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Discovery of Trifluoromethyl(pyrimidin-2-yl)azetidine-2-carboxamides as Potent, Orally Bioavailable TGR5 (GPBAR1) Agonists: Structure–Activity Relationships, Lead Optimization, and Chronic In Vivo Efficacy

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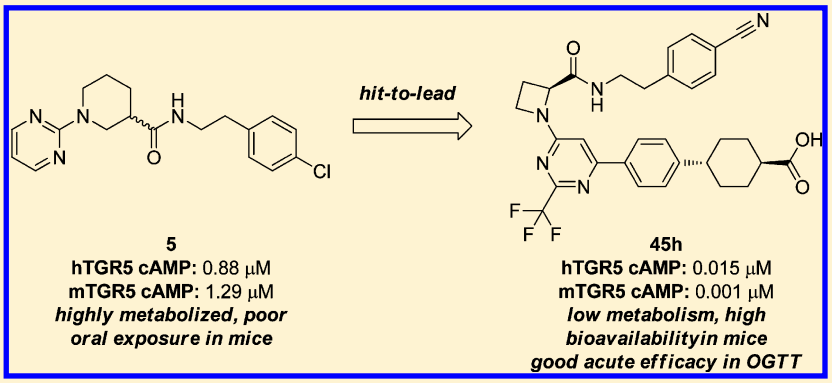

ABSTRACT: Activation of the G-protein coupled receptor (GPCR) Takeda G-protein receptor 5 (TGR5), also known as G-protein bile acid receptor 1 (GPBAR1), has been shown to play a key role in pathways associated with diabetes, metabolic syndrome, and autoimmune disease. Nipecotamide **5** was identified as an attractive starting point after a high-throughput screen (HTS) for receptor agonists. A comprehensive hit-to-lead effort culminated in the discovery of **45h** as a potent, selective, and bioavailable TGR5 agonist to test in preclinical metabolic disease models. In genetically obese mice (ob/ob), **45h** was as effective as a dipeptidyl peptidase-4 (DPP-4) inhibitor at reducing peak glucose levels in an acute oral glucose tolerance test (OGTT), but this effect was lost upon chronic dosing.

INTRODUCTION

TGR5 is a G_s-coupled GPCR activated by both primary and secondary bile acids, particularly taurolithocholic acid. The pattern of expression and ability to increase cAMP levels are key determinants of its biological role.[1] Activation of TGR5 leads to secretion of glucagon-like peptide-1 (GLP-1) and other related incretins in gut enteroendocrine cells[2] and to decreases in LPS Th1 cytokines like TNF-α and interleukin-12 (IL-12) in monocytes/macrophages[3] and dendritic cells.[4] Therefore, TGR5 appears to be an attractive target in the treatment of type 2 diabetes, where strategies to enhance GLP-1[5] and mitigate chronic inflammation[6] show clinical benefit. The efforts of several pharmaceutical and biotech companies toward identifying TGR5 agonists has recently been reviewed,[7] with no known compounds yet reaching the clinic. More recently, several new compounds have been reported from Hoffman-LaRoche,[8] SIMM,[9] and Pfizer[10] (Figure 1, compounds **1**–**4**). Herein, we report on our efforts to identify systemically bioavailable agonists and their effects in a chronic disease model.

CHEMISTRY

The goal of the research program was to identify orally bioavailable TGR5 agonists with activity on the human and mouse receptors to enable testing in disease-relevant preclinical animal models. An HTS of 1.5 million compounds was performed to identify starting points that were active on both human and mouse TGR5, as demonstrated by their ability to increase cellular cAMP in stable overexpressed cell lines. The majority of hits showed preferential activation of the human receptor, suggesting that the 83% sequence homology between

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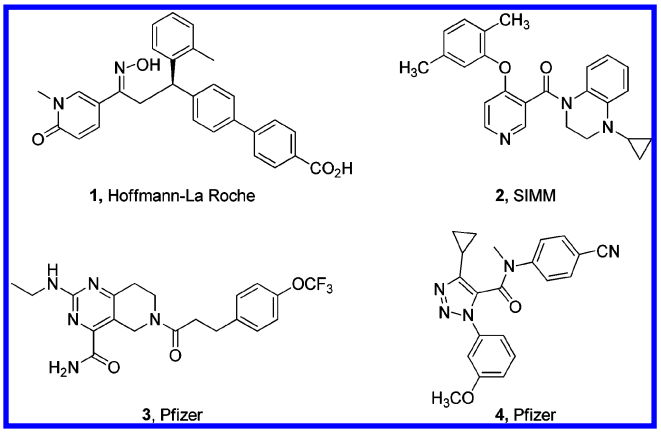
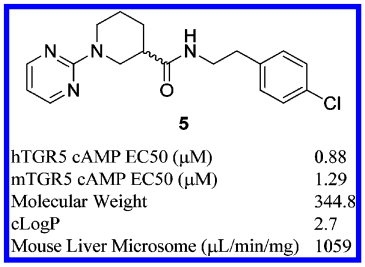


Figure 1. Reported TGR5 agonists.

human and mouse TGR5 was enough to confer significant ligand divergence. Furthermore, and perhaps not surprising given the endogenous ligand preference for lipophilic secondary bile acids, the majority of the hits were highly lipophilic compounds that were not ideal to initiate hit-to-lead efforts. However, nipecotamide **5** (Figure 2) was identified as an



5

hTGR5 cAMP EC50 (μM)	0.88
mTGR5 cAMP EC50 (μM)	1.29
Molecular Weight	344.8
cLogP	2.7
Mouse Liver Microsome (μL/min/mg)	1059

Figure 2. Profile of screening hit **5**.

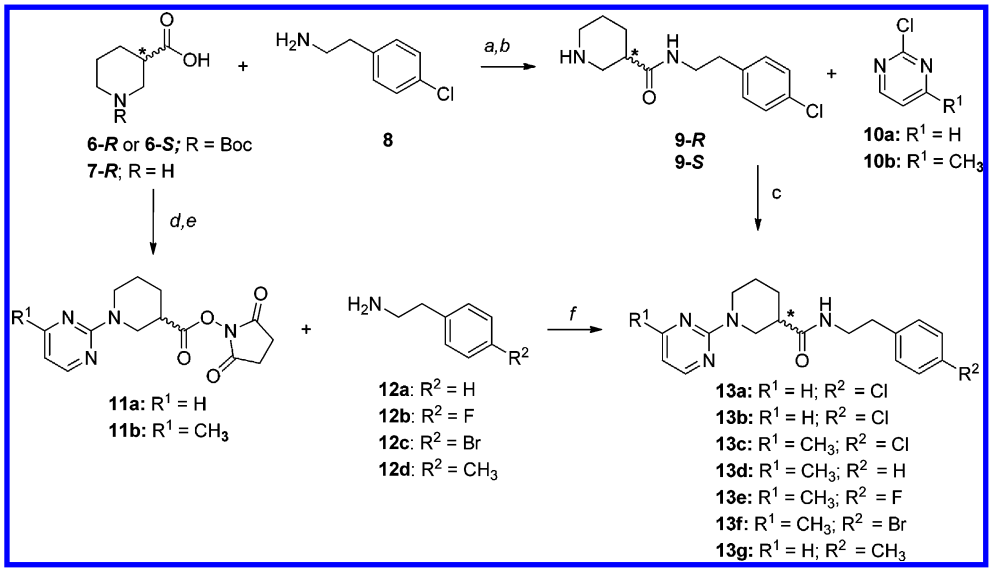
interesting starting point given its potency on both human (hTGR5) and mouse (mTGR5) isoforms, moderate molecular weight and clogP compared to other hit classes, and its potential synthetic tractability.

Because **5** was racemic, the initial objective of the hit-to-lead effort was to determine if the series exhibited any stereochemical preference. The syntheses began with combining the commercially available acids **6-*R*** and **6-*S*** with phenethylamine **8**, followed by Boc-deprotection to give intermediates **9-*R*** and **9-*S***. Addition to 2-chloropyrimidines **10a** and **10b** gave **13a−c**. Phenethylamine structure−activity relationships (SAR) were further expanded by treating **7-*R*** with **10a** or **10b**, then activating the acid as the *N*-hydroxysuccinimide ester (**11a** or **11b**), followed by treating with various phenyethylamines to yield **13d−g** (Scheme 1).

Synthesis of *N*-Heterocycles. After identifying the preferred stereochemistry of the piperidine-3-carboxamide and the substitution of the phenethyl side chain, we turned our attention toward investigating the SAR of the pyrimidine ring. The syntheses shown in Scheme 2 began with **9-*R*** or **15**, which was prepared via amide coupling of **14** with **6-*R***, followed by Boc-deprotection. Compounds **19a,c−g,i−j** were then prepared via nucleophilic aromatic substitution with the appropriate chloro-substituted heterocycle. Compound **19b** required a slightly different route that began with nucleophilic substitution with **9-*R*** on to **17**, followed by subsequent diazotization, conversion into the intermediate chloride, and Negishi coupling with methylzinc(II) chloride.[11] For compound **19h**, an alternative procedure was required due to the incompatibility of the *p*-cyano group under the Negishi conditions. Thus, **18** was treated with methyl magnesium-bromide in the presence of $Fe(acac)_3$, followed by nucleophilic substitution with **15**.[12]

In the next phase of the lead optimization effort, the *N*-methylpiperazine moiety was added as a potential solubilizing group to the methyl- and trifluoromethyl-substituted pyrimidine leads. The syntheses for the target compounds shown in Scheme 3 depended upon the desired position for the piperazine moiety relative to the nipecotamide core. Compounds **22a** and **22b** began with the nucleophilic addition of **20**

Scheme 1. Synthesis of (Pyrimidin-2-yl)piperidine-2-carboxamides[a]



[a]Reagents and conditions: (a) w/**6-*R*** or **6-*S***, HATU, DIEA; (b) TFA; (c) DIEA, iPrOH or DMA, 150 °C (d) w/**7-*R***, **10a** or **10b**, DIEA, iPrOH, 150 °C, EDCl, *N*-hydroxysuccinimide, DCM; (f) ACN, 25 °C. * denotes stereochemistry.

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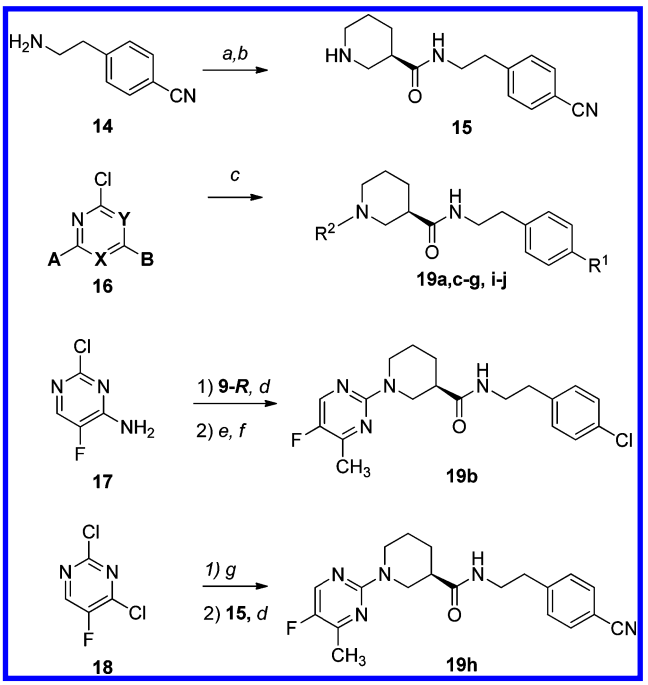
Scheme 2. Preparation of *N*-Heterocycle Analogues[a]



[a]Reagents and conditions: (a) (**6-*R***, EDCl, HOBt, DMF; (b) 4 M HCl in dioxane; (c) **9-*R*** or **15**, solvent DIEA, heat; (d) solvent, DIEA, heat; (e) tBuONO, $CuCl_2$, ACN, 0−25 °C; (f) $ClZnCH_3$, 5 mol % $Pd(PPh_3)_2Cl_2$, 25 °C; (g) CH_3MgBr, $Fe(acac)_3$, THF, 25 °C.

on to dichloropyrimidines **21a** and **21b**, followed by addition of piperidine **15** to yield the final compounds. The synthesis of compound **22c** began with the addition of **20** on to **21c** and then methylation via methyl magnesiumbromide and $Fe(acac)_3$.[12] The intermediate was subsequently treated with **15** to give the final compound. Finally, compounds **22d** and **22e** were prepared via nucleophilic aromatic substitutions of **15** on to **21d** or **21e** followed by the addition of **20**.

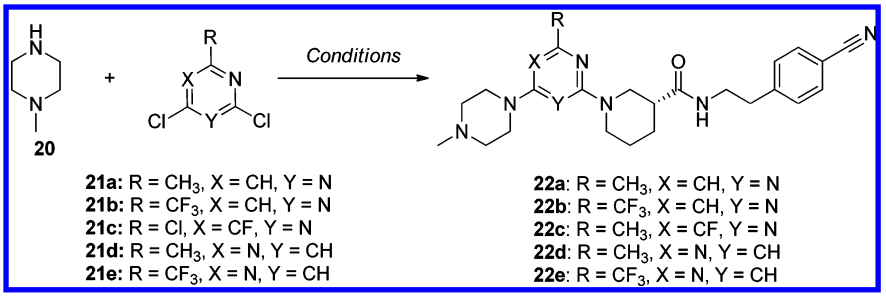
A strategy to reduce cLogP in the chemical series involved various substitutions on the piperazine ring and incorporating polar heterocycles as replacements for the phenyl group in the phenethyl side chain. In Scheme 4, piperidine **15** was added on to pyrimidine **21e** to yield the putative intermediate that was subsequently treated with Boc-piperazine, followed by deprotection to give **23a**. Subsequently, **23a** was subjected to reductive alkylation with 2-oxetanone to yield **23b**.

Next, we turned our attention to the installation of heterocycle replacements of the phenyl group. The synthesis shown in Scheme 4 of the pyridine side chain was accomplished in a one-pot procedure by nickel-assisted sodium borohydride reduction of nitrile **24** and concomitant Boc-protection.[13] Chloropyridine **25** was then subjected to palladium-catalyzed cyanation and Boc-deprotection to give **26**.[14] Compound **31a** was prepared in an analogous manner to **23a**, from **30** and **21e**; **31a** was then saponified to give **31b**. Amine **26** was coupled to **31b** and was followed by nucleophilic aromatic substitution with **20** to yield **32a**. Compound **32b** was prepared in a similar manner, **23a** from 1-Boc-piperazine, followed by deprotection. The synthesis of the pyrimidine isostere was accomplished via palladium-catalyzed cross-coupling of bromide **27** with potassium trifluoroborate **28**,[15] followed by Boc-deprotection to yield amine **29**. The 2-cyanopyrimidine was not stable to the nucleophilic aromatic substitution conditions, so **31a** was first treated with 1-ethylpiperazine, which was then saponified to yield the intermediate carboxylic acid and subjected to coupling with **29** to yield **32c**.

Ring size and position of the amide moiety was also investigated. Hence, the *R* and *S* piperidine-, azetidine-, and pyrrolidine-2-carboxylic acids were converted into the respective target compounds via HATU, EDCI, or T3P coupling with **14** or **26**, followed by Boc-deprotection, nucleophilic substitution on to **21e**, and then addition of a substituted piperazine to yield **34a−f**.

For the final phase of the lead optimization effort, a series of carboxylic acid-containing analogues were prepared and are summarized in Scheme 5. For the direct-linked acid analogues, nucleophilic substitution of **35a** and **35b** on to the desired chloropyrimidine intermediate **44**. The ether-linked analogues were derived from Mitsunobu reaction[6c] with alcohols **36a** and **36b** and phenols **37a** or **37b** to give the incipient intermediate, which was subsequently subjected to Boc- or Cbz-deprotection to yield piperidines **38a** and **38b**. The 4-phenylpiperidine intermediate **41** was synthesized via the Suzuki coupling[16] of pinacolborane **39** with phenylbromide **40**, hydrogenation of the alkene, and Boc-deprotection. The SAR investigation also included replacing the piperidine ring with a phenyl group. This was accomplished via conversion of phenylchlorides **42a** and **42b** into pinacolborane intermediates **43a** and **43b** with $Pd(dba)_2$ and PCy_3. Nucleophilic aromatic substitution of **44** with **38a**, **38b**, and **41**, followed by saponification, completed the syntheses of the acid-containing compounds **45a−f**. In an analogous fashion, Suzuki coupling with **43a** and **43b**, followed by saponification, yielded **45g** and **45h**, respectively.

Scheme 3. Preparation of Piperazine-Containing Analogues[a]



[a]Reagents and conditions: (a) w/**21a** (i) DIEA, EtOH, or ACN, 0−25 °C, (ii) **15**, DIEA, acetone/H_2O, 145 °C; (b) w/**21b** (i) DIEA, EtOH, or ACN, 0−25 °C, (ii) **15**, DIEA, NMP, 100 °C; (c) w/**21c** (i) DIEA, EtOH, or ACN, 0−25 °C, (ii) CH_3MgBr, $Fe(acac)_3$, THF, 25 °C, (iii) **15**, DIEA, DMF, 145 °C; (d) w/**21d** and **21e** (i) **15**, DIEA, iPrOH, 0−25 °C, (ii) **20**, ACN, 100 °C.

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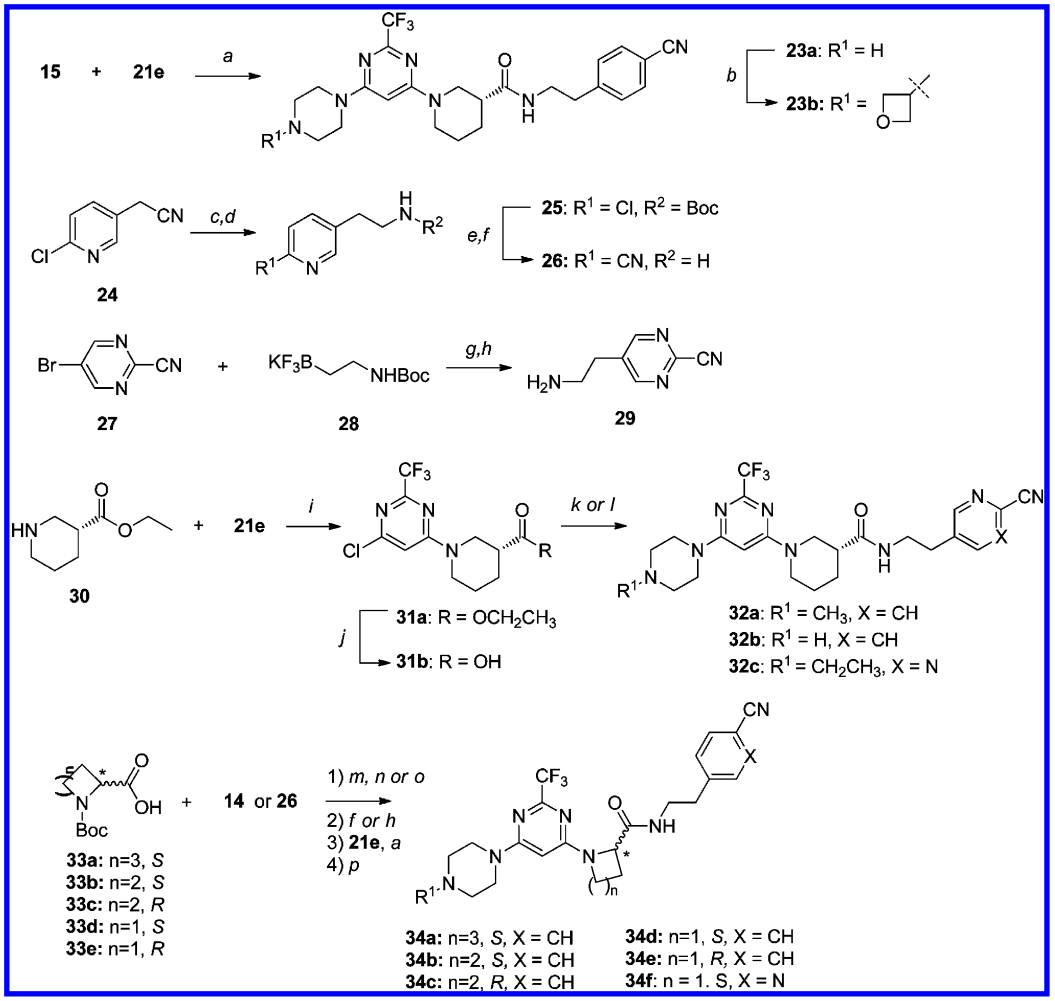
Scheme 4. Preparation of Core, Pyridylethyl, and Pyrimidylethyl Analogues[a]



[a]Reagents and conditions: (a) (i) ACN, DIEA, 0−25 °C, (ii) 1-Boc-piperazine, ACN, DIEA, 165 °C, (iii) 4 M HCl; (b) 2-oxetanone, $NaBH(OAc)_3$; (c) $NaBH_4$, $NiCl_2$−$6H_2O$; (d) Boc_2O; (e) $Pd(TFA)_2$, TrixiePhos, $Zn(CN)_2$, Zn; (f) 4 M HCl; (g) $Pd(OAc)_2$, RuPhos, Cs_2CO_3, 95 °C; (h) TFA; (i) 2-propanol, DIEA, 0−25 °C; (j) LiOH; (k) w/**31b** (1) **26**, HATU, (2) **20**, DMSO, 60 °C; (l) w/**31a** (1) 1-ethylpiperazine, 2-propanol, 80 °C, (2) LiOH, (3) **29**, HATU; (m) EDCI, HOBt, DIEA, DMF; (n) HATU, DIEA, DMF; (o) T3P; (p) R^1-piperazine, ACN or 2-propanol, heat.

RESULTS AND DISCUSSION

The percent efficacy for all compounds in this work were determined relative to a previously disclosed internal control that was found to be a potent and species cross-selective full agonist.[17] At the outset hit-to-lead effort, a clear preference for the *R*-enantiomer (**13a**) over the *S*-enantiomer (**13b**) was demonstrated, therefore all subsequent analogues in the nipecotamide series were made exclusively with this stereochemistry (Table 1). The addition of a methyl group to the pyrimidine ring at the 4-position (**13c**) yielded a ∼6-fold improvement in hTGR5 potency. The methyl-substituted pyrimidine was then employed as the heterocycle of choice to probe the SAR of the phenethyl amide region. The results showed that 4-Cl (**13c**) and 4-Br (**13f**) were preferred over 4-F (**13e**). Quite strikingly, nearly complete ablation of activity was observed by removal of the *p*-substituent (**13d**) and a loss of approximately 5-fold was observed by replacing the 4-Cl of **13a** with a 4-CH_3 (**13g**), suggesting perhaps that both lipophilicity and electronics play a key role in the activity in this series. Additional substitution patterns were also investigated, but *p*-substitution was strongly preferred and no clear advantage from substitution at other ring positions were observed.

The data in Table 2 show a strong preference for the (pyrimidin-2-yl)piperidine-3-carboxamide configuration. Among the 2-yl analogues, monosubstitution of the pyrimidine at the 5-position with fluorine (**19a**) was comparable to **13c**. Positing that the combination of the 5-fluoro and 4-methyl substituents could be additive led to **19b**, which showed improved potency by 10-fold and 4-fold on hTGR5 and mTGR5, respectively. By comparison, the pyrimidin-4-yl derivatives **19d** and **19e** were significantly less potent than their 2-yl congeners **13a** and **13c**.

We then turned our attention toward surveying substituted pyridines as replacements to the pyrimidine. While these compounds were generally less potent than their pyrimidine congeners, they did provide some insight into the potential binding mode for this series. First, the preference for the methyl substituent to be adjacent to the nitrogen on both heterocycles appeared to be important for gaining activity. Collectively, the matched pairs **13a**:**13c**, **19c**:**19f**, and **19d**:**19e**, yielded 6-, 4-, and 6-fold improvements, respectively, on hTGR5 when the methyl substituent was present adjacent to the ring nitrogen. By contrast, when the methyl substituent was located at the 4-position on the pyridine ring (**19g**), the compound was less

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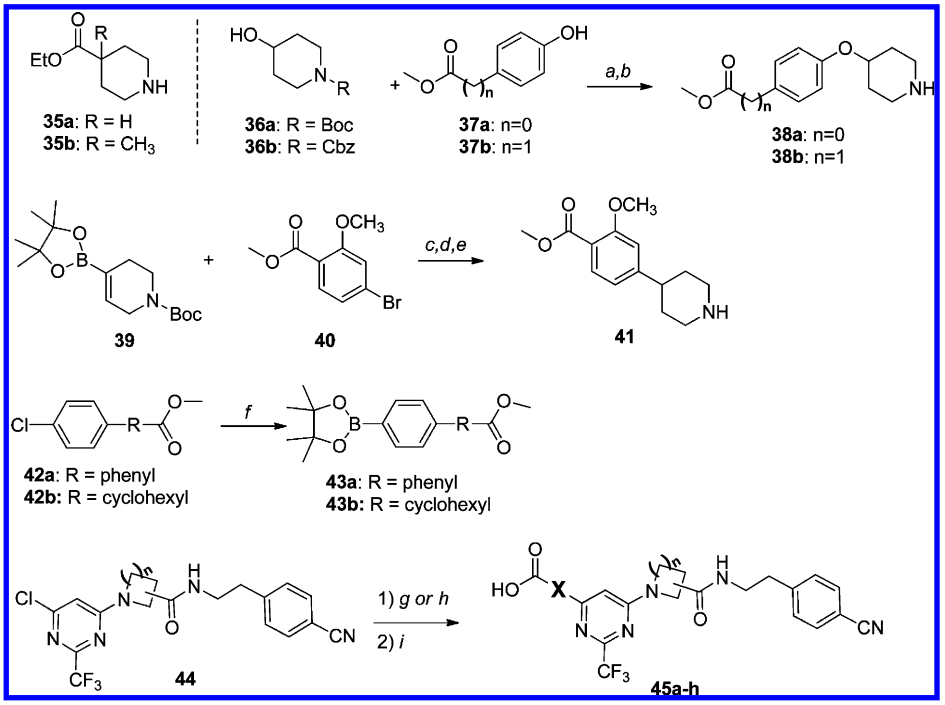
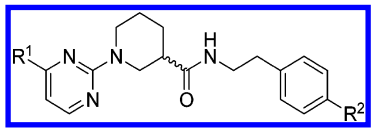
Scheme 5. Preparation of Acid-Containing Analogues[a]



[a]Reagents and conditions: (a) PPh_3, DIAD, THF; (b) w/**36a** 4 M HCl, w/**36b** H_2, Pd/C, EtOAc; (c) $Pd(PPh_3)_4$, $NaHCO_3$, dioxane; (d) H_2, Pd/C; (e) 4 M HCl/dioxane; (f) $Pd(dba)_2$, PCy_3, KOAc, dioxane; (g) **35a**, **35b**, **38a**, **38b**, or **41**, NMP, DIEA, 140 °C; (h) **43a** or **43b**, $Pd_2(dba)_3$, 5 M K_3PO_4, SPhos, dioxane; (i) 3 M LiOH.

Table 1. Initial SAR of Head, Tail, and Core Stereochemistry



			cAMP EC_{50} (μM)[a]	
no.	R^1	R^2	hTGR5	mTGR5
5[b]	H	Cl	0.88 ± 23	1.29 ± 32
13a[c]	H	Cl	0.35 ± 12	0.28 ± 16
13b[d]	H	Cl	>80	>80
13c[c]	CH_3	Cl	0.06 ± 0.01	0.14 ± 0.02
13d[c]	CH_3	H	5.8 ± 1.8	26.4 ± 8.9
13e[c]	CH_3	F	0.32 ± 0.06	0.75 ± 0.15
13f[c]	CH_3	Br	0.033 ± 0.002	0.088 ± 0.03
13g[c]	H	CH_3	4.3 ± 0.68	2.17 ± 0.55

[a]EC_{50} values are the average of at least two assay runs performed in triplicate ± SD; unless otherwise noted, all compounds are 100% full agonists relative to an internal control (see Materials and Methods). [b]Racemic. [c]*R*-Enantiomer. [d]*S*-Enantiomer.

potent by 15-fold and 35-fold on hTGR5 and mTGR5, respectively, compared to **13c**. Compound **19g** was also 4-fold and 7-fold less potent on hTGR5 and mTGR5 compared to pyridine **19f**. It seemed that retaining the methyl group adjacent to the nitrogen was key for maintaining favorable activity on both receptors.

When considering the mTGR5 SAR closely, it was apparent that compounds having a heterocycle ring nitrogen on the opposite face (N3) of the piperidine-3-carboxamide improved mTGR5 selectivity but at the expense of hTGR5 potency. SAR analysis of compounds **19e**, **19f**, and **13c** confirmed that the (pyrimidin-4-yl)piperidine-3-carboxamide subtype was more selective for mTGR5 than the (pyrimidin-2-yl)piperidine-3-carboxamide subtype; this was particularly evident with the 4-(trifluoromethyl)pyrimidine (**19i**) and 2(trifluoromethyl)-pyrimdine (**19j**) derivatives. Compounds **19i** and **19j** showed selectivity for hTGR5 and mTGR5 that was similar to their methyl counterparts **13c** and **19e**, respectively. While **19j** was weaker by approximately 2-fold on hTGR5 than **13c**, the nearly 6-fold improvement on mTGR5 was a desirable property that we were optimizing for as well. An important distinction for both **19i** and **19j** was the replacement of the 4-chloro group with a nitrile, R^1 = CN. This modification provided a nearly equipotent (**19h** vs **19b**) alternative to the R^1 = Cl analogues, but with lower cLogP. Moreover, the 4-trifluoromethyl (**19i**) and 2-trifluoromethyl (**19j**) pyrimidine substitution provided new optimization nodes with good hTGR5 and mTGR5 cAMP activity from which to explore further. While good potency and reliable SAR were achievable in the series to this point, compounds with acceptable solubility and metabolic stability remained elusive.

The SAR for compounds **22a**–**e** in Table 3 clearly showed a preference for the trifluoromethyl substituent, especially in combination with the piperazine group. Both **22b** and **22e** provided leads with low single-digit nanomolar potency on hTGR5 and mTGR5. All of the compounds in Table 3 were assayed in a high-throughput solubility assay at pH 6.8, and only **22b** showed poor solubility. The stronger cytochrome P450 3A4 (Cyp3A4) inhibition of **22b** and **22e** compared to the methyl counterparts (**22a** and **22d**, respectively) became a focus of the optimization campaign. While compound **22c** also demonstrated excellent potency compared to the CF_3-analogues and weaker Cyp3A4 inhibition, this compound had poorer microsomal stability than **22e** and was therefore deprioritized. Additionally, all of the compounds in this series

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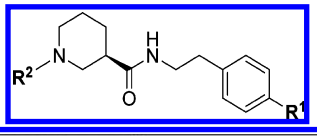
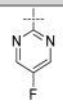
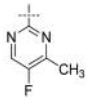
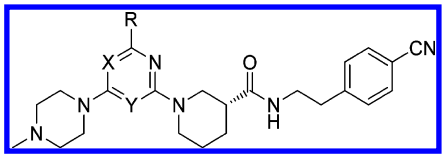
Table 2. *N*-Heteroaryl SAR



No.	R^1	R^2	cAMP EC_{50} (μM)[a] hTGR5	cAMP EC_{50} (μM)[a] mTGR5	No.	R^1	R^2	cAMP EC_{50} (μM)[a] hTGR5	cAMP EC_{50} (μM)[a] mTGR5
19a	Cl		0.095 ±0.01	0.16 ±0.01	**19f**	Cl		0.22 ±0.05	0.72 ±0.15
19b	Cl		0.008 ±0.001	0.038 ±0.006	**19g**	Cl		0.93 ±0.14	4.89 ±1.45
19c	Cl		0.930 ±0.08	1.40 ±0.08	**19h**	CN		0.013 ±0.004	0.063 ±0.021
19d	Cl		13.7 ±2.3	8.17 ±1.95	**19i**	CN		0.017 ±0.012	0.13 ±0.08
19e	Cl		2.10 ±0.28	0.12 ±0.007	**19j**	CN		0.14 ±0.02	0.023 ±0.001

[a]EC_{50} values are the average of at least two assay runs performed in triplicate ± SD; unless otherwise noted, all compounds are 100% full agonists relative to an internal control (see Materials and Methods).

Table 3. SAR of Piperazine-Containing Analogues



no.	R	X	Y	cAMP EC_{50} (μM)[a] hTGR5	cAMP EC_{50} (μM)[a] mTGR5	solubility (μM)[b,c]	Cyp3A4 IC_{50} (μM)[c]	hERG IC_{50} (μM)[c]	cLogP
22a	CH_3	CH	N	0.076 ± 0.031	0.19 ± 0.11	>175	>25	3.1	2.7
22b	CF_3	CH	N	0.002 ± 0.0004	0.003 ± 0.001	6.9	2	2.1	3.1
22c	CH_3	CF	N	0.004 ± 0.003	0.011 ± 0.005	110	7.6		2.8
22d	CH_3	N	CH	0.44 ± 0.12	0.17 ± 0.034	>175	>25		2.7
22e	CF_3	N	CH	0.003 ± 0.001	0.001 ± 0.0006	169	<1	3.2	3.1

[a]EC_{50} values are the average of at least two assay runs performed in triplicate ± SD; unless otherwise noted, all compounds are 100% full agonists relative to an internal control (see Materials and Methods). [b]High throughput thermodynamic solubility at pH 6.8. [c]Data from single assay run.

showed moderate to strong hERG inhibition, which also needed to be addressed in the next phase of the optimization.

As summarized in Table 4, compound **22e** was a potent full activator of functional cellular responses, including GLP-1 secretion from the murine GLUTag cell line (EC_{50} = 256 nM, 106% efficacy versus control) and inhibition of TNFα release from human peripheral blood mononuclear cells (PBMC) (IC_{50} = 92 nM, 89% efficacy versus control). In mouse, **22e** showed moderate oral bioavailability (24%), which made it an acceptable tool compound for early stage in vivo studies and additional profiling activities. Additional profiling showed that **22e** had high intrinsic clearance in microsomes for all species tested, especially rat. In rat, the compound exhibited poor bioavailability and high clearance (data not shown), consistent with the rat liver microsome (RLM) results, so the in vivo models were limited to the mouse unless high doses of **22e** were employed.

Extensive metabolite identification was performed in microsomes with **22e**, and the results suggested that the piperidine core, piperazine *N*-methyl, and phenethyl side chains were prone to oxidative metabolism. Unfortunately, early exploration of the piperidine core and phenethyl side chain proved to have particularly narrow SAR with no suitable alternatives identified at the time. Importantly, removal of the *N*-methyl substituent yielded compound **23a** (Table 5), which retained hTGR5 and mTGR5 activity and had improved intrinsic clearance (33 μL/min/mg) in mouse liver microsomes (MLM). Unfortunately, Cyp3A4 and hERG inhibition were not improved with this modification. It was clear after a survey of several alkyl substituents on the piperazine that the *N*-alkyl group contributed significantly to the poor stability in the series.

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Table 4. In Vitro Activity, ADME, and PK profiles of 22e

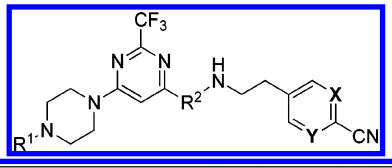
target functional assays (μM, % efficacy)		
GLP-1 secretion GLUTag	0.296 ± 0.046 (n = 202), 116	
PBMC LPS TNFα	0.090 ± 0.008 (n = 26), 86	
Microsomal Stability (μL/min/mg)		
mouse	214	
human	289	
rat	>500	
Cyp Inhibition (μM)		
3A4	<1	
2C9	4.7	
2D6	>10	
Off-Target Activity		
hERG (patch clamp)	IC_{50} = 6.7 μM	
Safety Pharm panel (110 assays)	>10 μM	
mouse pharmacokinetics		
route (dose mg/kg)	IV (5)	PO (20)
AUC (h·nM)	2667	2540
CL (mL/min/kg)	62	
V_{ss} (L/kg)	1.6	
C_{max} (nM)	4521	1067
$t_{1/2}$ (h)	0.36	1.35
F (%)		24

Interestingly, however, the hERG risk (>30 μM) could be mitigated with the *N*-oxetanyl group (**23b**)[18] and still retain target potency; however, Cyp3A4 inhibition and metabolic stability in MLMs (138 μL/min/mg) were not improved, so additional strategies needed to be explored.

It has been reported that compounds with a basic functional group and molecular weight >400 and cLogP > 4 are more likely to exhibit above average Cyp3A4 inhibition and high clearance,[19] which compound **22e** and related analogues resemble. The introduction of the trifluoromethyl group placed this series in a molecular weight (450−550) band that proved to be challenging for multiparameter optimization, particularly because analogues with cLogP < 4 did not significantly improve the ADME properties. Therefore, an SAR strategy to alter the structures and lower cLogP further was pursued in order to identify compounds with ADME properties necessary to progress into our preclinical animal models.

Several strategies toward reducing cLogP were considered with the chemical series. One strategy probed replacing the side chain phenyl ring with heterocyclic isosteres. The nitrile was retained as a substituent because it generally provided potent compounds while also maintaining lower cLogP than other substituents. The pyridine and pyrimidine analogues **32a** and **32c** have cLogPs lower than **22e** at 2.0 and 1.5, respectively,

Table 5. SAR of Tail and Carboxamide Analogues



No.	R^1	R^2	X	Y	cAMP EC_{50} (μM)[a] hTGR5	cAMP EC_{50} (μM)[a] mTGR5	Cyp3A4 IC_{50} (μM)[b]	hERG IC_{50} (μM)[b]	cLogP
23a	H		CH	CH	0.014 ±0.0004	0.013 ±0.001	<0.1	3.5	2.5
23b			CH	CH	0.001 ±0.0001	0.0005 ±0.00001	1.1	> 30	2.6
32a	CH_3		N	CH	0.006 ±0.004	0.003± 0.002	10	25	2.0
32b	H		N	CH	0.063± 0.012	0.048± 0.004	<0.3	20	1.4
32c	CH_2CH_3		N	N	0.038 ±0.018	0.017 ±0.006	5.2	18	1.5
34a	CH_3		CH	CH	0.020 ±0.004	0.031 ±0.004	0.4	2.3	3.5
34b	CH_3		CH	CH	0.008 ±0.002	0.004 ±0.001	2.2	13	2.9
34c	CH_3		CH	CH	0.274 ±0.011	0.160 ±0.011	0.7	5.5	2.9
34d	CH_3		CH	CH	0.004 ±0.001	0.002 ±0.0005	3	9.8	2.4
34e	CH_3		CH	CH	>8	3.54±1.6	0.8	6.0	2.4
34f	CH_2CH_3		N	CH	0.019 ±0.003	0.004 ±0.0005	7.8	>30	1.8

[a]EC_{50} values are the average of at least two assay runs performed in triplicate ± SD; unless otherwise noted, all compounds are 100% full agonists relative to an internal control (see Materials and Methods). [b]Data from single assay run.

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which likely contributed to the reduced Cyp3A4 and hERG inhibition in both cases; however, the switch to these moieties reduced target potency on hTGR5 and mTGR5 by >20-fold. Interestingly, despite the low cLogP (1.4) of the desmethyl compound **32b**, it was a highly potent Cyp3A4 inhibitor, like the phenyl analogue **23a**. Molecular docking studies with a model of Cyp3A4 provided evidence that **22e** is anchored into the active site of the enzyme via an hydrogen bond between the amide carbonyl and S119 and the CF_3 group fits into a hydrophobic pocket formed from F108, F213, F220, and F241 (Figure 3). The piperazine nitrogen of **22e** also engages E308 in a salt bridge, which may explain why the desmethyl piperazine analogues **23a** and **32b** were more potent Cyp3A4 inhibitors.

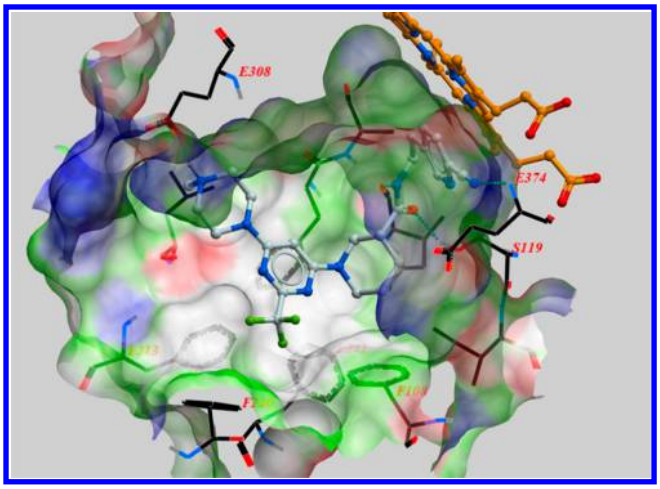

Figure 3. Compound **22e** docked into the Cyp3A4 active site.

The next series of compounds targeted replacement of the piperidine-3-carboxamide with progressively smaller and less lipophilic rings (**34a**−**34f**). Generally, the 2-substituted piperidine, pyrrolidine, and azetidine cores yielded compounds with acceptable target potency and, in some cases, improved in vitro ADME profiles. Stereochemical bias was observed in this series of compounds, and in all cases the *S*-enantiomer was preferred. Serendipitously, the more active *S*-enantiomer derivatives **34b** and **34d** were about 3-fold less potent as a Cyp3A4 inhibitors than the *R*-enantiomers **34c** and **34e**, respectively. Azetidines **34d** and **34f** showed promising target potency and solid evidence that Cyp3A4 and hERG inhibition could be mitigated by strategically reducing lipophilicity in this series. While this proved to be an effective strategy toward identifying compounds with good target selectivity, this series of molecules still suffered from poor microsomal stability and poor in vivo plasma exposure. Therefore, to obtain compounds with the desired balance of potency and systemic exposure, an alternative strategy was explored.

Several functional groups were considered as replacements for the *N*-alkylpiperazine group. The tactic that we chose was to replace the basic functional group with a carboxylic acid. Because carboxylic acids are polar and ionized at physiological pH, they generally show better metabolic stability than basic and neutral compounds. Moreover, they also have the added benefits of being poor substrates for the hERG channel and much weaker Cyp inhibitors.[20] These general properties of acids made them an attractive functional group[21] to explore, especially because the basic and neutral analogues presented us with several challenges. One caveat of carboxylic acids is that they generally have high plasma protein binding,[22] so high plasma exposure may need to be attained in order to overcome the potentially low free concentration of active compound.[23]

The first compound that was made in this series was isonipecotic acid derivative **45a** (Table 6). While not quite as potent as **22e**, it showed no Cyp inhibition and had good MLM stability (CL_{int} 16 μL/min/mg), consistent with the attractive features of acids outlined above. Unfortunately, **45a** had low plasma exposure. In Caco-2 cells, **45a** showed potential for efflux with P_{app} (A−B) of 1.36×10^{-6} cm/s and P_{app} (B−A) of 12.96×10^{-6}cm/s and B−A/A−B ratio of 9.53. The relatively unhindered nature of the acid and low calculated log *D* (cLogD 0.9 at pH 7.4) for a high molecular weight carboxylic acid were considered to be possible contributors to the poor permeability. To address this, a methyl group was installed adjacent to the carboxylic acid to yield **45b**. This compound showed similar potency to **45a** while still exhibited similar Cyp inhibition and metabolic profiles to its predecessor. Moreover, **45b** showed a nearly 10-fold improvement of plasma exposure but had nearly identical effects in Caco-2 cells (A−B 1.02×10^{-6} cm/s and B−A 13.51×10^{-6} cm/s) to **45a**. Nevertheless, the exposure for this compound was too low to progress into in vivo studies.

Balancing the moderately high molecular weight (>500) with the proper level of lipophilicity (cLogD) were important design elements for the carboxylic acid analogues. A thorough analysis of our data set revealed that absorption and oral exposure might be improved by increasing lipophilicity (e.g., cLogD at pH 7.4). Therefore, the 4-position of the piperidine ring was first explored by adding a phenyl ring via an ether or direct carbon−carbon bond (Table 6). Compounds **45c** and **45d** showed good potency and species cross-reactivity. In accordance with our hypothesis, both compounds showed improved plasma exposure (area under curve (AUC) 5018 h·nM and 14221 h·nM, respectively) in the mouse using a snapshot PK study protocol.[24] More importantly, the azetidine analogue showed high plasma exposure in the mouse, perhaps due to a lower potential for efflux (A−B 5.73 cm/s and B−A 6.79 cm/s for **45d** vs A−B 2.12 and 6.4 cm/s for **45c**) and high parallel artificial membrane permeability assay (PAMPA) bioavailability (89%). While both of these compounds had slightly higher intrinsic clearance in MLM (CL_{int} 46 and 53 μL/min/mg, respectively) than **45a** and **45b**, their improved plasma exposure was likely due to improved intestinal absorption, perhaps due to a more compact conformation. Direct attachment of the phenyl ring bearing the acid to the piperidine led to **45f**, which was potent on both hTGR5 and mTGR5 and had good MLM stability (CL_{int} 14 μL/min/mg) but unfortunately showed only moderate exposure (AUC 3742 h·nM) relative to the other acid-containing analogues. In Caco-2 cells, **45f** showed efflux potential with A−B of 2.7 cm/s and B−A of 12.9 cm/s (B−A/A−B 4.7). Overall, because the properties of the azetidine-2-carboxamide appeared to be superior to the piperidine congener, subsequent analogues employed the former as the key segment.

For compounds **45e**−**45h**, plasma AUC correlated well with increasing cLogD. Ultimately, this effort led to the identification of compounds **45g** and **45h** as potent, cross-selective agonists having good MLM stability and excellent plasma exposure, with **45h** providing a slightly more potent agonist on hTGR5; therefore, **45h** was chosen for more extensive profiling. (Table 7) Compound **45h** was potent in the GLP-1 and PBMC functional assays, showed no Cyp or hERG inhibition, and was

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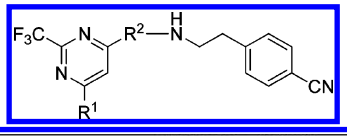
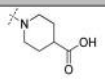
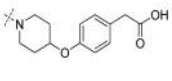
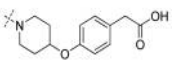
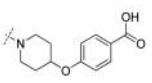
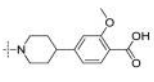
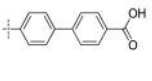
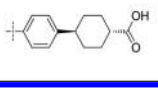
Table 6. SAR of Acid-Containing Analogues



No.	R^1	R^2	cAMP EC_{50} (μM)[a] hTGR5	cAMP EC_{50} (μM)[a] mTGR5	MLM[b]	AUC[c]	cLogD
45a			0.026 ±0.01	0.010 ±0.003	15	108	0.9
45b			0.012 ±0.005	0.004 ±0.0008	27	1046	1.2
45c			0.014 ±0.01	0.002 ±0.0007	46	5018	2.4
45d			0.030 ±0.003	0.002 ±0.0002	52	14221	2.0
45e			0.018 ±0.002	0.001 ±0.0004	33	7526	1.9
45f			0.011 ±0.005	0.0005 ±0.0003	14	3742	2.0
45g			0.025 ±0.004	0.002 ±0.0005	<7	13908	3.2
45h			0.015 ±0.002	0.001 ±0.0001	<7	11393	3.0

[a] EC_{50} values are the average of at least two assay runs performed in triplicate ± SD; unless otherwise noted, all compounds are 100% full agonists relative to an internal control (see Materials and Methods). [b] CL_{int} μL/min/mg, data from single assay run. [c] Snapshot PK (0−5 h, pooled samples), (20 mg/kg) AUC (h·nM).

Table 7. In Vitro Activity, ADME and PK Profile of 45h

target functional assays (μM, % efficacy)		
GLP-1 secretion GLUTag	0.046 ± 0.006 (n = 10), 80	
PBMC LPS TNFα	0.215 ± 0.06 (n = 3), 99	
FXR	>10 μM	
Cyp inhibition (μM) 3A4, 2C9, 2D6	All >25	
hERG (patch clamp)	IC_{50} >30 μM	
log D pH 6.8	2.5	
% plasma protein binding (human)	99.9	
mouse pharmacokinetics		
route dose (mg/kg)	IV (5)	PO (20)
AUC (h·nM)	13925	53750
CL (mL/min/kg)	10.4	
V_{ss} (L/kg)	1.31	
C_{max} (nM)	12002	9750
$t_{1/2}$ (h)	4.14	11.7
F (%)		96.5

nearly completely bioavailable in mouse. In a safety panel screen of 20 receptors, **45h** showed only moderate inhibition of human phosphodiesterase type 4D (hPDE4D) of 4.6 μM, while all other receptors tested were >30 μM. This compound was then taken forward in a chronic in vivo study.

The effects of compound **45h** on the secretion of total GLP-1 levels in wild-type C57BL/6 male mice was determined as an in vivo screening method prior to testing in a chronic model. Adult mice were deprived of food overnight and dosed orally with either vehicle, a control (compound **22e**), or **45h** 30 min prior to an oral glucose bolus (Figure 4). Both compound **22e** and **45h** produced a robust GLP-1 increase, with **45h** showing a near maximal effect at 3 mg/kg. This encouraging result prompted us to test **45h** in chronic disease models, particularly

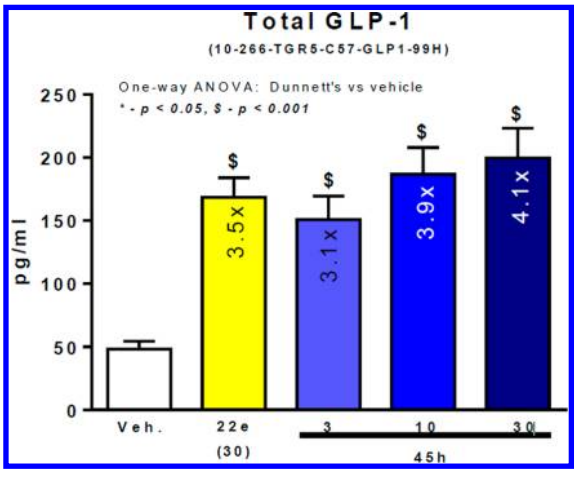


Figure 4. Effect of **22e** and **45h** on in vivo GLP-1 levels in C57/Bl6 mice.

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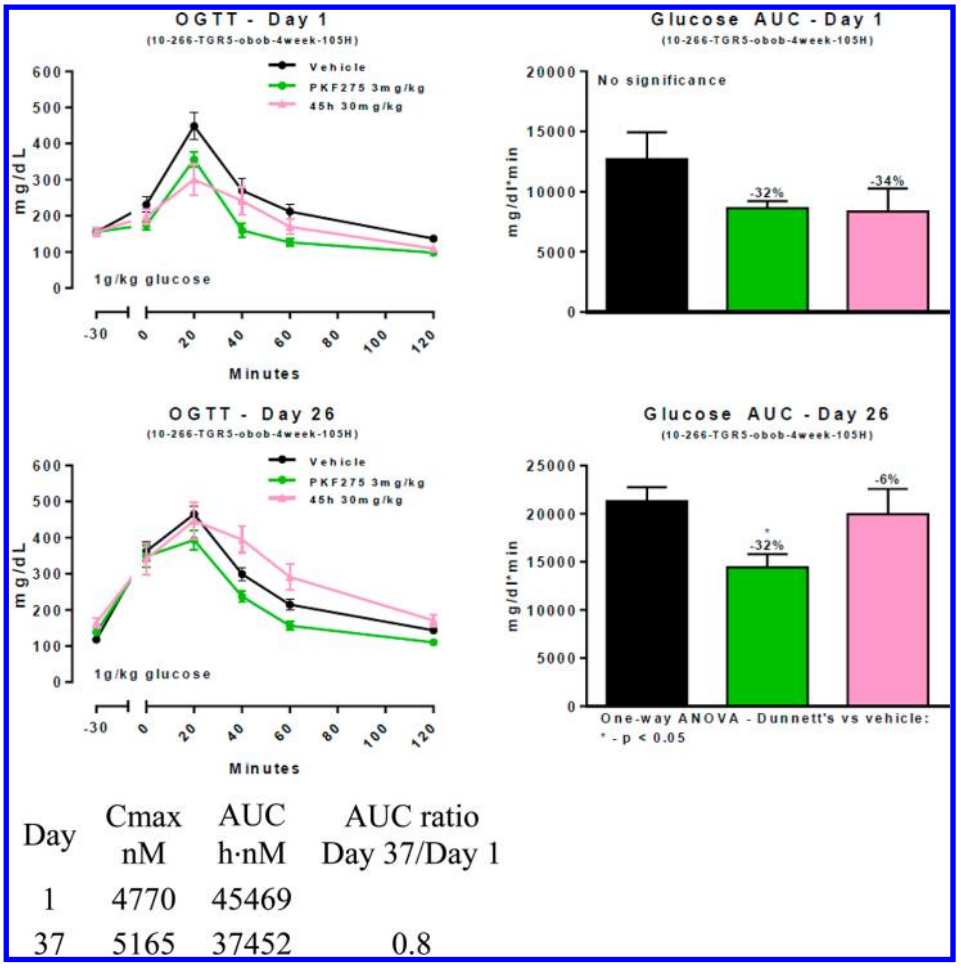

Day	Cmax nM	AUC h·nM	AUC ratio Day 37/Day 1
1	4770	45469	
37	5165	37452	0.8

Figure 5. Efficacy and pharmacokinetics of **45h** in 5-week ob/ob study at 30 mg/kg qd.

an examination of the effect on glucose excursion in an oral glucose tolerance test (OGTT).

Genetically obese (ob/ob) mice have elevated basal glucose levels and possess an inflammatory cytokine profile that has been shown to be comparable to the human disease. During a series of pilot studies with **22e**, we found that the compound provided a similar increase of GLP-1 levels in the ob/ob model as it did in wild types (data not shown). This result, combined with the robust GLP-1 effect of **45h** in the wild type, justified testing the compound in ob/ob mice for 5 weeks to determine if chronic TGR5 agonism would improve glucose disposal and reduce inflammatory cytokine levels, the two major purported effects of receptor activation. For this chronic study, a 30 mg/kg dose of **45h** was chosen in order to maximize the coverage of the compound during the course of the study. Interestingly, an OGTT on day 1 showed nearly equivalent efficacy to the DPP4 inhibitor PKF275.[25] However, after 26 days of dosing qd with **45h**, the efficacy in the OGTT was ablated (Figure 5). In addition to a lack of robust efficacy on glycemic control, **45h** showed no significant change in insulin levels or inflammatory cytokines in white adipose tissue (not shown). Importantly, **45h** maintained plasma exposure throughout the dosing period, showing equivalent exposure on day 1 and day 37. Therefore, it is possible that the lack of efficacy may be due to receptor internalization and down-regulation leading to tachyphylaxis.

CONCLUSION

In conclusion, extensive lead optimization was carried out from an HTS hit (**5**), which ultimately led to the potent, species cross-selective compound **45h**. Upon leading up to the selection of testing compound **45h** in acute and chronic disease models of obesity, we screened several of the lead compounds mentioned in this manuscript for their ability to produce robust GLP-1 increases in nondiseased C57 mice as well as for their ability to decrease glucose levels in OGTT experiments in nondiseased and diseased mice (diet-induced obese, ob/ob, and db/db). Compound **45h** met all of our selection criteria for testing in chronic disease models in which a modest effect on reducing peak glucose levels in ob/ob mice was observed that was equivalent to a DPP4 inhibitor on day 1 (not statistically significant). However, the effect with **45h** was lost upon chronic dosing despite nearly equivalent compound exposure at the beginning and end of the study and was confirmed in a second study with the compound. Recently, sustained TGR5 agonism has been reported to cause elevated heart rate and blood pressure in the dog, which would likely limit the applicability of a systemic agonist to treat metabolic disease.[10a,c,26] Moreover, concern has been raised about the effects of TGR5 activation on gall bladder function.[27] Indeed, pilot studies in dogs with compound **22e** led to changes in gall bladder contractility and heart rate (Nguyen et al., manuscript in preparation). Taken together, there would seem to be a number of toxicological and therapeutic issues that limit the

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utility of systemic TGR5 agonists for treatment of metabolic disease.

MATERIALS AND METHODS

Experimental Protocols (Biology). *TGR5 Cellular cAMP Assay.* Jurkat cells overexpressing human or mouse TGR5 were seeded at 50000 cells/25 μL/well in 384-well plates (Greiner) in AIM V media (Life Technologies) supplemented with 1 mM IBMX (Sigma) and 10 mM HEPES. Immediately after plating, 200 nL of compounds in DMSO were transferred using the Pintool (GNF systems). Following a 1 h at 37 °C, cryptate and D2 conjugates from the cAMP HiRange HTRF kit (Cisbio) were added (12.5 μL each). Plates were then incubated 1 h at rt and emissions at 620 nM and 665 nM were read on the Envision (Perkin-Elmer) as suggested by the manufacturer. Data was expressed as a ratio of emission at 665/620 and then normalized to a DMSO alone control. Dose−response data points were curve fitted using the standard logistic regression model, implemented with an in-house developed Matlab (mathworks.com) program. The percent efficacy was then determined based on *N*-(3,5-dichlorophenyl)-*N*-(1-(2-methoxyphenyl)ethyl)-3-methylisonicotinamide[17] as the internal control. The resultant EC_{50} values correspond to the compound concentration that displaces 50% of the binding of the d_2-labeled cAMP.

FXR Reporter Gene Assay. 293T cells stably expressing GAL4-luciferase-FXR receptor were maintained in Dulbecco's Modified Eagles Medium (Hyclone) containing 10% fetal bovine serum (Sigma), 1% penicillin/streptomycin (Hyclone), and 200 μg/mL hygromycin (Hyclone). Cells were detached and seeded into 384-well white plates (Greiner Bio-one) at 4000 cells/50 μL/well in Dulbecco's Modified Eagles Medium (Hyclone) containing 3% charcoal/dextran treated fetal bovine serum (Sigma) and 1% penicillin/streptomycin. Following 24 h incubation at 37 °C, 50 nL of 1000× compound diluted in DMSO was transferred by Pintool (GNF Systems) into the assay plate and incubated for 24 h at 37 °C. Then 25 μL of Bright Glo (Promega) was added per well and flash luminescence was detected on the Viewlux (PerkinElmer Life Sciences). Dose−response data points were curve fitted using the standard logistic regression model, implemented with an in-house developed Matlab (mathworks.com) program. The resultant IC_{50} values correspond to the compound concentration that inhibited activity by 50%.

GLUTag GLP-1 Secretion Assay. Murine GLUTag cells were maintained in Dulbecco's Modified Eagles Medium (Hyclone) containing 10% fetal bovine serum and 1% penicillin/streptomycin. On day one of the assay, cells from a confluent flask were detached using 0.25% trypsin (Life Technologies) and seeded in 96-well poly-D-lysine plates (BD Biosciences) at 42000 cells/150 μL/well in maintenance medium. After incubation at 37 °C overnight, medium was changed to phenol red free DMEM with low glucose (Sigma) and then incubated for another 24 h at 37 °C. GLUTag cells were then washed and incubated in glucose free EBSS buffer with 0.1% BSA (Sigma) at 37 °C to starve, followed by two additional EBSS buffer washes. Following the last wash, EBSS buffer containing 15 mM glucose, DPP4 inhibitor PKF275 (600 nM final concentration[28]), and agonist compounds was added to the cells and incubated for another 2 h at 37 °C to allow for GLP-1 secretion. Then 6 μL of supernatant was transferred to HEK293 cells stably expressing a GLP-1 luciferase reporter construct, seeding the morning of GLUTag cell treatment at 12000 cells/40 μL/well in a 384-well plate (Greiner). After overnight incubation, 15 μL of 25% Bright Glo (Promega) was added to the reporter cells, incubated at rt for 2 min, and luminescence was then read on Envision per the manufacturer's instructions (Perkin-Elmer). Data were normalized to a DMSO alone control, and the percent efficacy was then determined based on *N*-(3,5-dichlorophenyl)-*N*-(1-(2-methoxyphenyl)ethyl)-3-methylisonicotinamide.[17] Dose−response data points were curve fitted using the standard logistic regression model, implemented with an in-house developed Matlab (mathworks.com) program. The resultant EC_{50} values correspond to the compound concentration that induced half-maximal activity.

PBMC LPS Assay. Peripheral blood mononuclear cells (PBMC) were isolated from healthy donors using Ficoll Hypaque per the manufacturer's recommendation (GE Healthcare). Cells were seeded at 30000 cells/10 μL/well in a 384-well proxiplate (Perkin-Elmer) in AIM V media (Life Technologies) supplemented with 3% FBS (Hyclone) and 1× Pen/Strep/Glutamine. Following an overnight incubation at 37 °C, 50 nL of compound in DMSO was added per well by Pintool (GNF systems). Plates were incubated for 30 min at 37 °C, followed by the addition of 2 μL/well of 6× LPS (EC_{80} final, determined during LPS dose titration for each donor) and incubation overnight at 37 °C. To detect TNFα secretion by HTRF, 2.5 μL/well anti-TNFα cryptate, then 2.5 μL/well of anti-TNFα XL665 were added (Cisbio) and subsequently incubated for 3 h at rt. The emissions at 620 nM and 665 nM were read on the Envision (Perkin-Elmer) as suggested by the manufacturer. Data was expressed as a ratio of emission at 665/620 and subsequently normalized to a DMSO alone control, and the percent inhibition was then determined based on *N*-(3,5-dichlorophenyl)-*N*-(1-(2-methoxyphenyl)ethyl)-3-methylisonicotinamide.[17] Dose−response data points were curve fitted using the standard logistic regression model, implemented with an in-house developed Matlab (mathworks.com) program. The resultant IC_{50} values correspond to the compound concentration that inhibited activity by 50%.

Animal Studies. All aspects of animal care, use, and welfare for all animals used for in vivo studies were performed in compliance with U.S. Department of Agriculture regulations and the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Furthermore, all animals were handled in accordance with Novartis Animal Care and Use Committee protocols and regulations. For mouse studies, animals were obtained from the Jackson laboratories (C57BL/6) and Harlan (ob/ob).

Mouse in Vivo GLP-1 Secretion Assay. Male C57BL/6 mice were fasted overnight and then dosed orally with 10, 30, or 100 mg/kg of **22e**. After 30 min, animals were challenged with 3 g/kg oral dextrose (Sigma). For assessment of GLP-1 secretion, plasma was collected at 2 min postchallenge into EDTA coated tubes containing 8 μL of a DPP4 inhibitor solution (EMD Millipore) and GLP-1 levels were measured by ELISA according to the manufacturer's instructions (Meso Scale Discovery). Data was analyzed using one-way ANOVA followed by Dunnett's post-test in Graphpad Prism. Values of $p < 0.050$ were considered statistically significant. Data shown is the average ± SEM of eight animals.

4-Week ob/ob Study Oral Glucose Tolerance Test (OGTT). Male ob/ob mice (Harlan) were dosed once-daily (SID) for 4 weeks with vehicle, PKF275 30 mg/kg, or **45h** 30 mg/kg as the *N*-methyl-D-glucamine salt. On day 1 and after 4 weeks, mice were overnight fasted then orally dosed with vehicle or compound. After 30 min, all mice were orally challenged with 1 g/kg dextrose (Sigma) and blood glucose measured via tail tip by hand-held glucometer (Embrace). Blood glucose was measured predose, preglucose dose, and 20, 40, 60, and 120 min postglucose challenge. AUC was calculated and data analyzed using one-way ANOVA followed by Dunnett's post-test in Graphpad Prism. Values of $p < 0.050$ were considered statistically significant. Data shown is the average ± SEM of eight animals.

Experimental Protocols (Metabolite Identification). In vitro incubations in liver microsomes from mouse, rat, dog, monkey, and human were used. Stock solutions of trifluoromethyl(pyrimidin-2-yl)azetidine-2-carboxamide analogues (TGR5 agonists) at 10 mmol/L were prepared in DMSO. For the characterization of in vitro metabolites, compounds were incubated at 37 °C for 60 min with liver microsomes from mouse, rat, dog, monkey, and human in individual incubations. All incubations were conducted on the same date using the same batch of compound material. A 4 μL stock solution (0.5 mM in ACN/water) of TGR5 agonists were added to an enzyme reaction mixture (50 μL liver microsomes, containing 1 mg protein/mL with phosphate buffer) and preincubated for 3 min at 37 °C. After preincubation, the final reaction was started by addition of 46 μL of the NADPH-regenerating system or containing NADPH cofactors. After 60 min, the reaction was stopped with 200 μL of ice-cold ACN. The reaction mixture was stored at −20 °C. The 20 μM

concentration is used for incubation because the detection of metabolites is very challenging at 1 μM initial substrate levels.

Prior to use, the mixture was centrifuged (10000*g*, 5 min) and 200 μL of supernatant was used without prior evaporation and transferred to a clean 96-well plate. From these samples, 5 μL aliquots were used for analysis.

Samples were then analyzed by HPLC-MS/MS. High resolution chromatography was performed using the Acquity UPLC and a 1.0 mm × 50 mm C18 RP column (1.8 μm particles). The mobile phases consisted of 0.1% formic acid in water for buffer A and 0.1% formic acid in ACN for buffer B. A standard binary gradient was used for reverse phase chromatography.

Computational Modeling of Cyp3A4. Flexible docking was performed using Glide 5.8 (Schrodinger, Inc., Portland, OR, 2012). The CYP3A4 coordinates were taken from the Protein Data Bank (2J0D.pdb). The grid box was centered on the cocrystallized ligand erythromycin and extended 10 Å from the center, with the outer box extending an additional 20 Å. The ligand was docked using the standard precision (SP) algorithm and scored using GlideScore.

Experimental Protocols (Chemistry). Unless otherwise noted, materials were obtained from commercial suppliers and were used without purification. Removal of solvent under reduced pressure (i.e., en vacuo) refers to distillation using Buchi rotary evaporator attached to a vacuum pump (~3 mmHg). Products obtained as solids or high boiling oils were dried under vacuum (~1 mmHg). Purification of compounds by HPLC was achieved using a Waters 2487 series with Ultra 120 5 μm C_{18}Q column with a linear gradient from 10% solvent A (ACN with 0.035% TFA) in solvent B (water with 0.05% TFA) to 90% A in 7.5 min, followed by 2.5 min elution with 90% A. ^{1}H NMR spectra were recorded on Bruker XWIN-NMR (400 or 600 MHz). Proton resonances are reported in parts per million (ppm) downfield from tetramethylsilane (TMS). ^{1}H NMR data are reported as multiplicity (s singlet, d doublet, t triplet, q quartet, quint quinted, sept septed, dd doublet of doublets, dt doublet of triplet, br s broad singlet), number of protons, and coupling constant in hertz (Hz). For spectra obtained in $CDCl_3$, DMSO-d_6, and CD_3OD, the residual protons (7.27, 2.50, and 3.31 ppm, respectively) were used as the reference.

Analytical thin-layer chromatography (TLC) was performed on commercial silica plates (Merck 60-F 254, 0.25 mm thickness); compounds were visualized by UV light (254 nm). Flash chromatography was performed using ISCO CombiFlash cartridges. The purity and quantitative analysis were determined with a Waters ZQ 2000 LC/MS system, which employed an Acquity UPLC system with PAL autosampler, Waters 3100 single quadrupole mass spectrometer, Waters Acquity 2.1 mm × 50 mm C18 column (1.8 μM), flow rate set at 0.4 mL/min, gradient 5−95%, 0.5−15 min eluting with mobile phase A (water +0.05% formic acid) and mobile phase B (ACN + 0.035% formic acid). Unless otherwise stated, all compounds reported in this manuscript were tested at >95% HPLC purity by internal quality control protocols.

Unless otherwise stated, all purifications performed via preparative HPLC used the general conditions: C_{18} (19 mm × 50 mm), 10−90% ACN/water, 0.05% TFA, 100 mL/min.

*(R)-N-(4-Chlorophenethyl)-1-(pyrimidin-2-yl)piperidine-3-carboxamide (**13a**).* (*R*)-1-Boc-nipecotic acid (0.115 g, 0.5 mmol) and HATU (209 mg, 0.55 mmol) were dissolved in ACN (2 mL) and treated with DIEA (0.16 mL, 1.0 mmol, 2 equiv) and 4-chlorophenethylamine (0.077 mL, 0.55 mmol), and the reaction was stirred at ambient temperature overnight. The reaction was concentrated and partitioned between ether and water. The organic was concentrated, and purification of the residue by flash chromatography (0−10% methanol/DCM) gave the intermediate. The material was then dissolved in DCM (2 mL) and treated with excess TFA (1 mL). The reaction was complete after 1 h and then concentrated to dryness and carried forward crude into the next step. In a 5 mL microwave reaction vessel was combined (*R*)-*N*-(4-chlorophenethyl)piperidine-3-carboxamide 2,2,2-trifluoroacetate (130 mg, 0.5 mmol), DMA (2 mL), DIEA (0.25 mL, 1.5 mmol), and 2-chloropyrimidine (63 mg, 0.55 mmol). The vessel was sealed and irradiated by microwave at 150 °C for 30 min. Upon completion of the reaction, the mixture was cooled to rt and diluted with water (2 mL), precipitate was collected by filtration, and the title compound was obtained as a white solid (50 mg, 29%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.23 (d, J = 4.7, 2H), 7.22−7.16 (m, 2H), 7.02 (d, J = 8.4, 2H), 6.44 (t, J = 4.7, 1H), 6.28 (s, 1H), 4.07 (dd, J = 3.7, 13.8, 1H), 3.88 (s, 1H), 3.76 (dd, J = 7.4, 13.8, 1H), 3.62−3.33 (m, 4H), 2.71 (dd, J = 6.8, 12.5, 2H), 2.40−2.27 (m, 1H), 2.16−2.00 (m, 1H), 1.81 (s, 1H). HPLC-MS $C_{18}H_{21}ClN_4O$ (M + H$^+$): 345.1, 6.91 min. The *S*-enantiomer **13b** was synthesized in an identical manner starting from (*S*)-1-Boc-nipecotic acid and has spectral data that matches **13a**.

*(R)-N-(4-Chlorophenethyl)-1-(4-methylpyrimidin-2-yl)piperidine-3-carboxamide (**13c**).* To a microwave reaction tube was added (*R*)-*N*-(4-chlorophenethyl)piperidine-3-carboxamide (120 mg, 0.33 mmol), 2-propanol (1.2 mL), DIEA (122 μL, 0.7 mmol), and 2-chloro-4-methylpyrimidine (**10b**). The vessel was sealed and heated to 150 °C for 30 min. The solvent removed en vacuo, and the residue was purified by flash chromatography (12 g ISCO, 0−10% methanol/DCM) to yield a pale-yellow oil, which was then repurified by preparative HPLC to give 5 mg (4%) of the title compound. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03 (d, J = 5.0, 1H), 7.13−7.10 (m, 2H), 6.94 (dd, J = 2.3, 8.8, 2H), 6.56 (s, 1H), 6.27 (d, J = 5.0, 1H), 3.88 (d, J = 5.0, 2H), 3.71−3.64 (m, 2H), 3.50 (td, J = 6.6, 13.2, 1H), 3.38−3.25 (m, 1H), 2.74−2.59 (m, 2H), 2.37−2.27 (m, 1H), 2.22 (s, 3H), 2.08 (td, J = 6.4, 13.1, 1H), 1.79−1.70 (m, 1H), 1.51−1.45 (m, 2H). HPLC-MS $C_{19}H_{23}ClN_4O$ (M + H$^+$): 359.1, 6.72 min.

*(R)-2,5-Dioxopyrrolidin-1-yl 1-(4-Methylpyrimidin-2-yl)-piperidine-3-carboxylate (**11b**).* In a 35 mL microwave reaction vessel was combined (*R*)-nipecotic acid (1.07 g, 8.2 mmol), 2-propanol (10 mL), DIEA (1.43 mL, 8.2 mmol), and 2-chloro-4-methylpyrimidine (1.06 g, 8.2 mmol). The vessel was sealed and heated to 150 °C for 30 min. The solvent was removed en vacuo, and the residue was then dissolved in water (5 mL), acidified with 0.1 M HCl, and extracted with DCM (3 × 10 mL), dried over sodium sulfate, filtered, and concentrated to give 1.27 g (70%) of a pale-yellow oil that was carried forward without further purification. The crude intermediate was dissolved in DCM (30 mL) and treated with EDCI (1.21 g, 6.3 mmol). After 5 min, *N*-hydroxysuccinimide (791 mg, 6.8 mmol) was added and the mixture was allowed to stir for 18 h. The reaction mixture was then quenched with water (30 mL), and the layers were partitioned. The aqueous layer was washed once with DCM (30 mL). and the combined organics were washed with 10% citric acid (aq) (20 mL) and water (20 mL), dried over sodium sulfate, filtered, and concentrated to yield 1.21 g (66%) of the title compound that was used without purification. The same procedure was employed to prepare **11a**.

*General Method A for Compounds **13d**−**g**. (R)-1-(4-Methylpyrimidin-2-yl)-N-phenethylpiperidine-3-carboxamide (**13d**).* A stock solution of **11b** was prepared with a concentration of 0.3 M in ACN. Phenethylamine (50 μL, 0.4 mmol) was dispensed into the reaction vial and was subsequently dosed with 2 mL of ACN and 350 μL of the stock solution of **11b**. The reaction mixture was kept stirring until **11b** was consumed, and then the reaction mixture was quenched with water (2 mL), extracted with ethyl acetate (2 × 20 mL), and concentrated to dryness. The residue was purified via preparative HPLC to give (*R*)-1-(4-methylpyrimidin-2-yl)-*N*-phenethylpiperidine-3-carboxamide (33 mg, 96%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03 (d, J = 5.2, 1H), 7.23−7.15 (m, 2H), 7.15−7.09 (m, 1H), 7.08−7.03 (m, 2H), 6.48−6.36 (br, 1H), 6.26 (d, J = 5.2, 1H), 4.10−4.04 (m, 1H), 3.95−3.85 (m, 1H), 3.72−3.64 (m, 1H), 3.53−3.35 (m, 3H), 2.75−2.62 (m, 2H), 2.34−2.26 (m, 1H), 2.23 (s, 3H), 2.08−1.95 (m, 1H), 1.84−1.72 (m, 1H), 1.58−1.38 (m, 2H). HPLC-MS $C_{19}H_{24}N_4O$ (M + H$^+$): 325.2, 5.56 min.

*(R)-N-(4-Fluorophenethyl)-1-(4-methylpyrimidin-2-yl)piperidine-3-carboxamide (**13e**).* **13e** was prepared by general method A in 74% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 (d, J = 4.9, 1H), 7.07−6.97 (m, 2H), 6.96−6.84 (m, 2H), 6.56 (s, 1H), 6.31 (d, J = 5.0, 1H), 3.96 (dd, J = 3.8, 13.9, 1H), 3.88 (dd, J = 6.6, 13.9, 1H), 3.78−3.65 (m, 2H), 3.53 (td, J = 6.8, 13.2, 1H), 3.37 (dt, J = 6.9, 12.6, 1H), 2.70 (tq, J = 6.9, 13.9, 2H), 2.41−2.34 (m, 1H), 2.27 (s, 3H), 2.11 (td, J = 7.4,

12.5, 1H), 1.83−1.74 (m, 1H). HPLC-MS $C_{19}H_{23}FN_4O$ (M + H^+): 343.1, 5.86 min.

*(R)-N-(4-Bromophenethyl)-1-(4-methylpyrimidin-2-yl)piperidine-3-carboxamide (**13f**).* **13f** was prepared by general method A in 62% isolated yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03 (d, J = 5.0, 1H), 7.26 (d, J = 8.3, 2H), 6.89 (d, J = 8.3, 2H), 6.60 (s, 1H), 6.27 (d, J = 5.0, 1H), 3.87 (d, J = 5.1, 2H), 3.67 (s, 2H), 3.49 (dt, J = 6.6, 13.1, 1H), 3.31 (td, J = 6.8, 12.6, 1H), 2.63 (dq, J = 7.0, 13.9, 2H), 2.37−2.27 (m, 1H), 2.22 (s, 3H), 2.07 (dd, J = 6.6, 13.0, 1H), 1.73 (dd, J = 5.4, 12.7, 1H), 1.48 (dd, J = 5.9, 11.5, 2H). HPLC-MS $C_{19}H_{23}BrN_4O$ (M + H^+): 402.1, 1.91 min (4 min method).

*(R)-N-(4-Methylphenethyl)-1-(pyrimidin-2-yl)piperidine-3-carboxamide (**13g**).* **13g** prepared by general method A in 43% isolated yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.18 (d, J = 4.7, 2H), 7.00 (d, J = 8.0, 2H), 6.95 (d, J = 8.1, 2H), 6.37 (t, J = 4.7, 1H), 6.05 (s, 1H), 4.22−4.14 (m, 1H), 4.01−3.97 (m, 1H), 3.57 (dd, J = 8.2, 13.7, 1H), 3.43−3.33 (m, 3H), 2.68−2.63 (m, 2H), 2.29−2.21 (m, 4H), 1.99−1.96 (m, 1H), 1.81−1.76 (m, 1H), 1.55−1.52 (m, 1H), 1.45−1.42 (m, 1H). HPLC-MS $C_{19}H_{24}N_4O$ (M + H^+): 325.2, 6.75 min.

*(R)-N-(4-Cyanophenethyl)piperidine-3-carboxamide (**15**).* (*R*)-1-Boc-nipecotic acid (57 g, 249 mmol), EDCI (52.5 g, 274 mmol), and HOBT (37.0 g, 274 mmol) were dissolved in DMF (600 mL) and stirred for 60 min at ambient temperature. DIEA (95 mL, 548 mmol) and 4-(2-aminoethyl)benzonitrile hydrochloride (**14**) (50 g, 274 mmol) were sequentially added, and the reaction was stirred at ambient temperature overnight. The reaction was concentrated and partitioned between ethyl acetate (200 mL) and water (200 mL). The aqueous layer was extracted twice with ethyl acetate (100 mL), and the combined organic extracts were washed consecutively with 100 mL of 10% citric acid (2×), bicarbonate (2×), water (2×), and brine (1×). The extracts were dried over magnesium sulfate, filtered, and concentrated to give crude (*R*)-*tert*-butyl 3-((4-cyanophenethyl)-carbamoyl)piperidine-1-carboxylate (89 g, 99%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60 (d, J = 8.3, 2H), 7.31 (d, J = 8.3, 2H), 3.75 (s, 1H), 3.72−3.59 (m, 1H), 3.58−3.44 (m, 2H), 3.30−3.18 (m, 1H), 3.15−2.96 (m, 1H), 2.88 (t, J = 7.2, 2H), 2.24 (s, 1H), 1.96−1.82 (m, 1H), 1.78 (s, 1H), 1.68 (s, 2H), 1.55 (s, 1H), 1.44 (s, 9H). HPLC-MS calculated $C_{20}H_{27}N_3O_3$ (M + H^+-tBu): 302.2, 1.38 min (2.5 min method). (*R*)-*tert*-Butyl 3-((4-cyanophenethyl)-carbamoyl)piperidine-1-carboxylate was dissolved in ethyl acetate (475 mL) and was cooled to 0 °C, then 4 M HCl in dioxane (190 mL, 760 mmol) was added slowly and the mixture was stirred to completion. At 1 h the solution began to precipitate and at 90 min a viscous oil fell out of solution which impeded stirring. Then 100 mL of DCM was added and the mixture was allowed to stand overnight then evaporated to dryness to give a white foam. The salt was converted into the free base by dissolving in water and neutralizing with potassium carbonate (s) until pH ∼10, then extracted with DCM (2 × 300 mL), followed by chloroform/2-propanol (2 × 150 mL). The organic extracts were dried over sodium sulfate, filtered through a thin pad of Celite, and concentrated to dryness to yield **17g**. The pH was then adjusted to pH 12 and extracted with 3/1 chloroform/2-propanol (2 × 250 mL), combined with the initial extract, dried over sodium sulfate, filtered through a thin pad of Celite, and concentrated to dryness to give **15** as a white to off-white solid (54 g, 84%, 2 steps). ^{1}H NMR (400 MHz, CD_3OD) δ 7.66 (d, J = 8.2, 2H), 7.41 (d, J = 8.2, 2H), 2.88 (t, J = 7.1, 4H), 2.73−2.47 (m, 2H), 2.29 (m, 1H), 1.68 (m, 5H).

*(R)-N-(4-Chlorophenethyl)-1-(5-fluoropyrimidin-2-yl)piperidine-3-carboxamide (**19a**): General Method B.* To a 5 mL reaction vial with a screw cap was added **15** (100 mg, 0.37 mmol), 2-propanol (2 mL), DIEA (122 μL, 0.7 mmol, 2 equiv), and 2-chloro-5-fluoropyrimidine (54 μL, 0.44 mmol). The vessel was sealed and heated to 100 °C for 2 h. The solvent was removed en vacuo and the residue purified by flash chromatography (24 g ISCO, 0−10% methanol/DCM) to yield the title compound (93 mg, 69%) as a pale-yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.17 (s, 2H), 7.25 (d, J = 8.3, 2H), 7.09 (d, J = 8.3, 2H), 6.06 (s, 1H), 4.21−4.12 (m, 1H), 3.99 (s, 1H), 3.67−3.39 (m, 4H), 2.81−2.77 (m, 2H), 2.40−2.29 (m, 1H), 2.11−1.98 (m, 1H), 1.88−1.85 (m, 1H), 1.68−1.64 (m,, 1H), 1.56−1.53 (m, 1H). HPLC-MS $C_{18}H_{20}ClFN_4O$ (M + H^+): 363.1, 8.46 min.

*(R)-N-(4-Chlorophenethyl)-1-(5-fluoro-4-methylpyrimidin-2-yl)-piperidine-3-carboxamide (**19b**).* (*R*)-1-(4-Amino-5-fluoropyrimidin-2-yl)-*N*-(4-chlorophenethyl)piperidine-3-carboxamide (prepared by general method B) (50 mg, 0.13 mmol) and $CuCl_2$ (21 mg, 0.16 mmol) were added into 1 mL of ACN and cooled to 0 °C. After *tert*-butyl nitrite (19 μL, 0.16 mmol) was added, the reaction mixture was warmed to rt and left overnight. The mixture was concentrated, and the residue was purified by preparative HPLC (10−90% ACN/water, 0.05% TFA, 100 mL/min) to give (*R*)-1-(4-chloro-5-fluoropyrimidin-2-yl)-*N*-(4-chlorophenethyl)piperidine-3-carboxamide. (*R*)-1-(4-Chloro-5-fluoropyrimidin-2-yl)-*N*-(4-chlorophenethyl)piperidine-3-carboxamide (50 mg, 0.13 mmol), methylzinc chloride (95 μL, 2.0 M solution in THF, 0.19 mmol), and $Pd(PPh_3)_2Cl_2$ (4.4 mg, 0.006 mmol) were added into 1 mL of THF. After flushing with argon, the mixture was stirred at rt overnight. The reaction was quenched with aqueous NH_4Cl and purified by preparative HPLC (10−90% ACN/water, 0.05% TFA, 100 mL/min) to give (*R*)-*N*-(4-chlorophenethyl)-1-(5-fluoro-4-methylpyrimidin-2-yl)piperidine-3-carboxamide (9 mg, 19%). ^{1}H NMR (400 MHz, CD_3OD) δ 7.27−7.23 (m, 3H), 7.20−7.17 (m, 2H), 6.55 (dd, J = 2.6, 9.1, 1H), 4.10−4.02 (m, 1H), 3.94−3.90 (m, 1H), 3.47 (dt, J = 7.0, 13.8, 1H), 3.39 (dd, J = 6.8, 13.7, 1H), 3.01 (dd, J = 10.0, 12.9, 1H), 2.92−2.83 (m, 1H), 2.79 (t, J = 7.1, 2H), 2.39 (ddd, J = 3.9, 8.9, 14.1, 1H), 2.31 (d, J = 3.0, 2H), 1.89−1.80 (m, 1H), 1.79−1.66 (m, 2H), 1.64−1.49 (m, 2H).

*(R)-N-(4-Chlorophenethyl)-1-(pyridin-2-yl)piperidine-3-carboxamide (**19c**).* **19c** was prepared by general method B in 38% yield. ^{1}H NMR (400 MHz, CD_3OD) δ 7.98−7.92 (m, 1H), 7.47−7.41 (m, 1H), 7.21−7.14 (m, 2H), 7.12−7.06 (m, 2H) 6.68 (d, J = 8.8, 1H), 6.57−6.51 (m, 1H), 4.09−4.02 (m, 1H), 3.98−3.91 (m, 1H), 3.31 (td, J = 7.2, 2.8, 2H), 2.93 (dd, J = 13.2, 10.4, 1H), 2.86−2.77 (m, 1H), 2.68 (t, J = 7.2, 2H), 2.34−2.24 (m, 1H), 1.81−1.73 (m, 1H), 1.70−1.57 (m, 2H), 1.51−1.38 (m, 1H). HPLC-MS $C_{19}H_{22}ClN_3O$ (M + H^+): 344.0, 0.69 min (2 min).

*(R)-N-(4-Chlorophenethyl)-1-(pyrimidin-4-yl)piperidine-3-carboxamide (**19d**).* **19d** was prepared by general method B in 41% yield. ^{1}H NMR (400 MHz, CD_3OD) δ 8.34 (s, 1H), 8.00 (s, 1H), 7.22−7.16 (m, 2H), 7.13−7.07 (m, 2H), 6.66 (d, J = 6.3, 1H), 4.34 (s, 1H), 4.18 (d, J = 11.6, 1H), 3.31 (d, J = 7.1, 2H), 3.06−2.86 (m, 2H), 2.71−2.67 (m, 2H), 2.23 (tt, J = 4.0, 10.8, 1H), 1.86−1.76 (m, 1H), 1.76−1.63 (m, 2H), 1.47−1.35 (m, 1H). HPLC-MS $C_{18}H_{21}ClN_4O$ (M + H^+): 345.1, 4.56 min.

*(R)-N-(4-Chlorophenethyl)-1-(2-methylpyrimidin-4-yl)piperidine-3-carboxamide (**19e**).* **19e** was prepared by general method B in 63% yield. ^{1}H NMR (400 MHz, CD_3OD) δ 7.89 (d, J = 6.5, 1H), 7.17 (dd, J = 4.3, 6.3, 2H), 7.09 (d, J = 8.4, 2H), 6.47 (d, J = 6.6, 1H), 4.32 (s, 1H), 4.15 (s, 1H), 3.39−3.25 (m, 2H), 3.00 (dd, J = 10.6, 13.3, 1H), 2.97 (s, 1H), 2.77−2.65 (m, 2H), 2.34−2.27 (m, 3H), 2.23 (tt, J = 4.0, 10.7, 1H), 1.85−1.77 (m, 1H), 1.76−1.62 (m, 2H), 1.38 (ddd, J = 8.3, 14.7, 16.5, 1H). HPLC-MS $C_{19}H_{23}ClN_4O$ (M + H^+): 359.1, 4.79 min.

*(R)-N-(4-Chlorophenethyl)-1-(6-methylpyridin-2-yl)piperidine-3-carboxamide (**19f**).* **19f** was prepared by general method B in 41% yield. ^{1}H NMR (400 MHz, CD_3OD) δ 7.30 (dd, J = 7.3, 8.4, 1H), 7.17−7.11 (m, 2H), 7.08−7.02 (m, 2H), 6.40 (t, J = 8.4, 2H), 4.06−3.98 (m, 1H), 3.86 (dt, J = 3.9, 12.8, 1H), 3.36 (dt, J = 7.0, 14.0, 1H), 3.33−3.23 (m, 1H), 2.98 (dd, J = 9.9, 13.0, 1H), 2.85−2.74 (m, 1H), 2.72−2.62 (m, 2H), 2.34−2.21 (m, 4H), 1.80−1.38 (m, 4H). HPLC-MS $C_{20}H_{24}ClN_3O$ (M + H^+): 358.1, 5.19 min.

*(R)-N-(4-Chlorophenethyl)-1-(4-methylpyridin-2-yl)piperidine-3-carboxamide (**19g**).* **19g** was prepared by general method B in 8% yield. ^{1}H NMR (400 MHz, CD_3OD) δ 7.78 (d, J = 5.5, 1H), 7.20−7.14 (m, 2H), 7.10 (d, J = 8.4, 2H), 6.64 (s, 1H), 6.48 (d, J = 5.4, 1H), 3.99−3.88 (m, 2H), 3.32 (t, J = 6.6, 2H), 3.07−3.01 (m,, 1H), 2.93−2.91 (m, 1H), 2.69 (t, J = 7.1, 2H), 2.38−2.27 (m, 1H), 2.21 (s, 3H), 1.78 (s, 1H), 1.74−1.57 (m, 2H), 1.48 (s, 1H). HPLC-MS $C_{20}H_{24}ClN_3O$ (M + H^+): 358.2, 5.24 min.

*(R)-N-(4-Cyanophenethyl)-1-(5-fluoro-4-methylpyrimidin-2-yl)-piperidine-3-carboxamide (**19h**).* A solution of 2,4-dichloro-5-fluoropyrimidine (1g, 6 mmol) and $Fe(acac)_3$ (317 mg, 0.69 mmol) in dry THF (15 mL) was cooled to −78 °C and was subsequently treated with methyl magnesiumbromide (3.4 mL, 3 M in diethyl ether)

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via dropwise addition. After 1 h, the reaction was quenched with saturated ammonium chloride (2 mL) and extracted with diethyl ether. dried over magnesium sulfate, and filtered. Purification by flash chromatography (24 g cartridge, 0−30% ethyl acetate/hexanes) gave 596 mg (68%) of 2-chloro-5-fluoro-4-methylpyrimidine as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.35 (d, J = 0.9, 1H), 2.54 (d, J = 2.5, 3H). Caution should be taken when removing solvent en vacuo, as the product is volatile. The title compound was then prepared by general method B, starting from (*R*)-*N*-(4-cyanophenethyl)piperidine-3-carboxamide and the pyrimidine prepared above in 36% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.99 (d, J = 1.6, 1H), 7.53 (d, J = 8.3, 2H), 7.22 (d, J = 8.2, 2H), 6.35 (s, 1H), 3.98 (dd, J = 3.6, 13.8, 1H), 3.86−3.71 (m, 2H), 3.67−3.51 (m, 2H), 3.44 (dd, J = 6.2, 13.1, 1H), 2.85 (dq, J = 7.0, 17.9, 2H), 2.42−2.34 (m, 1H), 2.33 (d, J = 2.5, 3H), 2.07 (d, J = 8.2, 1H), 1.81 (s, 1H), 1.55 (d, J = 5.6, 2H). HPLC-MS $C_{20}H_{22}FN_5O$ (M + H$^+$): 367.2, 2.07 min (4 min method).

*(R)-N-(4-Cyanophenethyl)-1-(2-(trifluoromethyl)pyrimidin-4-yl)-piperidine-3-carboxamide (**19i**).* **19i** was prepared by general method B in 80% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.50 (d, J = 4.8, 1H), 7.55 (d, J = 8.2, 2H), 7.24 (d, J = 8.2, 2H), 6.76 (d, J = 4.8, 1H), 6.23 (s, 1H), 4.13 (s, 1H), 3.99 (s, 1H), 3.79 (d, J = 5.9, 1H), 3.74−3.57 (m, 2H), 3.52−3.34 (m, 1H), 2.92−2.82 (m, 2H), 2.45−2.35 (m, 1H), 2.14 (s, 1H), 1.87 (s, 1H), 1.60 (q, J = 4.6, 2H). HPLC-MS $C_{20}H_{20}F_3N_5O$ (M + H$^+$): 404.2, 8.53 min.

*(R)-N-(4-Cyanophenethyl)-1-(4-(trifluoromethyl)pyrimidin-2-yl)-piperidine-3-carboxamide (**19j**).* **19j** was prepared by general method B in 50% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.31 (d, J = 6.3, 1H), 7.56 (d, J = 8.2, 2H), 7.26 (d, J = 8.1, 2H), 6.58 (d, J = 6.3, 1H), 6.35−6.13 (m, 1H), 4.02 (d, J = 10.4, 1H), 3.96−3.75 (m, 2H), 3.71−3.58 (m, 1H), 3.49 (s, 1H), 3.34 (s, 1H), 2.98−2.80 (m, 2H), 2.41 (s, 1H), 2.22−2.08 (m, 1H), 1.89 (s, 1H), 1.62 (s, 2H). HPLC-MS $C_{20}H_{20}F_3N_5O$ (M + H$^+$): 404.2, 7.20 min.

*(R)-N-(4-Cyanophenethyl)-1-(4-methyl-6-(4-methylpiperazin-1-yl)pyrimidin-2-yl)piperidine-3-carboxamide (**22a**).* A solution of 2,4-dichloro-6-methylpyrimidine (326 mg, 2 mmol) in ethanol (4 mL) was treated with DIEA (0.35 mL, 2 mmol), and the mixture was cooled to −10 °C, when a solution of 1-methylpiperazine (0.22 mL, 2 mmol) in ethanol (1 mL) was added dropwise. The cooling bath was removed, and the mixture was slowly warmed to rt. After 2 h, the reaction was complete and the solvent was removed en vacuo and was purified by flash chromatography (12 g, 0−10% methanol/DCM) to give 320 mg of 2-chloro-4-methyl-6-(4-methylpiperazin-1-yl)-pyrimidine. 2-Chloro-4-methyl-6-(4-methylpiperazin-1-yl)pyr-imidine (35 mg, 0.15 mmol) was dissolved in DMA (1 mL) and treated with DIEA (26 μL, 0.15 mmol) and **15** (46 mg, 0.18 mmol). The mixture was heated to 150 °C for 2 h, cooled to rt, and subsequently purified by preparative HPLC to give the title compound as a white solid in 41% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60 (s, 1H), 7.44 (d, J = 8.2, 2H), 7.12 (d, J = 8.2, 2H), 5.73 (s, 1H), 4.45−4.43 (m, 1H), 4.20−4.18 (m, 1H), 3.78 (dd, J = 6.6, 13.4, 1H), 3.59 (s, 4H), 3.42−3.41 (m, 1H), 3.29−3.18 (m, 2H), 2.83 (dd, J = 6.9, 13.8, 1H), 2.74 (dd, J = 6.8, 13.6, 1H), 2.50−2.47 (m, 5H), 2.36−2.30 (m, 4H), 2.17 (s, 3H), 1.78−1.70 (m, 1H), 1.57−1.50 (m, 1H), 1.29−1.24 (m, 1H). HPLC-MS $C_{25}H_{33}N_7O$ (M + H$^+$): 448.2, 2.62 min.

*(R)-N-(4-Cyanophenethyl)-1-(4-(4-methylpiperazin-1-yl)-6-(trifluoromethyl)pyrimidin-2-yl)piperidine-3-carboxamide (**22b**).* **20** (0.079 mL, 0.71 mmol) was dissolved in *N*-methylpyrrolidinone (2.5 mL) and cooled to 0 °C. The mixture was then treated with DIEA (0.17 mL, 0.95 mmol) and 2,4-dichloro-6-(trifluoromethyl)pyrimidine (**21b**) (114 mg, 0.52 mmol). The mixture was warmed to rt over 30 min. To this was then added **15**-HCl salt (140 mg, 0.48 mmol), and the mixture was heated to 100 °C for 17 h. The reaction was cooled, diluted with water, extracted with ethyl acetate, and the organic layer was washed with water, dried over magnesium sulfate, and concentrated to dryness. It was purified by ISCO (24 g, 0−10% methanol/DCM) to yield the title compound as a white solid (51 mg, 21%). ^{1}H NMR (400 MHz, CD_3OD) δ 7.63 (d, J = 8.1, 2H), 7.38 (d, J = 8.1, 2H), 6.33 (s, 1H), 4.47 (s, 2H), 3.69 (s, 4H), 3.59−3.48 (m, 1H), 3.41 (d, J = 6.8, 1H), 3.21−3.11 (m, 1H), 3.05 (s, 1H), 2.96−2.84 (m, 2H), 2.52 (t, J = 5.0, 4H), 2.35 (s, 4H), 1.75 (d, J = 6.8, 4H), 1.55−1.41 (m, 1H). HPLC-MS $C_{25}H_{30}F_3N_7O$ (M + H$^+$): 502.2, 5.66 min.

*(R)-N-(4-Cyanophenethyl)-1-(5-fluoro-4-methyl-6-(4-methylpiperazin-1-yl)pyrimidin-2-yl)piperidine-3-carboxamide (**22c**).* To a solution of 2,4,6-trichloro-5-fluoropyrimidine (**21c**) (149 mg, 0.74 mmol) in ethanol (2 mL) was added DIEA (131 μL, 0.75 mmol) and **20** (81 μL, 0.73 mmol). The mixture was heated to 80 °C until complete by LC-MS, then cooled to rt, diluted with water (2 mL), extracted with DCM (5 mL), dried over magnesium sulfate, filtered, and concentrated to give 2,4-dichloro-5-fluoro-6-(4-methylpiperazin-1-yl)pyrimidine as an off-white solid (152 mg, 79%) that was carried forward without further purification. 2,4-Dichloro-5-fluoro-6-(4-methylpiperazin-1-yl)pyrimidine (100 mg, 0.37 mmol) and ferric acetylacetonate (20 mg, 0.056 mmol) were dissolved in THF (2 mL) and *N*-methylpyrroldinone (0.1 mL). The solution was cooled to 0 °C and treated with methylmagnesium bromide (3 M in diethyl ether, 211 μL, 0.63 mmol). The reaction was allowed to warm to rt over 1 h and was complete by LC-MS. The reaction was quenched with water, extracted with ethyl acetate, dried over magnesium sulfate, filtered, and concentrated then purified by flash chromatography (12 g ISCO, 0−7% gradient methanol/DCM) to give 2-chloro-5-fluoro-4-methyl-6-(4-methylpiperazin-1-yl)pyrimidine as a colorless oil that crystallized on standing (74 mg, 80%). The title compound was then prepared as described in general method B by combining 2-chloro-5-fluoro-4-methyl-6-(4-methylpiperazin-1-yl)pyrimidine with **15** to give a 6% isolated yield after preparative HPLC purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 (d, J = 8.2, 2H), 7.08 (d, J = 8.2, 2H), 6.99 (s, 1H), 4.06 (dd, J = 4.9, 14.0, 1H), 3.83−3.78 (m, 1H), 3.72−3.55 (m, 5H), 3.45 (dd, J = 3.4, 14.1, 1H), 3.33−3.17 (m, 2H), 2.84−2.64 (m, 2H), 2.47−2.33 (m, 5H), 2.26 (s, 3H), 2.15−2.10 (m, 4H), 1.69−1.62 (m, 1H), 1.47−1.45 (m, 1H), 1.37−1.28 (m, 1H). HPLC-MS $C_{25}H_{32}FN_7O$ (M + H$^+$): 466.3, 1.1 min (3 min method).

*(R)-N-(4-Cyanophenethyl)-1-(2-methyl-6-(4-methylpiperazin-1-yl)pyrimidin-4-yl)piperidine-3-carboxamide (**22d**).* A solution of **15**-HCl salt (123 mg, 0.41 mmol) in 2-propanol (1.5 mL) and DIEA (0.18 mL, 1 mmol) was cooled to 0 °C. To this was added 4,6-dichloro-2-methylpyrimidine **21d** (68 mg, 0.41 mmol). After warming to rt over 30 min, **15** was added and the mixture was heated to 160 °C in a microwave reaction vessel for 15 min. The crude was purified by preparative HPLC to yield the title compound (16 mg, 9%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.57 (s, 1H), 7.46 (d, J = 8.2, 2H), 7.15 (d, J = 8.2, 2H), 5.33 (s, 1H), 4.32 (d, J = 14.1, 1H), 3.72−3.50 (m, 5H), 3.37 (ddd, J = 4.9, 13.0, 17.1, 3H), 3.20 (t, J = 9.3, 1H), 2.87 (dt, J = 6.8, 13.7, 1H), 2.77 (dt, J = 6.9, 14.0, 1H), 2.46 (dt, J = 4.6, 8.5, 5H), 2.34 (s, 3H), 2.28 (s, 3H), 2.21 (s, 1H), 1.78−1.66 (m, 1H), 1.52 (s, 1H), 1.38 (s, 1H). HPLC-MS $C_{25}H_{33}N_7O$ (M + H$^+$): 448.2, 2.58 min.

*(R)-N-(4-Cyanophenethyl)-1-(2-methyl-6-(4-methylpiperazin-1-yl)pyrimidin-4-yl)piperidine-3-carboxamide (**22e**).* A solution of **15**-HCl salt (360 mg, 1.22 mmol) in 2-propanol (4 mL) and DIEA (0.64 mL, 3.7 mmol) was cooled to 0 °C. To this was added 4,6-dichloro-2-trifluoromethylpyrimidine (265 mg, 1.22 mmol). After warming to rt over 15 min, **20** (0.67 mL, 6.1 mmol) was added and the mixture was heated to 160 °C in a microwave reaction vessel for 15 min. The solvent was removed en vacuo, and the crude was suspended in water. The precipitate was collected by filtration and purified by flash chromatography (40 g, 0−7% methanol/DCM, 0.1% TEA) to give the title compound (381 mg, 62%) as a white solid. ^{1}H NMR ($CDCl_3$) δ 7.45 (d, J = 8.2, 2H), 7.17 (d, J = 8.2, 2H), 7.1 (br s, 1H), 5.5 (s, 1H), 4.27 (dd, J = 14.2, 4.6, 1H), 3.70−3.59 (m, 5H), 3.48−3.26 (m, 4H), 2.91−2.84 (m, 1H), 2.8.1−2.74 (m, 1H), 2.49 (t, J = 5.1, 4H), 2.46−2.42 (m, 1H), 2.34 (s, 3H), 2.30−2.22 (m, 1H), 1.78−1.70 (m, 1H), 1.62−1.53 (m, 1H), 1.50−1.40 (m, 1H). ^{13}C NMR ($CDCl_3$) δ 176.2, 164.9, 164.5, 146.6, 133.3, 131.1, 119.9, 111.2, 83.1, 55.5, 47.7, 46.2, 45.9, 44.8, 43.8, 44.2, 36.5, 29.3, 25.1. HPLC-MS $C_{25}H_{30}F_3N_7O$ (M + H$^+$): 502.3, 5.70 min. Melting point 169−171 °C.

(R)-1-(6-Chloro-2-(trifluoromethyl)pyrimidin-4-yl)-N-(4-cyanophenethyl)piperidine-3-carboxamide. A 3-neck 3 L round-bottom flask was fitted with an overhead stirrer and an addition funnel. Compound **15** (54 g, 210 mmol) was dissolved in a mixture of ACN (900 mL) and DIEA (105 mL) at rt. The mixture was then cooled to 0

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°C. A solution of **21e** (50 g, 231 mmol) in ACN (diluted to volume up to 100 mL) was added via an addition funnel over 30 min. During the addition, the mixture thickened considerably, and an additional 200 mL of ACN was added (total 1100 mL). Upon complete addition, the mixture was allowed to stir for 4 h, stirring was stopped, and the material was allowed to settle and precipitate for 1 h and was then collected by filtration as a white solid (85.8 g, 93%). ^{1}H NMR (400 MHz, DMSO) δ 8.03 (s, 1H), 7.76 (d, J = J =7.9, 2H), 7.42 (d, J = 8.0, 2H), 7.25 (s, 1H), 4.67−4.41 (m, 1H), 4.14−3.89 (m, 1H), 3.33−3.30 (m, 3H obscured by solvent), 3.06 (t, J = 11.2, 1H), 2.81 (t, J = 6.9, 2H), 2.39−2.21 (m, 1H), 1.85−1.68 (m, 3H), 1.43−1.40 (m, 1H). HPLC-MS $C_{20}H_{19}ClF_3N_5O$ (M + H$^+$): 438.2, 1.27 min (3 min method).

(R)-N-(4-Cyanophenethyl)-1-(6-(piperazin-1-yl)-2-(trifluoromethyl)pyrimidin-4-yl)piperidine-3-carboxamide (***23a***). A solution of (*R*)-1-(6-chloro-2-(trifluoromethyl)pyrimidin-4-yl)-*N*-(4-cyanophenethyl)piperidine-3-carboxamide (150 mg, 0.34 mmol) in 2-propanol (3 mL) was treated with DIEA (0.06 mL, 0.34 mmol) and 1-Boc-piperazine (316 mg, 1.7 mmol). The mixture was heated 165 °C for 25 min. The solvent was removed and then purified by flash chromatography (24 g ISCO cartridge, 0−8% methanol/DCM). The isolated product was then dissolved in DCM (1 mL) and treated with 4 M HCl in dioxane (1 mL, 4 mmol). After 1 h, the solvent was removed en vacuo, yielding the title compound as the HCl salt (135 mg, 76%). ^{1}H NMR (400 MHz, CD_3OD) δ 7.54−7.49 (m, 2H), 7.27 (d, J = 8.2, 2H), 5.74 (s, 1H), 4.20 (d, J = 13.3, 1H), 4.06−3.98 (m, 1H), 3.57−3.48 (m, 4H), 3.41 (dt, J = 6.8, 13.6, 1H), 3.32 (dd, J = 6.8, 13.6, 1H), 3.05 (dd, J = 10.0, 13.4, 1H), 2.96−2.85 (m, 1H), 2.83−2.74 (m, 6H), 2.31−2.19 (m, 1H), 1.81−1.56 (m, 3H), 1.43−1.39 (m, 1H). HPLC-MS $C_{24}H_{28}F_3N_7O$ (M + H$^+$): 488.2, 5.70 min.

(R)-N-(4-Cyanophenethyl)-1-(6-(4-(oxetan-3-yl)piperazin-1-yl)-2-(trifluoromethyl)pyrimidin-4-yl)piperidine-3-carboxamide (***23b***). Compound **23a** (135 mg, 0.25 mmol) was dissolved in THF (1 mL) and treated with acetic acid (0.043 mL, 0.75 mmol) and 3-oxetanone (0.03 mL, 0.5 mmol). The mixture was heated to 55 °C for 1 h under an atmosphere of nitrogen. To this was then added sodium triacetoxyborohydride (106 mg, 0.5 mmol). Heating was continued at 65 °C for 3 h. The mixture was then cooled to rt and quenched with 10% citric acid (1 mL). Water (1 mL) was added, and the aqueous layer was extracted with ethyl acetate (3 × 3 mL). The combined organics were dried over magnesium sulfate, filtered, and concentrated. Purified by flash chromatography (12 g ISCO cartridge, 0−8% methanol/DCM, 0.1% TEA). The product was further purified by preparative HPLC to give the title compound (35 mg, 25%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 (d, J = 8.2, 2H), 7.14 (d, J = 8.2, 2H), 7.02 (s, 1H), 5.43 (s, 1H), 4.68 (t, J = 6.6, 2H), 4.62 (t, J = 6.2, 2H), 4.29−4.25 (m, 1H), 3.73−3.58 (m, 5H), 3.57−3.47 (m, 1H), 3.41 (dd, J = 3.4, 14.4, 1H), 3.35−3.29 (m, 3H), 2.88 (dt, J = 7.1, 14.2, 1H), 2.82−2.72 (m, 1H), 2.47−2.37 (m, 5H), 2.32−2.21 (m, 1H), 1.72 (ddd, J = 4.2, 9.4, 17.9, 1H), 1.56 (d, J = 4.1, 1H), 1.51−1.37 (m, 1H). HPLC-MS $C_{27}H_{32}F_3N_7O_2$ (M + H$^+$): 544.3, 5.87 min.

5-(2-Aminoethyl)picolinonitrile Hydrochloride (***26***). 2-(6-Chloro-3-pyridinyl)acetonitrile (**24**) (0.68 g, 4.46 mmol), di-*tert*-butyl dicarbonate (1.95 g, 8.92 mmol), and nickel chloridehexahydrate (1.06 g, 4.46 mmol) were added to methanol (30 mL) at rt. Sodium borohydride (0.50 g, 13.37 mmol) was added portionwise to the reaction mixture. The reaction mixture was allowed to stir at rt for 3 h. After filtration on a pad of Celite and concentration en vacuo, 50 mL of ethyl acetate was added to the crude material, which was washed twice with 50 mL of water. The organic phase was dried over sodium sulfate, filtered, and concentrated en vacuo. The crude product was purified by flash chromatography (ethyl acetate/hexane = 30/70) to give 0.56 g (49%) of *tert-butyl*-2-(6-chloro-3-pyridinyl) ethylcarbamate HPLC-MS $C_{12}H_{17}ClN_2O_2$, (M + H$^+$) 257.1). A reaction vessel was then charged with *tert-butyl*-2-(6-chloro-3-pyridinyl) ethylcarbamate (0.5 g, 1.9 mmol), palladium trifluoroacetate (64 mg, 0.19 mmol), racemic-2-di-*t*-butylphosphino-1,1′-binaphthyl (155 mg, 0.39 mmol), $Zn(CN)_2$ (181 mg, 1.6 mmol), and zinc dust (25 mg, 0.39 mmol). The vessel was evacuated and backfilled with nitrogen. DMA (5 mL) was added via syringe. The reaction was stirred at rt for 5 min and then 100 °C for 3 h. After being cooled down to rt, the reaction was diluted into ethyl acetate and washed with brine. The organic phase was concentrated, and the crude product was purified by flash chromatography, eluting with 40% ethyl acetate in hexane to yield *tert*-butyl (2-(6-chloropyridin-3-yl)ethyl)carbamate (**25**) (0.26 g, 55%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.59 (d, J = 1.2, 1H), 7.98 (d, J = 7.6, 1H), 7.89 (dd, J = 8.0, 2.4, 1H), 6.95 (t, J = 5.6, 1H), 3.24−3.19 (m, 2H), 2.82 (t, J = 6.8, 2H), 1.32 (s, 9H). $C_{13}H_{17}N_3O_2$ (M + H$^+$) 248.2. To the solution of *tert*-butyl (2-(6-cyanopyridin-3-yl)ethyl)carbamate (**25**) (0.25 g, 1 mmol) in DCM (4 mL) was added 4 M HCl in dioxane (0.5 mL, 2 mmol). The reaction was stirred at ambient temperature for 16 h. The solvent was removed en vacuo to give the title compound **26**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.71−8.68 (m, 1H), 8.13 (s, 3H8.03−7.99 (m, 2H), 3.17−3.08 (m, 2H), 3.06−3.00 (m, 2H). HPLC-MS $C_8H_9N_3$ (M + H$^+$) 148.2.

5-(2-Aminoethyl)pyrimidine-2-carbonitrile (***29***). Potassium (2-((*tert*-butoxycarbonyl)-amino)ethyl) trifluoroborate (92 mg, 0.5 mmol), 5-bromopyrimidine-2-carbonitrile (125 mg, 0.5 mmol), $Pd(OAc)_2$ (11 mg, 0.05 mmol), 2-dicyclohexylphosphino-2′,6′-diisopropoxybiphenyl (RuPhos) (47 mg, 0.1 mmol), and Cs_2CO_3 (487 mg, 1.5 mmol) were mixed in a mixture solvent of toluene (2.5 mL) and water (0.25 mL). The reaction was complete after being heated at 90 °C for 10 h. After being cooled down to rt, the reaction mixture was diluted with ethyl acetate and washed with saturated sodium bicarbonate and brine. The organic phase was dried over sodium sulfate, and solvent was removed by rotary evaporation. The residue was purified by flash chromatography, eluting with 50% ethyl acetate/hexanes to give *tert*-butyl (2-(5-cyanopyrimidin-2-yl)ethyl)-carbamate as an off-white solid (11 mg, 9%). Next, a solution of *tert*-butyl (2-(5-cyanopyrimidin-2-yl)ethyl)carbamate (11 mg, 0.044 mmol) in DCM (0.5 mL) was treated with TFA (0.05 mL). The reaction was stirred at rt for 1 h. The solvent and excess TFA were removed by rotary evaporation, and the crude product was used directly in the next step without further purification.

(R)-N-(2-(6-Cyanopyridin-3-yl)ethyl)-1-(6-(4-methylpiperazin-1-yl)-2-(trifluoromethyl)pyrimidin-4-yl)piperidine-3-carboxamide (***32a***). To a solution of **21e** (4.92 g, 22.8 mmol) in 2-propanol (100 mL) at 0 °C were added (*R*)-ethyl piperidine-3-carboxylate (3.6 g, 22.9 mmol) and TEA (4.6 g, 45.6 mmol). The reaction was warmed to rt and stirred for 1 h. The reaction mixture was diluted with ethyl acetate, washed with saturated sodium bicarbonate and brine, and dried over sodium sulfate. The organic phase was passed through a pad of silica gel to give (*R*)-ethyl 1-(6-chloro-2-(trifluoromethyl)-pyrimidin-4-yl)piperidine-3-carboxylate (**31a**) as an oil (5.6 g, 73%). Then, to a 100 mL round-bottom flask containing **31a** (5.52 g, 16.4 mmol) and lithium hydroxide monohydrate (0.76 g, 18 mmol) were added THF (30 mL), methanol (10 mL), and water (10 mL). The reaction was stirred at 60 °C for 1 h. After cooling to 0 °C, the reaction was neutralized with 1N HCl (19.6 mL). The solid was collected by filtration to give (*R*)-1-(6-chloro-2-(trifluoromethyl)pyrimidin-4-yl)-piperidine-3-carboxylic acid (**31b**) as a white solid (4.8 g, 95%). To a solution of **31b** (0.88 g, 2.8 mmol) in DMF (10 mL) were added HATU (1.12 g, 2.9 mmol), DIEA (1.1 g, 8.5 mmol), and **26** (0.42 g, 2.8 mmol). The reaction was stirred at rt for 2 h. The reaction mixture was diluted with ethyl acetate, washed with saturated sodium bicarbonate and brine, and dried over sodium sulfate. The organic phase was dried, and the residue was purified by flash chromatography to give (*R*)-1-(6-chloro-2-(trifluoromethyl)-pyrimidin-4-yl)-*N*-(2-(6-cyanopyridin-3-yl)ethyl)piperidine-3-carboxamide as an off-white solid (1.1 g, 89%). Then, to a solution of (*R*)-1-(6-chloro-2-(trifluoromethyl)pyrimidin-4-yl)-*N*-(2-(6-cyanopyridin-3-yl)ethyl)-piperidine-3-carboxamide (44 mg, 0.1 mmol) in DMSO (0.3 mL) was added **20** (20 mg, 0.2 mmol), and the mixture was heated to 60 °C for 16 h. The reaction was cooled to rt and purified by HPLC to give the title compound (40 mg, 80%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.64−8.59 (m, 1H), 8.02 (t, J = 5.7, 1H), 7.97 (dd, J = 0.7, 7.9, 1H), 7.89 (dd, J = 2.1, 8.0, 1H), 6.12 (s, 1H), 4.32 (s, 2H), 3.75 (s, 3H), 3.50−3.25 (m, 6H), 2.95−2.77 (m, 5H), 2.66 (d, J = 15.2, 3H), 2.21 (tt, J = 3.7, 11.2, 1H), 1.73 (ddd, J = 7.6, 10.0, 14.6, 2H), 1.55 (qd, J =

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3.5, 12.4, 1H), 1.34 (qd, J = 4.0, 12.5, 1H). HPLC-MS $C_{24}H_{29}F_3N_8O$ (M + H$^+$): 503.2, 5.01 min.

(R)-N-(2-(6-Cyanopyridin-3-yl)ethyl)-1-(6-(piperazin-1-yl)-2-(trifluoromethyl)pyrimidin-4-yl)piperidine-3-carboxamide (**32b**). Compound **32b** was synthesized in a manner similar to compound **32a** from *tert*-butyl piperazine-1-carboxylate (20 mg, 0.1 mmol) and (*R*)-1-(6-chloro-2-(trifluoromethyl)pyrimidin-4-yl)-*N*-(2-(6-cyanopyridin-3-yl)ethyl)piperidine-3-carboxamide (40 mg, 0.09 mmol). Following removal of the DIEA by evaporation, a 1:1 solution of DCM:TFA was added and mixture was stirred for 1 h, purified by preparative HPLC, and converted into the free base by filtration through Si-carbonate column to yield the title compound (19.7 mg, 44%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.61 (d, J = 1.2, 1H), 8.02 (t, J = 5.6, 1H), 7.98 (d, J = 8.0, 1H), 7.90 (dd, J = 8.0, 2.0, 1H), 6.10 (s, 1H), 4.32 (m, 2H), 3.82 (t, J = 5.2, 4H), 3.38 (m, 2H), 3.18 (m, 5H), 2.94−2.83 (m, 4H), 2.23−2.18 (m, 1H), 1.80−1.70 (m, 2H), 1.57−1.53 (m, 1H), 1.36−1.32 (m, 1H). HPLC-MS $C_{23}H_{27}F_3N_8O$ (M + H$^+$): 489.2, 4.93 min.

(R)-N-(2-(2-Cyanopyrimidin-5-yl)ethyl)-1-(6-(4-ethylpiperazin-1-yl)-2-(trifluoromethyl)pyrimidin-4-yl)piperidine-3-carboxamide (**32c**). To a solution of **31a** (3.85 g, 11.4 mmol) in 2-propanol (50 mL) was added 1-ethylpiperazine (2.6g, 22.8 mmol). The reaciton was heated to 80 °C for 16 h. The solvent was removed en vacuo, and the crude product was purified by flash chromatography to give (*R*)-ethyl 1-(6-(4-ethylpiperazin-1-yl)-2-(trifluoro-methyl)pyrimidin-4-yl)-piperidine-3-carboxylate as an oil (3.9 g, 82%). Then, to a 100 mL round-bottom flask containing (*R*)-ethyl 1-(6-(4-ethylpiperazin-1-yl)-2-(trifluoromethyl)-pyrimidin-4-yl)piperidine-3-carboxylate (3.9 g, 9.4 mmol) were added THF (30 mL), methanol (10 mL), and water (10 mL) followed by lithium hydroxide monohydrate (0.5 g, 11.9 mmol). The reaction was heated to 60 °C for 16 h. The reaction was cooled to 0 °C and neutralized with 1N HCl (12 mL). The solid was collected by filtration to give (*R*)-1-(6-(4-ethylpiperazin-1-yl)-2-(trifluoromethyl)-pyrimidin-4-yl)piperidine-3-carboxylic acid as a white solid (3.3 g, 91%). To the solution of (*R*)-1-(6-(4-ethylpiperazin-1-yl)-2-(trifluoromethyl)-pyrimidin-4-yl)piperidine-3-carboxylic acid (15 mg, 0.04 mmol) in DMF (0.3 mL) was added DIEA (12.5 mg, 1 mmol), HATU (15 mg, 0.04 mmol), and **29** (6 mg, 0.04 mmol). The reaction was stirred at rt for 2 h. The reaction was then diluted with DMSO (0.5 mL) and purified by preparative HPLC to give the title compound (10 mg, 48%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.83 (s, 2H), 7.94 (t, J = 5.6, 1H), 5.92 (s, 1H), 4.27−4.17 (m, 2H), 3.51 (m, 4H), 3.35 (m, 2H), 2.82−2.69 (m, 4H), 2.34−2.26 (m, 6H), 2.17−2.10 (m, 1H), 1.70−1.60 (m, 2H), 1.48−1.44 (m, 1H), 1.28−1.23 (m, 1H), 0.98−0.94 (t, J = 6.8, 3H). HPLC-MS $C_{24}H_{30}F_3N_9O$ (M + H$^+$): 518.2, 5.20 min.

(2S)-N-[2-(4-Cyanophenyl)ethyl]-1-[6-(4-methylpiperazin-1-yl)-2-(trifluoromethyl)pyrimidin-4-yl]piperidine-2-carboxamide (**34a**). **34a** was synthesized in a manner similar to compound **22e** in 70% isolated yield. ^{1}H NMR (400 MHz, CD_3OD) δ 7.49 (d, J = 8.4, 2H), 7.26 (d, J = 8.4, 2H), 5.8 (s, 1H), 5.15 (s, 1H), 3.90 (d, J = 13.2, 1H), 3.6 (m, 4H), 3.41−3.33 (m, 2H), 3.02−2.95 (m, 1H), 2.78 (t, J = 6.8, 2H), 2.47 (t, J = 5.2, 4H), 2.28 (s, 3H), 2.05 (m, 1H), 1.63−1.51 (m, 3H), 1.44−1.29 (m, 2H). HPLC-MS $C_{25}H_{30}F_3N_7O$ (M + H$^+$): 502.2, 6.12 min.

(2S)-N-[2-(4-Cyanophenyl)ethyl]-1-[6-(4-methylpiperazin-1-yl)-2-(trifluoromethyl)pyrimidin-4-yl]pyrrolidine-2-carboxamide. (**34b**). Compound **34b** was synthesized in a manner similar to compound **22e** on 1 mmol scale in 50% isolated yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.98 (s, 1H), 7.72 (d, J = 8.4, 2H), 7.38 (d, J = 8.4, 2H), 5.91 (s, 1H), 4.52−4.45 (m, 3H), 3.53−3.50 (m, 3H), 3.30−3.03 (m, 7H), 2.84 (s, 3H), 2.77 (b, 2H), 2.09−2.08 (b, 1H), 1.91−1.75 (m, 3H). HPLC-MS $C_{24}H_{28}F_3N_7O$ (M + H$^+$): 488.2, 5.37 min.

(2R)-N-[2-(4-Cyanophenyl)ethyl]-1-[6-(4-methylpiperazin-1-yl)-2-(trifluoromethyl)pyrimidin-4-yl]pyrrolidine-2-carboxamide. Compound **34c** was obtained via chiral HPLC (16 mL/min 80/20 hexane/2-propanol, 21 mm × 250 mm Lux-Celluclose-2 colmun, 35 min elution run time) of *N*-[2-(4-cyanophenyl)ethyl]-1-[6-(4-methylpiperazin-1-yl)-2-(trifluoro-methyl)pyrimidin-4-yl]pyrrolidine-2-carboxamide that was synthesized in a manner similar to compound **22e**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.98 (s, 1H), 7.72 (d, J = 8.4, 2H), 7.38 (d, J = 8.4, 2H), 5.92 (s, 1H), 4.52−4.44 (m, 3H), 3.53−3.50 (m, 3H), 3.30−3.03 (m, 7H), 2.84 (s, 3H), 2.77 (b, 2H), 2.09−2.08 (b, 1H), 1.91−1.76 (m, 3H). HPLC-MS $C_{24}H_{28}F_3N_7O$ (M + H$^+$): 488.2, 5.35 min.

(S)-1-(6-Chloro-2-(trifluoromethyl)pyrimidin-4-yl)-N-(4-cyanophenethyl)azetidine-2-carboxamide. (*S*)-1-(*tert*-Butoxycarbonyl)azetidine-2-carboxylic acid (2.01 g, 10 mmol) and 5-(2-aminoethyl)benzontrile (2.0 g, 11 mmol) were dissolved in ethyl acetate (20 mL), pyridine (2.7 mL, 34 mmol), and DIEA (1.9 mL, 11 mmol). The mixture was cooled to 0 °C when 1-propanephosphonic anhydride (T3P, 50% w/w in ethyl acetate, 12.7 mL, 20 mmol) was added slowly over 3 min. After 1 h, 10% citric acid solution (30 mL) was added, followed by ethyl acetate (20 mL). The layers were partitioned and the aqueous layer was extracted once with ethyl acetate (20 mL), and the combined organics were washed with 10% citric acid (2 × 20 mL) and then brine (20 mL). Dried over magnesium sulfate, filtered and concentrated. Finally, 2.8 g (85%) of (*S*)-*tert*-butyl 2-((4-cyanophenethyl)carbamoyl)azetidine-1-carboxylate was obtained as a white solid that was carried forward without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.58 (d, J = 8.2, 2H), 7.32 (d, J = 8.3, 2H), 4.60 (s, 1H), 3.87 (d, J = 8.3, 1H), 3.72 (dd, J = 8.1, 14.3, 1H), 3.55 (dd, J = 6.8, 13.2, 2H), 2.90 (dd, J = 6.9, 13.9, 2H), 2.40 (s, 2H), 1.42 (s, 9H). The crude material from two batches of the previous step were combined and were dissolved in DCM (50 mL) and treated with 4 M HCl in dioxane (14 mL) and stirred for 20 h. Concentrated to dryness and then re-evaporated 3× from methanol to yield (*S*)-*N*-(4-cyanophenethyl)azetidine-2-carboxamide hydrochloride (4.7 g, 88%). ^{1}H NMR (400 MHz, CD_3OD) δ 7.71−7.64 (m, 2H), 7.45 (d, J = 8.2, 2H), 4.93 (d, J = 8.9, 1H), 4.11 (dd, J = 9.5, 18.3, 1H), 3.91 (dd, J = 10.0, 16.3, 1H), 3.55 (tdd, J = 6.5, 12.8, 18.9, 2H), 2.95 (td, J = 3.7, 7.0, 2H), 2.83−2.70 (m, 1H), 2.40 (ddd, J = 7.9, 12.1, 17.6, 1H). (*S*)-*N*-(4-Cyanophenethyl)azetidine-2-carboxamide hydrochloride (4.7 g, 17 mmol) was suspended in ACN (60 mL) and treated with DIEA (8.9 mL, 51 mmol). The mixture was cooled to 0 °C when **21e** (4.1 g, 18.7 mmol) was added. The mixture was then allowed to stir to rt overnight. Approximately 75% of the solvent was removed by evaporation and then diluted with ethyl acetate (70 mL), washed with 10% citric acid (2 × 30 mL), and brine (30 mL), dried over magnesium sulfate, filtered, and concentrated. The crude was suspended in ethyl acetate and filtered to give a white solid (2.4 g). The filtrate was then charged with excess Celite and concentrated to dryness for dryloading for flash chromatography (80g ISCO, 30−100% ethyl acetate/hexanes) to yield an additional 1.3 g. The mixed fractions were repurified by the same method to obtain an additional 0.69 g for a combined yield of 4.39 g (63%) of (*S*)-1-(6-chloro-2-(trifluoromethyl)pyrimidin-4-yl)-*N*-(4-cyanophenethyl)azetidine-2-carboxamide as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.35−8.16 (m, 2H), 7.74 (s, 2H), 7.42 (s, 2H), 4.87−4.70 (m, 1H), 4.10−4.03 (m, 2H), 2.92−2.75 (m, 2H), 2.67−2.58 (m, 1H), 2.21−2.08 (m, 1H) (peaks at 3.3 and 2.5 ppm partially obs by solvent). HPLC-MS $C_{18}H_{15}ClF_3N_5O$ (M + H$^+$): 410.2, 3.19 min (4.5 min method).

(2S)-N-[2-(4-Cyanophenyl)ethyl]-1-[6-(4-methylpiperazin-1-yl)-2-(trifluoromethyl)pyrimidin-4-yl]azetidine-2-carboxamide (**34d**). Compound **34d** was synthesized in a manner similar to compound **22e** in 55% yield. ^{1}H NMR (400 MHz, CD_3OD) δ 7.50 (d, J = 8.4, 2H), 7.28 (d, J = 8.4, 2H), 5.27 (s, 1H), 4.82 (m, 1H), 3.97 (m, 1H), 3.84 (m, 1H), 3.69−3.45 (m, 6H), 2.95−2.85 (m, 3H), 2.52 (m, 5H), 2.37 (s, 3H). HPLC-MS $C_{23}H_{26}F_3N_7O$ (M + H$^+$): 474.2, 5.16 min.

(2R)-N-[2-(4-Cyanophenyl)ethyl]-1-[6-(4-methylpiperazin-1-yl)-2-(trifluoromethyl)pyrimidin-4-yl]azetidine-2-carboxamide (**34e**). Compound **34e** was synthesized in a manner similar to compound **22e** in 34% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.43 (s, 1H), 7.43 (m, 2H), 7.21 (m, 2H), 5.22 (s, 1H), 4.75 (m, 1H), 3.89 (m, 1H), 3.75 (m, 1H), 3.50 (m, 3H), 2.85 (m, 3H), 2.58 (m, 1H), 2.43 (m, 5H), 2.30 (m, 3H), 1.05 (m, 2H). HPLC-MS $C_{23}H_{26}F_3N_7O$ (M + H$^+$): 474.2, 5.16 min.

(2S)-N-[2-(6-Cyanopyridin-3-yl)ethyl]-1-[6-(4-ethylpiperazin-1-yl)-2-(trifluoromethyl)pyrimidin-4-yl]azetidine-2-carboxamide (**34f**).

Compound **34f** was synthesized in a manner similar to compound **32c** in 63% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.52 (s, 1H), 8.15 (t, J = 5.6, 1H), 7.85−7.79 (m, 2H), 5.52 (s, 1H), 4.53 (m, 1H), 3.91−3.80 (m, 2H), 3.47 (b, 4H), 3.37−3.30 (m, 2H), 2.80 (t, J = 6.4, 2H), 2.47 (m, 1H), 2.34−2.27 (m, 6H), 2.10−2.01 (m, 1H), 0.97−0.93 (t, J = 7.2, 3H). HPLC-MS $C_{23}H_{27}F_3N_8O$ (M + H$^+$): 489.2, 4.50 min.

*Methyl 4-(N-Boc-piperidin-4-yloxy) benzoate (**38a**).* A mixture of *N*-boc-4-hydroxypiperidine (201 mg, 1 mmol), methyl 4-hydroxybenzoate (215 mg, 1 mmol), and triphenylphosphine (514 mg, 2 mmol) in THF (10 mL) was cooled to 0 °C and then treated with a solution of DEAD (358 mg, 2 mmol) in THF (1 mL) via dropwise addition. The reaction temperature was slowly raised to rt and stirred for 2 h. The solvent was removed en vacuo, and the crude product was purified by flash chromatography to give *tert*-butyl 4-(4-(methoxycarbonyl)phenoxy)piperidine-1-carboxylate (214 mg, 63%), which was subsequently treated with 4 M HCl solution in dioxane (2 mL). After stirring 2 h at rt, the solvent was removed by vacuum and the product was lyophilized overnight to yield the hydrochloride salt. (150 mg, 99%). ^{1}H NMR (400 MHz, CD_3OD) δ 7.98 (d, J = 8.9, 2H), 7.08 (d, J = 8.9, 2H), 4.85 (tt, J = 6.5, 3.5, 1H), 3.87 (s, 3H), 3.41 (td, J = 9.0, 4.5, 2H), 3.34−3.13 (m, 3H), 2.24 (m, 2H), 2.05 (m, 2H). ^{13}C NMR (101 MHz, MeOD) δ 168.18, 162.13, 132.77, 124.23, 116.46, 69.81, 52.45, 41.88, 28.30.

*Methyl 2-(4-(Piperidin-4-yloxy)phenyl)acetate (**38b**).* A mixture of benzyl 4-hydroxypiperidine-1-carboxylate (1.18 g, 5 mmol), methyl 2-(4-hydroxyphenyl)acetate (0.83 g, 5 mmol), and triphenylphosphine (1.57 g, 6 mmol) in THF (10 mL) was cooled to 0 °C and then treated with a solution of diisopropyl azodicarboxylate (DIAD) (0.75 mL, 6 mmol, 1.2 equiv 95%) in THF (1 mL) via dropwise addition. The reaction temperature was slowly raised to rt and stirred for 2 h. The solvent was removed by rotary evaporator, and the crude product was purified by flash chromatography to give benzyl 4-(4-(2-methoxy-2-oxoethyl)phenoxy)piperidine-1-carboxylate (0.924 g, 47%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 (d, J = 4.5, 4H), 7.34 (ddt, J = 8.4, 5.7, 2.9, 1H), 7.21 (d, J = 8.6, 2H), 6.88 (d, J = 8.7, 2H), 5.16 (s, 2H), 4.49 (tt, J = 6.8, 3.4, 1H), 3.83−3.71 (m, 2H), 3.70 (d, J = 1.4, 3H), 3.58 (s, 2H), 3.48 (ddd, J = 13.3, 7.2, 4.0, 2H), 2.02−1.85 (m, 2H), 1.81 (m 2H). The obtained intermediate (244 mg) was dissolved in ethyl acetate (8 mL) andPd/C (10%, 24 mg) was added under a stream of nitrogen. The reaction vessel was backfilled with hydrogen gas and was stirred under hydrogen atmosphere for 4 h. The catalyst was removed by filtration and was washed by methanol (3 × 10 mL), and then the solvent was removed en vacuo. After drying overnight, the title compound was obtained as a light-yellow solid (156 mg, 98%). HPLC-MS: 100% (ELSD), (M + H$^+$): 250.

*1-{6-[(3R)-3-{[2-(4-Cyanophenyl)ethyl]carbamoyl}piperidin-1-yl]-2-(trifluoromethyl)-pyrimidin-4-yl}piperidine-4-carboxylic Acid (**45a**).* (*R*)-1-(6-Chloro-2-(trifluoromethyl)pyrimidin-4-yl)-*N*-(4-cyanophenethyl)piperidine-3-carboxamide (200 mg, 0.457 mmol), ethyl piperidine-4-carboxylate (141 mg, 0.914 mmol), and diisopropylethyl amine (159 uL, 0.914 mmol) were dissolved in 2-propanol (50 mL) in a sealed tube. The reaction mixture was then heated at 130 °C for 20 h. The solvents were removed en vacuo, and the residue was purified by flash chromatography (40 g ISCO, 0−10% DCM/methanol) to yield the intermediate ester (212 mg, 85%). ^{1}H NMR (400 MHz, CD_3OD) δ 7.63 (d, J = 8.1, 2H), 7.38 (d, J = 8.1, 2H), 5.85 (s, 1H), 4.35 (m, 3H), 4.15 (m, 3H), 3.42 (m, 3H), 3.17 (m, 1H), 3.03 (m, 3H), 2.89 (m, 2H), 2.64 (m, 1H), 2.34 (m, 1H), 1.71 (m, 7H), 1.26 (t, J = 7.1, 3H). HPLC-MS $C_{28}H_{33}F_3N_6O_3$ (M + H$^+$) 559.2, 3.297 min (4.5 min method). (*R*)-Ethyl 1-(6-(3-((4-cyanophenethyl)-carbamoyl)piperidin-1-yl)-2-(trifluoromethyl)pyrimidin-4-yl)-piperidine-4-carboxylate (81.2 mg, 0.145 mmol) was dissolved in THF (4 mL) and ethanol (0.5 mL), then treated with lithium hydroxide (3M, 0.15 mL, 0.435 mmol). The reaction was stirred at rt overnight. Upon completion, the reaction was quenched with 6 N HCl to pH 3, and the residue was taken up in ethyl acetate (3×), and the combined organic layers were dried over magnesium sulfate, filtered, and concentrated to yield the title compound (115 mg, 48%). ^{1}H NMR (400 MHz, CD_3OD) δ 7.63 (d, J = 8.2, 2H), 7.39 (d, J = 8.2, 2H), 5.85 (s, 1H), 4.33 (m, 3H), 4.11 (m, 1H), 3.50 (m, 3H), 3.17 (m, 1H), 3.03 (m, 2H), 2.89 (s, 2H), 2.61 (m, 1H), 2.34 (m, 1H), 2.01 (m, 2H), 1.68 (m, 5H), 1.28 (m, 1H). HPLC-MS $C_{26}H_{29}F_3N_6O_3$ (M + H$^+$) 531.2, 8.25 min.

*1-{6-[(3R)-3-{[2-(4-Cyanophenyl)ethyl]carbamoyl}piperidin-1-yl]-2-(trifluoromethyl)-pyrimidin-4-yl}-4-methylpiperidine-4-carboxylic Acid (**45b**).* was prepared in a manner similar to **45a** from **35b** in 29% yield. ^{1}H NMR (400 MHz, CD_3CN) δ 7.64 (m, 2H), 7.36 (m, 2H), 6.64 (m, 1H), 5.77 (m, 1H), 4.02 (m, 3H), 3.44 (m, 2H), 3.21 (m, 3H), 2.99 (m, 1H), 2.86 (m, 2H), 2.26 (m, 2H), 2.07 (m, 2H), 1.99 (m, 1H), 1.74 (m, 4H), 1.45 (m, 2H), 1.24 (s, 3H). HPLC-MS $C_{27}H_{31}F_3N_6O_3$ (M + H$^+$) 545.0, 8.84 min.

*2-{4-[(1-{6-[(3R)-3-{[2-(4-Cyanophenyl)ethyl]carbamoyl}-piperidin-1-yl]-2-(trifluoromethyl)pyrimidin-4-yl}piperidin-4-yl)oxy]-phenyl}acetic Acid (**45c**).* **45c** was prepared in a manner similar to **45a** from **38b** in 66% yield. ^{1}H NMR (400 MHz, CD_3OD) δ 7.51 (d, J = 8.4, 2H), 7.27 (d, J = 8.0, 2H), 7.13 (d, J = 8.8, 2H), 6.84 (d, J = 8.8, 2H) 5.73 (s, 1H), 4.55−4.49 (m, 1H), 4.16 (dd, J = 2.8, 13.2, 1H), 3.98−3.80 (m, 3H), 3.54−3.44 (m, 5H), 3.38−3.29 (m, 1H), 3.17 (dd, J = 9.6, 13.6, 1H), 2.95 (dt, J = 2.8, 13.2, 1H), 2.88−2.74 (m, 2H), 2.34−2.26 (m, 1H), 1.99−1.90 (m, 3H), 1.85−1.59 (m, 5H), 1.54−1.42 (m, 1H). HPLC-MS $C_{33}H_{35}F_3N_6O_4$ (M + H$^+$) 637.3, 9.67 min.

*(S)-2-(4-((1-(6-(2-((4-Cyanophenethyl)carbamoyl)azetidin-1-yl)-2-(trifluoromethyl)pyrimidin-4-yl)piperidin-4-yl)oxy)phenyl)acetic Acid (**45d**).* **45d** was prepared in a manner similar to **45a** from **38b** and (*S*)-1-(6-chloro-2-(trifluoromethyl)pyrimidin-4-yl)-*N*-(4-cyanophenethyl)azetidine-2-carboxamide in 59% yield. ^{1}H NMR (400 MHz, CD_3OD) δ 8.51 (s, 1H), 7.54 (d, J = 8.3, 2H), 7.36 (d, J = 8.3, 2H), 7.21 (d, J = 8.6, 2H), 6.98−6.89 (m, 2H), 5.59 (s, 1H), 4.75−4.61 (m, 2H), 4.09−3.86 (m, 4H), 3.67−3.55 (m, 3H), 3.50−3.39 (m, 1H), 2.89 (t, J = 6.9, 2H), 2.61−2.40 (m, 2H), 2.12−1.96 (m, 4H), 1.85−1.69 (m, 2H). HPLC-MS $C_{31}H_{31}F_3N_6O_4$ (M + H$^+$) 608.2, 1.56 min (2 min method).

*4-[(1-{6-[(2S)-2-{[2-(4-Cyanophenyl)ethyl]carbamoyl}azetidin-1-yl]-2-(trifluoromethyl)pyrimidin-4-yl}piperidin-4-yl)oxy]benzoic Acid (**45e**).* To a mixture of **38a** and (*S*)-1-(6-chloro-2-(trifluoromethyl)-pyrimidin-4-yl)-*N*-(4-cyanophenethyl)azetidine-2-carboxamide (75 mg, 0.3 mmol) dissolved in NMP (6 mL) was added excess DIEA (0.2 mL). The reaction was stirred at 120 °C overnight in a seal tube. The reaction was quenched by a saturated aqueous solution of sodium bicarbonate and then was extracted by ethyl acetate (3 × 15 mL). The combined organic phase was washed by water (2 × 10 mL), saturated sodium bicarbonate (10 mL), and brine (10 mL). After drying over sodium sulfate, the solvent was removed and the crude product was dissolved in a mixture of THF/methanol/water (2 mL, 3/1/1). Excess lithium hydroxide (200 mg) was then added. The reaction was stirred at rt for 2 h and was quenched by saturated solution of sodium bisulfate (10 mL). The solution was extracted with a mixture of (2-propanol/chloroform: 1/3, 3 × 20 mL). The combined organic phase was washed with water (20 mL) and brine (20 mL) and was dried over sodium sulfate, and the solvent was removed en vacuo. The crude product was then purified by flash chromatography (24 g ISCO, 0−10% methanol/DCM) to give the title compound (86 mg, 73%). ^{1}H NMR (400 MHz, CD_3OD) δ 8.51(m, 1H), 8.08 (d, J = 7.8, 2H), 7.46 (m, J = 7.8, 2H), 7.26 (d, J = 7.8, 2H), 6.98 (d, J = 7.8, 2H), 5.32 (s, 1H). 4.82 (m, 1H), 4.74 (m, 1H), 4.12(m, 1H), 4.02−3.77 (m, 4H); 3.77−3.55 (m, 2H), 3.49 (m, 1H), 3.25−2.64 (m, 3H), 2.48 (m, 1H), 2.34−2.00 (m, 3H), 1.95 (m, 1H). HPLC-MS $C_{30}H_{30}F_4N_6O_4$ (M + H$^+$) 595.2, 9.08 min.

*4-(1-{6-[(2S)-2-{[2-(4-Cyanophenyl)ethyl]carbamoyl}azetidin-1-yl]-2-(trifluoromethyl)-pyrimidin-4-yl}piperidin-4-yl)-2-methoxybenzoic Acid (**45f**).* A mixture of methyl 4-bromo-2-methoxybenzoate (490 mg, 2.0 mmol), *tert*-butyl 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-5,6-dihydropyridine-1(2*H*)-carboxylate (927 mg, 3.0 mmol), sodium bicarbonate (504 mg, 6.0 mmol), and $Pd(PPh_3)_4$ (231 mg, 0.2 mmol) in dioxane (5.0 mL) and water (2.0 mL) in a sealed tube was heated 130 °C for 30 min. After cooling to rt, the reaction mixture was diluted with water (20 mL) and extracted with ethyl acetate (3 × 40 mL), the combined extracts were then dried over $MgSO_4$, filtered, and the residue purified by flash chromatography (10−30% ethyl acetate/hexanes) to give *tert*-butyl 4-(3-methoxy-4-(methoxycarbonyl)phenyl)-5,6-dihydropyridine-1(2*H*)-carboxylate

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(713 mg, 99%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81 (d, J = 8.0, 1H), 7.00 (dd, J = 1.6, 8.0, 1H), 6.96 (d, J = 1.6, 1H), 6.15 (m, 1H), 4.12 (m, 2H), 3.95 (s, 3H), 3.90 (s, 3H), 3.67 (t, J = 5.2, 2H), 2.55 (m, 2H), 1.51 (s, 9H). HPLC-MS $C_{19}H_{26}NO_5$ (M + H$^+$) 348.18, 2.19 min (3.5 min method). *tert*-Butyl 4-(3-methoxy-4-(methoxycarbonyl)-phenyl)-5,6-dihydropyridine-1(2*H*)-carboxylate (705 mg, 2.03 mmol) was dissolved in methanol (20 mL), and 10% Pd/C (200 mg) was added. The mixture was then stirred overnight under H_2 atmosphere. The catalyst was removed by filtration, and the solvent was removed en vacuo to afford the product (623 mg, 84%, two steps). HPLC-MS $C_{19}H_{28}NNaO_5$ (M + Na$^+$) 372.18, 2.21 min (3.5 min). A solution of *tert*-butyl 4-(3-methoxy-4-(methoxy-carbonyl)phenyl)-piperidine-1-carboxylate (250 mg, 0.715 mmol) in dioxane was treated with 4N HCl solution in dioxane (6.0 mL) overnight at rt; solvent was removed en vacuo to afford **41**. HPLC-MS $C_{14}H_{20}NO_3$ (M + H$^+$) 250.1, 1.11 min (3.5 min method).

A mixture of **41** (178 mg, 0.72 mmol), (*S*)-1-(6-chloro-2-(trifluoromethyl)pyrimidin-4-yl)-*N*-(4-cyanophenethyl)-azetidine-2-carboxamide (195 mg, 0.477 mmol), and DIEA (0.65 mL, 4.77 mmol) in NMP (6.0 mL) was heated to 140 °C for 2 h. The reaction mixture was then cooled to 35 °C and treated with lithium hydroxide (2 M, 2.4 mL) for 6 h. The reaction mixture was brought pH 3.0 with 2N HCl. This material was then purified by reverse phase HPLC to yield the title compound (150 mg, 34%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.56 (s, 1H), 8.02 (d, J = 8.0 Hz, 1H), 7.42 (m, 2H), 7.19 (m, 2H), 6.92 (dd, J = 1.6, 8.4 Hz, 1H), 6.85 (d, J = 1.2 Hz,1H), 5.35 (s, 1H), 4.92 (t, J = 8.0 Hz, 1H), 4.54 (m, 2H), 4.01 (s, 3H), 3.90 (m,2H), 3.58 (m, 1H), 3.47 (m, 1H), 3.05 (m, 2H), 2.87 (m, 3H), 2.67 (m, 1H), 2.54(m, 1H), 1.97 (m, 2H), 1.72 (m, 2H). HPLC-MS $C_{31}H_{32}F_3N_6O_4$ (M + H$^+$) 609.2, 9.13 min.

*4-(4-{6-[(2S)-2-{[2-(4-Cyanophenyl)ethyl]carbamoyl}azetidin-1-yl]-2-(trifluoromethyl)-pyrimidin-4-yl}phenyl)benzoic Acid (**45g**).* To a 100 mL pressure vessel was added [1,1′-bis(diphenylphosphino)-ferrocene]dichloropalladium(II) (1.63 g, 1.56 mmol, 10 mol %), potassium acetate (5.6 g, 57.5 mmol), methyl 4′-bromo-[1,1′-biphenyl]-4-carboxylate (4.5 g, 15.6 mmol), and bis(pinacolato)-diboron (4.8 g, 18.6 mmol). The vessel was evaculated and backfilled with argon then charged with dioxane (45 mL). The vessel was then purged with argon, sealed, and heated to 90 °C for 18 h. Upon completion, the mixture was cooled to rt, diluted with DCM (40 mL), and filtered on Celite. Water was added to the filtrate, and the layers were partitioned. The aqueous layer was extracted with DCM (2 × 30 mL) then dried over sodium sulfate, filtered, and concentrated. The crude material was then subjected to flash chromatography (330 g ISCO, 5−30% ethyl acetate/hexanes) to yield methyl 4′-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-[1,1′-biphenyl]-4-carboxylate (**41a**) (3.2 g, 62%).

(*S*)-1-(6-Chloro-2-(trifluoromethyl)pyrimidin-4-yl)-*N*-(4-cyanophenethyl)azetidine-2-carboxamide (3.1 g, 7.5 mmol), **43a** (3.2 g, 9.43 mmol), dicyclohexyl(2′,6′-dimethoxy-[1,1′-biphenyl]-2-yl)-phosphine (207 mg, 0.51 mmol), and tris(dibenzylideneacetone)-dipalladium(0) (69 mg, 0.075 mmol) were combined in a 100 mL pressure vessel. The vessel was evacuated and backfilled with argon twice. To this mixture was added dry dioxane (30 mL) and aqueous potassium phosphate (5 M, 8 mL, 30 mmol). The vessel was evacuated and backfilled once more, then heated to 95 °C for 3 h. The crude reaction mixture was filtered on Celite while the product remained in solution. Upon filtering the hot solution, the product precipitated. The Celite was washed with ethyl acetate (40 mL), and the Celite cake was suspended in ethyl acetate and heated to reflux and refiltered. The mixture was allowed to stand overnight and the product precipitated and was collected in four crops of white solid to yield (*S*)-methyl 4′-(6-(2-((4-cyanophenethyl)carbamoyl)azetidin-1-yl)-2-(trifluoromethyl)pyrimidin-4-yl)-[1,1′-biphenyl]-4-carboxylate (4.06 g, 93%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.18 (dd, J = 8.4, 12.6, 4H), 7.77 (dd, J = 8.4, 20.3, 4H), 7.51 (d, J = 8.2, 2H), 6.74 (s, 1H), 5.04−4.92 (m, 1H), 4.23−4.14 (m, 1H), 4.13−4.03 (m, 1H), 3.98 (s, 3H), 3.63 (s, 2H), 2.93 (s, 3H), 2.72−2.52 (m, 2H). HPLC-MS $C_{32}H_{26}F_3N_5O_3$ (M + H$^+$) 586.2, 2.36 min (3.5 min method). (*S*)-Methyl 4′-(6-(2-((4-cyanophenethyl)carbamoyl)azetidin-1-yl)-2-(trifluoromethyl)pyrimidin-4-yl)-[1,1′-biphenyl]-4-carboxylate (10.5 g, 17.9 mmol) was suspended in THF (50 mL) and methanol (50 mL) and treated with lithium hydroxide (3M, 13 mL, 38 mmol) and heated to at 50 °C for 1 h and then at 70 °C for 30 min. The reaction mixture was diluted with DCM, acidified with 10% citric acid, and poured into a separatory funnel. The aqueous layer was extracted with DCM (2 × 30 mL), and the combined organics were washed with water, then dried over magnesium sulfate, filtered, and concentrated to yield the title compound (9.47g, 93%). ^{1}H NMR (400 MHz, $CDCl_3$ CD_3OD) δ 8.03 (t, J = 8.7, 4H), 7.62 (dd, J = 8.4, 20.3, 4H), 7.37 (d, J = 8.1, 2H), 7.16 (d, J = 8.2, 2H), 6.65 (s, 1H), 4.82 (s, 1H), 4.13−3.96 (m, 2H), 3.49−3.39 (m, 2H), 2.80−2.60 (m, 3H), 2.51 (br s, 1H). HPLC-MS $C_{31}H_{24}F_3N_5O_3$ (M + H$^+$) 572.3, 9.13 min.

*4-(4-{6-[(2S)-2-{[2-(4-Cyanophenyl)ethyl]carbamoyl}azetidin-1-yl]-2-(trifluoromethyl)pyrimidin-4-yl}phenyl)cyclohexane-1-carboxylic Acid (**45h**).* To a 350 mL pressure vessel was added bis(dibenzylideneacetone)palladium(0) (605 mg, 1.05 mmol), tricyclohexylphosphine (708 mg, 2.52 mmol), and dioxane (25 mL). The mixture was stirred under Ar (g) for 30 min and was then charged with bis(pinacolato)diboron (12.85 g, 50.6 mmol), potassium acetate (6.77 g, 69 mmol), and *trans*-methyl 4-(4-chlorophenyl)-cyclohexanecarboxylate (11.6 g, 46 mmol) in 70 mL of dioxane. The vessel was then evacuated and backfilled with Ar (g) twice. The vessel was capped and heated to 120 °C for 1 h and then 100 °C overnight. It was cooled to rt, diluted with DCM (40 mL), and filtered on Celite to remove palladium. Water was added to the filtrate, and layers were partitioned. The aqueous layer was extracted with DCM, then dried, filtered, and concentrated by 75%. Then 10% ethyl acetate/hexanes was added (~200 mL) and reduced volume by 50% en vacuo and the product began to precipitate. The precipitate was collected by filtration to yield a brownish-gray solid. The crude material was dissolved in DCM and loaded on to a bed of silica gel and washed successively with 20% ethyl acetate/hexanes, 30% ethyl acetate/hexanes, and then 50% ethyl acetate/hexanes to give a white solid. A second crop was also collected from the original mother liquor. The mother liquor was then evaporated to dryness, and the material was subjected to flash chromatography (330 g ISCO, 5−30% ethyl acetate/hexanes). Mixed fractions were pooled, evaporated, and repurified under the same conditions. Clean fractions were combined with the original precipitate to yield **43b** (10.5 g, 67%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75 (d, J = 8.0, 2H), 7.21 (d, J = 8.0, 2H), 3.69 (s, 3H), 2.53 (m, 1H), 2.36 (m, 1H), 2.10 (m, 2H), 1.97 (m, 2H), 1.61- 1.42 (m, 4H), 1.33 (s, 12H).

Dicyclohexyl(2′,6′-dimethoxy-[1,1′-biphenyl]-2-yl)phosphine (487 mg, 1.2 mmol) and tris(dibenzylideneacetone)dipalladium(0) (162 mg, 0.18 mmol) were combined in a 350 mL pressure vessel. To this was added dry dioxane (20 mL), and the mixture was stirred for 20 min. In a separate flask were dissolved (*S*)-1-(6-chloro-2-(trifluoromethyl)pyrimidin-4-yl)-*N*-(4-cyanophenethyl)azetidine-2-carboxamide (7.2 g, 17.7 mmol) and **43b** (7.32 g, 21.3 mmol) in dioxane (80 mL). The starting materials were then added to the preformed catalyst, and the flask was rinsed (3 × 10 mL) with dry dioxane. Potassium phoshate tribasic (5 M, 30 mL) was then added, and the vessel was evacuated and backfilled with Ar (g) and heated to 90 °C for 3 h. The mixture was allowed to cool and stand overnight and diluted with ethyl acetate (100 mL) and brine (40 mL). The aqueous layer was extracted with ethyl acetate, and the combined organics were washed with brine (2×) and then dried over magnesium sulfate, filtered, concentrated en vacuo, and purified by flash chromatography (330 g ISCO, 20−100% ethyl acetate/hexanes) to give *trans*-methyl 4-(4-(6-((*S*)-2-((4-cyanophenethyl)carbamoyl)-azetidin-1-yl)-2-(trifluoromethyl)-pyrimidin-4-yl)phenyl)-cyclohexanecarboxylate. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.31−8.05 (m, 1H), 8.01 (d, J = 8.3, 2H), 7.49 (d, J = 8.2, 2H), 7.36 (d, J = 8.3, 2H), 7.27 (d, J = 9.4, 3H), 6.66 (s, 1H), 4.95 (s, 1H), 4.19−4.09 (m, 1H), 4.05 (s, 1H), 3.72 (s, 3H), 3.60 (dd, J = 6.4, 12.4, 2H), 3.07−2.97 (m, 1H), 2.91 (dd, J = 7.1, 9.5, 2H), 2.61 (d, J = 11.7, 2H), 2.41 (t, J = 11.9, 1H), 2.15 (d, J = 10.8, 2H), 2.02 (d, J = 11.1, 2H), 1.82−1.47 (m, 6H), 1.30−1.25 (m, 1H).

trans-Methyl 4-(4-(6-((S)-2-((4-Cyanophenethyl)carbamoyl)-azetidin-1-yl)-2-(trifluoro-methyl)pyrimidin-4-yl)phenyl)-

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cyclohexanecarboxylate. *trans*-Methyl 4-(4-(6-(((*S*)-2-((4-cyanophenethyl)carbamoyl)azetidin-1-yl)-2-(trifluoro-methyl)-pyrimidin-4-yl)phenyl)cyclohexanecarboxylate (12.6 g, 21.3 mmol) was dissolved in methanol (50 mL) and THF (50 mL) and was then charged with lithium hydroxide (3 M, 14 mL, 43 mmol). The mixture was heated to 40 °C for 1 h. Upon cooling to rt, the mixture was treated with 50 mL of 1 M HCl. The mixture was diluted with ethyl acetate, and the resulting white precipitate was collected by filtration. The filtrate was then extracted with ethyl acetate, washed with brine, dried, filtered, and concentrated to a white solid. The original precipitate and the solid collected from the extracted filtrate were stirred in ACN for 20 min, and the solid was collected by filtration. The resulting filtrate was then concentrated to dryness and subjected to flash chromatography on 220 g of ISCO (0−100% DCM/ACN) to give **45h** (11.4 g, 92%). ^{1}H NMR (400 MHz, CD_3OD) δ 8.09−7.99 (m, 2H), 7.60−7.49 (m, 2H), 7.40 (d, J = 8.2, 3H), 6.99−6.81 (m, 2H), 4.88−4.82 (m, 1H), 4.28−4.09 (m, 3H), 3.60−3.50 (m, 2H), 2.92 (m, 2H), 2.80−2.58 (m, 3H), 2.48−2.36 (m, 2H), 2.19−2.09 (m, 2H), 2.03−1.95 (m, 2H), 1.61 (s, 4H). ^{13}C NMR (101 MHz, DMSO) δ 175.58, 168.73, 162.18, 160.48, 149.02, 144.31, 132.35, 130.86, 128.64, 126.07, 125.84, 117.76, 107.80, 98.15, 61.44, 58.60, 53.75, 41.64, 40.81, 38.95, 33.83, 31.42, 19.59. HPLC-MS $C_{31}H_{30}F_3N_5O_3$ ($M + H^+$) 578.2, 9.15 min. Melting point 249−252 °C.

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Notes

The authors declare no competing financial interest.

ABBREVIATIONS USED

IBMX, 3-isobutyl-1-methylxanthine; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; HTRF, homogeneous time-resolved fluorescence; FXR, farnesoid-X-recptor; DMEM, Dulbecco's Modified Eagle Medium; DPP4, dipeptidylpeptidase-4; HEK293, human embryonic kidney-293 cells; GLP-1, glucagon-like peptide-1; FBS, fetal bovine serum; DIEA, diisopropylethylamine; HATU, 1-[bis(dimethylamino)-methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxide hexafluorophosphate; EDCI, 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide; HOBT, hydroxybenzotriazole-hydrate; ACN, acetonitrile; TEA, triethylamine; TrixiePhos, racemic-2-di-*t*-butylphosphino-1,1′-binaphthyl; RuPhos, 2-dicyclohexylphosphino-2′,6′-diisopropoxybiphenyl; Sphos, 2-dicyclohexylphosphino-2′,6′-dimethoxy-1,1′-biphenyl

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