6.1	121	70
19	Cl		6.4	75	82
20	Cl		6.3	92	
21	Cl		6.2	103	21
22	Cl		6.5	120	73
23	Cl		6.5	136	62

Table 1 (*continued*)

Compound	R^1	R^2	mGlu2 pEC_{50}[a]	mGlu2 E_{MAX}[a] (%)	HLM[b] (%)
24	Cl		5.7	88	
25	Cl		6.8	90	
26	Cl		5.9	64	46
27	Cl		6.4	81	
28	Cl		6.6	170	45
29	H		<5	66	
30	H		5.1	81	
31	CN		<4.3	–	

[a] Values are means of three experiments.
[b] HLM data refer to % of compound metabolized after 15 min at 5 μM concentration.

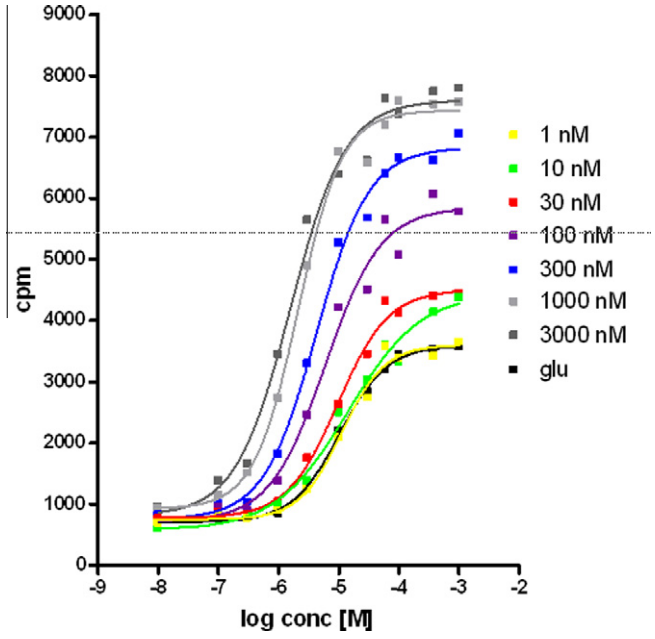


Figure 3. Glutamate (glu) concentration response curve in presence of varying concentration of compound **28**, demonstrating an approximate 5.6-fold-shift in glu EC_{50} at 3 μM concentration of **28**.

In summary, a novel series of 6,8-disubstituted isoquinolones with mGluR2 PAM activity has been presented. Initial SAR studies from the HTS hit **5** resulted in compounds with improved potency in a GTPγS assay. Some initial analogues combined good potency and good in vitro metabolic stability in HLM. Compound **28** was identified as the most promising hit from this exploration and proved to be a positive allosteric modulator of mGluR2 by its ability to potentiate the in vitro CRC of glutamate. Moreover, compound **28** showed a similar if not better in vitro pharmacology profile than one of the prototypical mGlu2 PAMs known to date, LY487379.[23] Further evaluation of **28** and SAR refinement are underway and will be reported in due course.

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References and notes

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15. The effect of these compounds on the [^{35}S]-GTPγS binding induced by 4 μM glutamate (~EC_{20}) was characterized using a CHO cell line expressing the human mGlu2 receptor.
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19. Calculated pK_a values: **7**, pK_a = 8.5; 8, pK_a = 4.1; 9, pK_a = 4.0.
20. Metabolite identification studies revealed that oxidation of the *N*-propyl chain is the preferred metabolic pathway for most of these compounds.
21. For an analysis of liphophilicicty across different GluR2 PAM chemical series see Ref. 10.
22. Compound **28** was tested at 3 μM for agonist or antagonist activity on mGlu receptors in fluorescent Ca^{2+} assays using HEK293 cells expressing human mGluR1, 3–8.
23. LY487379 increases Glu-induced [^{35}S]-GTPγS signaling with a pEC_{50} of 6.6 and E_{MAX} of 169%. Moreover, in our assay, LY487379 only caused a minimal shift in the CRC of Glu, with >10 μM needed for a five-fold decrease in Glu EC_{50}.