Journal of Medicinal Chemistry

ARTICLE

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1-[(1-Methyl-1*H*-imidazol-2-yl)methyl]-4-phenylpiperidines as mGluR2 Positive Allosteric Modulators for the Treatment of Psychosis

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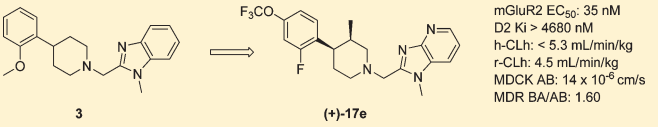
Supporting Information

ABSTRACT: A novel series of mGluR2 positive allosteric modulators (PAMs), 1-[(1-methyl-1*H*-imidazol-2-yl)methyl]-4-phenylpiperidines, is herein disclosed. Structure−activity relationship studies led to potent, selective mGluR2 PAMs with excellent pharmacokinetic profiles. A representative lead compound (+)-**17e** demonstrated dose-dependent inhibition of methamphetamine-induced hyperactivity and mescaline-induced scratching in mice, providing support for potential efficacy in treating psychosis.



INTRODUCTION

Group II metabotropic glutamate receptors (mGluR2 and 3) are widely expressed in the forebrain and localized presynaptically, serving as inhibitors of neurotransmission.[1] Activation of these receptors by orthosteric agonists, such as LY-354,740 [(1*S*,2*S*,5*R*,6*S*)-2-aminobicyclo[3.1.0]hexane-2,6-dicarboxylate monohydrate], led to robust activity in a number of animal models that have been used to predict antipsychotic activity.[2] Consistent with these animal studies, LY-2,140,023, a methionine amide pro-drug of the selective mGluR2/3 agonist, LY-404,039 [(−)-(1*R*,4*S*,5*S*,6*S*)-4-amino-2-sulfonylbicyclo[3.1.0]-hexane-4,6-dicarboxylic acid], was shown to be efficacious in improving both positive and negative symptoms in a recent 4 week phase IIb schizophrenia trial.[3] Subsequently, preclinical studies revealed that the antipsychotic effect of mGluR2/3 agonists is absent in mGluR2 knockout mice but not mGluR3 knockout mice, suggesting mGluR2 as the main receptor responsible for the antipsychotic efficacy.[4]

Because of the high degree of homology at the orthosteric sites of group II mGluRs, selective mGluR2 agonists have been difficult to design. Positive allosteric modulators (PAMs) provide an opportunity to achieve potentially greater subtype selectivity due to significant sequence differences in the transmembrane domain where modulators bind.[5] Additional advantages of PAMs include the potential for reduced receptor desensitization[6] and structurally diverse lead compounds with physicochemical properties suitable for passive brain penetration. Several structural classes of mGluR2 PAMs have been reported,[7] including recent disclosures by our group.[8] Herein, we describe a novel series of 1-[(1-methyl-1*H*-imidazol-2-yl)methyl]-4-phenylpiperidines as mGluR2 PAMs, which demonstrated robust in vivo efficacy in rodent psychosis models.[9]

In an effort to obtain chemical matter with mGluR2 subtype selectivity and central nervous system (CNS) druglike properties, we initiated an in-house high-throughput screen (HTS) of over 1.2 million compounds using a mGluR2 functional fluorescence imaging plate reader (FLIPR) assay that uses a stable human embryonic kidney (HEK) cell line expressing the rat mGluR2 receptor.[10] Multiple hits were identified including phenyl piperazine **1** and pyrimidinyl piperidine **2** (Figure 1). Compound **1** demonstrated reasonable potency but had a high molecular weight (MWt), whereas compound **2** had good overall physicochemical properties but weaker potency. Combining fragments from both chemotypes led to an exciting new aryl piperidine series exemplified by compound **3**. This series offered the potential to possess many CNS druglike characteristics, including low MWt and cLogP, potent in vitro activity, excellent passive permeability as measured by MDCK (Madin−Darby canine kidney) AB, and no multiple drug-resistant (MDR) liability.[11] Upon further profiling in in vitro absorption, distribution, metabolism, and excretion (ADME) assays and a selectivity panel of CNS receptors,[12] compound **3** was found to have high in vitro clearance in rat and human liver microsomes (*r*-CLh, >65.5 mL/min/kg; *h*-CLh, 17.3 mL/min/kg) and potent D_2 affinity (K_i = 426 nM) (Figure 1). Subsequent testing in D_2 functional assay confirmed that compound **3** was a D_2 antagonist (EC_{50} = 951 nM).[13] It is well known that D_2 antagonists, such as haloperidol, have potent effects in preclinical psychosis animal models.[14] Thus, carrying D_2 antagonism as an off-target activity could confound the outcomes from in vivo efficacy models and prevent a clear read on a given compound's antipsychotic efficacy that is truly mediated by the mGluR2 PAM activity. Hence, our

Received: November 1, 2010
Published: March 02, 2011

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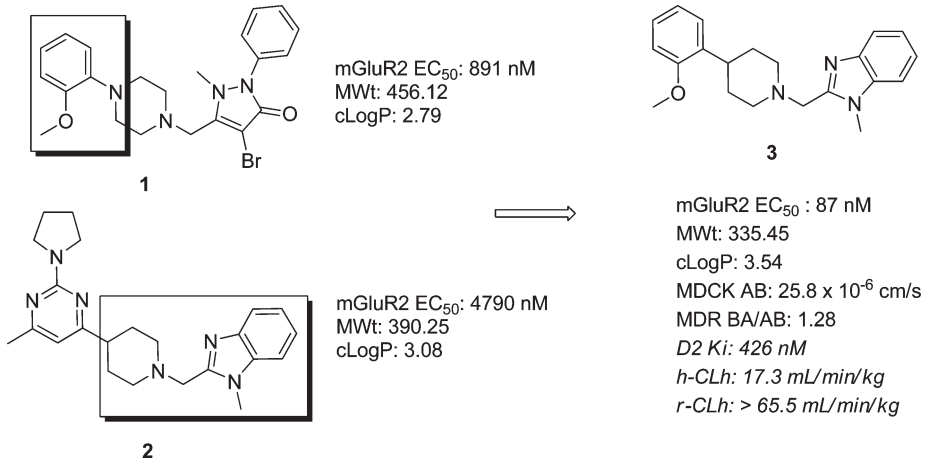


Figure 1. Origin of the 1-[(1-methyl-1*H*-imidazol-2-yl)methyl]-4-phenylpiperidine series.

chemistry efforts were focused on developing a structure−activity relationship (SAR) around compound **3**, seeking to improve potency, increase microsomal stability, and remove the D_2 affinity.

CHEMISTRY

The syntheses of analogues **7a**−**q** (Table 1) are illustrated in Scheme 1. Substituted 4-aryl piperidines **6** were readily prepared either from 4-bromopyridine via a Suzuki coupling with various aryl boronic acids followed by hydrogenation of the pyridine ring or from Boc-protected piperidinone **4** via a four-step synthetic sequence involving enol triflate formation, Suzuki coupling, hydrogenation of resulting olefin intermediate **5**, and subsequent Boc removal. Finally, reductive amination of **6** with imidazolyl aldehydes **8a**−**e**[8a] provided analogues **7a**−**q** bearing structural variations on R^1 and the aryl group.

Analogues **13**−**16** bearing various substituents on the piperidine ring (Table 2) were synthesized via routes outlined in Scheme 2. Hydroboration of olefin **9**[15] followed by debenzylation gave piperidine intermediate **10a** (*trans*-/*cis*-isomer 7:3), which upon standard reductive amination with imidazolyl aldehyde **8b** yielded *trans*-3-hydroxyl-4-phenyl piperidine analog **13**. 2-Methyl-4-phenyl piperidine analogue **14** was synthesized through a three-step sequence from 2-methyl-4-bromopyridine involving a Suzuki coupling, hydrogenation of the pyridine ring, and reductive amination, similar to what is described in Scheme 1. Syntheses of analogues **15** and **16** started with a Grignard addition to *N*-benzyl ketones **11** and **12**. The resulting tertiary alcohols were methylated and submitted to hydrogenation under strongly acidic conditions, which reduced the olefin and removed the benzyl group in one-pot to give substituted piperidines **10c** and **10d**. Standard reductive amination then afforded desired analogues **15** and **16**.

cis-3-Methyl-4-phenyl piperidine analogues **17a**−**e** were prepared from readily available 4-bromo-3-methyl pyridine as illustrated in Scheme 3. Suzuki coupling with aryl boronic acids followed by hydrogenation at elevated temperature gave 3-methyl-substituted piperidines **18a**−**e** in high yields (>90%). Reductive amination of **18a**−**e** with imdazolyl aldehyde **8b** afforded the desired racemic analogues **17a**−**e**. The corresponding (+)- and (−)-enantiomers were separated by chiral separation using Chiralpak AD columns. Alternatively, chiral separation of racemic piperidine precursors, **18a**−**e**, yielded (+)- and (−)-enantiomers, which upon reductive amination gave the corresponding (+)- and (−)-enantiomers of final analogues **17a**−**e**. The absolute stereochemistry of the active (+)-enantiomers was determined to be (*R*,*R*)- via X-ray analysis of the ditoluoyl-L-tartaric acid salt of the corresponding amine precursors as exemplified by (+)-**18d** (Scheme 3).

RESULTS AND DISCUSSION

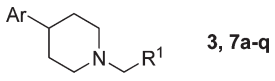
We began our SAR studies by exploring substitutions on the terminal phenyl ring of compound **3** (Table 1). Halogens and simple alkyls (**7b**−**e**,**h**−**j**) were preferred, and substitution at the ortho- or para-position appeared optimal for potency (**7b** and **7d** vs **7c**). While a wide range of substituents were tolerated, polar groups, such as nitrile (**7f**) or methyl amide (**7g**), in general led to a significant loss of activity. The high microsomal clearance issue identified in the lead **3** was effectively addressed by strategically placing substituents at the para-position (**7h** vs **7b**), possibly by blocking a site-specific metabolism. Analogues bearing para-substitutions (**7d** and **7i**) consistently had improved microsomal stability as compared to their ortho-substituted counterparts (**7b** and **7j**). Lowering the lipophilicity by incorporating a polar pyridyl nitrogen (**7k**−**n**) at the benzimidazole ring led to a further decrease in microsomal clearance, and **7k** had the best balance of potency (EC_{50} = 48 nM) and microsomal stability ($h\text{-}CL_h$ = <5.3 mL/min/kg). Combining the optimal moieties yielded the most promising ortho, para-disubstituted analogues, exemplified by **7o**−**q**, which had superior potency and microsomal stability profiles as compared to the original lead **3** (Figure 1).

While progress had been made in improving potency and microsomal stability, D_2 binding remained an unresolved issue. Simple halogens such as −F and −Cl, which were optimal for mGluR2 potency, also introduced the most potent D_2 activities (**7b**, **7d**, and **7h**). Converting the benzimidazole moiety to the aza-benzimidazoles had no effect on D_2 selectivity (**7i** vs **7k** and **7l**). While selected bulky aryl substituents, such as trifluoromethyl or alkoxy (**7i** and **7o**), demonstrated a slight reduction in D_2 affinity, a general approach to reduce D_2 affinity that allows the incorporation of optimal aryl substituents for mGluR2 potency such as simple halogens would be preferred.

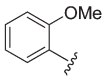
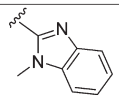
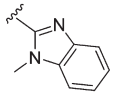
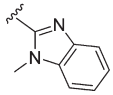
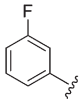
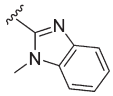
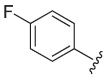
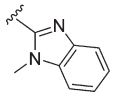
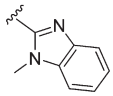
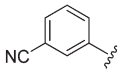
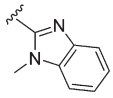
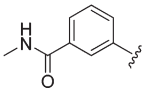
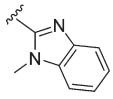
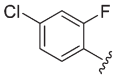
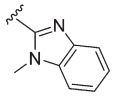
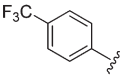
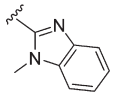
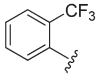
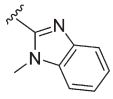
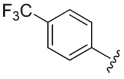
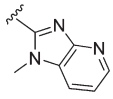
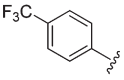
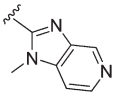
Comparing compound **7d** (D_2 K_i = 24 nM) to known D_2 antagonists, such as haloperidol (Figure 2), revealed close resemblance in the aryl piperidine region. We reasoned that

dx.doi.org/10.1021/jm101414h |*J. Med. Chem.* 2011, 54, 1724–1739

Table 1. mGluR2 Functional Activity, D_2 Binding Affinity, and in Vitro Human Microsomal Clearances of Analogues 3 and 7a−q

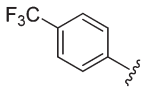
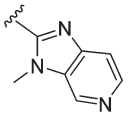
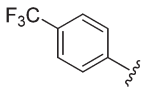
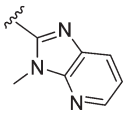
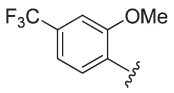
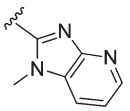
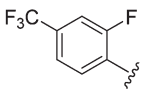
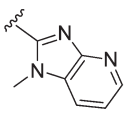
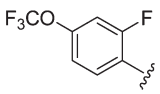
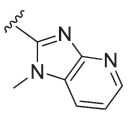


3, 7a-q

Compd	Ar	R^1	*r*-mGluR2 EC_{50} (nM)[a]	D_2 Ki (nM)[b]	*h*-CLh (mL/min/kg)[c]
3			87 (n=3, 20.5-290)	426	17.3
7a			212 (n=2, 7.86-5750)	ND[d]	15.7
7b			22 (n=167, 17.5-26.3)	93	15.8
7c			3,310 (n=3, 328-33400)	ND	12.0
7d			159 (n=7, 62.7-405)	24	11.2
7e			144 (n=3, 53.6-182)	ND	17.6
7f			1,620 (n=3, 341-7580)	ND	ND
7g			>10,000	ND	ND
7h			7.6 (n=5, 2-28.8)	91	9.0
7i			78 (n=6, 24.8-244)	561	8.6
7j			109 (n=2, 75.4-144)	ND	16.0
7k			48 (n=51, 35.2-65.2)	404	< 5.3
7l			168 (n=3, 25-814)	671	5.9

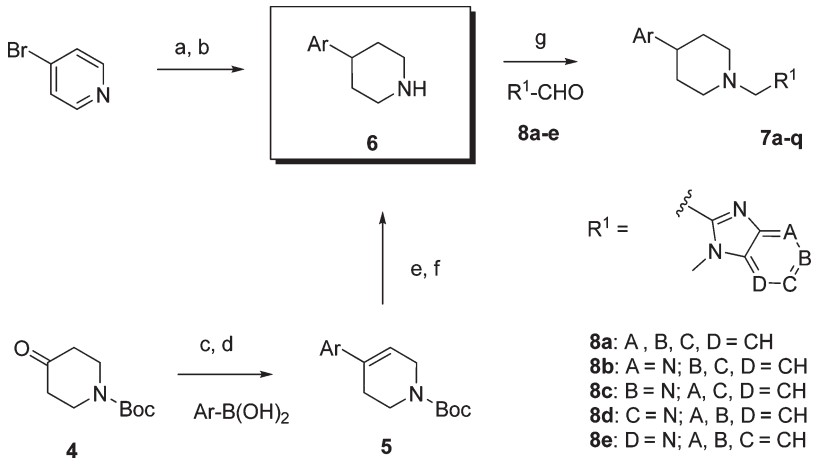
dx.doi.org/10.1021/jm101414h |*J. Med. Chem.* 2011, 54, 1724–1739

Table 1. Continued

Compd	Ar	R^1	*r*-mGluR2 EC_{50} (nM)[a]	D_2 Ki (nM)[b]	*h*-CLh (mL/min/kg)[c]
7m			196 (n=3, 86.7-442)	ND	7.0
7n			285 (n=4, 89-915)	ND	< 5.3
7o			13 (n=9, 5.96-27.7)	2,430	< 5.3
7p			25.1 (n=12, 15.2-41.4)	1,780	< 5.3
7q			25.0 (n=9, 5.9-75)	1,180	6.7

[a] EC_{50} values obtained from rat mGluR2 receptor transfected HEK cell line using FLIPR method in the presence of glutamate (EC_{10-20}); geometric mean (95% confidence interval); an EC_{50} value >10 000 indicates that no curve was noted in the dose−response up to 10 μM. [b] K_i values obtained from a D_2 binding assay using rat corpus striatum tissue prep with [^{3}H]-spiperone as the radioligand. [c] Predicted hepatic clearance (*h*-CLh) from human liver microsomal stability assay. [d] ND, not determined.

Scheme 1[a]

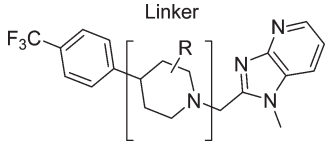


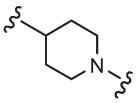
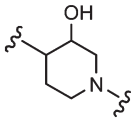
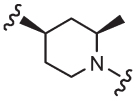
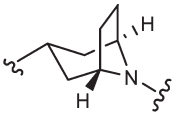
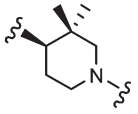
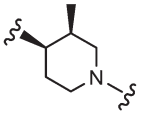
[a] Reagents and conditions: (a) $Pd(PPh_3)_4$, Ar−$B(OH)_2$, $NaHCO_3$, DME/H_2O, reflux; 4 N HCl in dioxane, CH_2Cl_2, then triturated with Et_2O. (b) PtO_2, H_2 (40 psi), MeOH, room temperature. (c) LDA, $PhN(CF_3SO_2)_2$, THF, −78 °C to room temperature. (d) $PdCl_2$dppf, Ar−$B(OH)_2$, Na_2CO_3, THF/H_2O (2:1), 50 °C. (e) Pd/C or PtO_2, H_2 (40 psi), EtOH, room temperature. (f) 2 N HCl in Et_2O, Et_2O, room temperature. (g) Compound **8a**−**e**, Et_3N, $MgSO_4$, then $Na(OAc)_3BH$, CH_2Cl_2, room temperature.

suitable substitutions on the piperidine linker could induce a conformational change of the terminal phenyl ring, which in turn could influence D_2 affinity. Computer modeling (Figure 2b) suggested that significant conformational changes in the terminal phenyl region could be induced by incorporation of a *cis*-3-methyl substitution (Figure 2a, compound B vs compound A), with the torsional angle between the terminal phenyl ring and the piperidine ring shifted from 118.1° to 138.5°. As a comparison, *cis*-2-methyl substitution was predicted to have no impact on the conformation of the terminal phenyl ring (Figure 2a, compound C vs compound A), therefore offering no potential differentiation in D_2 affinity.

dx.doi.org/10.1021/jm101414h |*J. Med. Chem.* 2011, 54, 1724–1739

Table 2. SAR of Substitutions on the Piperidine Linker



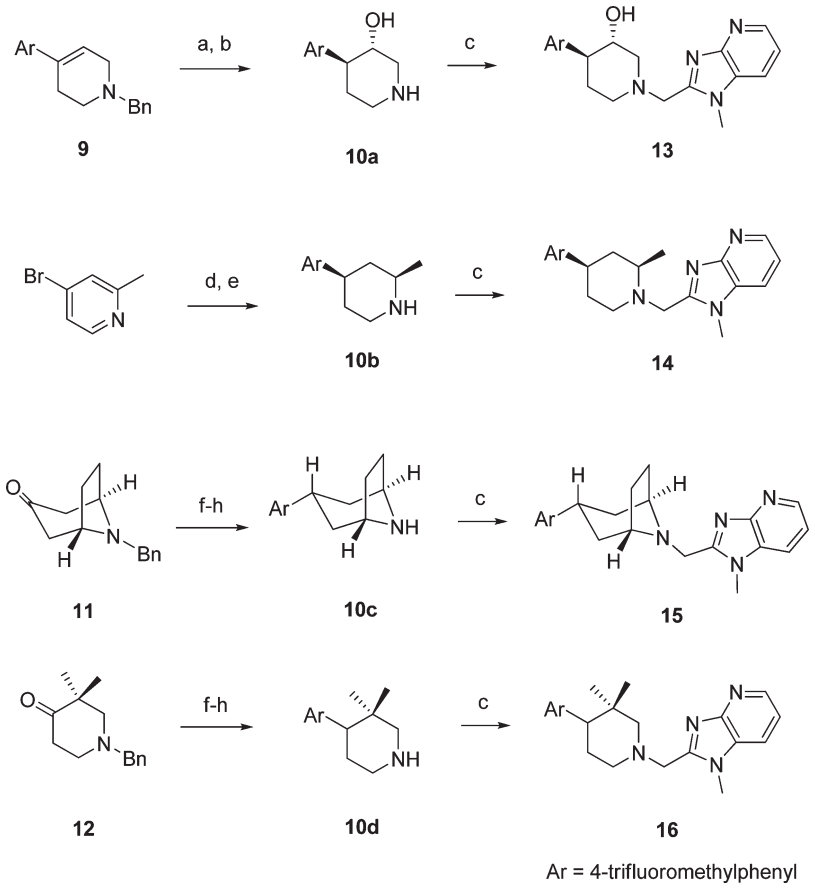
Compound	Linker	*r*-mGluR2 EC_{50} (nM)[a]	D_2 Ki (nM)[b]
7k		48 (n=51, 35.2-65.2)	404
13		>10,000	ND[c]
14		454 (n=3, 84-2450)	589
15		184 (n=5, 94.3-358)	355
16		745 (n=3, 345-1560)	>2,800
17a		111 (n=3, 26.4-465)	>2,800
	(+)-isomer	33 (n=6, 11.3-98.7)	>2,800
	(-)-isomer	>1,990 (n=3, 59.3-66700)	>2,800

[a] EC_{50} values obtained from rat mGluR2 receptor transfected HEK cell line using FLIPR method in the presence of glutamate (EC_{10-20}); geometric mean (95% confidence interval); an EC_{50} value >10 000 indicates that no curve was noted in the dose−response up to 10 μM. [b] K_i values obtained from a D_2 binding assay using rat corpus striatum tissue prep with [^{3}H]-spiperone as the radioligand; a K_i value >2800 nM indicates that no curve was noted in the dose−response up to 2800 nM. [c] ND, not determined.

To test this hypothesis, a group of analogues bearing substitutions at the 2- or 3-position of the piperidine linker were examined (Table 2). Polar groups, such as hydroxyl (**13**), led to complete loss of mGluR2 activity. Substitutions at the 2-position had no effect on D_2 affinity as evidenced by analogue **14** and **15**, consistent with the prediction of the computer modeling. On the other hand, substitutions at the 3-position decreased D_2 binding (**16** and **17a**, K_i > 2800 nM), while still maintaining reasonable mGluR2 activity. The *cis*-3-methyl-4-phenyl analogue **17a** was particularly exciting, as its (+)-enantiomer, obtained upon chiral separation, was equipotent (EC_{50} = 33 nM) to the parent piperidine analogue **7k**, with no D_2 affinity (K_i > 2800 nM). It is also worth noting that the mGluR2 activity of **17a** resides mainly in its (+)-enantiomer, as the corresponding (−)-enantiomer was much weaker.

We next examined whether the D_2 selectivity of **17a** could be expanded to analogues with aryl substituents that offer optimized mGluR2 activity and microsomal stability (Table 3). We were gratified to see that 3-methyl substitution proved to be a general and effective way to remove the undesired D_2 affinity, even for analogues bearing simple halogen substitutions (**17b**). In all cases, (+)-enantiomers were not only much more potent (up to >100-fold) but also more stable in human liver microsome than their corresponding (−)-enantiomers

Scheme 2[a]



[a] Reagents and conditions: (a) $NaBH_4$, $BF_3 \cdot Et_2O$, diglyme; then H_2O_2, NaOH. (b) Pd/C(10%), ammonium formate, MeOH, reflux. (c) Compound **8b**, Et_3N, $MgSO_4$, then $Na(OAc)_3BH$, CH_2Cl_2, room temperature. (d) Ar−$B(OH)_2$, $Pd(PPh_3)_4$, $NaHCO_3$, DME/H_2O (1:1); 4 N HCl in dioxane, DCM, then triturated with Et_2O. (e) PtO_2, H_2 (40 psi), 70 °C. (f) 1-Bromo-4-trifluoromethyl benzene, Mg, THF, 35−40 °C; then **11** or **12**, Et_2O, 5 °C to room temperature. (g) NaH, MeI, DMF, 45 °C. (h) Concentrated HCl, 10% Pd/C (10%), MeOH, H_2 (40 psi), 85 °C.

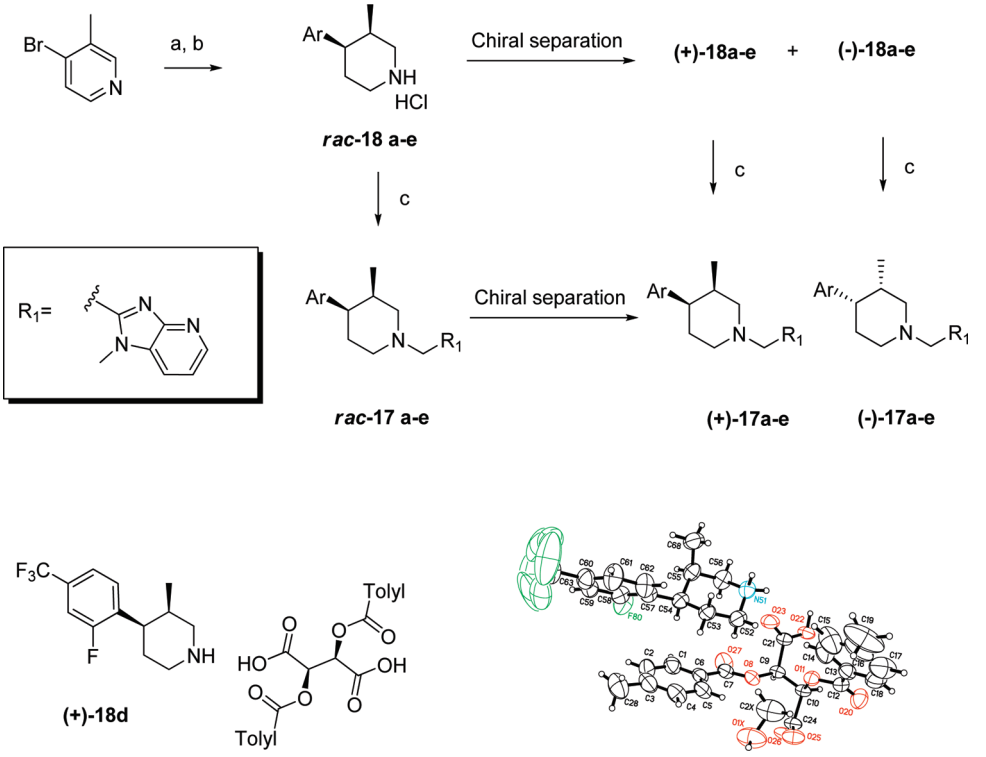
and had excellent D_2 selectivity that was lacking in the original lead.

A selected cohort of representative compounds, **7k** and (+)-**17c**−**e**, were evaluated in a FLIPR assay using a HEK cell line transfected with human mGluR2 receptor (*h*-mGluR2). As shown in Table 4, the EC_{50} values generated of these representative compounds in the *h*-mGluR2 assay were comparable to the corresponding *r*-mGluR2 values with no significant species difference observed. The *cis*-2-methyl substitution on the central piperidine ring played an essential role in reducing the D_2 affinities of analogues (+)-**17c**−**e**, improving the selectivity to over 100-fold from less than 10-fold observed with earlier analogues (**3** and **7k**). In subsequent pharmacokinetic (PK) studies, analogues (+)-**17c**−**e** also demonstrated the favorable PK profiles that were comparable to compound **7k** (Table 4), including high permeability (MDCK AB > 10×10^{-6} cm/s), no P-glycoprotein (P-gp) liability (MDR BA/AB < 2.5), and excellent brain penetrations in both mice and rats. However, the brain fractions unbound (Fu_brain) of analogues (+)-**17c**−**e** were low (0.3−0.6%), which appeared to be a general trait of the potent compounds in this structure series. The improved in vitro microsomal stability of analogues **7k**, (+)-**17d**, and (+)-**17e** translated into low in vivo clearances very nicely, giving rat clearances (*r*-CLh) ranging from 4.5 to 7.5 mL/min/kg. The low in vivo clearances coupled with the high permeabilities predicted good oral bioavailabilities, which were confirmed (50−100%) in subsequent oral PK studies, with excellent exposures achieved in brain. The nice balance of potencies, selectivities,[16] and in vivo PK properties rendered analogues (+)-**17c**−**e** as useful tools to evaluate the in vivo pharmacology of mGluR2 PAM activity in rodent psychosis models.

Two rodent psychosis models, inhibition of methamphetamine (MAP)-induced hyperlocomotor activity and mescaline-induced scratching (MIS), were used to evaluate the in vivo efficacy of this new class of mGluR2 PAMs. The results of compound (+)-**17e**, which are representative of this compound series, are described herein. Chronic use of MAP generates symptoms in human that mimic acute psychosis.[17] MAP induces hyperlocomotor activity in mice, the inhibition of which has been used as a measurement to predict antipsychotic activity.[18] As illustrated in Figure 3, compound (+)-**17e** showed robust and dose-responsive reduction of the MAP-induced hyperlocomotor activity with a minimally effective dose (MED) of 17.8 mg/kg. While the specific experiment was not performed with (+)-**17e**, the inhibitory effect of (+)-**17c** was effectively blocked by an mGluR2/3 antagonist, (2*S*,1′*S*,2′*S*)-2-(9-xanthylmethyl)-2-(2′-carboxycyclopropyl)-glycine (LY-341,495),[19] indicating such an effect was mediated by mGluR2 potentiation (data not shown).

dx.doi.org/10.1021/jm101414h |*J. Med. Chem.* 2011, 54, 1724–1739

Scheme 3[a]



[a] Reagents and conditions: (a) Ar−B(OH)$_2$, Pd(PPh$_3$)$_4$, NaHCO$_3$, DME/H$_2$O (1:1); 4 N HCl in dioxane, DCM, then triturated with Et$_2$O. (b) PtO$_2$, H$_2$ (40 psi), EtOH, 50 °C. (c) Compound **8b**, Et$_3$N, MgSO$_4$, then Na(OAc)$_3$BH, CH$_2$Cl$_2$, room temperature.

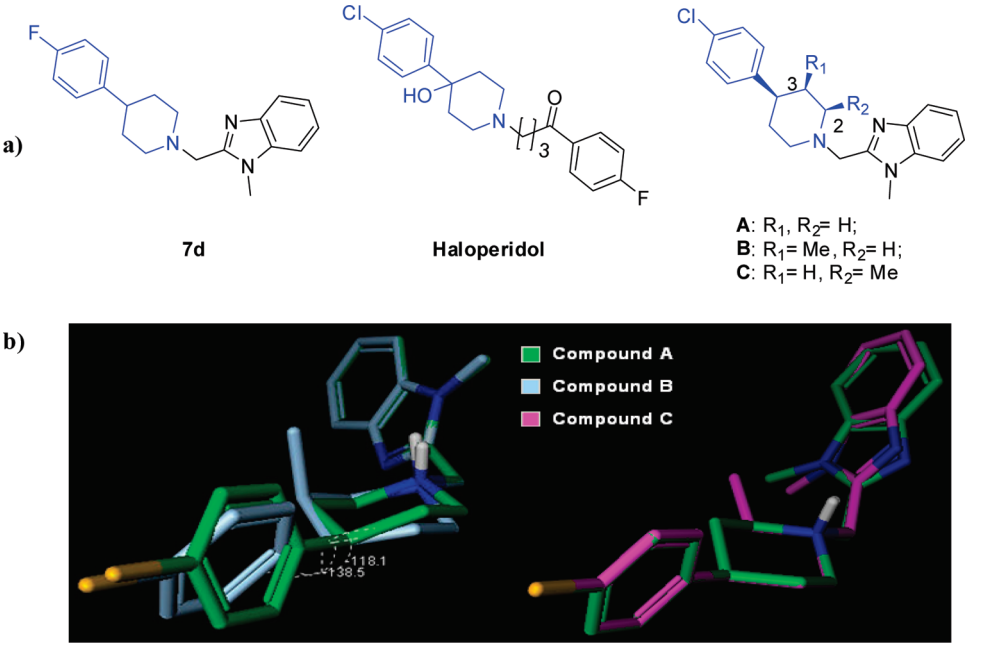
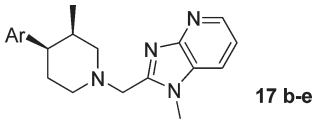


Figure 2. (a) Structure comparison of lead compounds to haloperidol; (b) comparison of potential conformation changes induced by *cis*-3-methyl or *cis*-2-methyl substitutions. Ligand minimizations were performed using Schrodinger's MacroModel software, and the resulting conformers were superimposed to provide the best overlap.

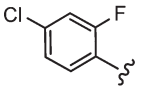
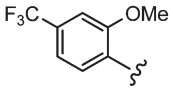
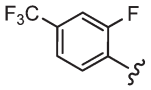
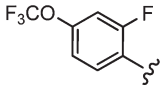
Compound (+)-**17e** was also examined in a MIS assay (Figure 4). This behavioral assay is a good measure of prefrontal cortical activity linked to the cortical-thalamic loop.[20] It is broadly published that 5-HT$_{2A}$ agonists like mescaline or 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane hydrochloride (DOI) act presynaptically to cause glutamate release from

dx.doi.org/10.1021/jm101414h |*J. Med. Chem.* 2011, 54, 1724–1739

Table 3. SAR of *cis*-3-Methyl-4-aryl Piperidine Analogues 17b−e



17 b-e

Compound	Ar	*r*-mGluR2 EC_{50} (nM)[a]	D_2 Ki (nM)[b]	*h*-CLh (mL/min/kg)[c]
(+)-**17b**		28 (n=15, 9.24-90.1)	1,620	< 9.5
(-)-**17b**		>1,810 (n=3, 46-71400)	ND[d]	15.9
(+)-**17c**		22 (n=16, 10.6-44.9)	>5,260	< 5.3
(-)-**17c**		787 (n=10, 480-1290)	ND	12.5
(+)-**17d**		9 (n=10, 5-17.2)	>5,260	8.4
(-)-**17d**		993 (n=10, 531-1860)	ND	10.5
(+)-**17e**		35 (n=15, 18.2-69.2)	>4,680	< 5.3
(-)-**17e**		818 (n-14, 530-1260)	ND	9.8

[a] EC_{50} values obtained from mGluR2 receptor transfected HEK cell line using FLIPR method in the presence of glutamate (EC_{10-20}); geometric mean (95% confidence interval); an EC_{50} value >10 000 indicates that no curve was noted in the dose−response up to 10 μM. [b] K_i values obtained from a D_2 binding assay using rat corpus striatum tissue prep with [^{3}H]-spiperone as the radioligand. [c] Predicted hepatic clearance (*h*-CLh) from human liver microsomal stability assay. [d] ND, not determined.

Table 4. In Vitro and in Vivo PK Properties of Representative Compounds

	7k	(+)-**17c**	(+)-**17d**	(+)-**17e**
r-mGluR2 EC_{50}	48 nM	22 nM	9 nM	35 nM
h-mGluR2 EC_{50}[a]	43 nM	76 nM	3 nM	31 nM
D_2 selectivity	8×	>239×	>584×	>133×
MDCK AB (10^{-6} cm/s)[b]	30	19	26	14
MDR BA/AB[c]	1.4	1.5	1.7	1.6
B/P (mouse)[d]	ND[j]	1.7	1.9	1.2
B/P (rat)[e]	5	8	7	4
Fu_brain[f]	ND	0.6%	0.5%	0.3%
in vivo *r*-CLh (mL/min/kg)[g]	5.4	ND	7.5	4.5
oral bioavailability[h]	100%	ND	74%	50%
C_{max_brain} (nM)/po dose (mg/kg)[i]	5213/10	1579/5	3125/10	1068/3

[a] EC_{50} values obtained from human mGluR2 receptor transfected HEK cell line using FLIPR method in the presence of glutamate (EC_{10-20}). [b] MS-based quantification of the basal/apical transfer rate of a test compound at 1 μM across contiguous monolayers from MDCK cells. [c] Ratio from the MS-based quantification of apical/basal and basal/apical transfer rates of a test compound at 1 μM across contiguous monolayers from MDR1-transfected MDCK cells. [d] Determined from PK in mice 60 min post 10 mg/kg subcutaneous dosing. [e] Determined from PK in rats 60 min post 3.2 mg/kg subcutaneous dosing. [f] Fraction unbound in rat brain. [g] Observed plasma clearance in rats following a single 0.5 [(+)-**17e**] or 1 mg/kg [**7k** and (+)-**17d**] iv bolus administration. [h] Estimated from rats following 3 [(+)-**17e**] or 10 mg/kg [**7k** and (+)-**17d**] oral dose as the ratio of dose-normalized AUC after oral and intravenous administration. [i] Determined in rats following a single 3 [(+)-**17e**], 5 [(+)-**17c**], or 10 mg/kg [**7k** and (+)-**17d**] oral dose. [j] ND, not determined.

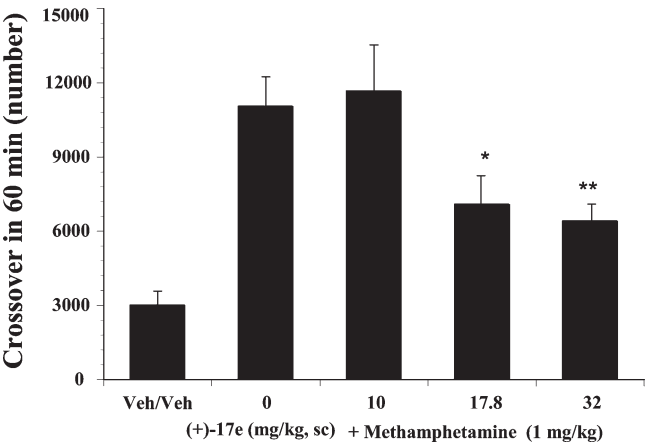


Figure 3. Inhibition of MAP-induced hyperlocomotor activity by (+)-**17e** in mice. Mice (n = 6/group) were dosed with test compound (sc) concurrently with MAP (1 mg/kg, sc). Data are presented as the group mean (±SEM) recorded for the total duration of the 60 min test period. Data were analyzed by a one-way ANOVA followed by Fisher's PLSD. *P < 0.05 and **P < 0.01 as compared to vehicle/MAP-treated mice.

thalamocortical neurons.[21] Intervention of this response was observed with typical and atypical antipsychotics, such as haloperidol and clozapine, and the mGluR2/3 agonist LY354740. To evaluate the effect of mGluR2 PAM (+)-**17e**, mice were dosed 20 min prior to oral mescaline, and the behavior was analyzed for a total of 12 min. The mGluR2 PAM inhibited MIS in a dose-responsive fashion (Figure 4) with an MED of 10 mg/kg (sc). At

dx.doi.org/10.1021/jm101414h |*J. Med. Chem.* 2011, 54, 1724–1739

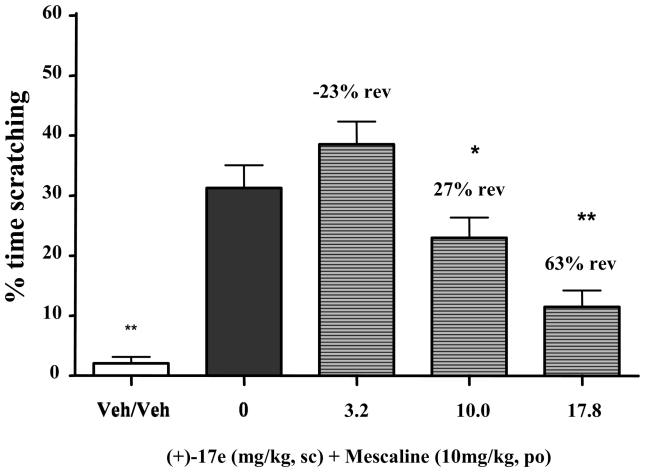


Figure 4. Inhibition of MIS by (+)-**17e** in mice CD-1 mice (n = 4/group) were predosed with test compound (sc) 20 min prior to receiving mescaline (10 mg/kg, po). Fifteen minutes later, the mice were placed in glass beakers for videotaping, and the animals were viewed every 30 s for a total of 12 min. Data were presented as % time scratching analyzed by a one-way ANOVA followed by Bonferroni's/Dunnett's posthoc tests. $^{*}P < 0.05$ and $^{**}P < 0.01$ as compared to vehicle/mescaline-treated mice.

this dose, compound (+)-**17e** had plasma and brain exposures of 15018 and 19644 nM (45 min postdose), respectively. The fraction unbound in mouse brain was measured as 0.3%, which gave an estimated [brain]free of 59 nM, similar to its in vitro mGluR2 EC_{50} of 35 nM. Noteworthy in these models, no additional mGluR2 agonist was added, so the effects of compound (+)-**17e** were solely due to the potentiated response of the endogenous glutamate.

CONCLUSION

In conclusion, a series of 1-[(1-methyl-1*H*-imidazol-2-yl)methyl]-4-phenylpiperidines have been developed as novel mGluR2 PAMs. The high clearance and selectivity issues of the original lead were successfully addressed through systematic SAR studies to yield a group of lead compounds [(+)-**17c**−**e**] that have excellent potency, selectivity, and PK profiles. Compound (+)-**17e** was found to be active in inhibiting MAP-induced hyperlocomotor activity and MIS in mice at a free brain exposure similar to its in vitro EC_{50}, providing evidence that an mGluR2 PAM could serve as a potential treatment for schizophrenia. However, the required dose and total plasma exposure were high due to low fractions unbound generally observed with these compounds (Table 4). Future efforts will be focused on improving mGluR2 potency while increasing fraction unbound in brain as an effort to achieve a lower efficacious dose.

EXPERIMENTAL SECTION

Biology. *mGluR2 FLIPR Functional Assay.* The increases in intracellular calcium release mediated by mGluR2 were measured in HEK 293 cells stably coexpressing the rat mGluR2 receptor and the Gα15 G protein using FLIPR technology (Molecular Devices). Cells were grown in a media containing Dulbecco's modified Eagle's medium (DMEM) high glucose with glutamine and sodium pyruvate (Gibco), 10% (v/v) heat-inactivated fetal bovine serum (FBS, Gibco), 500 μg/mL G418 (Gibco), and 3 μg/mL blasticidin (Invitrogen).

Two days prior to the testing, cells were plated on polystyrene 384-well plates (Becton Dickinson) at a density of approximately 15000 cells per well in a volume of 50 μL per well. One day prior to the assay, the growth media were removed from the plates and replaced with a media containing DMEM high glucose without glutamine, sodium pyruvate (Gibco), and 10% (v/v) dialyzed FBS (Gibco). On the day of the assay, an assay buffer was made containing 145 mM NaCl, 10 mM glucose, 5 mM KCl, 1 mM $MgSO_4$, 10 mM HEPES [4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid], and 2 mM $CaCl_2$, with the pH adjusted to 7.4 with 1 N NaOH. DMSO and P104 (15.8% stock solution) were added to the assay buffer used for compound plates for final concentrations of 0.18 and 0.006%, respectively.

A 4 μM dye loading media was prepared by adding a 1 mM (DMSO) stock solution of Fluo-4 am (Molecular Probes) containing 10% pluronic F-127 (Molecular Probes) to DMEM high glucose. A 250 mM stock solution of probenecid (Sigma) in equal volumes of 1 N NaOH and the assay buffer were then added to the dye media for a final concentration of 2.5 mM. Glutamic-pyruvic transaminase (GPT, Sigma, 3 units/mL) and 3 mM sodium pyruvate were added to the dye media to reduce extracellular glutamate in the assay wells.

Growth media were removed from the cell plates, and the dye loading media containing the 4 μM Fluo-4 am were added (50 μL/well). The plates were incubated for 1 h at 37 °C and 5% CO_2, after which the dye loading media were removed, and the plates were washed three times with the assay buffer using an Embla plate washer (Molecular Devices), leaving 30 μL assay buffer per well. Plates were incubated for 10−15 min at room temperature. Fifteen microliters of test compounds was added by FLIPR at four times their final concentrations. Intracellular calcium release was measured in the FLIPR by collecting five baseline samples, one sample every 2 s, before compound addition. After compound addition, from 5 to 40 samples were collected, one sample every 2 s. Plates were then incubated at room temperature for at least 20 min. Fifteen microliters of an EC_{10-20} concentration of glutamate at four times the final concentration was then performed by FLIPR using a similar read protocol (40 samples, one sample every 2 s, after compound addition). The specific concentration of glutamate needed to achieve an EC_{10-20} response was determined for each assay, typically ranging between 320 nM and 1 μM. Data for the second FLIPR addition were then analyzed. The ratio of peak fluorescence to baseline was calculated for each well. EC_{50} values for the positive modulators were then calculated using a sigmoidal four-parameter curve fit using the EC_{10-20} glutamate concentration as a negative control and an EC_{100} concentration of glutamate (100 μM) as a positive control.

[^{3}H]-Spiperone (Dopamine-2) Homogenate Binding Assay (Rat Striatum). One gram of rat corpus striatum by wet weight was homogenized with a polytron in 40 mL of buffer containing 20 mM HEPES, 118 mM NaCl, 4.5 mM KCl, 2.5 mM $CaCl_2$, and 1.2 mM $MgSO_4$, pH 7.5. The homogenate was centrifuged at 50000*g* for 10 min at 4 °C, and the resulting pellet was homogenized and centrifuged again as described above. The final pellet was resuspended to 5.6 mg/mL concentration in the same buffer. The plates were incubated in a shaking water bath in the dark at 37 °C for 15 min with 100 μL of drugs or vehicle for total binding, 100 μL of [^{3}H]-spiperone (0.2 nM), and 800 μL of tissue preparation. The reaction was terminated by filtration using 0.5% polyethylene imine-treated Whatman GF/B glass fiberfilters (Brandel Biomedical Research & Development Laboratories, Inc., Gaithersburg, MD) on a Skatron cell harvester (Molecular Devices Corp., Sunnyvale, CA) with ice-cold buffer. Filters were then counted on a Beta plate counter using Betaplate Scint. (Wallac Inc., Gaithersburg, MD). Data are expressed as IC_{50} values (concentration that inhibits 50% of the specific binding) or as an apparent K_i, $IC_{50}/1 + [L]/K_D$, where [L] = ligand concentration, and K_D = affinity constant for [^{3}H]-spiperone determined in a separate experiment.

Measurement of Fractions Unbound in Brain. The unbound fraction of each compound was determined in brain tissue homogenate using a 96-well equilibrium dialysis method as described by Kalvass et al.[22] with the following exceptions. Brain homogenates were prepared from freshly harvested rat brains following dilution with a 4-fold volume of phosphate

buffer and spiked with 1 μM compound. The homogenates were dialyzed against an equal volume (150 uL) of phosphate buffer at 37 °C for 6 h. Following the incubation, equal volumes (50 uL) of brain homogenate and buffer samples were collected and mixed with 50 μL of buffer or control homogenate, respectively, for preparation of mixed matrix samples. All samples were then precipitated with internal standard in acetonitrile (200 μL), vortexed, and centrifuged. Supernatants were analyzed using an LC-MS/MS assay. A dilution factor of 5 was applied to the calculation of brain fraction unbound.

Chemistry. *General Methods.* Solvents and reagents were of reagent grade and were used as supplied by the manufacturer. All reactions were run under a N_2 atmosphere. Organic extracts were routinely dried over anhydrous Na_2SO_4. Concentration refers to rotory evaporation under reduced pressure. Chromatography refers to flash chromatography using disposable RediSepR_f 4−120 g silica columns or Biotage disposable columns on a CombiFlash Companion or Biotage horizon automatic purification system. Microwave reactions were carried out in a microwave reactor manufactured by Smithcreator of Personal Chemistry. Purification by mass-triggered HPLC was carried out using Waters XTerra PrepMS C_{18} columns, 5 μm, 30 mm × 100 mm steel. Compounds were presalted as TFA salts and diluted with 1 mL of dimethylsulfoxide. Samples were purified by mass-triggered collection using a mobile phase of 0.1% trifluoroacetic acid in water and acetonitrile with a starting gradient of 100% aqueous to 100% acetonitrile over 10 min at 20 mL per minute flow rate. All target compounds were analyzed using ultrahigh-performance liquid chromatography/ultraviolet/evaporative light scattering detection coupled to time-of-flight mass spectrometry (UHPLC/UV/ELSD/TOFMS). Unless otherwise noted, all tested compounds were found to be ≥95% pure by this method.

UHPLC/MS Analysis. The UHPLC was performed on a Waters ACQUITY UHPLC system (Waters, Milford, MA), which was equipped with a binary solvent delivery manager, column manager, and sample manager coupled to ELSD and UV detectors (Waters). Detection was performed on a Waters LCT premier XE mass spectrometry (Waters). The instrument was fitted with an Acquity BEH (Bridged Ethane Hybrid) C18 column (30 mm × 2.1 mm, 1.7 μm particle size, Waters) operated at 60 °C.

Reductive Amination Method A (Singleton Format). To a stirred solution of the hydrochloride salts of amines (**6**) (1 equiv) in CH_2Cl_2 under N_2 at room temperature was added triethylamine (4 equiv), $MgSO_4$ (10 equiv), and aldehydes (**8a**−**e**) (1 equiv). The reaction mixture was stirred for 30 min, and then, $NaBH(OAc)_3$ (1.5 equiv) was added. The mixture was stirred at room temperature overnight and was then diluted with methylene chloride and washed with water and brine and dried with Na_2SO_4. The solvent was removed in vacuo, and the residue was purified by flash chromatography to give the desired products. To make the hydrochloride salts, the free bases were dissolved in MeOH, and a 4 N HCl solution in dioxane was added. The mixture was stirred for 10 min, and the solvent was then removed in vacuo to give the hydrochloride salts of the desired product as white solids.

Reductive Amination Method B (Library Format). Stock solutions (0.25 M) of amines (**6**) and aldehydes (**8a**−**e**) in DCE were prepared. When applicable, the amine or the aldehyde salt forms were neutralized by addition of 4 equiv of DIPEA. A 0.25 M fine suspension of $NaBH(OAc)_3$ in anhydrous DMF/DCE mixture (20/80) was prepared. To each vial was added a solution of amine (**6**) (0.2 mL) followed by a solution of aldehyde (**8a**−**e**) (0.2 mL) and the $NaBH(OAc)_3$ suspension (0.5 mL) to each vial. The vials were capped and shaken at room temperature for 16 h. An additional 0.5 mL of the $NaBH(OAc)_3$ suspension was added to each vial, the vials were vortexed, capped, and shaken at room temperature for 16 h. The solvent was removed in vacuo. DMSO (1 mL) and water (0.1 mL) were added to each vial. The samples were vortexed for 1 h, and concentrated NH_4OH (0.05 mL) was added to each vial. The samples were filtered and directly submitted to mass-triggered HPLC purification, and the compounds were characterized by UHPLC and MS.

*2-((4-(2-Methoxy-phenyl)piperidin-1-yl)methyl)-1-methyl-1H-benzo[d]imidazole (**3**)*

Step 1. 4-Bromopyridine (2.62 g, 16.3 mmol), 2-methoxyphenyl boronic acid (2.50 g, 16.3 mmol), and tetrakis (triphenylphosphine) palladium (0) (1.89 g, 1.63 mmol) were combined in DME (100 mL) and H_2O (33 mL) under N_2 at room temperature. The reaction mixture was then heated to reflux at 85 °C for 17 h. After it was cooled to room temperature, the mixture was partitioned between brine (300 mL) and ethyl acetate (300 mL). The organic layer was separated and dried over anhydrous Na_2SO_4. The solvent was evaporated in vacuo, and the residue was purified by flash chromatography (silica gel, 1:1 EtOAc: hexane) to give 2.21 g of 4-(2-methoxyphenyl)pyridine as a colorless oil, which crystallized under high vacuum. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 8.6 (m, 2H), 7.5 (m, 2H), 7.3−7.4 (m, 2H), 7.0−7.1 (m, 2H), 3.8 (s, 3H). MS (ES^+): *m*/*z* 186.1 (M + 1). The product was converted to the HCl salt by dissolving the residue in EtOAc (20 mL) and adding 1 M HCl in diethyl ether (20 mL). The solvent was removed in vacuo to 2.6 g of an off-white solid after drying under high vacuum.

Step 2. 4-(2-Methoxyphenyl)pyridine hydrochloride salt (1.0 g) was dissolved in methanol (23 mL), and platinum(IV) oxide (499 mg) was added. The mixture was then shaked on a Parr shaker under hydrogen (40 psi) at room temperature for 90 min. An additional 500 mg of platinum(IV) oxide was added, and the mixture was again placed on the Parr shaker for additional 2 h. The reaction mixture was then filtered through a pad of Celite, and the cake was rinsed several times with methanol. The filtrate was evaporated in vacuo to give 1.0 g of 4-(2-methoxyphenyl)piperidine hydrochloride salt as a white solid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 9.5−9.7 (broad d, 2H), 7.2 (m, 2H), 6.9 (m, 1H), 6.8−6.9 (d, 1H), 3.8 (s, 3H), 3.6 (d, 2H), 3.1−3.2 (m, 1H), 3.0 (q, 2H), 2.1−2.2 (m, 2H), 2.0 (d, 2H). MS (ES^+): *m*/*z* 192.0 (M + 1).

Step 3. The hydrochloride salt of the title compound was prepared by reductive amination method A from 4-(2-methoxyphenyl)piperidine hydrochloride salt and 1-methyl-1*H*-benzo[*d*]imidazole-2-carbaldehyde (**8a**). ^{1}H NMR (CD_3OD, 400 MHz): δ 7.94 (m, 2H), 7.69 (m, 2H), 7.20 (m, 2H), 6.94 (m, 2H), 5.05 (s, 2H), 4.24 (s, 3H), 3.83 (m, 2H), 3.81 (s, 3H), 3.47 (t, 2H, *J* = 11 Hz), 3.30 (m, 1H), 2.22 (q, 2H, *J* = 12 Hz)), 2.09 (d, 2H, *J* = 14 Hz). MS (ES^+): *m*/*z* 336.5 (M + 1). UHPLC: 100% (UV), 100% (ELSD).

*1-Methyl-2-((4-phenylpiperidin-1-yl)methyl)-1H-benzo[d]-imidazole (**7a**).* The title compound was prepared by reductive amination method B (library format) from commercially available 4-phenyl piperidine hydrochloride salt and 1-methyl-1*H*-benzo[*d*]imidazole-2-carbaldehyde (**8a**). MS (ES^+): *m*/*z* 306.2 (M + 1). UHPLC: 100% (UV), 98% (ELSD).

*2-((4-(2-Fluorophenyl)piperidin-1-yl)methyl)-1-methyl-1H-benzo-[d]imidazole (**7b**).* The hydrochloride salt of the title compound was prepared by following reductive amination method A from commercially available 4-(2-fluoro-phenyl)piperidine hydrochloride and 1-methyl-1*H*-benzo[*d*]imidazole-2-carbaldehyde (**8a**) in 72% overall yield. ^{1}H NMR (CD_3OD, 400 MHz): δ 7.59 (d, 1H, *J* = 7.9 Hz), 7.49 (d, 1H, *J* = 7.9 Hz), 7.20−7.28 (m, 3H), 7.18 (m, 1H), 7.07 (t, 1H, *J* = 7 Hz), 6.98 (dd, 1H, *J* = 11, 8 Hz), 3.93 (s, 3H), 3.85 (s, 2H), 3.00 (d, 2H, *J* = 12 Hz), 2.86 (m, 1H), 2.30 (m, 2H), 1.78 (m, 4H). MS (ES^+): *m*/*z* 324.1 (M + 1). UHPLC: 100% (UV), 100% (ELSD).

*2-((4-(3-Fluorophenyl)piperidin-1-yl)methyl)-1-methyl-1H-benzo-[d]imidazole (**7c**).* The title compound was prepared by reductive amination method B (library format) from commercially available 4-(3-fluoro-phenyl)piperidine hydrochloride and 1-methyl-1*H*-benzo-[*d*]imidazole-2-carbaldehyde (**8a**). MS (ES^+): *m*/*z* 324.1 (M + 1). UHPLC: 97% (UV), 100% (ELSD).

*2-((4-(4-Fluorophenyl)piperidin-1-yl)methyl)-1-methyl-1H-benzo-[d]imidazole (**7d**)*

Step 1. To a stirred solution of diisopropylamine (7 mL) in THF (150 mL) at −78 °C was added a solution of *n*-butyl lithium in hexanes (20 mL, 2.5 M). After 1 h, *tert*-butyl 4-oxo-1-piperidinecarboxylate (10

g) was added. After an additional 1.5 h, *N*-phenyltrifluoromethanesulfonimide (19.65 g) was added, and the mixture was allowed to warm to room temperature. After it was stirred for 16 h, the solvent was removed in vacuo, and the resulting residue was used without further purification.

Step 2. To a stirred solution of *tert*-butyl 4-trifluoromethanesulfonate-1-(1,2,3,6-tetrahydropyridine) carboxylate (8.3 g, crude) in EtOH (85 mL) and water (15 mL) under N_2 at room temperature were added 4-fluorophenylboronic acid (3.5 g) and tetrakis(triphenylphosphine)-palladium(0) (2.89 g). The mixture was then heated to 90 °C and stirred for 16 h. The reaction mixture was then cooled to room temperature, and the solvent was removed in vacuo. The residue was suspended in water (100 mL), and the mixture was extracted with ethyl acetate (100 mL × 2). The combined organic layers were washed with brine, dried over sodium sulfate, filtered, and concentrated in vacuo. The residue was purified by flash chromatography (silica gel, 0−20% EtOAc in hexanes) gave 3.2 g of *tert*-butyl 4-(4-fluorophenyl)-1-(1,2,3,6-tetrahydropyridine) carboxylate as a brownish oil. ^{1}H NMR (400 MHz, CD_3OD): δ 7.23−7.44 (m, 3H), 7.04 (t, 1H), 6.04 (m, 1H), 4.03 (m, 2H), 3.61 (m, 2H), 2.49 (m, 2H), 1.47 (s, 9H).

Step 3. A mixture of *tert*-butyl 4-(4-fluorophenyl)-1-(1,2,3,6-tetrahydropyridine) carboxylate (3.2 g) and Pd/C (60 mg) in ethanol (20 mL) was shaken in a Parr apparatus under 40 psi of hydrogen. After 16 h, the mixture was purged with nitrogen, filtered through Celite, and concentrated in vacuo to give 3.2 g of *tert*-butyl 4-(4-fluorophenyl)-1-piperidinecarboxylate as a yellow oil. To this residue was added 4 M HCl/dioxane (10 mL), and the mixture was stirred at room temperature for 2 h. The solvent was removed in vacuo to give 2.5 g of 4-(4-fluorophenyl)pyperidine hydrochloride as a white solid. ^{1}H NMR (400 MHz, CD_3OD): δ 7.23−7.39 (m, 3H), 7.05 (t, 1H), 3.47−3.50 (m, 2H), 3.09−3.16 (m, 2H), 2.88−2.94 (m, 1H), 2.03−2.08 (m, 2H), 1.82−1.93 (m, 2H). MS (ES^+): *m*/*z* 180.1 (M + 1).

Step 4. The hydrochloride salt of the title compound was prepared by reductive amination method A from 4-(4-fluoro-phenyl)piperidine hydrochloride and 1-methyl-1*H*-benzo[*d*]imidazole-2-carbaldehyde (**8a**) in 83% yield. NMR (400 MHz, CD_3OD): 7.90 (m, 2H), 7.68 (m, 2H), 7.28 (m, 2H), 7.03 (m, 2H), 5.03 (s, 2H), 4.24 (s, 3H), 3.80 (m, 2H), 3.45 (m, 2H), 2.95 (m, 1H), 2.20 (m, 2H), 2.18 (m, 2H). MS (ES^+): *m*/*z* 324.1 (M + 1). UHPLC: 100% (UV), 100% (ELSD).

*2-((4-(2-Chlorophenyl)piperidin-1-yl)methyl)-1-methyl-1H-benzo-[d]imidazole (**7e**).* The title compound was prepared by reductive amination method B (library format) from commercially available 4-(2-chloro-phenyl)piperidine hydrochloride and 1-methyl-1*H*-benzo-[*d*]imidazole-2-carbaldehyde (**8a**). MS (ES^+): *m*/*z* 340.2 (M + 1). UHPLC: 100% (UV), 99% (ELSD).

*3-(1-((1-Methyl-1H-benzo[d]imidazol-2-yl)methyl)piperidin-4-yl)-benzonitrile (**7f**).* The title compound was prepared by reductive amination method B (library format) from commercially available 3-(piperidin-4-yl)benzonitrile and 1-methyl-1*H*-benzo[*d*]imidazole-2-carbaldehyde (**8a**). MS (ES^+): *m*/*z* 331.2 (M + 1). UHPLC: 100% (UV), 98% (ELSD).

*N-Methyl-3-(1-((1-methyl-1H-benzo[d]imidazol-2-yl)methyl)-piperidin-4-yl)benzamide (**7g**).* The title compound was prepared by reductive amination method B (library format) from commercially available *N*-methyl-3-(piperidin-4-yl)benzamide and 1-methyl-1*H*-benzo[*d*]imidazole-2-carbaldehyde (**8a**). MS (ES^+): *m*/*z* 363.2 (M + 1). UHPLC: 100% (UV), 100% (ELSD).

*2-((4-(4-Chloro-2-fluorophenyl)piperidin-1-yl)methyl)-1-methyl-1H-benzo[d]imidazole (**7h**).* *tert*-Butyl 4-(4-chloro-3-fluorophenyl)-1-(1,2,3,6-tetrahydropyridine) carboxylate was prepared by the method described in the synthesis of **7d** (steps 1 and 2) from commercially available 4-chloro-3-fluorophenyl boronic acid. A mixture of *tert*-butyl 4-(3-chloro-4-fluorophenyl)-1-(1,2,3,6-tetrahydropyridine) carboxylate (465 mg, 1.49 mmol) and PtO_2 (20 mg) in methanol (8 mL) was shaken in a Parr apparatus at room temperature under 45 psi of hydrogen. After 1 h, the mixture was purged with nitrogen, filtered through Celite, and concentrated in vacuo to give 443 mg of *tert*-butyl 4-(3-chloro-4-fluorophenyl)-1-piperidinecarboxylate as a yellow oil. The residue was the treated with 4 N HCl in dioxane (3 mL) to yield 375 mg of 4-(4-chloro-3-fluorophenyl)pyperidine hydrochloride. MS (ES^+): *m*/*z* 255, 257 (M + CH_3CN). The free base of the title compound was prepared by reductive amination method A from 4-(4-chloro-3-fluorophenyl)pyperidine hydrochloride in 87% yield. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.72 (dd, 1H, *J* = 7.0, 1.5 Hz), 7.31 (dd, 1H, *J* = 7.0, 1.5 Hz), 7.20−7.25 (m, 3H), 7.07 (t, 1H, *J* = 7.8 Hz), 7.01 (dd, 1H, *J* = 12, 7.8 Hz), 3.87 (s, 3H), 3.84 (s, 2H), 3.00 (d, 2H, *J* = 12 Hz), 2.82 (m, 1H), 2.30 (m, 2H), 1.75 (m, 4H). MS (ES^+): *m*/*z* 358.2 (M + 1). UHPLC: 100% (UV), 100% (ELSD).

*1-Methyl-2-((4-(4-(trifluoromethyl)phenyl)piperidin-1-yl)methyl)-1H-benzo[d]imidazole (**7i**).* The title compound was prepared by reductive amination method B (library format) from commercially available 4-(4-trifluoromethyl-phenyl)piperidine hydrochloride salt and 1-methyl-1*H*-benzo[*d*]imidazole-2-carbaldehyde (**8a**). MS (ES^+) *m*/*z* 374.2 (M + 1). UHPLC: 100% (UV), 98% (ELSD).

*1-Methyl-2-((4-(2-(trifluoromethyl)phenyl)piperidin-1-yl)methyl)-1H-benzo[d]imidazole (**7j**).* The title compound was prepared by reductive amination method B (library format) from commercially available 2-trifluoromethylphenyl piperidine hydrochloride and 1-methyl-1*H*-benzo[*d*]imidazole-2-carbaldehyde (**8a**). MS (ES^+): *m*/*z* 374.2 (M + 1). UHPLC: 100% (UV), 100% (ELSD).

*1-Methyl-2-((4-(4-(trifluoromethyl)phenyl)piperidin-1-yl)methyl)-1H-imidazo[4,5-b]pyridine (**7k**).* The hydrochloride salt of the title compound was prepared by reductive amination method A from commercially available 4-(4-trifluoromethyl-phenyl)piperidine hydrochloride salt and 1-methyl-1*H*-imidazo[4,5-*b*]pyridine-2-carbaldehyde hydrochloride hydrate (**8b**). ^{1}H NMR (CD_3OD, 400 MHz): δ 8.60 (dd, 1H, *J* = 5.4, 1.3 Hz), 8.45 (dd, 1H, *J* = 8.1, 1.3 Hz), 7.63 (m, 3H), 7.51 (d, 2H, *J* = 8.3 Hz), 4.03 (m, 2H), 4.02 (s, 3H), 3.64 (s, 2H), 3.47 (m, 2H), 3.10 (m, 1H), 2.20 (m, 4H). MS (ES^+): *m*/*z* 375.1 (M + 1). UHPLC: 100% (UV), 100% (ELSD).

*1-Methyl-2-((4-(4-(trifluoromethyl)phenyl)piperidin-1-yl)methyl)-1H-imidazo[4,5-c]pyridine (**7l**).* The free base of the title compound was prepared by reductive amination method A from commercially available 4-(4-trifluoromethyl-phenyl)piperidine hydrochloride salt and 1-methyl-1*H*-imidazo[4,5-*c*]pyridine-2-carbaldehyde dihydrochloride dihydrate (**8c**). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 9.04 (s, 1H), 8.44 (d, 1H, *J* = 5.8 Hz), 7.54 (d, 2H, *J* = 8.3 Hz), 7.32 (m, 3H), 3.93 (s, 3H), 3.88 (s, 2H), 3.00 (d, 2H, *J* = 11.3 Hz), 2.60 (m, 1H), 2.31 (m, 2H), 1.84 (m, 2H), 1.75 (m, 2H). MS (ES+): *m*/*z* 375.1 (M + 1). UHPLC: 100% (UV), 100% (ELSD).

*3-Methyl-2-((4-(4-(trifluoromethyl)phenyl)piperidin-1-yl)methyl)-3H-imidazo[4,5-c]pyridine (**7m**).* The free base of the title compound was prepared by reductive amination method A from commercially available 4-(4-trifluoromethyl-phenyl)piperidine hydrochloride salt and 3-methyl-3*H*-imidazo[4,5-*c*]pyridine-2-carbaldehyde (**8d**). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 9.00 (s, 1H), 8.43 (d, 1H, *J* = 5.8 Hz), 7.71 (d, 1H, *J* = 5.8 Hz), 7.54 (d, 2H, *J* = 7.8 Hz), 7.32 (d, 2H, *J* = 7.8 Hz), 4.01 (s, 3H), 3.95 (s, 2H), 3.05 (d, 2H, *J* = 11.6 Hz), 2.60 (m, 1H), 2.39 (m, 2H), 1.80 (m, 4H). MS (ES^+): *m*/*z* 375.2 (M + 1). UHPLC: 99% (UV), 100% (ELSD).

*3-Methyl-2-((4-(4-(trifluoromethyl)phenyl)piperidin-1-yl)methyl)-3H-imidazo[4,5-b]pyridine (**7n**).* The free base of the title compound was prepared by reductive amination method A from commercially available 4-(4-trifluoromethyl-phenyl)piperidine hydrochloride salt and 3-methyl-3*H*-imidazo[4,5-*b*]pyridine-2-carbaldehyde hydrochloride hydrate (**8e**). ^{1}H NMR (CD_3OD, 400 MHz): δ 8.35 (d, 1H, *J* = 4.8 Hz), 8.0 (dd, 1H, *J* = 8.0, 1.3 Hz), 7.54 (d, 2H, *J* = 8.2 Hz), 7.40 (d, 2H, *J* = 8.2 Hz), 7.29 (dd, 1H, *J* = 8.0, 4.8 Hz), 3.98 (s, 3H), 3.89 (s, 2H), 3.02 (d, 2H, *J* = 11.6 Hz), 2.65 (m, 1H), 2.33 (m, 2H), 1.82 (m, 4H). MS (ES^+): *m*/*z* 375.2 (M + 1). UHPLC: 100% (UV), 100% (ELSD).

*2-((4-(2-Methoxy-4-(trifluoromethyl)phenyl)piperidin-1-yl)methyl)-1-methyl-1H-imidazo[4,5-b]pyridine (**7o**).* To a stirred solution of 1-methoxy-3-(trifluoromethyl)benzene (9.8 mL, 68 mmol) in THF (50 mL) under N_2 at 0 °C was added *n*-BuLi (1.6 M in hexanes, 45 mL, 68 mmol) dropwise. The reaction mixture was stirred at 0 °C for 2 h, and then, triisopropylborate (11.6 mL, 68 mmol) was added. The reaction mixture was slowly warmed up to room temperature and stirred overnight. A solution of 10% HCl in water (50 mL) was added, and the mixture was stirred for 1 h. The mixture was extracted with CH_2Cl_2 (100 mL × 3). The organic layers were combined, washed with brine, and dried over Na_2SO_4. The solvent was removed in vacuo to give 8.14 g of 2-methoxy-4-(trifluoromethyl)phenylboronic acid as a viscous oil.

2-Methoxy-4-trifluoromethyl phenylpiperidine was prepared from 2-methoxy-4-(trifluoromethyl)phenylboronic acid and 4-bromopyridine by the method described in the synthesis of **3** (steps 1 and 2), and the free base of the title compound was prepared by reductive amination method A from 2-methoxy-4-trifluoromethyl phenylpiperidine and 1-methyl-1*H*-imidazo[4,5-*b*]pyridine-2-carbaldehyde hydrochloride hydrate (**8b**). ^{1}H NMR (CD_3OD, 400 MHz): δ 8.40 (d, 1H, J = 5.0 Hz), 8.0 (d, 1H, J = 7.9 Hz), 7.34 (m, 2H), 7.19 (d, 1H, J = 7.9 Hz), 7.12 (s, 1H), 3.99 (s, 3H), 3.92 (s 2H), 3.87 (s, 3H), 3.03 (m, 3H), 2.33 (m, 2H), 1.77 (m, 4H). MS (ES^+): m/z 405.2 (M + 1). UHPLC: 100% (UV), 100% (ELSD).

*2-((4-(2-Fluoro-4-(trifluoromethyl)phenyl)piperidin-1-yl)methyl)-1-methyl-1H-imidazo[4,5-b]pyridine (**7p**).* The free base of the title compound was prepared from 2-fluoro-4-(trifluoromethyl)phenylboronic acid and 4-bromopyridine by the method described in the synthesis of **3** with 1-methyl-1*H*-imidazo[4,5-*b*]pyridine-2-carbaldehyde hydrochloride hydrate (**8b**). ^{1}H NMR (CD_3OD, 400 MHz): δ 8.40 (dd, 1H, J = 5.0, 1.6 Hz), 8.0 (d, 1H, J = 8.3 Hz), 7.51(m, 1H), 7.43 (m, 1H), 7.34 (m, 2H), 3.99 (s, 3H), 3.95 (s 2H), 3.07 (d, 2H, J = 11.2 Hz), 2.96 (m, 1H), 2.37 (m, 2H), 1.97 (m, 2H), 1.83 (m, 2H). MS (ES^+): m/z 393.2 (M + 1). UHPLC: 100% (UV), 100% (ELSD).

*2-((4-(2-Fluoro-4-(trifluoromethoxy)phenyl)piperidin-1-yl)methyl)-1-methyl-1H-imidazo[4,5-b]pyridine (**7q**).* The free base of the title compound was prepared from 2-fluoro-4-(trifluoromethoxy)phenylboronic acid and 4-bromopyridine by the method described in the synthesis of **3** with 1-methyl-1*H*-imidazo[4,5-*b*]pyridine-2-carbaldehyde hydrochloride hydrate (**8b**). ^{1}H NMR (CD_3OD, 400 MHz): δ 8.39 (dd, 1H, J = 5.0, 1.5 Hz), 8.0 (dd, 1H, J = 8.3, 1.5 Hz), 7.36(m, 2H), 7.38 (t, 1H, J = 10 Hz), 7.35 (dd, 1H, J = 8.3, 5.0 Hz), 7.04 (t, 1H, J = 10 Hz), 3.99 (s, 3H), 3.92 (s, 2H), 3.03 (d, 2H, J = 12.0 Hz), 2.89 (m, 1H), 2.35 (m, 2H), 1.80 (m, 4H). MS (ES^+): m/z 409.0 (M + 1). UHPLC: 100% (UV), 100% (ELSD).

*1-Benzyl-4-(4-(trifluoromethyl)phenyl)-1,2,3,6-tetrahydropyridine (**9**).* To a stirred solution of 1-bromo-4-(trifluoromethyl)benzene (1.84 mL, 13 mmol) in THF (5 mL) under N_2 at −78 °C was added BuLi (5.5 mL, 2.5 M in hexanes, 14 mmol) dropwise. The reaction mixture turned into a yellow and then dark orange solution upon stirring for 15 min. A solution of 1-benzylpiperidin-4-one (246 mg, 13 mmol) in THF (4 mL) was then added dropwise. The resulting mixture was stirred at −78 °C for 30 min, then allowed to warm up to room temperature, and stirred for additional 3 h. The reaction was quenched with water, and the mixture was extracted with EtOAc (10 mL × 2). The organic layer was washed with water and brine and dried over Na_2SO_4. The solvent was removed in vacuo, and the residue was purified with flash chromatography (silica gel, 10−40% EtOAc in hexanes) to give 238 mg of 1-benzyl-4-(4-(trifluoromethyl)phenyl)-1,2,3,6-tetrahydropyridine as a viscous oil. ^{1}H NMR (CD_3OD, 400 MHz): δ 7.66 (s, 4H), 7.30−7.40 (m, 5H), 6.26 (s, br, 1H), 3.68 (s, 2H), 3.20 (m, 2H), 2.77 (m, 2H), 2.60 (m, 2H). MS (APCI): m/z 318.3 (M + 1).

*4-(4-(Trifluoromethyl)phenyl)piperidin-3-ol (**10a**)*

Step 1. To a stirred solution of 1-benzyl-4-(4-(trifluoromethyl)-phenyl)-1,2,3,6-tetrahydropyridine (300 mg, 0.95 mmol) in diglyme (1 mL) under N_2 at room temperature was added $NaBH_4$ (57 mg, 1.51 mmol). The reaction mixture was then cooled to 0 °C, and a solution of $BF_3 \cdot Et_2O$ (0.24 mL, 1.89 mmol) in diglyme (0.25 mL) was added dropwise. After the addition was complete, the reaction mixture was warmed to room temperature and stirred for 2 h. H_2O (0.1 mL) was added very slowly to the mixture, followed by 6 N NaOH aqueous solution (0.25 mL). The reaction mixture was then heated to 65 °C, and 30% H_2O_2 (0.18 mL, 1.89 mmol) was added slowly. The mixture was stirred at 65 °C for additional 45 min and then was cooled to room temperature. The mixture was diluted with CH_2Cl_2 and washed with water and brine and dried over Na_2SO_4. The solvent was removed in vacuo to give 298 mg of 1-benzyl-4-(4-(trifluoromethyl)phenyl)-piperidin-3-ol as a mixture of diastereomers. MS (APCI): m/z 336.3 (M + 1).

Step 2. To a stirred solution of 1-benzyl-4-(4-(trifluoromethyl)-phenyl)piperidin-3-ol (298 mg, 0.9 mmol) in MeOH (10 mL) were added 10% Pd/C (40 mg) and ammonium formate (673 mg, 10.7 mmol). The reaction mixture was then heated to reflux for 6 h. The mixture was then cooled to room temperature and filtered through a pad of Celite, and the cake was further washed with MeOH (50 mL). The filtrate was concentrated in vacuo to give 219 mg of 4-(4-(trifluoromethyl)phenyl)piperidin-3-ol as a mixture of diastereomers (70/30 *trans*/*cis*- ratio). The major *trans*-diastereomer: ^{1}H NMR (CD_3OD, 400 MHz): δ 7.55 (d, 2H, J = 7.9 Hz), 7.42 (d, 2H, J = 7.9 Hz), 3.27 (m, 1H), 3.20 (m, 2H), 3.00 (m, 1H), 2.60 (m, 1H), 2.42 (m, 2H), 1.75 (m, 2H). MS (APCI): m/z 246.2 (M + 1).

*3-[4-(Trifluoromethyl)phenyl]-8-azabicyclo[3.2.1]octane Hydrochloride (**10c**)*

Step 1. A solution of 1-bromo-4-(trifluoromethyl)benzene (188 g, 0.935 mol) in ether (1.2 L) was added dropwise to a suspension of magnesium (22 g, 0.9 mol) in THF (200 mL) at such a rate that the temperature of the mixture was 35−40 °C. The mixture was stirred at 35 °C for 1 h in a flask equipped with a reflux condenser. The reaction mass was cooled to 0 °C, and a solution of 8-benzyl-8-azabicyclo-[3.2.1]octan-3-one (155 g, 0.72 mol) in ether (200 mL) was added dropwise at 5 °C. The reaction mixture was stirred overnight at room temperature. A 5% solution of sodium hydroxide (200 mL) was added dropwise. The mixture was stirred, and the organic layer was separated. The suspension of salts was extracted with ether (400 mL). The combined organic fractions were extracted with a solution of HCl (150 mL) in water (300 mL). The aqueous layer was separated and basified with a solution of sodium hydroxide (120 g) in water (300 mL), and the product was extracted with ether (300 mL × 3). The extract was dried with sodium sulfate and concentrated in vacuo. The residue was purified by flash chromatography (silica gel, CH_2Cl_2/Et_2O 9:1) to give 8-benzyl-3-[4-(trifluoromethyl)phenyl]-8-azabicyclo[3.2.1]octan-3-ol (95.1 g, 36.5%).

Step 2. A solution of 8-benzyl-3-[4-(trifluoromethyl)phenyl]-8-azabicyclo[3.2.1]octan-3-ol (95.1 g, 0.263 mol) in DMF (300 mL) was added dropwise under cooling with ice to a suspension of NaH (44 g, 60% suspension in oil, 1.1 mol) in DMF (1 L). The mixture was stirred at 45 °C for 3 h and cooled, and a solution of methyl iodide (41 mL, 0.295 mol) in ether (50 mL) was added dropwise. The mixture was stirred at room temperature overnight. The larger part of DMF was evaporated, and the residue was diluted with water (1 L). The reaction mixture was extracted with ethyl acetate (300 mL × 2). The extract was washed with water and brine and dried over Na_2SO_4. The solvent was removed in vacuo, and the residue was diluted with THF (50 mL) and ether (950 mL). A 4 M solution of HCl in dioxane (75 mL, 0.3 mol) was added dropwise. The formed crystals were separated by filtration and dried to give 8-benzyl-3-methoxy-3-[4-(trifluoromethyl)phenyl]-8-azabicyclo[3.2.1]octane (107 g, 98%).

Step 3. 8-Benzyl-3-methoxy-3-[4-(trifluoromethyl)phenyl]-8-azabicyclo[3.2.1]octane (107 g, ∼0.26 mol) and concentrated HCl (3 mL)

were added to a suspension of 10% Pd/C (17 g) in methanol (1 L) in argon. The system was flushed with hydrogen, and the mixture was hydrogenated under a pressure of 40 psi at 84 °C for 5 days. The mixture was cooled and filtered, and methanol was evaporated. The residue was treated with a solution of NaOH (25 g) in water (100 mL) and extracted with dichloromethane (100 mL × 2). The combined organic layers were washed with water and brine and dried over Na_2SO_4. The solvent was removed in vacuo, and the residue was dissolved in a mixture of THF (200 mL) and ether (200 mL). A 4 N solution of HCl in dioxane (53 mL) was added. The formed crystals were separated by filtration to give 3-[4-(trifluoromethyl)phenyl]-8-azabicyclo[3.2.1]octane hydrochloride (**10c**) (53.6 g, 70%). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 9.15 (br, s, 2H), 7.68 (s 4H), 3.95 (m, 2H), 3.40 (m, 1H), 2.45 (m, 2H), 2.20 (m, 2H), 1.88 (m, 2H), 1.55 (m, 2H). MS (ES^+): m/z 256.1 (M + 1). UHPLC: 96% (UV), 98% (ELSD).

*3,3-Dimethyl-4-(4-(trifluoromethyl)phenyl)piperidine Hydrochloride (**10d**).* The title compound was prepared by the method described in the synthesis of **10c**. ^{1}H NMR (CD_3OD, 400 MHz): δ 7.56 (d, 2H, J = 7.9 Hz), 7.35 (d, 2H, J = 7.9 Hz), 3.18 (m, 1H), 2.6−2.75 (m, 4H), 2.18 (m, 1H), 1.50 (m, 1H), 0.88 (s, 3H), 0.74 (s, 3H). MS (ES^+): m/z 299.2 (M + CH_3CN).

*1-((1-Methyl-1H-imidazo[4,5-b]pyridin-2-yl)methyl)-4-(4-(trifluoromethyl)phenyl)piperidin-3-ol (**13**).* The free base of the title compound was prepared by reductive amination method A from *trans*-4-(4-(trifluoromethyl)phenyl)piperidin-3-ol (**10a**) and 1-methyl-1*H*-imidazo[4,5-*b*]pyridine-2-carbaldehyde hydrochloride hydrate (**8b**). The *trans*-diastereomer: ^{1}H NMR (CD_3OD, 400 MHz): δ 8.37 (dd, 1H, J = 3.3, 1.2 Hz), 8.0 (m, 1H), 7.50−7.70 (m, 3H), 7.42 (d, 2H, J = 8.4 Hz), 7.32 (m, 1H), 3.97 (s, 3H), 3.93 (s, 2H), 3.78 (m, 1H), 3.12 (m, 1H), 2.91 (m, 1H), 2.78 (m, 1H), 2.68 (m, 1H), 2.52 (m, 1H), 2.25 (m, 1H), 2.10 (m, 1H), 1.80 (m, 1H). MS (APCI): m/z 391.1 (M + 1). UHPLC: 95% (UV), 86% (ELSD).

*1-Methyl-2-((cis-2-methyl-4-(4-(trifluoromethyl)phenyl)piperidin-1-yl)methyl)-1H-imidazo[4,5-b]pyridine (**14**)*

Step 1. 4-Bromo-2-methylpyridine (209 mg, 1.16 mmol), 4-trifluoromethylphenyl boronic acid (221 mg, 1.16 mmol), sodium bicarbonate (293 mg, 3.48 mmol), and tetrakis (triphenylphosphine) palladium(0) (18.4 mg, 0.012 mmol) were combined in DME (10 mL) and H_2O (2 mL) under N_2 at room temperature. The reaction mixture was then heated to reflux at 85 °C for 17 h. After it was cooled to room temperature, the mixture was diluted by water and extracted with ethyl acetate (50 mL × 2). The combined organic layer was washed with water and brine and dried over anhydrous Na_2SO_4. The solvent was removed in vacuo, and the residue was purified by flash chromatography (silica gel, 30% EtOAc in hexanes) to give 240 mg of 2-methyl-4-(4-(trifluoromethyl)phenyl)pyridine as a viscous oil. The residue was then dissolved in CH_2Cl_2 (5 mL), and 4 N HCl in dioxane was added (1 mL). The mixture was stirred at room temperature for 10 min, and the solvent was concentrated in vacuo. The resulting solid was suspended in cold ethylether and filtered to yield 250 mg of 2-methyl-4-(4-(trifluoromethyl)phenyl)pyridine hydrochloride salt as pure white solid. MS (APCI): m/z 238.3 (M + 1).

Step 2. 2-Methyl-4-(4-(trifluoromethyl)phenyl)pyridine hydrochloride salt (250 mg, 0.92 mmol) was dissolved in methanol (10 mL), and platinum(IV) oxide (20 mg) was added. The mixture was then shaken on a Parr shaker under hydrogen (45 psi) at 50 °C for 5 h. The reaction mixture was then filtered through a pad of Celite, and the cake was rinsed several times with methanol. The filtrate was evaporated in vacuo to give 253 mg of 2-methyl-4-(4-(trifluoromethyl)phenyl)piperidine hydrochloride salt (**10b**) as a white solid. MS (APCI): m/z 244.3 (M + 1).

Step 3. The free base of the title compound was prepared by reductive amination method A with 2-methyl-4-(4-(trifluoromethyl)phenyl)-piperidine hydrochloride salt (**10b**) and 1-methyl-1*H*-imidazo[4,5-*b*]-pyridine-2-carbaldehyde hydrochloride hydrate (**8b**). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.42 (dd, 1H, J = 5.5, 1.0 Hz), 7.98 (d, 1H, J = 8.0 Hz), 7.53 (d, 2H, J = 8.9 Hz), 7.30 (m, 3H), 4.40 (d, 1H, J = 13.7 Hz), 3.91 (s, 3H), 3.49 (d, 1H, J = 13.3 Hz), 2.84 (m, 1H), 2.68 (m, 1H), 2.50 (m, 1H), 2.24 (m, 1H), 1.86 (m, 1H), 1.70 (m, 1H), 1.58 (m, 2H), 1.20 (d, 3H, J = 6.2 Hz). MS (ES^+): m/z 389.2 (M+1). UHPLC: 100% (UV), 100% (ELSD).

*1-Methyl-2-((3-(4-(trifluoromethyl)phenyl)-8-azabicyclo[3.2.1]-octan-8-yl)methyl)-1H-imidazo[4,5-b]pyridine (**15**).* The hydrochloride salt of the title compound was prepared by reductive amination method A from 3-[4-(trifluoromethyl)phenyl]-8-azabicyclo[3.2.1]-octane hydrochloride (**10c**) and 1-methyl-1*H*-imidazo[4,5-*b*]pyridine-2-carbaldehyde hydrochloride hydrate (**8b**). ^{1}H NMR (CD_3OD, 400 MHz): δ 8.53 (dd, 1H, J = 5.0, 1.2 Hz), 8.30 (d, 1H, J = 8.3 Hz), 7.79 (d, 2H, J = 8.3 Hz), 7.68 (d, 2H, J = 8.3 Hz), 7.53 (dd, 1H, J = 8.3, 5.0 Hz), 4.78 (m, 1H), 4.45 (m, 2H), 3.95 (s, 3H), 3.46 (m, 1H), 2.80 (m, 4H), 2.28 (m, 2H), 1.88 (m, 2H). MS (APCI): m/z 401.1 (M + 1). UHPLC: 100% (UV), 100% (ELSD).

*2-((3,3-Dimethyl-4-(4-(trifluoromethyl)phenyl)piperidin-1-yl)-methyl)-1-methyl-1H-imidazo[4,5-b]pyridine (**16**).* The free base of the title compound was prepared by reductive amination method A from 3,3-dimethyl-4-(4-(trifluoromethyl)phenyl)piperidine hydrochloride (**10d**) and 1-methyl-1*H*-imidazo[4,5-*b*]pyridine-2-carbaldehyde hydrochloride hydrate (**8b**). ^{1}H NMR (CD_3OD, 400 MHz): δ 8.39 (dd, 1H, J = 5.5, 1.2 Hz), 8.01 (d, 1H, J = 8.0 Hz), 7.53 (d, 2H, J = 8.9 Hz), 7.35 (m, 3H), 4.03 (s, 3H), 3.87 (s, 2H), 3.00 (d, 1H, J = 8.3 Hz), 2.53 (m, 2H), 2.23 (m, 2H), 2.18 (m, 1H), 1.56 (m, 1H), 0.89 (s, 3H), 0.71 (s, 3H). MS (APCI): m/z 403.4 (M + 1). UHPLC: 100% (UV), 97% (ELSD).

*1-Methyl-2-((cis-3-methyl-4-(4-(trifluoromethyl)phenyl)piperidin-1-yl)methyl)-1H-imidazo[4,5-b]pyridine (**17a**)*

Step 1. 4-Bromo-3-methylpyridine (1.36 g, 7.89 mmol), 4-trifluoromethylphenyl boronic acid (1.50 g, 7.89 mmol), sodium bicarbonate (2.65 g, 31.6 mmol), and tetrakis (triphenylphosphine) palladium(0) (912 mg, 0.79 mmol) were combined in DME (40 mL) and H_2O (10 mL) under N_2 at room temperature. The reaction mixture was then heated to reflux at 85 °C for 17 h. After it was cooled to room temperature, the mixture was diluted by water and extracted with ethyl acetate. The organic layers were combined and washed with water and brine and dried over anhydrous Na_2SO_4. The solvent was removed in vacuo, and the residue was purified by flash chromatography (silica gel, 30% EtOAc in hexanes) to give 1.42 g (76% yield) of 3-methyl-4-(4-(trifluoromethyl)phenyl)pyridine as a viscous oil. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.52 (s, 1H), 8.47 (d, 1H, J = 5 Hz), 7.69 (d, 2H, J = 8.3 Hz), 7.42 (d, 2H, J = 7.9 Hz), 7.10 (d, 1H, J = 5 Hz), 2.24 (s, 3H). MS (ES^+): m/z 238.1 (M + 1). The free base was dissolved in CH_2Cl_2 (10 mL), and 4 N HCl in dioxane was added (2 mL). The mixture was stirred at room temperature for 10 min, and the solvent was concentrated in vacuo. The resulting solid was suspended in cold ethyl ether and filtered to yield 1.58 g of the hydrochloride salt as a white solid, which was used in the next step.

Step 2. 2-Methyl-4-(4-(trifluoromethyl)phenyl)pyridine hydrochloride salt (1.27 g, 4.68 mmol) was dissolved in ethanol (20 mL), and platinum-(IV) oxide (50 mg) was added. The mixture was then shaken on a Parr shaker under hydrogen (45 psi) at 50 °C overnight. The reaction mixture was then filtered through a pad of Celite, and the cake was rinsed several times with methanol. The filtrate was evaporated in vacuo to give 1.25 g of *cis*-3-methyl-4-(4-(trifluoromethyl)phenyl)piperidine hydrochloride salt (**18a**) as a white solid. MS (APCI): m/z 244.1 (M + 1).

Step 3. The free base of the title compound was prepared by reductive amination method A with *cis*-3-methyl-4-(4-(trifluoromethyl)phenyl)-piperidine hydrochloride salt (**18a**) and 1-methyl-1*H*-imidazo[4,5-*b*]pyridine-2-carbaldehyde hydrochloride hydrate (**8b**). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.52 (dd, 1H, J = 5.0, 1.7 Hz), 7.65 (d, 1H, J = 8.0 Hz), 7.52 (d, 2H, J = 8.3 Hz), 7.23 (m, 2H), 7.18 (m, 1H), 3.92

(s, 3H), 3.86 (m, 3H), 3.02 (m, 1H), 2.95 (m, 1H), 2.78 (m, 1H), 2.51 (m, 1H), 2.26 (m, 1H), 2.00−2.20 (m, 2H), 1.60 (m, 1H), 0.72 (d, 3H, J = 7.0 Hz). MS (ES^+): m/z 389.1 (M + 1).

Step 4. Two enantiomers were separated by chiral HPLC from 368 mg of the racemic **17a** using a Chiralcel OD column (5 cm × 50 cm) with 80/20 heptane/EtOH as the mobile phase at a flow rate of 145 mL/min. The individual enantiomers were then dissolved in DCM and treated with 4 N HCl in dioxane (3 equiv). The solvent was removed in vacuo, and the resulting solids were triturated with ethylether and dried under vacuum. (+)-**17a**: retention time, 21.94 min. MS (ES^+): m/z 389.1 (M + 1). UHPLC: 100% (UV), 100% (ELSD). Optical rotation: $[\alpha]_D^{20}$ + 25.9° (c 0.57, MeOH). (−)-**17a**: retention time, 26.31 min. MS (ES^+): m/z 389.1 (M + 1). UHPLC: 100% (UV), 100% (ELSD). Optical rotation: $[\alpha]_D^{20}$ − 26.2° (c 0.62, MeOH).

*1-Methyl-2-((cis-3-methyl-4-(4-chloro-2-fluorophenyl)piperidin-1-yl)methyl)-1H-imidazo[4,5-b]pyridine (**17b**).* The free base of the title compound was prepared by the method described for the synthesis of **17a** from commercially available 4-chloro-2-fluorophenyl boronic acid. ^{1}H NMR (CD_3OD, 400 MHz): δ 8.91 (d, 1H, J = 8.3 Hz), 8.74 (d, 1H, J = 6.8 Hz), 7.92 (m, 1H), 7.22 (m, 3H), 5.10 (s, 2H), 4.12 (s, 3H), 4.00 (m, 2H), 3.60 (m, 3H), 2.65(m, 1H), 2.55 (m, 1H), 2.02 (m, 1H), 1.03 (d, 3H, J = 7.1 Hz). MS (ES^+): m/z 373.1 (M + 1). Two enantiomers were separated by chiral HPLC from 1.46 g of the racemic **17b** using a Chiralcel OJ-H column (3 cm × 25 cm) with 83/17 CO_2/MeOH as the mobile phase at flow rate of 65 mL/min. The individual enantiomers were then dissolved in DCM and treated with 4 N HCl in dioxane (3 equiv). The solvent was removed in vacuo, and the resulting solids were triturated with ethylether and dried under vacuum. (+)-**17b**: retention time, 11.50 min. MS (ES^+): m/z 373.1 (M + 1). UHPLC: 100% (UV), 100% (ELSD). Optical rotation: $[\alpha]_D^{20}$ + 27.1° (c 0.37, MeOH). (−)-**17b**: retention time, 13.46 min. MS (ES^+): m/z 373.1 (M + 1). UHPLC: 100% (UV), 100% (ELSD). Optical rotation: $[\alpha]_D^{20}$ − 26.7 (c 0.28, MeOH).

*1-Methyl-2-((cis-3-methyl-4-(4-trifluoromethyl-2-methoxy)-phenyl)piperidin-1-yl)methyl)-1H-imidazo[4,5-b]pyridine (**17c**).* The free base of the title compound was prepared by the method described for the synthesis of **17a** from 4-trifluormethyl-2-methoxyphenyl boronic acid. ^{1}H NMR (CD_3OD, 400 MHz): δ 8.90 (dd, 1H, J = 8.3, 1.2 Hz), 8.74 (dd, 1H, J = 5.8, 0.8 Hz), 7.92 (m, 1H), 7.30 (m, 2H), 7.23 (s, 1H), 5.09 (s, 2H), 4.12 (s, 3H), 4.05 (m, 2H), 3.92 (s, 3H), 3.68 (m, 3H), 2.68 (m, 2H), 2.00 (m, 1H), 0.98 (d, 3H, J = 7.5 Hz). MS (ES^+): m/z 419.0 (M + 1). Two enantiomers were separated by chiral HPLC from 1.08 g of the racemic **17c** using a Chiralpak AD column (10 cm × 50 cm) with 85/15 heptane/EtOH as mobile phase at flow rate of 500 mL/min. The individual enantiomers were then dissolved in DCM and treated with 4 N HCl in dioxane (3 equiv). The solvent was removed in vacuo, and the resulting solids were triturated with ethylether and dried under vacuum. (+)-**17c**: retention time, 9.86 min. MS (ES^+): m/z 373.1 (M + 1). UHPLC: 100% (UV), 100% (ELSD). Optical rotation: $[\alpha]_D^{20}$ + 58.0° (c 0.47, MeOH). (−)-**17c**: retention time, 10.18 min. MS (ES^+): m/z 373.1 (M + 1). UHPLC: 100% (UV), 100% (ELSD). Optical rotation: $[\alpha]_D^{20}$ − 57.2° (c 0.56, MeOH).

*1-Methyl-2-((cis-3-methyl-4-(4-trifluoromethyl-2-fluoro)phenyl)-piperidin-1-yl)methyl)-1H-imidazo[4,5-b]pyridine (**17d**).* The free base of the title compound was prepared by the method described for the synthesis of **17a** from 4-trifluormethyl-2-fluorophenyl boronic acid. ^{1}H NMR (CD_3OD, 400 MHz): δ 8.88 (dd, 1H, J = 7.1, 0.8 Hz), 8.72 (d, 1H, J = 5.9 Hz), 7.91 (m, 1H), 7.53 (m, 2H), 7.48 (m, 1H), 5.08 (s, 2H), 4.10 (s, 3H), 4.05 (m, 2H), 3.64 (m, 3H), 2.68 (m, 1H), 2.60 (m, 1H), 2.05 (m, 1H), 1.04 (d, 3H, J = 7.4 Hz). MS (ES^+): m/z 407.1 (M + 1). Two enantiomers were separated by chiral HPLC from 2.94 g of the racemic **17d** using a Chiralpak AD column (10 cm × 50 cm) with 80/20 heptane/EtOH as mobile phase at flow rate of 500 mL/min. The individual enantiomers were then dissolved in DCM and treated with 4 N HCl in dioxane (3 equiv). The solvent was removed in vacuo, and the resulting solids were triturated with ethylether and dried under vacuum. (+)-**17d**: retention time, 8.12 min. MS (ES^+): m/z 407.1 (M + 1). UHPLC: 100% (UV), 100% (ELSD). Optical rotation: $[\alpha]_D^{20}$ + 18° (c 0.47, MeOH). (−)-**17d**: retention time, 10.18 min. MS (ES^+): m/z 407.1 (M + 1). UHPLC: 100% (UV), 100% (ELSD). Optical rotation: $[\alpha]_D^{20}$ − 18° (c 0.50, MeOH).

*Determination of the Absolute Stereochemistry of Active Enantiomer (+)-**17d**.* The racemic *cis*-4-(2-fluoro-4-(trifluoromethyl)phenyl)-3-methylpiperidine hydrochloride (**18d**) was prepared by the method described in the synthesis of **18a** from 4-trifluoromethyl-2-fluorophenyl boronic acid. ^{1}H NMR (CD_3OD, 400 MHz): δ 7.41−7.50 (m, 3H), 3.55 (m, 2H), 3.36 (m, 2H), 3.16 (m, 1H), 2.44 (m, 2H), 1.85 (m, 1H), 0.85 (d, 3H, J = 7.4 Hz). MS (ES^+): m/z 262.1 (M + 1). Two enantiomers were separated by chiral HPLC using a Chiralpak AD-H column (21 cm × 250 cm) with 90/10 CO_2/PrOH as the mobile phase at a flow rate of 65 mL/min. (+)-**18d**: retention time, 3.11 min. Pptical rotation: $[\alpha]_D^{20}$ + 78.1° (c 0.32, MeOH). (−)-**18d**: retention time, 3.92 min. Optical rotation: $[\alpha]_D^{20}$ − 77.5° (c 0.45, MeOH).

Following the reductive amination method A, (+)-**18d** yielded the active enantiomer (+)-**17d**. The absolute stereochemistry of (+)-**18d** was determined by X-ray crystallography of its (−)-ditoluoyl-L-tartaric acid salt, generated via the procedure shown below.

To a stirred solution of (+)-**18d** (220 mg, 0.842 mmol) in DCM/MeOH (9/1) at room temperature was added (−)-ditoluoyl-L-tartaric acid (325 mg, 0.842 mmol). The mixture was stirred for 30 min and concentrated in vacuo to give the desired (−)-ditoluoyl-L-tartaric acid salt of (+)-**18d**, which was recrystallized in MeOH to yield crystals suitable for X-ray crystallography.

Data collection was performed on a Bruker APEX, diffractometer at room temperature. Data collection consisted of three ω scans at low angle and three at high angle, each with a 0.5 step. In addition, 2 φ scans were collected to improve the quality of the absorption correction. The structure was solved by direct methods using SHELX software suite in the space group $P2(1)2(1)2$. The structure was subsequently refined by the full-matrix least-squares method, and the ORTEP representation is shown in Scheme 3. All nonhydrogen atoms were found and refined using anisotropic displacement parameters. The hydrogen atoms located on nitrogen and oxygen were found from the Fourier difference map and refined freely. The remaining hydrogen atoms were placed in calculated positions and were allowed to ride on their carrier atoms. The final refinement included isotropic displacement parameters for all hydrogen atoms. The CF_3 group was disordered and modeled freely as a 55:45 ratio between both positions. Most likely, this group is rotating in the structure. The absolute stereochemistry of (+)-**18d** was determined to be *cis*-(*R*,*R*)-4-(2-fluoro-4-(trifluoromethyl)phenyl)-3-methylpiperidine, from the known chiral centers on the acid. The crystal structure has been deposited in Cambridge Crystallography Data Centre (CCDC) and allocated with the deposition number CCDC 782631. On the basis of this result, the absolute stereochemistry of (+)-**17d** was determined to be 1-methyl-2-((*cis*-(*R*,*R*)-3-methyl-4-(4-trifluoromethyl-2-fluoro)phenyl)piperidin-1-yl)methyl)-1*H*-imidazo[4,5-*b*]pyridine.

*1-Methyl-2-((cis-3-methyl-4-(4-trifluoromethyl-2-fluoro)phenyl)piperidin-1-yl)methyl)-1H-imidazo[4,5-b]pyridine (**17e**).* The title compound was prepared by the method described for the synthesis of **17a** from 4-trifluormethoxy-2-fluorophenyl boronic acid. ^{1}H NMR (CD_3OD, 400 MHz): δ 8.90 (d, 1H, J = 7.8 Hz), 8.72 (d, 1H, J = 5.4 Hz), 7.90 (m, 1H), 7.40 (m, 1H), 7.13 (m, 2H), 5.10 (s, 2H), 4.13 (s, 3H), 4.00 (m, 2H), 3.66 (m, 3H), 2.68 (m, 1H), 2.55 (m, 1H), 2.01 (m, 1H), 1.03 (d, 3H, J = 6.6 Hz). MS (ES^+): m/z 423.4 (M + 1). Two enantiomers were separated by chiral HPLC from 2.94 g of the racemic **17e** using a Chiralpak AD column (10 cm × 50 cm) with 85/15 heptane/EtOH as the mobile phase at a flow rate of 500 mL/min. The individual enantiomers were then dissolved in DCM and treated with

4 N HCl in dioxane (3 equiv). The solvent was removed in vacuo, and the resulting solids were triturated with ethylether and dried under vacuum. (+)-**17e**: retention time, 9.19 min. MS (ES^+): m/z 423.4 (M + 1). UHPLC: 100% (UV), 100% (ELSD). Optical rotation: $[\alpha]_D^{20}$ + 19.4° (c 0.60, MeOH). (−)-**17e**: retention time, 11.38 min. MS (ES^+): m/z 423.4 (M + 1). UHPLC: 100% (UV), 100% (ELSD). Optical rotation: $[\alpha]_D^{20}$ − 19° (c 0.45, MeOH).

■ ASSOCIATED CONTENT

Supporting Information. X-ray report of (+)-**18d**. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ACKNOWLEDGMENT

We thank Joel Schachter and Diane Lang for the cell generation, Prof. Shigetada Nakanishi of Kyoto University for the cDNA supply, Rebecca O'Connor and Anne Schmidt for the D_2 selectivity data, Brian Samas and Ivan Samardjiev for X-ray analysis, and Pfizer ADME technology group for in vitro PK data. We also thank Dr. Brian O'Neill, Dr. Jotham Coe, and Dr. Christopher J. O'Donnell for their helpful suggestions and comments.

■ ABBREVIATIONS USED

ADME, absorption, distribution, metabolism, and excretion; CNS, central nervous system; DMEM, Dulbecco's modified Eagle's medium; DOI, 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane hydrochloride; ELSD, evaporative light scattering detection; FBS, fetal bovine serum; FLIPR, fluorescence imaging plate reader; HEK, human embryonic kidney; HTS, high-throughput screening; MAP, methamphetamine; MDCK, Madin−Darby canine kidney; MDR, multiple-drug resistant; MED, minimally effective dose; mGluR, metatropic glutamate receptor; MIS, mescaline-induced scratching; MS, mass spectrometry; MWt, molecular weight; PAM, positive allosteric modulators; PK, pharmacokinetics; P-gp, P-glycoprotein; po, per os; SAR, structure−activity relationship; sc, subcutaneous; UHPLC, ultrahigh-performance liquid chromatography

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