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3-(Imidazolyl methyl)-3-aza-bicyclo[3.1.0]hexan-6-yl)methyl ethers: A novel series of mGluR2 positive allosteric modulators

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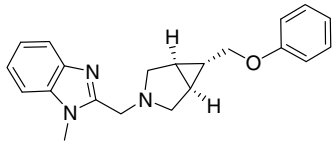
ABSTRACT

The synthesis and structure–activity relationship (SAR) of a novel series of 3-(imidazolyl methyl)-3-aza-bicyclo[3.1.0]hexan-6-yl)methyl ethers, derived from a high throughput screening (HTS), are described. Subsequent optimization led to identification of potent, metabolically stable and orally available mGluR2 positive allosteric modulators (PAMs).

The metabotropic glutamate receptors (mGluRs) belong to family C of GPCRs characterized by a large extracellular bi-lobed agonist binding site connected to a heptahelical transmembrane (7TM) domain through a cysteine-rich linker.[1] These receptors are typically subdivided into three groups based on structural homology and signal transduction pathways.[2] Group II are comprised of mGluR2 and mGluR3 which are both negatively coupled to adenylate cyclase. Efficacy observed in preclinical animal models and human clinical trials with known dual mGluR2/3 agonists (e.g., LY-354740) provides solid support of group II mGluRs as novel targets for the treatment of anxiety,[3] psychosis,[4] convulsive disorders,[5] Parkinson's disease,[6] neurodegeneration,[7] and pain.[8] Tremendous excitement in this field has been generated since the report that LY 2140023, a pro-drug of mGluR2/3 agonist LY-404039, demonstrated efficacy against positive and negative symptoms in a 4-week phase IIb schizophrenia trial.[9]

Due to the high degree of homology in the orthosteric sites of group II mGlu receptors, selective mGluR2 agonists have yet to be discovered. Moreover, known direct agonists are typically amino acid analogs,[10] which can limit their utility in pharmacological studies due to poor physicochemical properties and low CNS exposure. Positive allosteric modulators (PAMs), in comparison, bind in the transmembrane domain where there is much less homology among different subtypes, thus providing an opportunity to achieve subtype selectivity. Furthermore, the large number of structural diverse, non-amino acid allosteric modulators of mGluRs found to date[11] hold promise of identifying mGluR2 PAMs with suitable physicochemical properties for CNS exposure. Herein the discovery and structure–activity relationship of a novel series of mGluR2 PAMs, 3-(imidazolyl methyl)-3-aza-bicyclo[3.1.0]hexan-6-yl)methyl ethers, are described.

An in-house high throughput screening campaign using a functional FLIPR assay[12] yielded several leads featuring a [3.1.0] azabicyclic core, as represented by compound **1** (Fig. 1). Compound **1** displays potent functional activity (mGluR2 EC_{50} = 24 nM) and good brain penetration (brain/plasma = 2).[13] However, further pharmacokinetic (PK) profiling revealed high in vitro clearance in rat and human liver microsomes (*r*-CLh: >65.7 ml/min/kg; *h*-CLh: 14.2 ml/min/kg).[14] Moreover, compound **1** has low absorption (5%) and oral bioavailability (<2%),[15] potentially due to poor aqueous solubility of **1** observed during sample formulation. In light of these results, our chemistry efforts were focused on developing a structure–activity relationship around the 3-aza-bicyclo[3.1.0]hex-



1

Figure 1. The original HTS lead, compound **1**.

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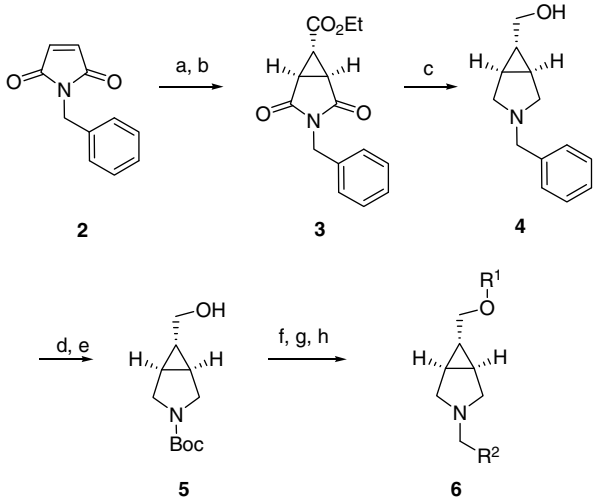
an-6-yl)methyl ether template to identify compounds with improved PK characteristics.

A general synthetic strategy of 3-aza-bicyclo[3.1.0]hexan-6-yl)-methyl ether analogs is illustrated in Scheme 1. Starting from *N*-benzylmaleimide **2**, room temperature cycloaddition with ethyl diazoacetate followed by thermolysis[16] gave cyclopropane **3**, which upon reduction yielded amino alcohol **4**. Benzyl removal via standard hydrogenation with Pd/C and subsequent Boc-protection afforded the key Boc amine intermediate **5**. A facile three-step protocol involving a Mitsunobu reaction, Boc removal and a reductive amination reaction provided rapid access to the desired products **6** bearing structural variations in the R^1 and R^2 positions.

Preliminary SAR at the R^2 position of **6** (Table 1) revealed *N*-methyl benzimidazole (MBI) as a key structural moiety for mGluR2 activity. Replacing MBI with alternative heteroaryls led to significant loss of potency, even with structurally similar benzothiazole (**6a**), imidazopyridine (**6b**), and quinoline (**6c**). *N*-methyl appeared to be the optimal side chain as a bulkier cyclopropyl group (**6d**) or a tricyclic benzimidazole moiety (**6e**) resulted in much lower mGluR2 activities. On the other hand, a range of substitutions on the terminal phenyl ring moiety of compound **1**, were well tolerated (**6f–k**). Simple substituents such as halogens and small alkyls were preferred, yielding compounds (**6g–k**) with improved or comparable in vitro potency to compound **1**. Subsequent pharmacokinetic evaluation revealed that the incorporation of *para*-substituents (**6h–k**) dramatically improved microsomal stability. In particular, *para*-CF_3 substitution (**6k**) led to a low turnover rate in human liver microsome (*h*-CLh <5.3 ml/min/kg), albeit with a threefold drop in potency.

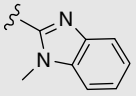
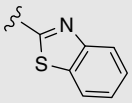
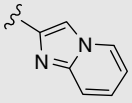
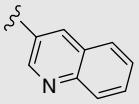
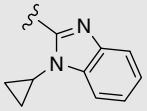
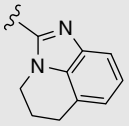
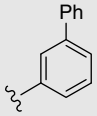
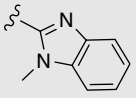
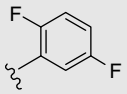
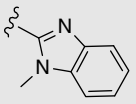
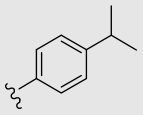
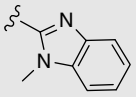
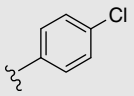
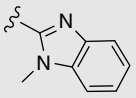
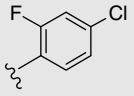
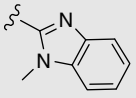
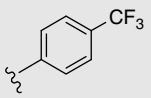
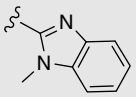
With clearance issues effectively addressed, we turned our attention to the low oral bioavailability associated with compound **1**. Compounds such as **6g** and **6f** were found to have the poor aqueous solubility similar to that of compound **1**. To address solubility as a component of bioavailability, we explored lowering *c*Log*P* through incorporation of pyridyl nitrogens within the R_1 and R_2 moieties of **6**.

Pyridyl ether analogs (**14a–c**, Table 2) were synthesized through the three-step protocol described in Scheme 1 using commercially available hydroxyl-pyridines. The syntheses of *N*-methyl aza-benzimidazoles are illustrated in Scheme 2. Starting from commercially available nitropyridine **7a**, the key aldehyde intermediate **9a** was obtained in four steps through a cycloaddition of diamino pyridine intermediate **8** and diethoxy ethanimidate **13**. Following a similar synthetic sequence, the three other regioisom-



Scheme 1. Reagent and conditions: (a) ethyl diazoacetate, Et_2O, rt; (b) neat, 200–230 °C, 36% for two steps; (c) $LiAlH_4$, THF, reflux; (d) Pd/C (10%), H_2 (40 psi), MeOH, rt; (e) Et_3N, Boc_2O, CH_2Cl_2, 74% for three steps; (f) R^1-OH, PPh_3, DEAD, THF, rt; (g) TFA, CH_2Cl_2; (h) R^2-CHO, Et_3N, $MgSO_4$, then $Na(OAc)_3BH$, CH_2Cl_2.

Table 1
Functional activities and in vitro human microsomal clearance data of representative mGluR2 PAMs

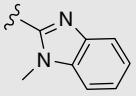
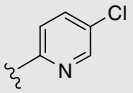
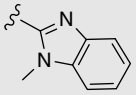
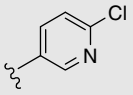
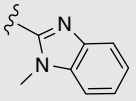
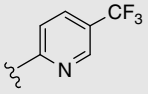
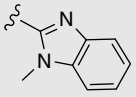
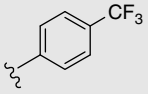
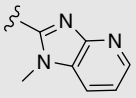
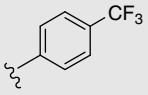
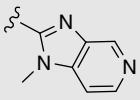
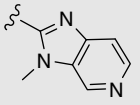
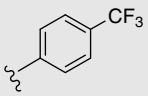
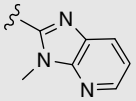
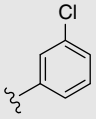
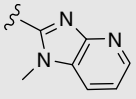
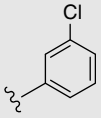
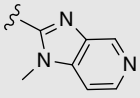
Compound	R^1	R^2	mGluR2 EC_{50} (nM)[a]	*h*-CLh (ml/min/kg)[b]
1			27	14.2
6a			>10,000	<5.3
6b			>10,000	ND[c]
6c			9750	<5.3
6d			3020	ND
6e			967	17.3
6f			113	11
6g			7	>18.7
6h			26	5.8
6i			26	6.6
6j			8	6.9
6k			85	<5.3

[a] EC_{50} values obtained from mGluR2 receptor transfected HEK cell line using FLIPR method in the presence of glutamate (EC_{10-20}); means of at least three experiments; an EC_{50} value >10,000 indicates that no curve was noted in the dose–response up to 10 μM.

[b] Predicted hepatic clearance (*h*-CLh) from human liver microsomal stability assay.

[c] ND, not determined.

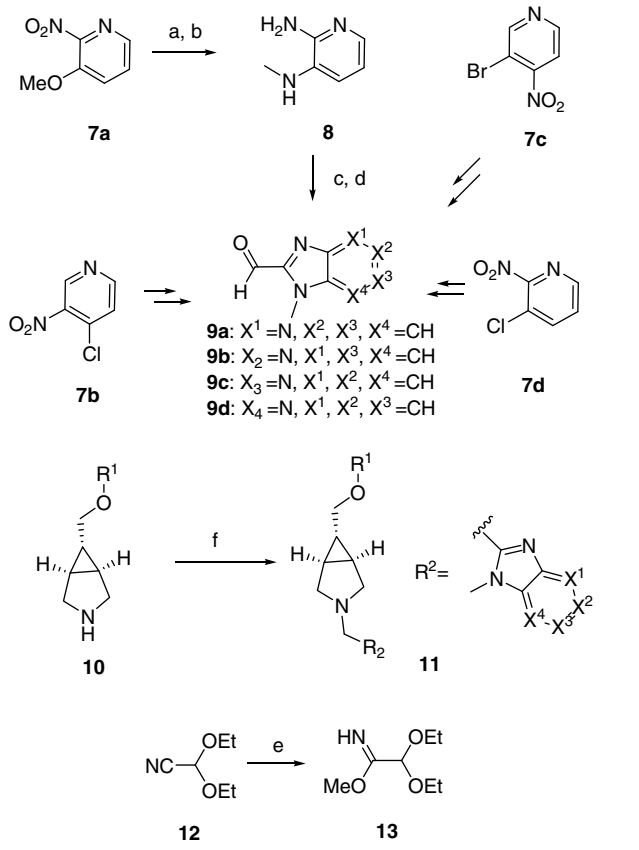
Table 2
Functional activities and human microsomal clearance data of pyridyl ether and aza-methylbenzimidazole analogs

Compound	R^1	R^2	mGluR2 EC_{50} (nM)[a]	*h*-CLh (ml/min/kg)[b]
1			27	14.2
14a			26	12.3
14b			511	ND[c]
14c			30	<5.3
15			20	9.3
16			251	10.5
17			2120	11.5
18			419	ND
19			64	10.5
20			86	<5.3

[a] EC_{50} values obtained from mGluR2 receptor transfected HEK cell line using FLIPR method in the presence of glutamate (EC_{10-20}); means of at least three experiments.
[b] Predicted hepatic clearance (*h*-CLh) from human liver microsomal stability assay.
[c] ND, not determined.

ers **9b–d** were synthesized from commercially available nitropyridines **7b–d**, respectively. Reductive amination of **9a–d** with secondary amine **10** gave the desired products **11** bearing *N*-methyl aza-benzimidazoles, exemplified by compounds **15–20** in Table 2.

To this end, the phenyl moiety of **6i** and **6k** was replaced with pyridyl groups (Table 2, **14a–c**). 2-Pyridyl was preferred as more than a 20-fold activity drop was observed when moving the nitrogen to the 3-position (**14a**–**14b**). Low to moderate predicted clearances were maintained in pyridyl ethers with *para*-substituents. **14c** was stable in the presence of human microsome (*h*-CLh <5.3 ml/min/kg) and provided mGluR2 potency similar to



Scheme 2. Reagent and conditions: (a) $MeNH_2$, EtOH, microwave (105 °C, 4 h), 57%; (b) Pd/C (10%), DME/MeOH (1:1), H_2 (40 psi), rt, 97%; (c) **13**, DME, AcOH, rt, then catalytic amount TsOH, reflux, 39%; (d) 4 N HCl aq, 60 °C, 96%; (e) Na, MeOH, rt, 68%; (f) **9a–d**, $MgSO_4$, Et_3N, CH_2Cl_2, then $Na(OAc)_3BH$.

compound **1** (EC_{50} = 30 nM). Incorporation of *N*-methyl aza-benzimidazole proved to be a fruitful SAR direction. The 4-aza and 5-aza-analogs, **15** and **16** (Scheme 2), displayed good potency, while compounds derived from the other two regioisomers (**17**, **18**) were generally much weaker. Interestingly, these aza-benzimidazole moieties yielded compounds generally with low to moderate clearances, even in the absence of *para*-substituents on the top phenyl ring (**19**, **20**).

Based on the in vitro microsomal clearance predictions, the in vivo pharmacokinetics of compounds **14a**, **14c**, and **19** were evaluated in jugular and portal vein cannulated rats. Improvements in clearance and fraction absorbed contributed to the improvement in bioavailability (Table 3). As an example, compound **14c** has a rat in vivo clearance of 28 ml/min/kg compared to 67 ml/min/kg for compound **1**.[18] Excellent absorption (70–90%,

Table 3
Rat in vivo PK evaluation

Compound	In vivo *r*-CLh[a] (ml/min/kg)	Fraction absorbed[b] (%)	Oral bioavailability[c] (%)
1	67	5	<2
14a	59	72	20
14c	28	—	27
19	28	84	79

[a] Observed plasma clearance in rats following a single 1 mg/kg iv bolus administration.
[b] Fraction absorbed determined using portal vein concentrations from rats following 5 mg/kg oral dose and clearance method. Fraction absorbed = $Cl_b{}^*AUC_{po,pv}/Dose_{po}$.[17]
[c] Determined from oral pharmacokinetics in rats following 5 mg/kg oral dose.

14a and **19**) together with low to moderate in vivo clearances led to good oral bioavailability (20–79%), a pronounced improvement over the original lead, compound **1**.

In conclusion, a series of 3-(imidazolyl methyl)-3-aza-bicyclo[3.1.0]hexan-6-yl)methyl ethers has been developed as novel mGluR2 positive allosteric modulators. The high clearance and low bioavailability issues of the original lead have been successfully addressed by introducing *para*-substitutions and incorporating pyridyl nitrogens. A group of lead compounds (**14a**, **14c**, and **19**) have been identified with excellent potency and pharmacokinetic profiles. These compounds have been profiled in in vivo efficacy models and the results will be reported in due course.

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12. The mGluR2 FLIPR functional assay uses a stable HEK cell line expressing the rat mGluR2 receptor and Gα15 G protein. Cells are plated in 384 well poly-D-lysine coated plates and incubated in glutamine free medium. Cells are loaded with Fluo-4 am containing Glutamic Pyruvic transaminase to reduce endogenous glutamate. Compound is added with the FLIPR, incubated at room temperature approximately 30 min, then tested against an approximate EC_{10} to EC_{20} glutamate challenge. Data is analyzed as a ratio of the peak fluorescence to the baseline after compound addition. Assay to assay variability in PAM potency required multiple experiments to determine potency, typically $n > 3$.
13. Whole brain and blood samples were collected from Sprague–Dawley rats at various times following administration of a single 10 mg/kg subcutaneous dose. Plasma and brain homogenate samples were quantitated using an LC/MS method and the brain to plasma ratio reported.
14. Compound **1** (1 μM) was incubated in rat or human liver microsome and monitored for substrate depletion using an LC/MS method. Hepatic clearance was determined by scaling of the observed half-life using the well stirred model.
15. Pharmacokinetic parameters were determined in rats following a 1 mg/kg intravenous or 5 mg/kg oral administration to male Sprague–Dawley rats. Whole blood was collected from a jugular (iv and oral) or portal vein (oral only) catheter and plasma samples analyzed using an LC/MS method. Pharmacokinetic parameters were calculated using a non-compartmental analysis of the plasma concentration versus time data.
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18. In vitro rat microsomal stability *r*-CLh of **14c** is 41 ml/min/kg, consistent with the in vivo measurement (within two folds).