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Article

Improvement of the Metabolic Stability of GPR88 Agonist RTI-13951-33: Design, Synthesis, and Biological Evaluation

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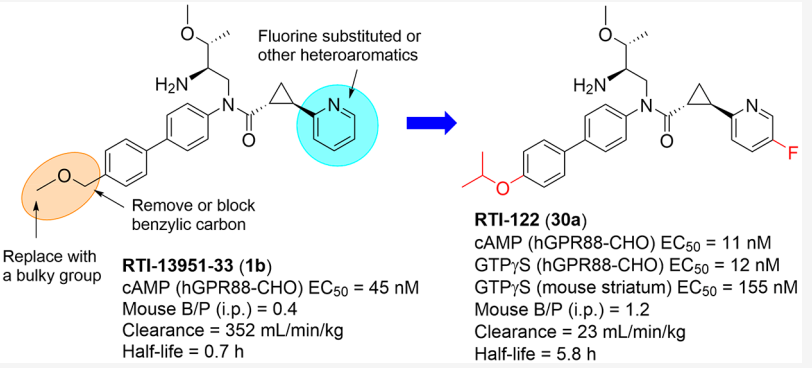

ABSTRACT: GPR88 is an orphan G protein-coupled receptor mainly expressed in the brain, whose endogenous ligand has not yet been identified. To elucidate GPR88 functions, our group has developed RTI-13951-33 (**1b**) as the first *in vivo* active GPR88 agonist, but its poor metabolic stability and moderate brain permeability remain to be further optimized. Here, we report the design, synthesis, and pharmacological characterization of a new series of RTI-13951-33 analogues with the aim of improving pharmacokinetic properties. As a result, we identified a highly potent GPR88 agonist RTI-122 (**30a**) (cAMP EC_{50} = 11 nM) with good metabolic stability (half-life of 5.8 h) and brain permeability (brain/plasma ratio of >1) in mice. Notably, RTI-122 was more effective than RTI-13951-33 in attenuating the binge-like alcohol drinking behavior in the drinking-in-the-dark paradigm. Collectively, our findings suggest that RTI-122 is a promising lead compound for drug discovery research of GPR88 agonists.

INTRODUCTION

Alcohol use disorder (AUD) is a chronic relapsing brain disorder, characterized by an impaired ability to stop or control alcohol use despite adverse consequences.[1] In the United States, alcohol is the third leading preventable cause of death, and the economic burden to society for AUD exceeds $240 billion annually.[2,3] Despite the huge impact of AUD on society, current pharmacotherapies for alcoholism, such as naltrexone and acamprosate, are far from adequate due to medication compliance issues and low efficacy.[4,5] Therefore, there are unmet needs for additional therapeutic agents based on novel targets for the treatment of AUD.

Emerging evidence suggests that G protein-coupled receptor 88 (GPR88) is involved in some of the abuse-related effects of alcohol addiction. GPR88 is an orphan $G\alpha_i$-coupled receptor with predominant expression in both striatonigral and striatopallidal pathways of the striatum.[6−8] Studies have revealed that GPR88 knockout (KO) mice displayed enhanced voluntary alcohol drinking and motivation compared with wild-type (WT) mice.[9] Additionally, magnetic resonance imaging of the same GPR88 KO mice showed significant weakening of mesocorticolimbic network connectional patterns.[9] Although the endogenous ligand of GPR88 remains unknown, we have previously reported that this orphan receptor can be activated by a synthetic agonist (1*R*,2*R*)-2-PCCA [**1a** (Figure 1)].[10] Unfortunately, 2-PCCA has poor brain permeability and nonspecific GTPγS binding activity in the mouse striatum, which limit its usefulness as an *in vivo* tool compound.[11] Later, we have carried out structure−activity relationship (SAR) studies around the 2-PCCA scaffold, which led to the identification of the first potent, selective, and brain-penetrant GPR88 agonist RTI-13951-33 (**1b**).[11,12] RTI-13951-33 significantly reduced alcohol self-administration in a dose-dependent manner in rats when administered via an intraperitoneal (i.p.) injection and at doses that did not affect

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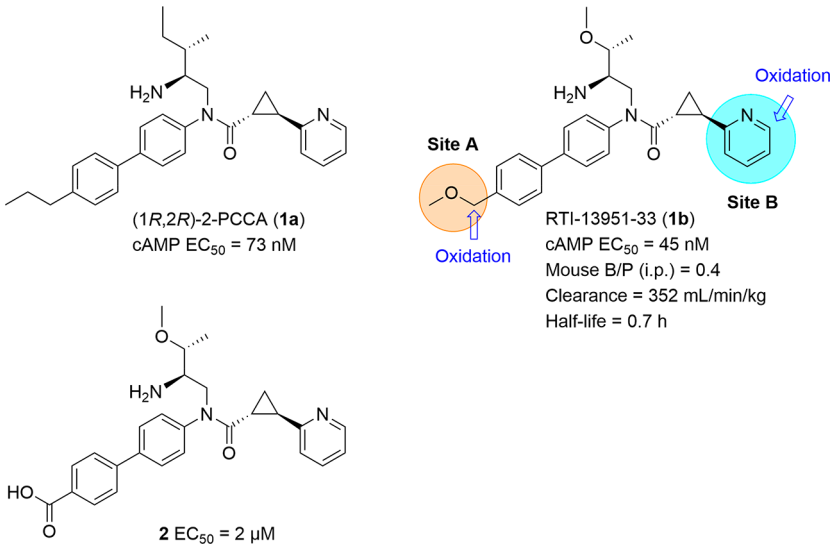
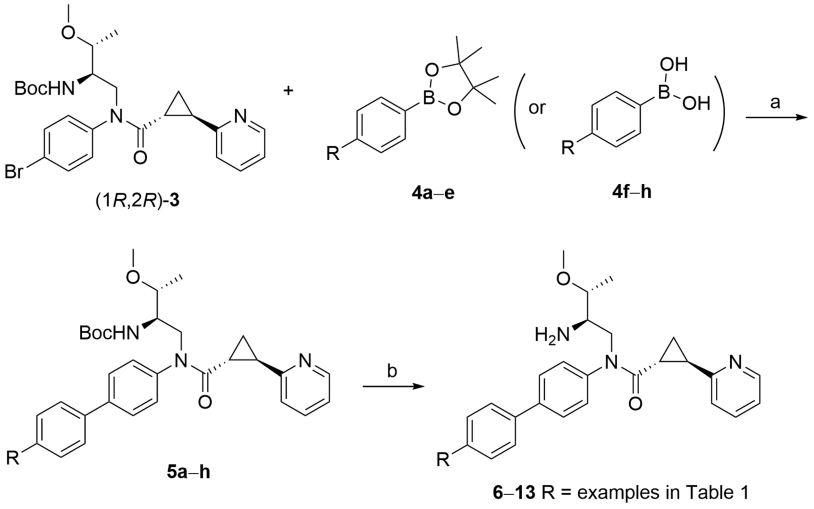


Figure 1. Structures of (1*R*,2*R*)-2-PCCA (**1a**), RTI-13951-33 (**1b**), and the major metabolite identified (**2**) and proposed metabolically labile sites for modifications.

Scheme 1. Synthesis of Target Compounds 6−13[a]



[a](a) Pd(dppf)Cl_2·DCM, K_3PO_4, DME/H_2O (3:1), microwave, 160 °C, 6 min; (b) 4 M HCl/dioxane, DCM, rt, 6 h.

locomotor activity or sucrose self-administration.[11] In mouse alcohol behavioral studies, RTI-13951-33 reduced both voluntary alcohol drinking and binge-like drinking in WT mice but not in GPR88 KO mice, confirming the GPR88-mediated alcohol drinking effects.[13] Moreover, RTI-13951-33 reduced alcohol-induced conditioned place preference, suggesting a reduction of alcohol reward.[13] Altogether, these findings support the development and pharmacological validation of GPR88 agonists as a potential therapeutic for the treatment of AUD.

Although RTI-13951-33 is a valuable tool for probing GPR88 activation *in vivo*, the compound's pharmacokinetic (PK) properties, such as brain permeability and metabolic stability, need to be further optimized for drug development of GPR88 agonists. In mouse PK studies, RTI-13951-33 (10 mg/kg, i.p.) demonstrated poor metabolic stability with a half-life of 0.7 h and a clearance (CL) of 352 mL min^{-1} kg^{-1} in plasma. In addition, the compound had moderate blood−brain barrier (BBB) permeability with a brain/plasma ratio of 0.4 at 30 min after injection in mice. To identify possible metabolic pathways, studies to determine major metabolites were performed. These studies revealed that a majority of metabolites were derived from oxidation of the benzylic methoxymethyl group at site A of RTI-13951-33, which ultimately led to the identification of a carboxylic acid metabolite **2** (Figure 1). Acid **2** is a weak agonist at GPR88 with an EC_{50} of 2 μM in the cAMP assay. This oxidative metabolism may be a contributing factor to the high clearance and moderate brain bioavailability seen in mice. In addition, the pyridine ring at site B is also known to be vulnerable to the oxidative metabolism.[14] To improve the PK properties of RTI-13951-33, we designed and synthesized a series of analogues by replacing or blocking metabolically labile functionalities at sites A and B. Compounds were characterized in cAMP accumulation and [^{35}S]GTPγS binding assays for their potency as GPR88 agonists and assessed for metabolic stability in mouse liver microsomes. Selected compounds were then screened through *in vitro* absorption, distribution, metabolism, and excretion (ADME) profiling before a PK study was performed to determine the brain bioavailability. Finally, the *in*

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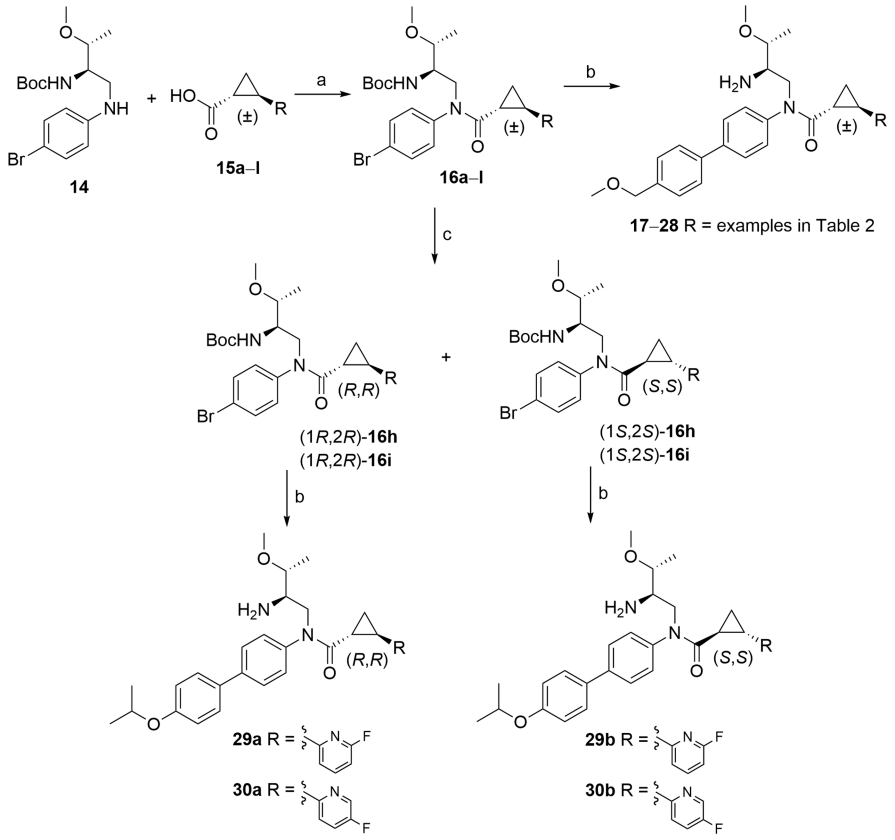
Scheme 2. Synthesis of Target Compounds 17−30[a]



[a](a) Acid **15**/oxalyl chloride/DCM/40 °C/2 h, concentrated, then **14**/Et_3N/DCM, rt, overnight; (b) (i) 4-(methoxymethyl)phenylboronic acid or 4-isopropoxyphenylboronic acid, Pd(dppf)Cl_2·DCM, K_3PO_4, DME/H_2O (3:1), microwave, 160 °C, 6 min; (ii) 4 M HCl/dioxane, DCM, rt, 6 h; (c) chiral HPLC separation.

vivo efficacy of the lead compound, **30a** (designated as RTI-122), was examined in a mouse model of binge-like alcohol intake.

RESULTS AND DISCUSSION

Chemistry. The newly designed GPR88 agonists were synthesized following methods detailed in our earlier publications.[10−12] All final products were characterized by NMR and MS and determined to be >95% pure by HPLC analyses. As shown in Scheme 1, Suzuki coupling of enantiomerically pure (1*R*,2*R*)-**3**[11] with arylboronic pinacol esters **4a−e**, prepared from aryl bromide, or with commercially available arylboronic acid **4f−h** under microwave conditions afforded intermediates **5a−h**, respectively. Removal of the Boc protecting group with 4 M HCl in dioxane provided **6−13**.

Synthesis of diastereomeric mixture **17−28** is depicted in Scheme 2. Reaction of aniline **14** with the acid chloride of racemic (±)-*trans*-2-arylcyclopropanecarboxylic acid **15**, prepared by cyclopropanation of aryl olefin with *tert*-butyldiazoacetate[10] or by cyclopropanation of *tert*-butyl (2*E*)-3-arylprop-2-enoate with trimethylsulfoxonium iodide,[15] gave amides **16a−l**. Suzuki coupling of **16a−l** with 4-(methoxymethyl)-phenylboronic acid, followed by Boc deprotection, provided **17−28**, respectively. Compounds **17−28** are 1:1 mixture of (1*R*,2*R*)- and (1*S*,2*S*)-diastereomers, differentiating at the configuration of *trans*-cyclopropane, determined by ^{1}H NMR and HPLC analyses. For the preparation of enantiomerically pure (1*R*,2*R*)-isomers (**29a** and **30a**) and (1*S*,2*S*)-isomers (**29b** and **30b**), the diastereomeric mixture of **16h** (or **16i**) was separated by chiral HPLC to afford a pair of pure isomers, (1*R*,2*R*)-**16h** and (1*S*,2*S*)-**16h** [or (1*R*,2*R*)-**16i** and (1*S*,2*S*)-**16i**]. The (*R*,*R*)- and (*S*,*S*)-configurations of the *trans*-cyclopropane ring were assigned on the basis of comparison of the ^{1}H NMR spectrum and HPLC retention time to those of the structurally similar analogues with known absolute configurations (see the Supporting Information).[10,11] Suzuki coupling of the individual isomer of **16h** or **16i** with 4-isopropoxyphenylboronic acid, followed by Boc deprotection, furnished target compounds **29a**, **29b**, **30a**, and **30b**.

Pharmacological Evaluation at GPR88. The Lance TR-FRET cAMP assay was used as the primary screen to determine the agonist potency (EC_{50}) at GPR88 as described previously.[11,16,17] The metabolic stability was assessed in mouse liver microsomes (MLMs). Because the major metabolite of RTI-13951-33 is derived from oxidative metabolism of the methoxymethyl group at site A (Figure 1), we first explored whether blocking or replacing this metabolically labile site would lead to an improvement of the metabolic stability while maintaining a high potency at GPR88. The results are summarized in Table 1. RTI-13951-33 was used as the reference compound in our SAR studies and had a cumulative average EC_{50} of 45 nM (n > 100).[17] In MLMs, the compound was rapidly metabolized with a short half-life of 2.2 min and a high CL of 643 μL min^{-1} (mg of protein)$^{-1}$, which is in agreement with the poor metabolic stability observed in mouse PK studies. Blocking the benzylic carbon with a methyl

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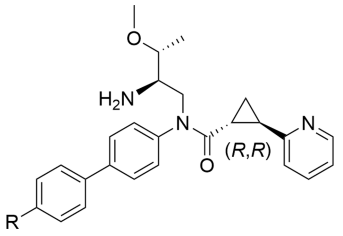
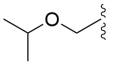
Table 1. Biological Data of RTI-13951-33 Analogues with Site A Modifications



Compound[a]	R	cAMP EC_{50} (nM)[b]	Mouse liver microsomes	
			$t_{1/2}$ (min)	CL (μL/min/mg)
RTI-13951-33		45.0 ± 1.8	2.2	643
6		27.8 ± 5.1	3.1	453
7		29.1 ± 4.0	ND	ND
8		67.0 ± 12.4	4.3	330
9		44.4 ± 1.4	2.5	564
10		36.1 ± 1.8	ND	ND
11		26.3 ± 1.2	2.5	552
12		24.7 ± 3.0	4.0	345
13		45.1 ± 8.7	2.8	494

[a]All compounds were tested as the HCl salt. [b]EC_{50} values are means ± SEM of at least three independent experiments performed in duplicate. ND = not determined.

group led to (*S*)-isomer **6** and (*R*)-isomer **7** with slight increases in both agonist activity (EC_{50} ∼ 30 nM) and liver microsomal stability (CL = 453 μL min^{-1} mg^{-1}) compared to those of RTI-13951-33. Replacing the methoxy group with a larger isopropoxy group in **8** further improved microsomal stability (half-life = 4.3 min; CL = 330 μL min^{-1} mg^{-1}); however, it suffered from a 2-fold loss of potency (67 nM vs 28 nM **6**). Substituting the methoxymethyl group in RTI-13951-33 with a tetrahydrofuran functionality resulted in equally potent compounds **9** and **10** (EC_{50} ∼ 40 nM) without an improvement of the microsomal stability. In a further optimization effort, removal of the benzylic carbon by employing an ethoxy group at site A afforded **11** with an EC_{50} of 26 nM, but with no improvement in microsomal stability. An improvement in both potency and metabolic stability was achieved by increasing the size of the alkoxy group to give isopropoxy analogue **12**, which had an EC_{50} of 25 nM and a CL of 345 μL min^{-1} mg^{-1} in MLMs. On the contrary, the cyclopropoxy group in **13** offered no advantage over the ethoxy group in terms of potency or microsomal stability. Overall, our findings suggest that phenyl position 4 at site A tolerates small- and medium-sized alkoxy groups without losing GPR88 activity, allowing for optimization of PK properties with appropriate structural modifications.

Next, we turned our attention to modifications of the pyridine ring at site B, as pyridine can be readily oxidized in the first-pass metabolism. In addition to N oxidation, the *ortho* position to the nitrogen is also susceptible to oxidation by aldehyde oxidase (AO) to give the corresponding 2-pyridone metabolite.[14] Successful lead optimization strategies to avoid or decrease the potential oxidative metabolism of pyridine typically include the removal of nitrogen, reduction of the ring size, blockage of the oxidation site, introduction of steric hindrance near the affected carbon, and modulation of electronic properties. However, modifications of the pyridine ring may also alter key pharmacophores and physicochemical properties leading to decreases in potency and/or drug-like properties. Indeed, SAR studies from our laboratory, as well as others, suggest that the aromatic ring at site B of RTI-13951-33 has limited tolerance for modifications.[10,11,18] Replacement of pyridine in (1*R*,2*R*)-2-PCCA (Figure 1), an analogue of RTI-13951-33, with a phenyl group led to a significant loss of agonist activity at GPR88.[10,18] To explore potential approaches to mitigating pyridine metabolism without sacrificing GPR88 activity, we carried out an SAR study at site B of RTI-13951-33 using a 1:1 diastereomeric mixture of (1*R*,2*R*)- and (1*S*,2*S*)-cyclopropyl isomers that can be readily synthesized for quick access to the structural diversity. Diastereomeric mixture **17** of

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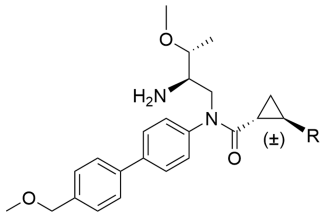
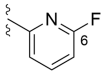
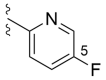
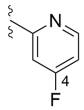
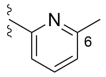
Table 2. Biological Data of RTI-13951-33 Analogues with Site B Modifications



Compound[a]	R	cAMP EC_{50} (nM)[b]	Compound[a]	R	cAMP EC_{50} (nM)[b]
17		115 ± 21	**23**		183 ± 10
18		4431 ± 942	**24**		39.0 ± 6.0
19		6490 ± 868	**25**		52.3 ± 6.4
20		536 ± 114	**26**		155 ± 14
21		732 ± 52	**27**		187 ± 9
22		226 ± 24	**28**		2350 ± 616

[a]All compounds were tested as the HCl salt. [b]EC_{50} values are means ± SEM of at least three independent experiments performed in duplicate.

RTI-13951-33 was also synthesized and tested for SAR comparison. As Table 2 shows, replacement of the 2-pyridyl group in **17** with a 3-pyridyl (**18**) or 4-pyridyl group (**19**) led to a >30-fold loss of activity. Pyrimidine **20** and pyrazine **21** are also not favored in this region with EC_{50} values of 536 and 732 nM, respectively. However, a smaller thienyl group seems to be tolerated as 2-thienyl analogue **22** (EC_{50} = 226 nM) and 3-thienyl analogue **23** (EC_{50} = 183 nM) are only 2-fold less potent than **17** (EC_{50} = 115 nM). Recently, the cryogenic electron microscopy (cryo-EM) structure of GPR88/(1*R*,2*R*)-2-PCCA revealed that the agonist occupies an allosteric binding pocket of GPR88.[19] The 2-pyridyl nitrogen forms a key hydrogen bond interaction with the backbone NH group of glycine 283 (G283) in the allosteric pocket, contributing to a significant amount of the agonist activity of 2-PCCA. Mutation of G283 into a bulky valine residue completely abolished the GPR88 activity. Given that the 2-pyridyl group is critical for activity, we performed a "fluorine walk" on the pyridine ring to determine which position is tolerant to further modifications. In general, the pyridine ring is tolerated with the fluorine substitution with the following preferred rank order: *o*-6-F **24** (EC_{50} = 39 nM) ≈ *m*-5-F **25** (EC_{50} = 52 nM) > *p*-4-F **26** (EC_{50} = 155 nM) ≈ *m*-3-F **27** (EC_{50} = 187 nM). Interestingly, *o*-6-methyl analogue **28** (EC_{50} = 2350 nM) is 60-fold less potent than 6-fluoro **24**, which is likely due to a steric clash between the methyl group and G283 in the binding pocket.

With the SAR information obtained above, we combined the isopropoxy group at site A with the 6-fluoro or the 5-fluoro group at site B in hopes of further honing the potency and metabolic stability. Compounds **29** and **30** were synthesized and tested as the pure (1*R*,2*R*)- or (1*S*,2*S*)-isomers. Their biological results, along with the data of RTI-13951-33 and (*S*,*S*)-RTI-13951-33 for comparison, are listed in Table 3. Consistent with the previous findings, the GPR88 agonist activity resides in the (1*R*,2*R*)-isomers (**29a** and **30a**), while the (1*S*,2*S*)-isomers (**29b** and **30b**) are significantly less potent.[10,11,18,20] These findings further confirm the assignment of the (*R*,*R*)-configuration to the cyclopropane ring in **29a** and **30a**. Compound **30a** had an EC_{50} of 11 nM and is 4-fold more potent than RTI-13951-33, being the most potent compound in this study. The GPR88 agonist activity was further confirmed in [^{35}S]GTPγS binding assays using membranes prepared from stable PPLS-HA-hGPR88-CHO cells.[12] RTI-13951-33 and **30a** had EC_{50} values of 65 and 12 nM, respectively, which are in agreement with their EC_{50} values obtained from cAMP accumulation assays.

We further characterized **30a** in the [^{35}S]GTPγS binding assay using mouse striatal membranes that endogenously express GPR88, with the goal of validating the activity of GPR88 agonists at both human and mouse receptors. Compound **30a** significantly enhanced [^{35}S]GTPγS binding activity with an EC_{50} of 155 ± 6 nM (E_{max} = 386% relative to the basal level) (Figure 2), which is 13-fold less potent than its EC_{50} of 12 nM obtained from the overexpressed human GPR88-CHO cell line. The discrepancy between the EC_{50} values is likely due to the sensitivity of the two different assay systems (native vs overexpressed system) or species differences (mouse vs human receptor). Compound **30a** at a concentration of 30 μM was inactive in striatal membranes prepared

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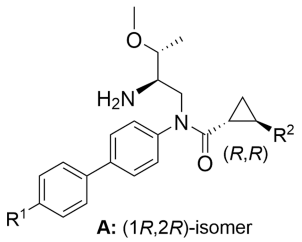
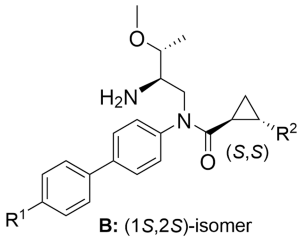
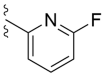
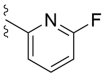
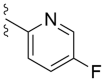
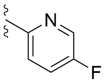
Table 3. Biological Data of RTI-13951-33 Analogues with Modifications at Sites A and B





Compound[a]	Structure	R^1	R^2	cAMP EC_{50} (nM)[b]	[^{35}S]GTPγS EC_{50} (nM)[b]
RTI-13951-33	A			45.0 ± 1.8	64.7 ± 1.2
(*S*,*S*)-RTI-13951-33	B			>10,000	>10,000
29a	A			54.8 ± 9.3	ND
29b	B			>10,000	ND
30a	A			10.8 ± 1.2	11.5 ± 2.3
30b	B			>10,000	9841 ± 1854

[a]All compounds were tested as the HCl salt. [b]EC_{50} values are means ± SEM of at least three independent experiments performed in duplicate. ND = not determined.

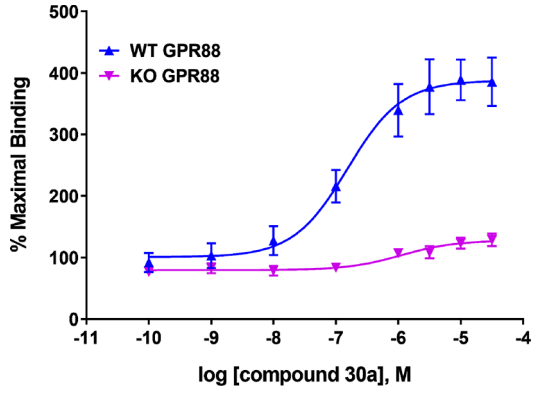


Figure 2. [^{35}S]GTPγS binding of **30a** in WT mouse striatal membranes vs GPR88 KO mouse striatal membranes. Compound **30a** significantly increases [^{35}S]GTPγS binding activity compared to the basal binding activity, which refers to binding in the absence of the agonist. Each data point is the mean ± SEM of at least three independent experiments performed in duplicate.

from GPR88 KO mice, demonstrating good selectivity for GPR88 G protein signaling activity in the mouse striatum.

***In Vitro* ADME and PK Profile of 30a.** Compound **30a** was assessed in a panel of ADME assays, including MDCK-MDR1 permeability, MLM stability, kinetic solubility, and plasma−protein binding (PPB) assays. As shown in Table 4, **30a** demonstrated good BBB permeability in the MDCK-MDR1 bidirectional transport assay with an apparent permeability P_{app} (A → B) of 2.8 × 10^{-6} cm/s and an efflux ratio ($P_{B\to A}/P_{A\to B}$) of 2.5.[21,22] Notably, **30a** had a half-life of 24.6 min and a CL of 56.4 μL min^{-1} mg^{-1} in MLMs, which are 10 times better than those of RTI-13951-33 (half-life = 2.2 min, and CL = 643 μL min^{-1} mg^{-1}). Compound **30a** had good aqueous solubility (kinetic solubility = 261 μM) but a high level of protein binding of 99.5% in mouse plasma, which needs to be further optimized.

Table 4. ADME Properties of 30a

	desired value	RTI-13951-33[a]	**30a**
MDCK-MDR1			
P_{app} (×10^{-6} cm/s), A → B	≥3		2.8
P_{app} (×10^{-6} cm/s), B → A			7.1
efflux ratio	<3		2.5
MLM			
$t_{1/2}$ (min)		2.2	24.6
CL (μL min^{-1} mg^{-1})	<60	643	56.4
kinetic solubility (μM)	>30	412	261
mouse PPB (%)	<95	93.9	99.5

[a]RTI-13951-33 had 13% permeability expressed in percent transported from the apical (A) to basolateral (B) side in the MDCK-MDR1 assay.[11]

The PK properties of **30a** were evaluated in mice using a single i.p. injection of 10 mg/kg, and the results are summarized in Table 5. Following injection, **30a** had good plasma exposure with a C_{max} of 662 ng/mL and an AUC_{0-inf} of 7292 ng·h/mL. In line with the MLM data, **30a** had good

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Table 5. PK Properties of 30a in Mice (10 mg/kg, i.p.)

	C_{max} (ng/mL)	t_{max} (h)	CL (mL min^{-1} kg^{-1})	$t_{1/2}$ (h)	AUC_{0-inf} (ng·h/mL)
plasma	662	2.0	23	5.8	7292
brain	630	4.0	20	5.5	8530
brain:plasma ratio					1.2

metabolic stability with a half-life of 5.8 h and a CL of 23 mL min^{-1} kg^{-1} in plasma. The brain concentration peaked at 4 h with a C_{max} of 630 ng/mL (1.3 μM). The overall brain:plasma AUC ratio (K_p) is 1.2, indicating good brain penetration. On the basis of the *in vitro* ADME and PK findings, compound **30a** was selected for *in vivo* efficacy evaluation.

Compound 30a Reduced Alcohol Drinking. Both genetic and pharmacological studies have suggested that GPR88 is a promising drug target for the treatment of alcohol addiction.[9,11,13] We previously demonstrated that mice pretreated with RTI-13591-33 (30 mg/kg, i.p.), the first brain-penetrant GPR88 agonist, were less likely to drink and seek alcohol.[13] Thus, we examined whether **30a** would achieve the same effects *in vivo*. As shown in Figure 3, RTI-13951-33 at a dose of 30 mg/kg significantly reduced alcohol intake [via one-way ANOVA, $F(2, 42) = 4.30$ and $p = 0.02$] in the drinking-in-the-dark (DID) procedure. Notably, pretreatment of **30a** at a dose of 10 mg/kg produced the same degree of attenuation of binge-like alcohol consumption as that of RTI-13951-33 at a dose of 30 mg/kg (in the Newman–Keuls post hoc test, $p < 0.05$ compared to saline-treated mice). No effect on water intake was detected (data not shown). The better *in vivo* potency of **30a** than of RTI-13951-33 is likely due to the improved potency and PK properties (metabolic stability and brain penetration) of **30a** compared to those of RTI-13951-33.

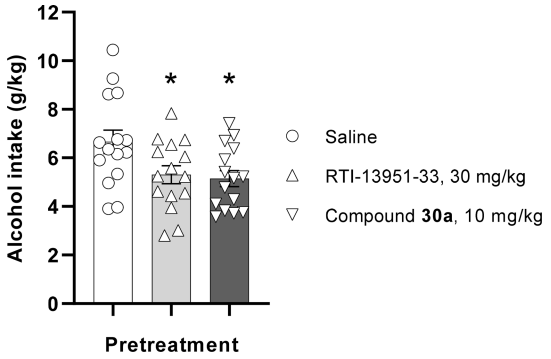

Figure 3. RTI-13951-33 and **30a** attenuated alcohol drinking in the drinking-in-the-dark (DID) procedure. C57BL/6J mice were subjected to a DID procedure for 2 weeks. On the test day, saline, RTI-13951-33 (30 mg/kg, i.p.), or **30a** (10 mg/kg, i.p.) was administered 1 h before the beginning of the 4 h session test (n = 15 per group). Data are presented as means ± SEM. *$p < 0.05$.

CONCLUSIONS

The orphan receptor GPR88 has recently attracted considerable attention as a novel drug target for the treatment of neuropsychiatric disorders, including Parkinson's disease, schizophrenia, and drug addiction. To date, the endogenous ligand for GPR88 has not been discovered. To characterize GPR88 signaling mechanisms and biological functions, our laboratory, as well as others, has carried out a medicinal chemistry campaign to develop GPR88 synthetic agonists.[10−12,16−18,23−25] RTI-13951-33 is the first-generation *in vivo* active agonist that has been shown to reduce alcohol drinking and seeking behaviors in mice and rats.[11,13] In the study presented here, we have conducted a lead optimization to further improve the potency and PK profile of RTI-13951-33. Our medicinal chemistry efforts led to the identification of RTI-122 (**30a**) that has EC_{50} values of 11 and 12 nM in the cell-based cAMP accumulation and [^{35}S]GTPγS binding assays, respectively, and has GPR88-specific agonist activity in mouse striatal tissues. In addition, RTI-122 has a favorable PK profile exhibiting good metabolic stability (half-life = 5.8 h) and brain permeability (brain:plasma ratio = 1.2) for *in vivo* assessment. Finally, RTI-122 at a dose of 10 mg/kg significantly attenuates binge-like alcohol drinking in mice when administrated intraperitoneally. Taken together, RTI-122 is an advanced lead compound for further development of GPR88 agonists for treating AUD and possibly other disease conditions mediated by GPR88. Future studies of RTI-122 include assessments of its oral bioavailability, off-target activity (e.g., hERG), toxicity, and behavioral effects in different alcohol drinking and seeking models.

EXPERIMENTAL SECTION

Chemistry. *General Methods.* All solvents and chemicals were reagent grade. Unless otherwise mentioned, all reagents and solvents were purchased from commercial vendors and used as received. Flash column chromatography was carried out on a Teledyne ISCO CombiFlash Rf system using prepacked columns. Solvents used include EtOAc, hexanes, MeOH, and DCM. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance DPX-300 (300 MHz) or a Bruker AVANCE III HD 700 MHz spectrometer and were determined in $CDCl_3$, DMSO-d_6, or CD_3OD with tetramethylsilane (TMS) (0.00 ppm) or solvent peaks as the internal reference. Chemical shifts are reported in parts per million relative to the reference signal, and coupling constant (J) values are reported in hertz. Nominal mass spectra were recorded using an Agilent InfinityLab MSD single-quadrupole mass spectrometer system (ESI). Thin layer chromatography (TLC) was performed on EMD precoated silica gel 60 F254 plates, and spots were visualized with ultraviolet light or iodine staining. All final compounds were >95% pure as determined by HPLC on a Waters 2695 Separation Module equipped with a Waters 2996 photodiode array detector and a Phenomenex Synergi 4 mm Hydro-RP 80A C18 250 mm × 4.6 mm column using a flow rate of 1 mL/min starting with 1 min at 5% solvent B, followed by a 15 min gradient from 5% to 95% solvent B, followed by 9 min at 95% solvent B (solvent A, H_2O with 0.1% TFA; solvent B, acetonitrile with 0.1% TFA and 5% H_2O; absorbance monitored at 280 nm). All of the synthesized target compounds were characterized by NMR, MS, and HPLC (>95% pure).

*tert-Butyl N-[(2R,3R)-3-Methoxy-1-(N-{4′-[(1S)-1-methoxyethyl]-[1,1′-biphenyl]-4-yl}-1-[(1R,2R)-2-(pyridin-2-yl)cyclopropyl]-formamido)butan-2-yl]carbamate (**5a**).* To a solution of 1-bromo-4-[(1*S*)-1-methoxyethyl]benzene (**4a**) (190 mg, 0.88 mmol) in 1,4-dioxane (10 mL) were added bis(pinacolato)diboron (336 mg, 1.33 mmol), the 1,1′-bis(diphenylphosphino)ferrocene palladium(II) dichloride DCM adduct (72 mg, 0.088 mmol), and potassium acetate (173 mg, 1.77 mmol). The reaction mixture was heated at 90 °C overnight, cooled, diluted with Et_2O, and filtered through a short pad of Celite, rinsing through with Et_2O. The solvent was removed under reduced pressure. The crude product was purified by chromatography on silica gel using 0–6% EtOAc in hexanes to give the corresponding pinacol boronic ester as a clear oil (155 mg, 67% yield). The boronic ester, bromide (1*R*,2*R*)-**3** (78 mg, 0.15 mmol), 1,1′-bis-(diphenylphosphino)ferrocene palladium(II) dichloride DCM adduct (12 mg, 0.015 mmol), and potassium phosphate (96 mg, 0.45 mmol) were combined in 1,2-dimethoxyethane (1.5 mL) and water (0.5 mL)

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and then heated by microwave irradiation to 160 °C for 6 min. The reaction mixture was cooled, poured into a 1 N NaOH solution (5 mL), and then extracted with DCM (3 × 10 mL). The combined extracts were dried (Na_2SO_4), and the solvents were removed under reduced pressure. The crude product was purified by chromatography on silica gel using 0−40% EtOAc in hexanes to give **5a** (68 mg, 79% yield) as a clear oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.27 (d, J = 4.5 Hz, 1H), 7.57−7.46 (m, 5H), 7.38 (d, J = 8.1 Hz, 2H), 7.31−7.24 (m, 2H), 7.21 (d, J = 7.7 Hz, 1H), 7.03−6.95 (m, 1H), 5.05 (d, J = 9.2 Hz, 1H), 4.39−4.26 (m, 2H), 3.93 (t, J = 10.1 Hz, 1H), 3.52 (dd, J = 3.9, 13.7 Hz, 1H), 3.40 (dd, J = 2.1, 6.2 Hz, 1H), 3.27 (s, 6H), 2.72−2.62 (m, 1H), 2.02−1.96 (m, 1H), 1.66−1.58 (m, 1H), 1.48 (d, J = 6.4 Hz, 3H), 1.48−1.41 (m, 1H), 1.43 (s, 9H), 1.13 (d, J = 6.4 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.8, 159.5, 156.2, 149.2, 143.0, 141.5, 140.2, 139.3, 135.8, 128.4, 128.1, 127.1, 126.7, 122.7, 120.9, 79.3, 78.9, 77.2, 56.6, 56.5, 53.3, 50.6, 28.4, 27.5, 24.9, 23.7, 17.7, 15.1; MS (ESI) m/z 574.2 $[M + H]^+$.

*tert-Butyl N-[(2R,3R)-3-Methoxy-1-(N-{4′-[(1R)-1-methoxyethyl]-[1,1′-biphenyl]-4-yl}-1-[(1R,2R)-2-(pyridin-2-yl)cyclopropyl]-formamido)butan-2-yl]carbamate (**5b**).* The procedure for the synthesis of **5a** was followed starting with 1-bromo-4-[(1*R*)-1-methoxyethyl]benzene (**4b**, 55 mg) to afford **5b** (66 mg, 45% yield) as a clear oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.27 (d, J = 4.1 Hz, 1H), 7.57−7.47 (m, 5H), 7.38 (d, J = 8.3 Hz, 2H), 7.32−7.25 (m, 2H), 7.21 (d, J = 7.9 Hz, 1H), 6.99 (dd, J = 5.2, 6.7 Hz, 1H), 5.05 (d, J = 9.2 Hz, 1H), 4.40−4.25 (m, 2H), 4.00−3.87 (m, 1H), 3.52 (dd, J = 4.1, 13.7 Hz, 1H), 3.40 (dq, J = 2.1, 6.2 Hz, 1H), 3.27 (s, 6H), 2.72−2.63 (m, 1H), 2.02−1.95 (m, 1H), 1.66−1.58 (m, 1H), 1.50−1.45 (m, 1H), 1.48 (d, J = 6.4 Hz, 3H), 1.43 (s, 9H), 1.13 (d, J = 6.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.8, 159.5, 156.2, 149.2, 143.0, 141.5, 140.2, 139.3, 135.8, 128.4, 128.1, 127.1, 126.7, 122.7, 120.9, 79.3, 78.9, 77.2, 56.6, 56.5, 53.3, 50.6, 28.4, 27.5, 24.9, 23.7, 17.7, 15.1; MS (ESI) m/z 574.2 $[M + H]^+$.

*tert-Butyl N-[(2R,3R)-3-Methoxy-1-(N-{4′-[(propan-2-yloxy)-methyl]-[1,1′-biphenyl]-4-yl}-1-[(1R,2R)-2-(pyridin-2-yl)-cyclopropyl]formamido)butan-2-yl]carbamate (**5c**).* The procedure for the synthesis of **5a** was followed starting with 1-bromo-4-[(propan-2-yloxy)methyl]benzene (**4c**, 60 mg) to afford **5c** (88 mg, 57% yield) as a clear oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.27 (d, J = 4.5 Hz, 1H), 7.55−7.46 (m, 5H), 7.44−7.38 (m, 2H), 7.31−7.24 (m, 2H), 7.21 (d, J = 7.7 Hz, 1H), 7.02−6.96 (m, 1H), 5.03 (d, J = 9.2 Hz, 1H), 4.55 (s, 2H), 4.31 (dd, J = 10.7, 13.6 Hz, 1H), 3.93 (t, J = 9.3 Hz, 1H), 3.79−3.65 (m, 1H), 3.52 (dd, J = 3.8, 13.6 Hz, 1H), 3.44−3.35 (m, 1H), 3.27 (s, 3H), 2.71−2.62 (m, 1H), 2.06−1.96 (m, 1H), 1.69−1.59 (m, 1H), 1.52−1.46 (m, 1H), 1.43 (s, 9H), 1.24 (d, J = 6.2 Hz, 6H), 1.13 (d, J = 6.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.8, 159.5, 156.2, 149.2, 141.5, 140.3, 139.2, 138.6, 135.8, 128.4, 128.1, 128.0, 127.0, 122.7, 120.9, 78.9, 77.2, 71.1, 69.7, 56.6, 53.3, 50.6, 28.4, 27.5, 24.9, 22.1, 17.7, 15.1; MS (ESI) m/z 588.2 $[M + H]^+$.

*tert-Butyl N-[(2R,3R)-3-Methoxy-1-(N-{4′-[(2S)-oxolan-2-yl]-[1,1′-biphenyl]-4-yl}-1-[(1R,2R)-2-(pyridin-2-yl)cyclopropyl]formamido)-butan-2-yl]carbamate (**5d**).* The procedure for the synthesis of **5a** was followed starting with (2*S*)-2-(4-bromophenyl)oxolane (**4d**, 94 mg) to afford **5d** (128 mg, 53% yield) as a white foam: ^{1}H NMR (700 MHz, $CDCl_3$) δ 8.26 (d, J = 4.1 Hz, 1H), 7.50 (d, J = 8.1 Hz, 5H), 7.40 (d, J = 8.1 Hz, 2H), 7.30−7.25 (m, 2H), 7.20 (d, J = 7.7 Hz, 1H), 7.04−6.85 (m, 1H), 5.03 (d, J = 9.3 Hz, 1H), 4.94 (t, J = 7.2 Hz, 1H), 4.31 (dd, J = 13.7, 10.7 Hz, 1H), 4.12 (dd, J = 14.7, 7.3 Hz, 1H), 4.01−3.89 (m, 2H), 3.52 (dd, J = 13.8, 4.0 Hz, 1H), 3.39 (qd, J = 6.2, 2.2 Hz, 1H), 3.25 (d, J = 16.1 Hz, 3H), 2.73−2.57 (m, 1H), 2.42−2.30 (m, 1H), 2.11−1.94 (m, 3H), 1.90−1.77 (m, 1H), 1.50−1.45 (m, 1H), 1.43 (s, 9H), 1.39 (d, J = 8.9 Hz, 1H), 1.13 (d, J = 6.3 Hz, 3H); ^{13}C NMR (175 MHz, $CDCl_3$) δ 176.9, 159.5, 149.2, 142.9, 141.4, 140.3, 131.0, 135.8, 128.4, 128.1, 127.0, 126.2, 122.7, 120.9, 80.4, 78.9, 76.5, 68.7, 56.6, 53.2, 50.6, 34.6, 28.4, 27.4, 26.1, 24.9, 17.7, 15.1; MS (ESI) m/z 586.2 $[M + H]^+$.

*tert-Butyl N-[(2R,3R)-3-Methoxy-1-(N-{4′-[(2R)-oxolan-2-yl]-[1,1′-biphenyl]-4-yl}-1-[(1R,2R)-2-(pyridin-2-yl)cyclopropyl]-formamido)butan-2-yl]carbamate (**5e**).* The procedure for the synthesis of **5a** was followed starting with (2*R*)-2-(4-bromophenyl)-oxolane (**4e**, 61 mg) to afford **5e** (90 mg, 57% yield) as a white foam: ^{1}H NMR (700 MHz, $CDCl_3$) δ 8.26 (d, J = 4.2 Hz, 1H), 7.50 (t, J = 5.8 Hz, 5H), 7.40 (d, J = 8.1 Hz, 2H), 7.27 (d, J = 10.7 Hz, 2H), 7.20 (d, J = 7.8 Hz, 1H), 6.99 (dd, J = 6.7, 5.3 Hz, 1H), 5.03 (d, J = 9.3 Hz, 1H), 4.94 (t, J = 7.2 Hz, 1H), 4.31 (dd, J = 13.7, 10.7 Hz, 1H), 4.12 (dd, J = 14.6, 7.4 Hz, 1H), 4.00−3.90 (m, 2H), 3.52 (dd, J = 13.8, 4.0 Hz, 1H), 3.39 (qd, J = 6.1, 2.1 Hz, 1H), 3.25 (d, J = 16.0 Hz, 3H), 2.73−2.56 (m, 1H), 2.43−2.28 (m, 1H), 2.12−1.94 (m, 3H), 1.90−1.79 (m, 1H), 1.50−1.45 (m, 1H), 1.43 (s, 9H), 1.38 (s, 1H), 1.13 (d, J = 6.3 Hz, 3H); ^{13}C NMR (175 MHz, $CDCl_3$) δ 172.9, 159.5, 156.2, 149.2, 142.9, 141.4, 140.2, 139.0, 135.8, 128.4, 128.1, 127.0, 126.1, 122.7, 120.9, 80.4, 78.9, 76.4, 68.7, 56.6, 53.2, 50.6, 34.6, 28.4, 27.4, 26.1, 24.9, 17.7, 15.1; MS (ESI) m/z 586.2 $[M + H]^+$.

*tert-Butyl N-[(2R,3R)-1-(N-{4′-Ethoxy-[1,1′-biphenyl]-4-yl}-1-[(1R,2R)-2-(pyridin-2-yl)cyclopropyl]formamido)-3-methoxybutan-2-yl]carbamate (**5f**).* A mixture of bromide (1*R*,2*R*)-**3** (52 mg, 0.1 mmol), 4-ethoxyphenylboronic acid (**4f**) (24.9 mg, 0.15 mmol), $Pd(dppf)Cl_2{\cdot}CH_2Cl_2$ (8.7 mg, 0.01 mmol), and K_3PO_4 (76 mg, 5.4 mmol) in dimethoxyethane (1 mL) and water (0.3 mL) was heated in a sealed vessel by microwave irradiation at 160 °C for 6 min. The resulting mixture was poured into a 1 N NaOH solution (5 mL) and extracted with CH_2Cl_2 (3 × 10 mL). The combined organic layers were dried (Na_2SO_4) and concentrated under reduced pressure. The crude product was purified by chromatography on silica gel using 0−20% EtOAc in hexanes to give **5f** (40 mg, 71% yield) as a white foam: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.27 (d, J = 4.1 Hz, 1H), 7.56−7.38 (m, 5H), 7.32−7.14 (m, 3H), 7.04−6.89 (m, 3H), 5.05 (d, J = 9.4 Hz, 1H), 4.39−4.22 (m, 1H), 4.08 (q, J = 7.5 Hz, 2H), 3.99−3.87 (m, 1H), 3.51 (dd, J = 3.8, 13.6 Hz, 1H), 3.43−3.34 (m, 1H), 3.26 (s, 3H), 2.72−2.62 (m, 1H), 2.06−1.96 (m, 1H), 1.67−1.56 (m, 1H), 1.52−1.34 (m, 13H), 1.13 (d, J = 6.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.9, 159.5, 158.7, 156.2, 153.4, 149.2, 140.9, 140.1, 135.8, 128.4, 128.1, 127.7, 122.7, 120.9, 114.8, 78.9, 63.5, 56.6, 53.3, 50.5, 46.8, 28.4, 27.4, 24.9, 17.7, 15.1, 14.9; MS (ESI) m/z 560.4 $[M + H]^+$.

*tert-Butyl N-[(2R,3R)-3-Methoxy-1-{N-[4′-(propan-2-yloxy)-[1,1′-biphenyl]-4-yl]-1-[(1R,2R)-2-(pyridin-2-yl)cyclopropyl]formamido}-butan-2-yl]carbamate (**5g**).* The procedure for the synthesis of **5f** was followed starting with bromide (1*R*,2*R*)-**3** (52 mg) and [4-(propan-2-yloxy)phenyl]boronic acid (**4g**, 28 mg) to afford **5g** (41 mg, 71% yield) as a white foam: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.27 (d, J = 4.1 Hz, 1H), 7.56−7.40 (m, 5H), 7.31−7.16 (m, 3H), 7.04−6.89 (m, 3H), 5.05 (d, J = 9.0 Hz, 1H), 4.67−4.51 (m, 1H), 4.31 (dd, J = 13.6, 10.5 Hz, 1H), 3.99−3.87 (m, 1H), 3.50 (dd, J = 4.1, 13.6 Hz, 1H), 3.44−3.34 (m, 1H), 3.26 (s, 3H), 2.72−2.62 (m, 1H), 2.06−1.94 (m, 1H), 1.78−1.69 (m, 1H), 1.66−1.55 (m, 1H), 1.43 (s, 9H), 1.37 (d, J = 6.0 Hz, 6H), 1.13 (d, J = 6.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.9, 159.5, 157.7, 156.2, 149.6, 149.2, 140.9, 140.2, 135.8, 128.4, 128.1, 127.7, 122.6, 120.9, 116.1, 78.9, 76.5, 70.0, 56.6, 53.3, 50.5, 28.4, 27.4, 24.9, 22.1, 17.7, 15.1; MS (ESI) m/z 574.4 $[M + H]^+$.

*tert-Butyl N-[(2R,3R)-1-(N-{4′-Cyclopropoxy-[1,1′-biphenyl]-4-yl}-1-[(1R,2R)-2-(pyridin-2-yl)cyclopropyl]formamido)-3-methoxybutan-2-yl]carbamate (**5h**).* The procedure for the synthesis of **5f** was followed starting with bromide (1*R*,2*R*)-**3** (52 mg) and (4-cyclopropoxyphenyl)boronic acid (**4h**, 28 mg) to afford **5h** (42 mg, 73% yield) as a white foam: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.27 (d, J = 4.0 Hz, 1H), 7.56−7.38 (m, 5H), 7.32−7.18 (m, 3H), 7.15−7.06 (m, 2H), 7.03−6.93 (m, 1H), 5.05 (d, J = 9.0 Hz, 1H), 4.31 (dd, J = 13.8, 10.5 Hz, 1H), 3.99−3.87 (m, 1H), 3.82−3.74 (m, 1H), 3.48 (dd, J = 4.1, 13.8 Hz, 1H), 3.43−3.35 (m, 1H), 3.26 (s, 3H), 2.72−2.62 (m, 1H), 2.06−1.95 (m, 1H), 1.67−1.57 (m, 1H), 1.50−1.35 (m, 10H), 1.13 (d, J = 6.2 Hz, 3H), 0.86−0.75 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.9, 159.5, 158.7, 156.3, 149.2, 140.1, 135.8, 133.0, 128.4, 128.0, 127.7, 122.7, 120.9, 115.4, 78.9, 56.6, 53.3, 50.9, 50.6, 28.4, 27.4, 24.9, 18.9, 17.7, 15.1, 6.2; MS (ESI) m/z 572.2 $[M + H]^+$.

*(1R,2R)-N-[(2R,3R)-2-Amino-3-methoxybutyl]-N-{4′-[(1S)-1-methoxyethyl]-[1,1′-biphenyl]-4-yl}-2-(pyridin-2-yl)cyclopropane-1-carboxamide Dihydrochloride (**6**).* To a solution of **5a** (62 mg, 0.11 mmol) in DCM (1 mL) was added 4 M HCl in 1,4-dioxane (0.14 mL, 0.55 mmol), and the resulting solution was stirred at room

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temperature for 6 h. The solvent was removed under reduced pressure. The resulting residue was triturated with hexanes to give **6** (59 mg, 83% yield) as a white solid: ^{1}H NMR (300 MHz, CD_3OD) δ 8.62 (d, J = 5.5 Hz, 1H), 8.35 (t, J = 7.6 Hz, 1H), 7.80 (t, J = 6.7 Hz, 1H), 7.72 (d, J = 7.9 Hz, 2H), 7.64−7.54 (m, 5H), 7.40 (d, J = 8.3 Hz, 2H), 4.39 (q, J = 6.4 Hz, 1H), 4.29−4.17 (m, 1H), 4.03 (dd, J = 4.3, 14.7 Hz, 1H), 3.62−3.50 (m, 1H), 3.40−3.25 (m, 1H), 3.34 (s, 3H), 3.23 (s, 3H), 3.04−2.94 (m, 1H), 2.25−2.14 (m, 1H), 2.03−1.92 (m, 1H), 1.69 (td, J = 5.6, 8.6 Hz, 1H), 1.42 (d, J = 6.4 Hz, 3H), 1.13 (d, J = 6.2 Hz, 3H); ^{13}C NMR (75 MHz, CD_3OD) δ 173.2, 157.3, 147.6, 144.8, 142.7, 142.6, 142.0, 140.0, 129.7, 128.2, 128.0, 125.9, 125.3, 80.6, 75.0, 57.4, 56.9, 56.8, 51.1, 27.2, 24.9, 24.0, 18.1, 15.6; MS (ESI) free base m/z 474.2 $[M + H]^+$; HPLC t_R 10.9 min.

*(1R,2R)-N-[(2R,3R)-2-Amino-3-methoxybutyl]-N-{4′-[(1R)-1-methoxyethyl]-[1,1′-biphenyl]-4-yl}-2-(pyridin-2-yl)cyclopropane-1-carboxamide Dihydrochloride (**7**).* The procedure for the synthesis of **6** was followed starting with **5b** (60 mg) to afford **7** (57 mg, 95% yield) as a white solid: ^{1}H NMR (300 MHz, CD_3OD) δ 8.62 (d, J = 5.7 Hz, 1H), 8.35 (t, J = 7.9 Hz, 1H), 7.80 (t, J = 6.8 Hz, 1H), 7.73 (d, J = 8.1 Hz, 2H), 7.64−7.52 (m, 5H), 7.42 (d, J = 8.1 Hz, 2H), 4.40 (q, J = 6.4 Hz, 1H), 4.30−4.19 (m, 1H), 4.08−3.97 (m, 1H), 3.61−3.50 (m, 1H), 3.40−3.25 (m, 1H), 3.35 (s, 3H), 3.24 (s, 3H), 3.05−2.94 (m, 1H), 2.26−2.15 (m, 1H), 2.04−1.93 (m, 1H), 1.70 (td, J = 5.5, 8.7 Hz, 1H), 1.43 (d, J = 6.4 Hz, 3H), 1.15 (d, J = 6.2 Hz, 3H); ^{13}C NMR (75 MHz, CD_3OD) δ 173.2, 157.3, 147.5, 144.9, 142.8, 142.7, 142.0, 140.0, 129.7, 128.2, 128.1, 125.9, 125.3, 80.6, 74.9, 57.3, 56.9, 56.8, 51.1, 27.1, 24.9, 24.0, 18.1, 15.6; MS (ESI) free base m/z 474.2 $[M + H]^+$; HPLC t_R 10.9 min.

*(1R,2R)-N-[(2R,3R)-2-Amino-3-methoxybutyl]-N-{4′-[(propan-2-yloxy)methyl]-[1,1′-biphenyl]-4-yl}-2-(pyridin-2-yl)cyclopropane-1-carboxamide Dihydrochloride (**8**).* The procedure for the synthesis of **6** was followed starting with **5c** (55 mg) to afford **8** (53 mg, 98% yield) as a white solid: ^{1}H NMR (300 MHz, CD_3OD) δ 8.59 (d, J = 5.5 Hz, 1H), 8.31 (t, J = 7.7 Hz, 1H), 7.77 (d, J = 6.8 Hz, 1H), 7.72 (d, J = 8.3 Hz, 2H), 7.61−7.52 (m, 5H), 7.44 (d, J = 8.1 Hz, 2H), 4.56 (s, 2H), 4.23 (dd, J = 7.3, 14.8 Hz, 1H), 4.02 (dd, J = 4.2, 14.8 Hz, 1H), 3.74 (quin, J = 6.1 Hz, 1H), 3.60−3.51 (m, 1H), 3.34 (s, 3H), 3.30−3.20 (m, 1H), 3.02−2.92 (m, 1H), 2.23−2.13 (m, 1H), 2.01−1.90 (m, 1H), 1.68 (td, J = 5.6, 8.7 Hz, 1H), 1.22 (d, J = 6.0 Hz, 6H), 1.14 (d, J = 6.2 Hz, 3H); ^{13}C NMR (75 MHz, CD_3OD) δ 173.4, 157.6, 147.1, 143.1, 142.8, 142.1, 140.5, 140.0, 129.8, 129.6, 128.1, 125.8, 125.3, 75.1, 72.9, 70.9, 57.5, 57.0, 51.2, 27.2, 25.2, 22.6, 18.2, 15.7; MS (ESI) free base m/z 488.0 $[M + H]^+$; HPLC t_R 11.7 min.

*(1R,2R)-N-[(2R,3R)-2-Amino-3-methoxybutyl]-N-{4′-[(2S)-oxolan-2-yl]-[1,1′-biphenyl]-4-yl}-2-(pyridin-2-yl)cyclopropane-1-carboxamide Dihydrochloride (**9**).* The procedure for the synthesis of **6** was followed starting with **5d** (122 mg) to afford **9** (116 mg, quantitative yield) as a white solid: ^{1}H NMR (700 MHz, CD_3OD) δ 8.51 (s, 1H), 8.16 (s, 1H), 7.68 (d, J = 7.5 Hz, 2H), 7.64−7.57 (m, 1H), 7.57−7.45 (m, 5H), 7.42 (d, J = 8.0 Hz, 2H), 4.91 (t, J = 7.3 Hz, 1H), 4.20 (dd, J = 14.8, 7.2 Hz, 1H), 4.13−4.06 (m, 1H), 4.06−3.98 (m, 1H), 3.98−3.87 (m, 1H), 3.64−3.51 (m, 1H), 3.36−3.32 (m, 4H), 3.00−2.81 (m, 1H), 2.38 (td, J = 12.6, 6.7 Hz, 1H), 2.16−2.09 (m, 1H), 2.08−2.00 (m, 2H), 1.95−1.85 (m, 1H), 1.85−1.73 (m, 1H), 1.63 (d, J = 4.5 Hz, 1H), 1.13 (d, J = 6.2 Hz, 3H); MS (ESI) free base m/z 486.2 $[M + H]^+$; HPLC t_R 10.7 min.

*(1R,2R)-N-[(2R,3R)-2-Amino-3-methoxybutyl]-N-{4′-[(2R)-oxolan-2-yl]-[1,1′-biphenyl]-4-yl}-2-(pyridin-2-yl)cyclopropane-1-carboxamide Dihydrochloride (**10**).* The procedure for the synthesis of **6** was followed starting with **5e** (75 mg) to afford **10** (64 mg, 90% yield) as a white solid: ^{1}H NMR (700 MHz, CD_3OD) δ 8.60 (d, J = 5.1 Hz, 1H), 8.31 (t, J = 7.1 Hz, 1H), 7.76 (t, J = 6.0 Hz, 1H), 7.71 (d, J = 7.6 Hz, 2H), 7.57 (d, J = 8.1 Hz, 5H), 7.43 (d, J = 8.1 Hz, 2H), 4.91 (t, J = 7.3 Hz, 1H), 4.21 (dd, J = 14.8, 7.2 Hz, 1H), 4.11 (dd, J = 14.8, 7.2 Hz, 1H), 4.07−3.99 (m, 1H), 3.94 (dd, J = 14.4, 7.6 Hz, 1H), 3.57 (dd, J = 7.4, 4.9 Hz, 1H), 3.36−3.32 (m, 4H), 2.98 (d, J = 3.2 Hz, 1H), 2.38 (td, J = 12.5, 6.9 Hz, 1H), 2.21−2.12 (m, 1H), 2.10−2.00 (m, 2H), 1.96 (dd, J = 8.3, 4.4 Hz, 1H), 1.80 (dq, J = 12.5, 8.1 Hz, 1H), 1.70−1.65 (m, 1H), 1.13 (d, J = 6.2 Hz, 3H); MS (ESI) free base m/z 486.2 $[M + H]^+$; HPLC t_R 10.6 min.

*(1R,2R)-N-[(2R,3R)-2-Amino-3-methoxybutyl]-N-{4′-ethoxy-[1,1′-biphenyl]-4-yl}-2-(pyridin-2-yl)cyclopropane-1-carboxamide Dihydrochloride (**11**).* The procedure for the synthesis of **6** was followed starting with **5f** (40 mg) to afford **11** (35 mg, 95% yield) as a white solid: ^{1}H NMR (300 MHz, CD_3OD) δ 8.42 (d, J = 6.0 Hz, 1H), 7.99 (t, J = 7.5 Hz, 1H), 7.62 (d, J = 6.0 Hz, 2H), 7.54−7.38 (m, 6H), 6.98 (d, J = 8.3 Hz, 2H), 4.27−4.13 (m, 1H), 4.11−3.99 (m, 3H), 3.76−3.63 (m, 1H), 3.61−3.47 (m, 1H), 3.34 (s, 3H), 2.87−2.75 (m, 1H), 2.14−2.02 (m, 1H), 1.88−1.77 (m, 1H), 1.62−1.52 (m, 1H), 1.40 (t, J = 7.5 Hz, 3H), 1.13 (d, J = 5.3 Hz, 3H); MS (ESI) free base m/z 460.2 $[M + H]^+$; HPLC t_R 11.4 min.

*(1R,2R)-N-[(2R,3R)-2-Amino-3-methoxybutyl]-N-[4′-(propan-2-yloxy)-[1,1′-biphenyl]-4-yl]-2-(pyridin-2-yl)cyclopropane-1-carboxamide Dihydrochloride (**12**).* The procedure for the synthesis of **6** was followed starting with **5g** (36 mg) to afford **12** (33 mg, 96% yield) as a white solid: ^{1}H NMR (300 MHz, CD_3OD) δ 8.46 (d, J = 4.9 Hz, 1H), 8.06 (t, J = 7.4 Hz, 1H), 7.62 (d, J = 7.2 Hz, 2H), 7.56−7.36 (m, 6H), 6.97 (d, J = 8.3 Hz, 2H), 4.69−4.57 (m, 1H), 4.26−4.11 (m, 1H), 4.08−3.93 (m, 1H), 3.78−3.62 (m, 1H), 3.61−3.48 (m, 1H), 3.34 (s, 3H), 2.90−2.77 (m, 1H), 2.15−2.04 (m, 1H), 1.91−1.80 (m, 1H), 1.66−1.51 (m, 1H), 1.33 (t, J = 6.0 Hz, 6H), 1.13 (d, J = 6.0 Hz, 3H); MS (ESI) free base m/z 474.2 $[M + H]^+$; HPLC t_R 11.8 min.

*(1R,2R)-N-[(2R,3R)-2-Amino-3-methoxybutyl]-N-{4′-cyclopropoxy-[1,1′-biphenyl]-4-yl}-2-(pyridin-2-yl)cyclopropane-1-carboxamide Dihydrochloride (**13**).* The procedure for the synthesis of **6** was followed starting with **5h** (40 mg) to afford **13** (37 mg, 98% yield) as a white solid: ^{1}H NMR (300 MHz, CD_3OD) δ 8.50 (d, J = 4.0 Hz, 1H), 8.27−8.12 (m, 1H), 7.72−7.26 (m, 8H), 7.02 (d, J = 8.1 Hz, 2H), 4.18−4.01 (m, 1H), 4.01−3.87 (m, 1H), 3.73−3.39 (m, 2H), 3.25 (s, 3H), 2.94−2.81 (m, 1H), 2.15−2.01 (m, 1H), 1.91−1.78 (m, 1H), 1.67−1.49 (m, 1H), 1.27−1.12 (m, 1H), 1.03 (t, J = 6.0 Hz, 3H), 0.75−0.51 (m, 4H); MS (ESI) free base m/z 472.4 $[M + H]^+$; HPLC t_R 10.8 min.

(±)-tert-Butyl [(2R,3R)-1-{(4-Bromophenyl)-[(1R,2R*)-2-(pyridin-2-yl)cyclopropanecarbonyl]amino}-3-methoxybutan-2-yl]-carbamate (**16a**).* The compound was synthesized following the procedure described in our previous publication:[11] ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.35−8.27 (m, 1H), 7.58−7.50 (m, 1H), 7.50−7.38 (m, 2H), 7.25−6.98 (m, 4H), 4.96 (d, J = 9.0 Hz, 1H), 4.35−4.20 (m, 1H), 3.92−3.78 (m, 2H), 3.55−3.45 (m, 1H), 3.45−3.32 (m, 1H), 3.26 and 3.25 (2s, 3H), 2.70−2.62 (m, 0.5H), 2.62−2.55 (m, 0.5H), 2.00−1.88 (m, 1H), 1.72−1.58 (m, 1H), 1.41 (s, 9H), 1.11 and 1.10 (2d, J = 6.0 Hz, 3H); MS (ESI) m/z 518.6 $[M + H]^+$ (^{79}Br), 520.5 $[M + H]^+$ (^{81}Br).

(±)-tert-Butyl N-[(2R,3R)-1-{N-(4-Bromophenyl)-1-[(1R,2R*)-2-(pyridin-3-yl)cyclopropyl]formamido}-3-methoxybutan-2-yl]-carbamate (**16b**).* (±)-(1R*,2R*)-2-(Pyridin-3-yl)cyclopropane-1-carboxylic acid (**15b**) was synthesized from 3-ethenylpyridine following the procedure described in our previous publication.[10] To a solution of **15b** (100 mg, 0.61 mmol) in DCM (5 mL) were added oxalyl chloride (105 μL, 1.2 mmol) and DMF (20 μL) at room temperature, and the mixture was stirred for 2 h. The reaction mixture was concentrated under reduced pressure, and the resulting acid chloride was dissolved in DCM (5 mL) and treated with **14** (251 mg, 0.67 mmol) and Et_3N (0.25 mL, 0.18 mmol). The mixture was stirred overnight at room temperature. Saturated $NaHCO_3$ (5 mL) was added, and the layers were separated. The aqueous layer was extracted with DCM (3 × 10 mL). The combined organic layers were washed with brine (3 × 10 mL), dried (Na_2SO_4), and concentrated under reduced pressure. The crude product was purified by chromatography on silica gel using 0−25% EtOAc in hexanes to afford **16b** (168 mg, 53% yield) as a white foam: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.40 (d, J = 3.0 Hz, 1H), 8.30 (s, 1H), 7.48 (d, J = 7.6 Hz, 2H), 7.23−7.00 (m, 4H), 5.10−4.81 (m, 1H), 4.36−4.04 (m, 1H), 4.00−3.65 (m, 1H), 3.63−3.42 (m, 1H), 3.42−3.30 (m, 1H), 3.26 (s, 3H), 2.70−2.34 (m, 1H), 1.77−1.48 (m, 2H), 1.48−1.34 (m, 9H), 1.17−1.04 (m, 4H); MS (ESI) m/z 518.0 $[M + H]^+$ (^{79}Br), 520.0 $[M + H]^+$ (^{81}Br).

(±)-tert-Butyl N-[(2R,3R)-1-{N-(4-Bromophenyl)-1-[(1R,2R*)-2-(pyridin-4-yl)cyclopropyl]formamido}-3-methoxybutan-2-yl]-carbamate (**16c**).* The procedure for the synthesis of **16b** was

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followed starting with (±)-(1*R**,2*R**)-2-(pyridin-4-yl)cyclopropane-1-carboxylic acid (**15c**, 100 mg) to afford **16c** (130 mg, 41% yield) as an off-white foam: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.40 (d, J = 4.7 Hz, 2H), 7.46 (d, J = 5.6 Hz, 2H), 7.13 (d, J = 8.3 Hz, 2H), 6.82 (dd, J = 10.0, 5.4 Hz, 2H), 4.91 (t, J = 9.9 Hz, 1H), 4.37−4.02 (m, 1H), 4.00−3.64 (m, 1H), 3.64−3.42 (m, 1H), 3.42−3.31 (m, 1H), 3.26 (d, J = 2.3 Hz, 3H), 2.60−2.31 (m, 1H), 1.85−1.50 (m, 2H), 1.49−1.32 (m, 9H), 1.18−0.95 (m, 4H); MS (ESI) m/z 518.4 $[M + H]^+$ (^{79}Br), 520.4 $[M + H]^+$ (^{81}Br).

(±)-tert-Butyl N-[(2R,3R)-1-{N-(4-Bromophenyl)-1-[(1R,2R*)-2-(pyrimidin-2-yl)cyclopropyl]formamido}-3-methoxybutan-2-yl]carbamate (**16d**).* The procedure for the synthesis of **16b** was followed starting with (±)-(1*R**,2*R**)-2-(pyrimidin-2-yl)-cyclopropane-1-carboxylic acid (**15d**, 142 mg), synthesized from pyrimidine-2-carboxaldehyde following the patent procedure,[15] to afford **16d** (90 mg, 20% yield) as a yellowish foam: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.50 (d, J = 4.8 Hz, 2H), 7.43 (d, J = 8.3 Hz, 2H), 7.20−6.98 (m, 3H), 4.98 (d, J = 9.4 Hz, 1H), 4.40−4.19 (m, 1H), 3.88−3.64 (m, 1H), 3.56−3.30 (m, 2H), 3.26 (s, 3H), 2.93−2.75 (m, 1H), 2.02−1.85 (m, 1H), 1.85−1.60 (m, 1H), 1.54−1.30 (m, 10H), 1.10 (d, J = 6.3 Hz, 3H); MS (ESI) m/z 519.0 $[M + H]^+$ (^{79}Br), 521.0 $[M + H]^+$ (^{81}Br).

(±)-tert-Butyl N-[(2R,3R)-1-{N-(4-Bromophenyl)-1-[(1R,2R*)-2-(pyrazin-2-yl)cyclopropyl]formamido}-3-methoxybutan-2-yl]carbamate (**16e**).* The procedure for the synthesis of **16b** was followed starting with (±)-(1*R**,2*R**)-2-(pyrazin-2-yl)cyclopropane-1-carboxylic acid (**15e**, 61 mg) to afford **16e** (105 mg, 54% yield) as a yellow foam: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.63−8.44 (m, 1H), 8.40−8.18 (m, 2H), 7.52−7.33 (m, 3H), 7.12 (d, J = 8.2 Hz, 2H), 4.93 (d, J = 9.5 Hz, 1H), 4.32−4.10 (m, 1H), 3.95−3.68 (m, 1H), 3.59−3.42 (m, 1H), 3.42−3.30 (m, 1H), 3.26 (s, 3H), 2.81−2.56 (m, 1H), 1.98−1.84 (m, 1H), 1.82−1.54 (m, 1H), 1.53−1.30 (m, 9H), 1.16−0.95 (m, 3H); MS (ESI) m/z 519.0 $[M + H]^+$ (^{79}Br), 521.0 $[M + H]^+$ (^{81}Br).

(±)-tert-Butyl N-[(2R,3R)-1-{N-(4-Bromophenyl)-1-[(1R,2R*)-2-(thiophen-2-yl)cyclopropyl]formamido}-3-methoxybutan-2-yl]carbamate (**16f**).* The procedure for the synthesis of **16b** was followed starting with (±)-(1*R**,2*R**)-2-(thiophen-2-yl)-cyclopropane-1-carboxylic acid (**15f**, 0.76 g) to afford **16f** (1.07 g, 45% yield) as a thick brown oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.46 (d, J = 7.8 Hz, 2H), 7.28−7.08 (m, 2H), 7.01−6.93 (m, 1H), 6.82−6.72 (m, 1H), 6.67−6.53 (m, 1H), 5.08 (dd, J = 23.0, 9.4 Hz, 1H), 4.44−4.15 (m, 1H), 3.96−3.67 (m, 1H), 3.62−3.28 (m, 3H), 3.28−3.09 (m, 3H), 2.81−2.50 (m, 1H), 1.70−1.54 (m, 2H), 1.51−1.29 (m, 9H), 1.10 (d, J = 6.6 Hz, 3H); MS (ESI) m/z 523.2 $[M + H]^+$ (^{79}Br), 525.2 $[M + H]^+$ (^{81}Br).

(±)-tert-Butyl N-[(2R,3R)-1-{N-(4-Bromophenyl)-1-[(1R,2R*)-2-(thiophen-3-yl)cyclopropyl]formamido}-3-methoxybutan-2-yl]carbamate (**16g**).* The procedure for the synthesis of **16b** was followed starting with (±)-(1*R**,2*R**)-2-(thiophen-3-yl)-cyclopropane-1-carboxylic acid (**15g**, 148 mg) to afford **16g** (300 mg, 65% yield) as an off-white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.48 (d, J = 8.5 Hz, 2H), 7.22−7.05 (m, 3H), 6.83−6.55 (m, 2H), 4.95 (t, J = 10.1 Hz, 1H), 4.38−4.16 (m, 1H), 3.92−3.70 (m, 1H), 3.57−3.41 (m, 1H), 3.41−3.29 (m, 1H), 3.29−3.16 (m, 3H), 2.69−2.36 (m, 1H), 1.67−1.45 (m, 3H), 1.45−1.33 (m, 9H), 1.11 (d, J = 6.2 Hz, 3H); MS (ESI) m/z 523.2 $[M + H]^+$ (^{79}Br), 525.2 $[M + H]^+$ (^{81}Br).

(±)-tert-Butyl N-[(2R,3R)-1-{N-(4-Bromophenyl)-1-[(1R,2R*)-2-(6-fluoropyridin-2-yl)cyclopropyl]formamido}-3-methoxybutan-2-yl]carbamate (**16h**).* The procedure for the synthesis of **16b** was followed starting with (±)-(1*R**,2*R**)-2-(6-fluoropyridin-2-yl)-cyclopropane-1-carboxylic acid (**15h**, 362 mg) to afford **16h** (751 mg, 70% yield) as a brown foam: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.61 (dd, J = 15.8, 7.7 Hz, 1H), 7.50−7.34 (m, 2H), 7.20−6.95 (m, 3H), 6.65 (d, J = 7.8 Hz, 1H), 4.92 (d, J = 9.3 Hz, 1H), 4.42−4.09 (m, 1H), 4.00−3.58 (m, 1H), 3.49 (d, J = 14.0 Hz, 1H), 3.36 (d, J = 6.3 Hz, 1H), 3.26 (s, 3H), 2.70−2.48 (m, 1H), 1.98−1.76 (m, 1H), 1.68−1.55 (m, 1H), 1.51−1.21 (m, 10H), 1.11 (d, J = 6.2 Hz, 3H); MS (ESI) m/z 536.0 $[M + H]^+$ (^{79}Br), 538.0 $[M + H]^+$ (^{81}Br).

*tert-Butyl N-[(2R,3R)-1-{N-(4-Bromophenyl)-1-[(1R,2R)-2-(6-fluoropyridin-2-yl)cyclopropyl]formamido}-3-methoxybutan-2-yl]carbamate [(1R,2R)-**16h**] and tert-Butyl N-[(2R,3R)-1-{N-(4-Bromophenyl)-1-[(1S,2S)-2-(6-fluoropyridin-2-yl)cyclopropyl]formamido}-3-methoxybutan-2-yl]carbamate [(1S,2S)-**16h**].* Diastereomeric mixture **16h** (450 mg) was separated into (1*R*,2*R*)-**16h** (220 mg, 49%) and (1*S*,2*S*)-**16h** (201 mg, 45%) by preparative HPLC using a ChiralPak IA column: mobile phase, 10% isopropanol/hexanes; flow rate, 10 mL/min; detection, 254 nm. The diastereomeric excess of both of separated compounds was determined to be >99% by HPLC [ChiralPak IA column; 10% isopropanol/hexanes; flow rate, 1 mL/min; detection, 254 nm; retention times of 9.5 min for (1*R*,2*R*)-**16h** and 16.6 min for (1*S*,2*S*)-**16h**]. (1*R*,2*R*)-**16h**: off-white foam; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.70−7.52 (m, 1H), 7.45 (d, J = 8.2 Hz, 2H), 7.11 (d, J = 8.1 Hz, 3H), 6.65 (dd, J = 8.1, 2.3 Hz, 1H), 4.92 (d, J = 8.9 Hz, 1H), 4.30−4.10 (m, 1H), 3.99−3.68 (m, 1H), 3.62−3.40 (m, 1H), 3.40−3.29 (m, 1H), 3.26 (s, 3H), 2.74−2.51 (m, 1H), 1.96−1.77 (m, 1H), 1.65−1.52 (m, 1H), 1.51−1.27 (m, 10H), 1.11 (d, J = 6.2 Hz, 3H); MS (ESI) m/z 536.0 $[M + H]^+$ (^{79}Br), 538.0 $[M + H]^+$ (^{81}Br). (1*S*,2*S*)-**16h**: off-white solid; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.61 (q, J = 8.0 Hz, 1H), 7.43 (d, J = 8.3 Hz, 2H), 7.07 (dd, J = 17.0, 7.1 Hz, 3H), 6.65 (d, J = 8.1 Hz, 1H), 4.93 (d, J = 8.8 Hz, 1H), 4.35−4.11 (m, 1H), 3.87−3.61 (m, 1H), 3.59−3.42 (m, 1H), 3.35 (d, J = 6.3 Hz, 1H), 3.25 (s, 3H), 2.66−2.43 (m, 1H), 1.97−1.76 (m, 1H), 1.76−1.60 (m, 1H), 1.41 (s, 10H), 1.10 (d, J = 6.2 Hz, 3H); MS (ESI) m/z 536.0 $[M + H]^+$ (^{79}Br), 538.0 $[M + H]^+$ (^{81}Br).

(±)-tert-Butyl N-[(2R,3R)-1-{N-(4-Bromophenyl)-1-[(1R,2R*)-2-(5-fluoropyridin-2-yl)cyclopropyl]formamido}-3-methoxybutan-2-yl]carbamate (**16i**).* The procedure for the synthesis of **16b** was followed starting with (±)-(1*R**,2*R**)-2-(5-fluoropyridin-2-yl)-cyclopropane-1-carboxylic acid (**15i**, 543 mg) to afford **16i** (1.05 g, 65% yield) as a white foamy solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.16 (s, 1H), 7.53−7.33 (m, 2H), 7.27−7.17 (m, 2H), 7.11 (d, J = 6.6 Hz, 2H), 4.94 (d, J = 9.4 Hz, 1H), 4.31−4.08 (m, 1H), 3.99−3.63 (m, 1H), 3.55−3.42 (m, 1H), 3.36 (d, J = 6.3 Hz, 1H), 3.26 (s, 3H), 2.72−2.47 (m, 1H), 1.97−1.74 (m, 1H), 1.61−1.52 (m, 1H), 1.49−1.28 (m, 10H), 1.11 (d, J = 6.2 Hz, 3H); MS (ESI) m/z 536.0 $[M + H]^+$ (^{79}Br), 538.0 $[M + H]^+$ (^{81}Br).

*tert-Butyl N-[(2R,3R)-1-{N-(4-Bromophenyl)-1-[(1R,2R)-2-(5-fluoropyridin-2-yl)cyclopropyl]formamido}-3-methoxybutan-2-yl]carbamate [(1R,2R)-**16i**] and tert-Butyl N-[(2R,3R)-1-{N-(4-Bromophenyl)-1-[(1S,2S)-2-(5-fluoropyridin-2-yl)cyclopropyl]formamido}-3-methoxybutan-2-yl]carbamate [(1S,2S)-**16i**].* Diastereomeric mixture **16i** (650 mg) was separated into (1*R*,2*R*)-**16i** (302 mg, 47%) and (1*S*,2*S*)-**16i** (299 mg, 46%) by preparative HPLC using a ChiralPak IA column: mobile phase, 12% isopropanol/hexanes; flow rate, 10 mL/min; detection, 220 nm. The diastereomeric excess of both of separated compounds was determined to be >99% by HPLC [ChiralPak IA column; 12% isopropanol/hexanes; flow rate, 1 mL/min; detection, 220 nm; retention times of 8.4 min for (1*R*,2*R*)-**16i** and 15.2 min for (1*S*,2*S*)-**16i**]. (1*R*,2*R*)-**16i**: off-white foam; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.15 (s, 1H), 7.44 (d, J = 8.3 Hz, 2H), 7.31−7.00 (m, 4H), 4.93 (d, J = 9.5 Hz, 1H), 4.31−4.12 (m, 1H), 3.99−3.69 (m, 1H), 3.58−3.42 (m, 1H), 3.42−3.30 (m, 1H), 3.26 (s, 3H), 2.76−2.49 (m, 1H), 1.97−1.71 (m, 1H), 1.59 (d, J = 5.2 Hz, 1H), 1.41 (s, 10H), 1.11 (d, J = 6.2 Hz, 3H); MS (ESI) m/z 536.0 $[M + H]^+$ (^{79}Br), 538.0 $[M + H]^+$ (^{81}Br). (1*S*,2*S*)-**16i**: off-white foam; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.16 (s, 1H), 7.43 (d, J = 8.3 Hz, 2H), 7.25−7.19 (m, 1H), 7.17−7.00 (m, 3H), 4.94 (d, J = 9.4 Hz, 1H), 4.31−4.09 (m, 1H), 3.87−3.63 (m, 1H), 3.50 (dd, J = 13.7, 3.9 Hz, 1H), 3.43−3.29 (m, 1H), 3.25 (s, 3H), 2.68−2.49 (m, 1H), 1.98−1.73 (m, 1H), 1.69−1.61 (m, 1H), 1.45−1.26 (m, 10H), 1.10 (d, J = 6.2 Hz, 3H); MS (ESI) m/z 536.0 $[M + H]^+$ (^{79}Br), 538.0 $[M + H]^+$ (^{81}Br).

*(±)-tert-Butyl N-[(2R,3R)-1-{N-(4-Bromophenyl)-1-[(1R,2R)-2-(4-fluoropyridin-2-yl)cyclopropyl]formamido}-3-methoxybutan-2-yl]carbamate (**16j**).* The procedure for the synthesis of **16b** was followed starting with (±)-(1*R**,2*R**)-2-(4-fluoropyridin-2-yl)-cyclopropane-1-carboxylic acid (**15j**, 91 mg) to afford **16j** (162 mg, 60% yield) as a brown foam: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.25 (dd, J = 8.5, 5.7 Hz, 1H), 7.43 (d, J = 7.4 Hz, 2H), 7.12 (d, J = 7.2 Hz,

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2H), 6.92 (ddd, J = 20.0, 9.7, 1.8 Hz, 1H), 6.83−6.68 (m, 1H), 4.94 (d, J = 9.5 Hz, 1H), 4.33−4.15 (m, 1H), 3.94−3.73 (m, 1H), 3.59−3.45 (m, 1H), 3.41−3.31 (m, 1H), 3.28−3.18 (m, 3H), 2.71−2.43 (m, 1H), 2.02−1.85 (m, 1H), 1.74−1.55 (m, 1H), 1.48−1.38 (m, 10H), 1.14−1.07 (m, 3H); MS (ESI) m/z 536.0 $[M + H]^+$ (^{79}Br), 538.0 $[M + H]^+$ (^{81}Br).

(±)-tert-Butyl N-[(2R,3R)-1-{N-(4-Bromophenyl)-1-[(1R,2R*)-2-(3-fluoropyridin-2-yl)cyclopropyl]formamido}-3-methoxybutan-2-yl]carbamate (**16k**).* The procedure for the synthesis of **16b** was followed starting with (±)-(1R*,2R*)-2-(3-fluoropyridin-2-yl)-cyclopropane-1-carboxylic acid (**15k**, 91 mg) to afford **16k** (121 mg, 45% yield) as a white foam: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.09 (d, J = 4.3 Hz, 1H), 7.49−7.35 (m, 2H), 7.35−7.23 (m, 1H), 7.21−7.08 (m, 2H), 7.08−6.96 (m, 1H), 5.03 (t, J = 8.6 Hz, 1H), 4.44−4.22 (m, 1H), 3.94−3.64 (m, 1H), 3.54−3.41 (m, 1H), 3.41−3.31 (m, 1H), 3.31−3.15 (m, 3H), 3.05−2.80 (m, 1H), 2.01−1.87 (m, 1H), 1.81−1.55 (m, 1H), 1.55−1.30 (m, 10H), 1.17−1.01 (m, 3H); MS (ESI) m/z 536.0 $[M + H]^+$ (^{79}Br), 538.0 $[M + H]^+$ (^{81}Br).

(±)-tert-Butyl N-[(2R,3R)-1-{N-(4-Bromophenyl)-1-[(1R,2R*)-2-(6-methylpyridin-2-yl)cyclopropyl]formamido}-3-methoxybutan-2-yl]carbamate (**16l**).* The procedure for the synthesis of **16b** was followed starting with (±)-(1R*,2R*)-2-(6-methylpyridin-2-yl)-cyclopropane-1-carboxylic acid (**15l**, 89 mg) to afford **16l** (186 mg, 70% yield) as a white foam: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.50−7.31 (m, 3H), 7.11 (t, J = 8.9 Hz, 2H), 6.98−6.74 (m, 2H), 4.97 (t, J = 10.3 Hz, 1H), 4.43−4.05 (m, 1H), 3.93−3.67 (m, 1H), 3.67−3.42 (m, 1H), 3.37 (d, J = 4.4 Hz, 1H), 3.30−3.03 (m, 3H), 2.66−2.41 (m, 1H), 2.41−2.18 (m, 3H), 1.95−1.78 (m, 1H), 1.68−1.57 (m, 1H), 1.41 (s, 10H), 1.11 (d, J = 6.2 Hz, 3H); MS (ESI) m/z 532.0 $[M + H]^+$ (^{79}Br), 534.2 $[M + H]^+$ (^{81}Br).

(±)-(1R,2R*)-N-[(2R,3R)-2-Amino-3-methoxybutyl]-N-[4′-(methoxymethyl)-[1,1′-biphenyl]-4-yl]-2-(pyridin-2-yl)cyclopropane-1-carboxamide Dihydrochloride (**17**).* The compound was synthesized following the procedure described in our previous publication:[11] ^{1}H NMR (300 MHz, CD_3OD) δ 8.55−8.45 (m, 1H), 8.20−8.15 (m, 1H), 7.78−7.38 (m, 10H), 4.49 (s, 2H), 4.45−4.30 (m, 0.5H), 4.30−4.15 (m, 0.5H), 4.12−3.95 (m, 0.5H), 3.95−3.80 (m, 0.5H), 3.78−3.62 (m, 1H), 3.62−3.50 (m, 1H), 3.40 (s, 3H), 3.35−3.25 (m, 3H), 3.00−2.82 (m, 1H), 2.20−2.08 (m, 1H), 1.95−1.80 (m, 1H), 1.70−1.55 (m, 1H), 1.22−1.08 (m, 3H); MS (ESI) free base m/z 460.2 $[M + H]^+$.

(±)-(1R,2R*)-N-[(2R,3R)-2-Amino-3-methoxybutyl]-N-[4′-(methoxymethyl)-[1,1′-biphenyl]-4-yl]-2-(pyridin-3-yl)cyclopropane-1-carboxamide Dihydrochloride (**18**).* Suzuki coupling of **16b** (90 mg) with 4-(methoxymethyl)phenylboronic acid was carried out using the procedure for **5f**, followed by Boc deprotection with 4 M HCl in dioxane as described for **6**, to afford **18** (58 mg, 63% yield) as an off-white solid: ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.76−8.55 (m, 1H), 8.13−7.97 (m, 1H), 7.79−7.52 (m, 10H), 7.39 (d, J = 6.0 Hz, 2H), 4.43 (s, 2H), 4.14−4.01 (m, 0.5H), 4.01−3.91 (m, 0.5H), 3.91−3.79 (m, 0.5H), 3.58−3.32 (m, 2.5H), 3.30 (s, 3H), 3.25−3.07 (m, 3H), 2.75−2.62 (m, 1H), 1.75−1.55 (m, 2H), 1.52−1.38 (m, 1H), 1.10 (d, J = 6.0 Hz, 1.5H), 1.07 (d, J = 6.0 Hz, 1.5H); MS (ESI) free base m/z 460.4 $[M + H]^+$.

(±)-(1R,2R*)-N-[(2R,3R)-2-Amino-3-methoxybutyl]-N-[4′-(methoxymethyl)-[1,1′-biphenyl]-4-yl]-2-(pyridin-4-yl)cyclopropane-1-carboxamide Dihydrochloride (**19**).* Suzuki coupling of **16c** (90 mg) with 4-(methoxymethyl)phenylboronic acid was carried out using the procedure for **5f**, followed by Boc deprotection with 4 M HCl in dioxane as described for **6**, to afford **19** (83 mg, 90% yield) as an off-white solid: ^{1}H NMR (300 MHz, CD_3OD) δ 8.58 (d, J = 5.8 Hz, 2H), 7.73−7.63 (m, 4H), 7.57 (d, J = 8.1 Hz, 2H), 7.50 (d, J = 8.1 Hz, 2H), 7.43 (d, J = 8.1 Hz, 2H), 4.49 (s, 2H), 4.28 (dd, J = 14.7, 7.6 Hz, 1H), 3.94 (dd, J = 14.8, 4.0 Hz, 1H), 3.75−3.45 (m, 2H), 3.40 (s, 3H), 3.35−3.25 (m, 3H), 2.91−2.80 (m, 1H), 2.15−1.94 (m, 1H), 1.70−1.55 (m, 1H), 1.37−1.23 (m, 1H), 1.19−1.10 (m, 3H); MS (ESI) free base m/z 460.4 $[M + H]^+$.

(±)-(1R,2R*)-N-[(2R,3R)-2-Amino-3-methoxybutyl]-N-[4′-(methoxymethyl)-[1,1′-biphenyl]-4-yl]-2-(pyrimidin-2-yl)cyclopropane-1-carboxamide Dihydrochloride (**20**).* Suzuki coupling of **16d** (85 mg) with 4-(methoxymethyl)phenylboronic acid was carried out using the procedure for **5f**, followed by Boc deprotection with 4 M HCl in dioxane as described for **6**, to afford **20** (74 mg, 85% yield) as a light-yellow solid: ^{1}H NMR (300 MHz, CD_3OD) δ 8.67−8.51 (m, 2H), 7.76−7.52 (m, 4H), 7.43 (d, J = 7.4 Hz, 4H), 7.26 (s, 1H), 4.50 (s, 2H), 4.30−4.10 (m, 1H), 4.05−3.87 (m, 1H), 3.78−3.46 (m, 2H), 3.40 (s, 3H), 3.34 (s, 3H), 2.87−2.70 (m, 1H), 2.20−2.09 (m, 1H), 1.83−1.71 (m, 1H), 1.64−1.48 (m, 1H), 1.20−1.04 (m, 3H); MS (ESI) free base m/z 461.2 $[M + H]^+$.

(±)-(1R,2R*)-N-[(2R,3R)-2-Amino-3-methoxybutyl]-N-[4′-(methoxymethyl)-[1,1′-biphenyl]-4-yl]-2-(pyrazin-2-yl)cyclopropane-1-carboxamide Dihydrochloride (**21**).* Suzuki coupling of **16e** (100 mg) with 4-(methoxymethyl)phenylboronic acid was carried out using the procedure for **5f**, followed by Boc deprotection with 4 M HCl in dioxane as described for **6**, to afford **21** (63 mg, 61% yield) as a light-yellow solid: ^{1}H NMR (300 MHz, CD_3OD) δ 8.68−8.52 (m, 1H), 8.39−8.23 (m, 2H), 7.74−7.51 (m, 4H), 7.43 (d, J = 7.5 Hz, 4H), 4.50 (s, 2H), 4.35−4.06 (m, 1H), 4.00−3.87 (m, 1H), 3.70−3.43 (m, 2H), 3.40 (s, 3H), 3.34 (s, 3H), 2.89−2.59 (m, 1H), 2.12−1.98 (m, 1H), 1.81−1.66 (m, 1H), 1.63−1.42 (m, 1H), 1.22−1.05 (m, 3H); MS (ESI) free base m/z 461.2 $[M + H]^+$.

(±)-(1R,2R*)-N-[(2R,3R)-2-Amino-3-methoxybutyl]-N-[4′-(methoxymethyl)-[1,1′-biphenyl]-4-yl]-2-(thiophen-2-yl)cyclopropane-1-carboxamide Hydrochloride (**22**).* Suzuki coupling of **16f** (300 mg) with 4-(methoxymethyl)phenylboronic acid was carried out using the procedure for **5f**, followed by Boc deprotection with 4 M HCl in dioxane as described for **6**, to afford **22** (121 mg, 42% yield) as a light-brown solid: ^{1}H NMR (300 MHz, CD_3OD) δ 7.72 (dd, J = 8.0, 3.8 Hz, 2H), 7.60 (dd, J = 8.1, 2.3 Hz, 2H), 7.50 (d, J = 7.3 Hz, 2H), 7.43 (d, J = 8.0 Hz, 2H), 7.10 (dd, J = 5.0, 4.0 Hz, 1H), 6.90−6.76 (m, 1H), 6.76−6.61 (m, 1H), 4.50 (s, 2H), 4.25 (td, J = 14.2, 7.5 Hz, 1H), 3.94 (td, J = 15.1, 3.9 Hz, 1H), 3.74−3.44 (m, 2H), 3.40 (s, 3H), 3.34 (s, 3H), 2.77−2.60 (m, 1H), 1.70 (dd, J = 8.1, 3.8 Hz, 2H), 1.34−1.19 (m, 1H), 1.16 (d, J = 6.0, 1.5H), 1.14 (d, J = 6.0, 1.5H); MS (ESI) free base m/z 465.2 $[M + H]^+$.

(±)-(1R,2R*)-N-[(2R,3R)-2-Amino-3-methoxybutyl]-N-[4′-(methoxymethyl)-[1,1′-biphenyl]-4-yl]-2-(thiophen-3-yl)cyclopropane-1-carboxamide Hydrochloride (**23**).* Suzuki coupling of **16g** (200 mg) with 4-(methoxymethyl)phenylboronic acid was carried out using the procedure for **5f**, followed by Boc deprotection with 4 M HCl in dioxane as described for **6**, to afford **23** (82 mg, 43% yield) as a light-yellow solid: ^{1}H NMR (300 MHz, CD_3OD) δ 7.74−7.63 (m, 2H), 7.59 (dd, J = 8.1, 2.7 Hz, 2H), 7.51−7.35 (m, 4H), 7.24 (dd, J = 7.7, 3.3 Hz, 1H), 6.95−6.88 (m, 1H), 6.72 (t, J = 5.9 Hz, 1H), 4.50 (s, 2H), 4.23 (td, J = 14.1, 7.4 Hz, 1H), 3.94 (td, J = 14.5, 4.2 Hz, 1H), 3.77−3.45 (m, 2H), 3.40 (s, 3H), 3.34 (s, 3H), 2.60−2.27 (m, 1H), 1.69−1.59 (m, 2H), 1.25−1.16 (m, 1H), 1.15 (d, J = 6.0, 1.5H), 1.14 (d, J = 6.0, 1.5H); MS (ESI) free base m/z 465.2 $[M + H]^+$.

(±)-(1R,2R*)-N-[(2R,3R)-2-Amino-3-methoxybutyl]-2-(6-fluoropyridin-2-yl)-N-[4′-(methoxymethyl)-[1,1′-biphenyl]-4-yl]-cyclopropane-1-carboxamide Dihydrochloride (**24**).* Suzuki coupling of **16h** (54 mg) with 4-(methoxymethyl)phenylboronic acid was carried out using the procedure for **5f**, followed by Boc deprotection with 4 M HCl in dioxane as described for **6**, to afford **24** (38 mg, 69% yield) as an off-white solid: ^{1}H NMR (300 MHz, CD_3OD) δ 7.75 (dd, J = 15.8, 8.0 Hz, 1H), 7.65 (d, J = 8.0 Hz, 2H), 7.57 (d, J = 8.1 Hz, 2H), 7.42 (d, J = 7.9 Hz, 4H), 7.19 (d, J = 7.4 Hz, 1H), 6.74 (d, J = 8.0 Hz, 1H), 4.50 (s, 2H), 4.23 (dd, J = 14.6, 7.3 Hz, 1H), 3.93 (d, J = 14.6 Hz, 1H), 3.68−3.45 (m, 2H), 3.40 (s, 3H), 3.37−3.20 (m, 3H), 2.67−2.54 (m, 1H), 2.05−1.92 (m, 1H), 1.75−1.61 (m, 1H), 1.49−1.34 (m, 1H), 1.17 (d, J = 6.0, 1.5H), 1.14 (d, J = 6.0, 1.5H); MS (ESI) free base m/z 478.2 $[M + H]^+$.

(±)-(1R,2R*)-N-[(2R,3R)-2-Amino-3-methoxybutyl]-2-(5-fluoropyridin-2-yl)-N-[4′-(methoxymethyl)-[1,1′-biphenyl]-4-yl]-cyclopropane-1-carboxamide Dihydrochloride (**25**).* Suzuki coupling of **16i** (107 mg) with 4-(methoxymethyl)phenylboronic acid was carried out using the procedure for **5f**, followed by Boc deprotection with 4 M HCl in dioxane as described for **6**, to afford **25** (68 mg, 62% yield) as an off-white solid: ^{1}H NMR (300 MHz, CD_3OD) δ 8.44−8.32 (m, 1H), 7.80−7.64 (m, 3H), 7.64−7.54 (m, 2H), 7.53−7.36 (m, 5H), 4.50 (s, 2H), 4.37−4.15 (m, 1H), 4.06−3.83 (m, 1H), 3.77−3.44 (m, 2H), 3.40 (s, 3H), 3.34 (s, 1.5H), 3.32

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(s, 1.5H), 2.88−2.73 (m, 1H), 2.08−1.97 (m, 1H), 1.83−1.69 (m, 1H), 1.57−1.41 (m, 1H), 1.15 (d, J = 6.0, 1.5H), 1.13 (d, J = 6.0, 1.5H); MS (ESI) free base m/z 478.2 $[M + H]^+$.

(±)-(1R,2R*)-N-[(2R,3R)-2-Amino-3-methoxybutyl]-2-(4-fluoropyridin-2-yl)-N-[4′-(methoxymethyl)-[1,1′-biphenyl]-4-yl]-cyclopropane-1-carboxamide Dihydrochloride (**26**).* Suzuki coupling of **16j** (107 mg) with 4-(methoxymethyl)phenylboronic acid was carried out using the procedure for **5f**, followed by Boc deprotection with 4 M HCl in dioxane as described for **6**, to afford **26** (93 mg, 85% yield) as an off-white solid: ^{1}H NMR (300 MHz, CD_3OD) δ 8.51−8.32 (m, 1H), 7.76−7.62 (m, 2H), 7.62−7.53 (m, 2H), 7.52−7.45 (m, 2H), 7.45−7.36 (m, 2H), 7.32−7.14 (m, 2H), 4.47 (s, 2H), 4.36−4.13 (m, 1H), 4.04−3.80 (m, 1H), 3.65−3.45 (m, 2H), 3.38 (s, 3H), 3.35−3.27 (m, 3H), 2.85−2.74 (m, 1H), 2.17−2.02 (m, 1H), 1.84−1.71 (m, 1H), 1.59−1.47 (m, 1H), 1.15−1.08 (m, 3H); MS (ESI) free base m/z 478.2 $[M + H]^+$.

(±)-(1R,2R*)-N-[(2R,3R)-2-Amino-3-methoxybutyl]-2-(3-fluoropyridin-2-yl)-N-[4′-(methoxymethyl)-[1,1′-biphenyl]-4-yl]-cyclopropane-1-carboxamide Dihydrochloride (**27**).* Suzuki coupling of **16k** (107 mg) with 4-(methoxymethyl)phenylboronic acid was carried out using the procedure for **5f**, followed by Boc deprotection with 4 M HCl in dioxane as described for **6**, to afford **27** (104 mg, 95% yield) as a white solid: ^{1}H NMR (300 MHz, CD_3OD) δ 8.16−8.03 (m, 1H), 7.73−7.50 (m, 5H), 7.49−7.37 (m, 4H), 7.35−.17 (m, 1H), 4.49 (s, 2H), 4.37−4.15 (m, 1H), 4.02−3.86 (m, 1H), 3.66−3.44 (m, 2H), 3.40 (s, 1.5H), 3.39 (s, 1.5H), 3.37−3.30 (m, 3H), 3.02−2.85 (m, 1H), 2.21−2.07 (m, 1H), 1.80−1.66 (m, 1H), 1.59−1.43 (m, 1H), 1.14 (d, J = 6.0 Hz, 3H); MS (ESI) free base m/z 478.2 $[M + H]^+$.

(±)-(1R,2R*)-N-[(2R,3R)-2-Amino-3-methoxybutyl]-N-[4′-(methoxymethyl)-[1,1′-biphenyl]-4-yl]-2-(6-methylpyridin-2-yl)-cyclopropane-1-carboxamide Dihydrochloride (**28**).* Suzuki coupling of **16l** (100 mg) with 4-(methoxymethyl)phenylboronic acid was carried out using the procedure for **5f**, followed by Boc deprotection with 4 M HCl in dioxane as described for **6**, to afford **28** (65 mg, 63% yield) as a white solid: ^{1}H NMR (300 MHz, CD_3OD) δ 8.15−8.06 (m, 1H), 7.74−7.65 (m, 2H), 7.64−7.50 (m, 5H), 7.49−7.33 (m, 2H), 7.23 (t, J = 8.8 Hz, 1H), 4.47 (s, 2H), 4.38−4.08 (m, 0.5H), 4.23−4.12 (m, 0.5H), 4.08−4.00 (m, 0.5H), 3.90−3.82 (m, 0.5H), 3.62−3.44 (m, 2H), 3.38 (s, 3H), 3.32 (s, 1.5H), 3.31 (s, 1.5H), 3.02−2.88 (m, 1H), 2.72 (s, 1.5H), 2.70 (s, 1.5H), 2.15−1.99 (m, 1H), 1.93−1.80 (m, 1H), 1.66−1.56 (m, 1H), 1.14 (d, J = 6.0 Hz, 1.5H), 1.09 (d, J = 6.0 Hz, 1.5H); MS (ESI) free base m/z 474.2 $[M + H]^+$.

*(1R,2R)-N-[(2R,3R)-2-Amino-3-methoxybutyl]-2-(6-fluoropyridin-2-yl)-N-[4′-(propan-2-yloxy)-[1,1′-biphenyl]-4-yl]cyclopropane-1-carboxamide Dihydrochloride (**29a**).* Suzuki coupling of (1*R*,2*R*)-**16h** (107 mg) with 4-isopropoxyphenylboronic acid was carried out using the procedure for **5f**, followed by Boc deprotection with 4 M HCl in dioxane as described for **6**, to afford **29a** (63 mg, 56% yield) as a white solid: ^{1}H NMR (300 MHz, CD_3OD) δ 7.75 (q, J = 8.0 Hz, 1H), 7.59 (d, J = 7.9 Hz, 2H), 7.49 (d, J = 8.7 Hz, 2H), 7.39 (d, J = 8.1 Hz, 2H), 7.19 (dd, J = 5.5, 2.0 Hz, 1H), 6.97 (d, J = 8.7 Hz, 2H), 6.74 (dd, J = 8.1, 2.1 Hz, 1H), 4.64 (dt, J = 12.1, 6.0 Hz, 1H), 4.22 (dd, J = 14.7, 7.3 Hz, 1H), 3.93 (dd, J = 14.7, 4.0 Hz, 1H), 3.57−3.41 (m, 1H), 3.32 (s, 3H), 3.32−3.23 (m, 1H), 2.65−2.54 (m, 1H), 2.04−1.94 (m, 1H), 1.72−1.62 (m, 1H), 1.47−1.38 (m, 1H), 1.33 (d, J = 6.0 Hz, 6H), 1.14 (d, J = 6.1 Hz, 3H); ^{13}C NMR (75 MHz, CD_3OD) δ 175.4, 164.8 (d, J_{C-F} = 237.0 Hz), 159.8 (d, J_{C-F} = 12.8 Hz), 159.4, 143.0 (d, J_{C-F} = 8.3 Hz), 142.5, 141.6, 133.2, 129.4, 129.1, 129.0, 120.70 (d, J_{C-F} = 3.8 Hz), 117.4, 107.7 (d, J_{C-F} = 36.8 Hz), 75.0, 71.1, 57.4, 56.9, 51.0, 28.1, 26.2, 22.4, 17.9, 15.6; MS (ESI) free base m/z 492.2 $[M + H]^+$; HPLC t_R 15.2 min.

*(1S,2S)-N-[(2R,3R)-2-Amino-3-methoxybutyl]-2-(6-fluoropyridin-2-yl)-N-[4′-(propan-2-yloxy)-[1,1′-biphenyl]-4-yl]cyclopropane-1-carboxamide Dihydrochloride (**29b**).* Suzuki coupling of (1*S*,2*S*)-**16h** (107 mg) with 4-isopropoxyphenylboronic acid was carried out using the procedure for **5f**, followed by Boc deprotection with 4 M HCl in dioxane as described for **6**, to afford **29b** (61 mg, 54% yield) as a white solid: ^{1}H NMR (300 MHz, CD_3OD) δ 7.76 (dd, J = 15.7, 8.0 Hz, 1H), 7.57 (d, J = 7.8 Hz, 2H), 7.48 (d, J = 8.7 Hz, 2H), 7.39 (d, J = 8.3 Hz, 2H), 7.21 (dd, J = 7.2, 2.0 Hz, 1H), 6.97 (d, J = 8.7 Hz, 2H), 6.75 (dd, J = 8.0, 2.3 Hz, 1H), 4.64 (dt, J = 12.0, 6.0 Hz, 1H), 4.25 (dd, J = 14.7, 7.7 Hz, 1H), 3.90 (dd, J = 14.7, 4.2 Hz, 1H), 3.57−3.39 (m, 1H), 3.31 (s, 3H), 3.30−3.21 (m, 1H), 2.70−2.55 (m, 1H), 2.04−1.88 (m, 1H), 1.71−1.56 (m, 1H), 1.48−1.36 (m, 1H), 1.33 (d, J = 6.0 Hz, 6H), 1.15 (d, J = 6.2 Hz, 3H); ^{13}C NMR (75 MHz, CD_3OD) δ 175.4, 164.8 (d, J_{C-F} = 237.0 Hz), 159.8 (d, J_{C-F} = 12.8 Hz), 159.4, 143.0 (d, J_{C-F} = 7.5 Hz), 142.5, 141.4, 133.2, 129.3, 129.1, 129.0, 120.70 (d, J_{C-F} = 3.8 Hz), 117.4, 107.7 (d, J_{C-F} = 36.8 Hz), 75.0, 71.1, 57.2, 56.8, 51.0, 28.2, 26.2, 22.4, 17.7, 15.6; MS (ESI) free base m/z 492.2 $[M + H]^+$; HPLC t_R 15.3 min.

*(1R,2R)-N-[(2R,3R)-2-Amino-3-methoxybutyl]-2-(5-fluoropyridin-2-yl)-N-[4′-(propan-2-yloxy)-[1,1′-biphenyl]-4-yl]cyclopropane-1-carboxamide Dihydrochloride (**30a**).* Suzuki coupling of (1*R*,2*R*)-**16i** (107 mg) with 4-isopropoxyphenylboronic acid was carried out using the procedure for **5f**, followed by Boc deprotection with 4 M HCl in dioxane as described for **6**, to afford **30a** (96 mg, 85% yield) as a white solid: ^{1}H NMR (300 MHz, CD_3OD) δ 8.32 (s, 1H), 7.73−7.55 (m, 3H), 7.55−7.27 (m, 5H), 6.97 (d, J = 8.1 Hz, 2H), 4.64 (dt, J = 11.5, 5.8 Hz, 1H), 4.29−4.07 (m, 1H), 3.98 (d, J = 14.1 Hz, 1H), 3.59−3.46 (m, 1H), 3.32 (s, 3H), 3.32−3.23 (m, 1H), 2.82−2.69 (m, 1H), 2.11−1.97 (m, 1H), 1.70−1.66 (m, 1H), 1.56−1.42 (m, 1H), 1.33 (d, J = 5.9 Hz, 6H), 1.13 (d, J = 4.7 Hz, 3H); ^{13}C NMR (75 MHz, CD_3OD) δ 174.9, 160.0 (d, J_{C-F} = 250.5 Hz), 159.4, 155.9 (d, J_{C-F} = 3.8 Hz), 142.5, 141.5, 136.4 (d, J_{C-F} = 27.0 Hz), 133.0, 129.5, 129.1, 129.0, 127.6 (d, J_{C-F} = 18.8 Hz), 125.2 (d, J_{C-F} = 4.5 Hz), 117.4, 75.0, 71.1, 57.4, 56.9, 51.1, 27.1, 26.3, 22.4, 18.1, 15.6; MS (ESI) free base m/z 492.2 $[M + H]^+$; HPLC t_R 14.6 min.

*(1S,2S)-N-[(2R,3R)-2-Amino-3-methoxybutyl]-2-(5-fluoropyridin-2-yl)-N-[4′-(propan-2-yloxy)-[1,1′-biphenyl]-4-yl]cyclopropane-1-carboxamide Dihydrochloride (**30b**).* Suzuki coupling of (1*S*,2*S*)-**16i** (107 mg) with 4-isopropoxyphenylboronic acid was carried out using the procedure for **5f**, followed by Boc deprotection with 4 M HCl in dioxane as described for **6**, to afford **30b** (97 mg, 86% yield) as a white solid: ^{1}H NMR (300 MHz, CD_3OD) δ 8.23 (s, 1H), 7.69−7.30 (m, 8H), 6.97 (d, J = 8.5 Hz, 2H), 4.64 (dt, J = 12.0, 6.0 Hz, 1H), 4.28 (dd, J = 14.6, 7.5 Hz, 1H), 3.89 (d, J = 14.0 Hz, 1H), 3.56−3.44 (m, 1H), 3.32 (s, 3H), 3.32−3.23 (m, 1H), 2.78−2.68 (m, 1H), 2.04−1.93 (m, 1H), 1.72−1.62 (m, 1H), 1.48−1.38 (m, 1H), 1.33 (d, J = 6.0 Hz, 6H), 1.15 (d, J = 5.8 Hz, 3H); ^{13}C NMR (75 MHz, CD_3OD) δ 175.3, 159.9 (d, J_{C-F} = 250.5 Hz), 159.4, 156.2 (d, J_{C-F} = 3.0 Hz), 142.5, 141.5, 137.3 (d, J_{C-F} = 25.5 Hz), 133.0, 129.5, 129.1, 129.0, 125.9 (d, J_{C-F} = 18.0 Hz), 124.8 (d, J_{C-F} = 4.5 Hz), 117.4, 75.0, 71.1, 57.3, 56.9, 51.0, 27.7, 26.1, 22.4, 17.8, 15.6; MS (ESI) free base m/z 492.2 $[M + H]^+$; HPLC t_R 15.1 min.

Pharmacology. *Materials.* Cell culture materials and general assay consumables were purchased from Fisher SSI. Forskolin, GDP, GTPγS, and binding buffer reagents were purchased from Sigma-Aldrich. The Lance *Ultra* kit (TRF0262), cAMP 96-well plates, GF/C filter plates, and [^{35}S]GTPγS were purchased from PerkinElmer.

Lance Ultra cAMP Assay Using Stable PPLS-HA-GPR88 CHO Cells. All cAMP assays were performed using our previously published methods.[12] Stimulation buffer containing 1× Hank's balanced salt solution (HBSS), 5 mM HEPES, 0.1% BSA stabilizer, and 0.5 mM final IBMX was prepared and titrated to pH 7.4 at room temperature. Serial dilutions of the test compounds (5 μL) and 300 nM forskolin (5 μL), both prepared at 4 times the desired final concentration in 2% DMSO/stimulation buffer, were added to a 96-well white 1/2 area microplate (PerkinElmer). A cAMP standard curve was prepared at 4 times the desired final concentration in stimulation buffer, and 5 μL was added to the assay plate. Stable PPLS-HA-GPR88 CHO cells were lifted with versene and spun at 270*g* for 10 min. The cell pellet was resuspended in stimulation buffer, and 4000 cells (10 μL) were added to each well, except for wells containing the cAMP standard curve. After incubation for 30 min at room temperature, Eu-cAMP tracer and u*LIGHT-anti-cAMP* working solutions were added per the manufacturer's instructions. After incubation at room temperature for 1 h, the TR-FRET signal (excitation at 337 nm) was read on a CLARIOstar multimode plate reader (BMG Biotech, Cary, NC).

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[^{35}S]GTPγS Binding Assay Using PPLS-HA-hGPR88-CHO Membranes. Membranes were prepared from our previously described PPLS-HA-hGPR88-CHO cells.[12] Cells were grown in culture dishes in DMEM/F12 supplemented with 10% FBS, 1× penicillin/streptomycin, and 400 μg/mL G418. Confluent monolayers of cells were lysed for 20 min in cold lysis buffer [2 mM HEPES (pH 7.4), 2 mM EDTA, 1 mM DTT, and 0.7× Halt protease inhibitor cocktail], pooled, and homogenized using a Wheaton glass homogenizer (15 strokes). The homogenate was centrifuged at 30000*g* for 20 min at 4 °C. The resulting pellet was resuspended in ice-cold storage buffer [50 mM HEPES (pH 7.4), 0.32 M sucrose, and 0.7× Halt protease inhibitor]. Aliquots were snap-frozen in liquid nitrogen and stored at −80 °C. The protein concentration was determined using a BCA assay. The assay was conducted in matrix tubes with the following components: 100 μL of assay buffer [20 mM HEPES (pH 7.4), 3 mM $MgCl_2$, 100 mM NaCl, and 0.1% BSA], 50 μL of compound serial dilutions (prepared at 10× in 10% DMSO/assay buffer), 50 μL of 10 μM GDP (prepared at 10× in assay buffer), 50 μL of 0.2 nM [^{35}S]GTPγS (prepared at 10× in assay buffer), and 250 μL of PPLS-HA-hGPR88-CHO membranes (20 μg/well). Nonspecific binding (NSB) was determined in the presence of 10 μM GTPγS (prepared at 10× in assay buffer), and total binding (TB) was determined in the presence of 10% DMSO/assay buffer. The tubes were incubated at room temperature for 60 min. The assay was terminated by rapid filtration through Unifilter-96 GF/C plates (PerkinElmer) presoaked for 1 h in wash buffer [20 mM HEPES (pH 7.4), 3 mM $MgCl_2$, and 100 mM NaCl] and washed three times with 1 mL of cold wash buffer. The plates were dried, and 50 μL of Microscint 20 was added. Plates were counted using a Wallac MicroBeta TriLux (PerkinElmer).

[^{35}S]GTPγS Binding Assay Using Mouse Striatal Membranes. Striatal membranes from WT mice or GPR88 KO mice were prepared following our previously published methods.[26,27] Briefly, brains from WT mice were quickly removed after cervical dislocation, and the whole striatal region was dissected, frozen, and stored at −80 °C until use. Membranes were prepared by homogenizing brain samples in an ice-cold 0.25 M sucrose solution (10 volumes, milliliters per gram of wet weight of tissue). The obtained suspensions were then centrifuged at 2500*g* for 10 min. Supernatants were collected and diluted 10 times in buffer containing 50 mM Tris-HCl (pH 7.4), 3 mM $MgCl_2$, 100 mM NaCl, and 0.2 mM EGTA and then centrifuged at 23000*g* for 30 min. The pellets were homogenized in 800 μL of an ice-cold sucrose solution (0.32 M), aliquoted, and kept at −80 °C. The protein concentration was determined using a Bradford assay. The assay was conducted in matrix tubes with the following components: 100 μL of assay buffer [50 mM Tris-HCl (pH 7.4), 3 mM $MgCl_2$, 100 mM NaCl, 0.2 mM EGTA, and 0.1% BSA], 50 μL of compound serial dilutions (prepared at 10× in 1% DMSO/assay buffer), 50 μL of 30 μM GDP (prepared at 10× in assay buffer), 50 μL of 0.2 nM [^{35}S]GTPγS (prepared at 10× in assay buffer), and 250 μL of striatal membranes (2 μg/well). NSB was determined in the presence of 10 μM GTPγS (prepared at 10× in assay buffer), and TB was determined in the presence of 1% DMSO/assay buffer. Basal binding refers to binding in the absence of the agonist. The tubes were incubated at room temperature for 60 min. The assay was terminated by rapid filtration through Unifilter-96 GF/C plates (PerkinElmer) presoaked for 1 h in wash buffer [25 mM Tris-HCl (pH 7.4), 5 mM $MgCl_2$, and 50 mM NaCl] and washed three times with 1 mL of cold wash buffer. The plates were dried, and 50 μL of Microscint 20 was added. Plates were counted using a Wallac MicroBeta TriLux (PerkinElmer).

Data Analysis. All nonlinear regression analysis was performed using GraphPad Prism (GraphPad Software Inc., San Diego, CA). For the cAMP assays, the TR-FRET signal (665 nm) was converted to femtomoles of cAMP by interpolating from the standard cAMP curve. The number of femtomoles of cAMP was plotted against the log of the compound concentration, and data were fit to a three-parameter logistic curve to generate EC_{50} values. For the [^{35}S]GTPγS binding assays, the percent maximal binding was plotted against the log of the compound concentration and data were fit to a three-parameter logistic curve to generate EC_{50} values.

Kinetic Solubility Assay. The kinetic solubility of compound **30a** was measured in phosphate-buffered saline (PBS) at pH 7.4 (Paraza Pharma Inc., Montreal, QC). Ten microliters of a test compound stock solution (20 mM DMSO) was combined with 490 μL of PBS (1 mM potassium phosphate monobasic, 3 mM sodium phosphate dibasic, and 155 mM sodium chloride buffer) to reach a targeted concentration of 400 μM. The solution was agitated on a VX-2500 multitube vortexer (VWR) for 2 h at room temperature. Following agitation, the samples were filtered on a glass-fiber filter (1 μm), and the eluates were diluted 400-fold with a 1:1 acetonitrile/water mixture. On each experimental occasion, nicardipine and imipramine were assessed as reference compounds for low and high solubility, respectively. All samples were assessed in triplicate and analyzed by LC-MS/MS using electrospray ionization against standards prepared in the same matrix.

Plasma−Protein Binding Assay. The plasma−protein binding of compound **30a** was determined in C57BL/6 mouse plasma using the high-throughput dialysis (HTD) device (Paraza Pharma Inc.). Test compound and controls (1 μM) were spiked in Wistar rat plasma and aliquoted in triplicate in a HTD 96-well plate, where the plasma and dialysate buffer were separated by a semipermeable cellulose membrane. Once sealed, the HTD plate was incubated at 37 °C and kept under light agitation for 6 h, until equilibrium was reached. Plasma and buffer samples were then extracted along with their corresponding standard curve samples using an ice-cold 1:1 acetonitrile/methanol mixture. After centrifugation, supernatants from both plasma- and buffer-containing samples were further diluted prior to being submitted to bioanalysis by LC-MS/MS. Acebutolol and warfarin served as weakly and strongly bound controls, respectively.

Liver Microsomal Stability Assay. The liver microsomal stability assessment was performed by Paraza Pharma Inc. Compounds were incubated with mouse liver microsomes at 37 °C for a total of 45 min. The reaction was performed at pH 7.4 in 100 mM potassium phosphate buffer containing 0.5 mg/mL mouse liver microsomal protein. Phase I metabolism was assessed by adding NADPH to a final concentration of 1 mM and collecting samples at 0, 5, 15, 30, and 45 min. All collected samples were quenched with ice-cold stop solution (1 μM labetalol and 1 μM glyburide in acetonitrile) and centrifuged to remove precipitated protein. Resulting supernatants were further diluted with H_2O. Diphenhydramine ($t_{1/2}$ = 9.3 min, and CL = 148 μL min^{-1} mg^{-1}) and verapamil ($t_{1/2}$ = 2.2 min, and CL = 630 μL min^{-1} mg^{-1}) were used as reference compounds. Samples were analyzed by LC-MS/MS, and calculations for half-life and *in vitro* clearance were accomplished using Microsoft Excel (see the Supporting Information for calculation methods and examples of crude data).

Bidirectional MDCK-MDR1 Permeability Assay. MDCK-MDR1 cells at passage 12 were seeded onto permeable polycarbonate supports in 12-well Costar Transwell plates and allowed to grow and differentiate for 3 days. On day 3, culture medium (DMEM supplemented with 10% FBS) was removed from both sides of the transwell inserts, and cells were rinsed with warm HBSS. After the rinse step, the chambers were filled with warm transport buffer [HBSS containing 10 mM HEPES and 0.25% BSA (pH 7.4)], and the plates were incubated at 37 °C for 30 min prior to transepithelial electric resistance (TEER) measurements.

The buffer in the donor chamber (apical side for the A-to-B assay, basolateral side for the B-to-A assay) was removed and replaced with the working solution (10 μM test article in transport buffer). The plates were then placed at 37 °C under light agitation. At designated time points (30, 60, and 90 min), an aliquot of transport buffer from the receiver chamber was removed and replenished with fresh transport buffer. Reactions were quenched with ice-cold acetonitrile containing an internal standard and then centrifuged to pellet protein. The resulting supernatants were further diluted with a 50:50 acetonitrile/water mixture (water only for atenolol) and subjected to LC-MS/MS analysis. The reported apparent permeability (P_{app}) values were calculated from a single determination. Atenolol and propranolol were tested as low- and moderate-permeability references,

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respectively. Bidirectional transport of digoxin was assessed to demonstrate Pgp activity and/or expression.

The apparent permeability (P_{app}, measured in centimeters per second) of a compound was determined according to the formula $P_{app} = (dQ/dt)/(AC_i \times 60)$, where dQ/dt is the net rate of appearance in the receiver compartment, A is the area of the Transwell measured in square centimeters (1.12 cm^2), C_i is the initial concentration of the compound added to the donor chamber, and 60 is the factor for converting minutes to seconds.

Pharmacokinetic Analysis. Male C57BL/6NCrl mice were procured from Charles River Laboratories at 8−9 weeks of age. Animals were allowed to acclimate to the vivarium for a period of 1 week. Each mouse was administered a single 10 mg/kg dose of **30a** through i.p. injection in a 10 mL/kg dosing volume. Doses were formulated in saline. Three animals were sacrificed at each of the selected time points (0.25, 0.5, 1, 2, 4, 6, 8, and 24 h) postdose. Blood was collected through the abdominal vena cava for plasma analysis, and whole-body perfusion was performed with phosphate-buffered saline (pH 7.4). Brains were collected following perfusion.

Sample Preparations. First, 40 μL of plasma, 10 μL of MeCN, and 150 μL of 100 ng/mL Reserpine in MeCN with 0.1% formic acid were vortexed and centrifuged at 4000 rpm for 10 min. Then, 50 μL of the supernatant was diluted with 50 μL of H_2O prior to LC-MS/MS analysis. Brain was homogenized with a 50:50 EtOH/H_2O mixture (1:5, v/v) using a Geno Grinder. Then, 40 μL of the homogenate, 10 μL of MeCN, and 150 μL of 100 ng/mL Reserpine in MeCN with 0.1% formic acid were vortexed and centrifuged at 4000 rpm for 10 min. Finally, 50 μL of the supernatant was diluted with 50 μL of H_2O prior to LC-MS/MS analysis.

LC-MS/MS Analysis. LC-MS/MS was conducted using an Applied Biosystems API 4000 instrument coupled with an Agilent 1100 HPLC system. Chromatography was performed with a Phenomenex Synergi Hydro C18 (150 mm × 3 mm, 4 μm) column with a C18 guard cartridge. The mobile phases were (A) 0.1% formic acid in H_2O and (B) 0.1% formic acid in MeCN. The initial conditions were 25% B and held for 0.5 min, followed by a linear gradient to 80% B over 10 min, before returning to the initial conditions.

Drinking-in-the-Dark Experiment. Male C57BL/6J mice were obtained from Jackson Laboratories and individually housed under a 12 h reversed light/dark cycle. Animals were maintained and experiments conducted in accordance with guidelines approved by the Regional Committee of Ethics in Animals Experiment of Strasbourg (CREMEAP, APAFIS 32224-202106301444378). Oral alcohol intake was determined using the DID paradigm for 2 weeks. The procedure was adapted from ref 28. Briefly, each week, drinking sessions were conducted 2 h every day during three consecutive days and 4 h on the fourth and final day, with one bottle containing tap water and the other containing alcohol diluted to 20% alcohol (v/v) in tap water. The bottles were weighed every day, and the mice were weighed at the beginning of the experiment. Animals were injected with saline, RTI-13951-33 (30 mg/kg, i.p.), or compound **30a** (10 mg/kg, i.p.) 1 h before being tested in the 4 h drinking session.

Drugs. The alcohol solution was prepared from absolute ethanol (190 proof) diluted to 20% alcohol (v/v). RTI-13951-33 (30 mg/kg) or compound **30a** (10 mg/kg) was dissolved in sterile 0.9% saline to be administered i.p. at 10 mL/kg.

Data Analysis. Data were analyzed with one-way ANOVA (GraphPad Prism). The significant main effect of the ANOVAs was further investigated with the Newman−Keuls post hoc test. Statistical significance was set at the $p < 0.05$ level.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jmedchem.2c01983.

^{1}H NMR, ^{13}C NMR, and HPLC data of selected compounds (PDF)

Molecular formula strings with biological data (CSV)

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

ADME, absorption, distribution, metabolism, and excretion; cAMP, cyclic adenosine monophosphate; ANOVA, analysis of variance; AO, aldehyde oxidase; AUC, area under the curve; AUD, alcohol use disorder; BBB, blood−brain barrier; BSA, bovine serum albumin; CHO, Chinese hamster ovary; CL, clearance; cryo-EM, cryogenic electron microscopy; DCM, dichloromethane; DID, drinking-in-the-dark; DME, 1,2-dichloroethane; DMEM, Dulbecco's modified Eagle's medium; DMSO, dimethyl sulfoxide; DTT, dithiothreitol; EDTA, ethylenediaminetetraacetic acid; EGTA, ethylene glycol-bis-(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; ESI, electrospray ionization; FBS, fetal bovine serum; GDP, guanosine diphosphate; GPR88, G protein-coupled receptor 88; GTP, guanosine triphosphate; HA, human influenza hemagglutinin; HBSS, Hank's balanced salt solution; HEPES, *N*-(2-hydroxyethyl)piperazine-*N*′-(2-ethanesulfonic acid); hERG, human ether-a-go-go-related gene; HPLC, high-performance liquid chromatography; HTD, high-throughput dialysis; i.p.,

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intraperitoneal; KO, knockout; MDCK, Madin-Darby canine kidney; MDR1, multidrug resistance protein 1; MLM, mouse liver microsome; NADPH, nicotinamide adenine dinucleotide phosphate; NMR, nuclear magnetic resonance; NSB, nonspecific binding; MS, mass spectroscopy; PBS, phosphate-buffered saline; 2-PCCA, (1*R*,2*R*)-2-(pyridin-2-yl)-cyclopropane carboxylic acid [(2*S*,3*S*)-2-amino-3-methylpentyl]-(4′-propylbiphenyl-4-yl)amide; Pgp, P-glycoprotein; PK, pharmacokinetic; PPB, plasma−protein binding; PPLS, prolactin leader sequence; SAR, structure−activity relationship; SEM, standard error of the mean; TB, total binding; TEER, transepithelial electric resistance; TFA, trifluoroacetic acid; TLC, thin layer chromatography; TMS, trimethylsilyl; TR-FRET, time-resolved fluorescence energy transfer; WT, wild type.

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