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Optimization of Potency and Pharmacokinetic Properties of Tetrahydroisoquinoline Transient Receptor Potential Melastatin 8 (TRPM8) Antagonists

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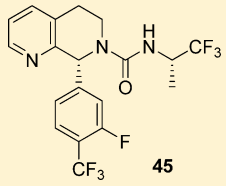
Supporting Information

ABSTRACT: Transient receptor potential melastatin 8 (TRPM8) is a nonselective cation channel expressed in a subpopulation of sensory neurons in the peripheral nervous system. TRPM8 is the predominant mammalian cold temperature thermosensor and is activated by cold temperatures ranging from 8 to 25 °C and cooling compounds such as menthol or icilin. TRPM8 antagonists are being pursued as potential therapeutics for treatment of pain and bladder disorders. This manuscript outlines new developments in the SAR of a lead series of 1,2,3,4-tetrahydroisoquinoline derivatives with emphasis on strategies to improve pharmacokinetic properties and potency. Selected compounds were profiled in two TRPM8 target-specific in vivo coverage models in rats (the icilin-induced wet dog shake model and the cold pressor test). Compound **45** demonstrated robust efficacy in both pharmacodynamic models with ED_{90} values <3 mg/kg.

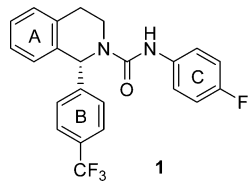


hTRPM8 IC_{50} = 17 nM
rTRPM8 IC_{50} = 25 nM
HLM = <14 μL/(min•mg)
RLM = <14 μL/(min•mg)
Rat iv CL = 0.09 L/h/kg
Rat Wet-Dog Shakes PD ED_{90} = 1.1 mg/kg

INTRODUCTION

Transient receptor potential melastatin type 8 (TRPM8) is a membrane bound ion channel and member of the TRP family of Ca^{2+} permeable nonselective, nonspecific cation channels.[1] TRPM8 is activated by cold temperatures (8−25 °C), exogenous activators menthol and icilin, and endogenous activators such as PIP_2 and lysophosphatidylcholine. TRPM8 is expressed primarily in the dorsal root ganglia (DRG) and trigeminal ganglia (TG),[2] key components of the peripheral nervous system. In recent years, TRPM8 has emerged as a potential target for treatment of pain disorders[3] as well as overactive and painful bladder syndromes.[4] Small molecule TRPM8 antagonists comprising a variety of chemotypes have been recently disclosed.[5−8] Although several series of potent TRPM8 antagonists have been described in patent and scientific literature, much less information has been revealed describing optimized TRPM8 antagonists suitable for advancement to preclinical or clinical studies.

We have previously reported our efforts toward synthesis and characterization of a series of fused piperidine TRPM8 inhibitors and described initial in vivo results in an icilin-induced rat wet dog shake (WDS) pharmacodynamic (PD) model.[5] Tetrahydroisoquinoline **1** (Figure 1), which showed moderate in vitro TRPM8 potency (hTRPM8 IC_{50} = 36 nM; rTRPM8 = 57 nM) as well as efficacy in the rat WDS model of TRPM8 inhibition, required improvements in potency and pharmacokinetic (PK) properties to be considered for further advancement to preclinical toxicology studies. Our efforts toward optimization of potency and PK parameters and in vivo results of optimized compounds in PD models are described below.



hTRPM8 IC_{50} = 36 nM
rTRPM8 IC_{50} = 57 nM
HLM = 17 μL/(min•mg)
RLM = 42 μL/(min•mg)
Rat iv CL = 2.9 L/h/kg

Figure 1. Tetrahydroisoquinoline TRPM8 antagonist (**1**) used as starting point for SAR investigations. (HLM, human liver microsomes; RLM, rat liver microsomes, iv, intravenous; CL, clearance).

CHEMISTRY

Initial investigations necessitated the synthesis of the tetrahydroisoquinoline cores **5a** and **5b** (Scheme 1).[5] Acid chloride condensation or carboxylic acid coupling onto phenethylamine **2** provided benzamides **3a** and **3b**. Cyclization by Bischler−Napieralski isoquinoline synthesis[9] was accomplished by treatment of benzamides **3a** and **3b** with P_2O_5 and polyphosphoric acid at 165 °C or with P_2O_5 and $POCl_3$ in toluene at reflux, giving 3,4-dihydroisoquinolines **4a** and **4b**. The dihydroisoquinolines were then reduced with sodium

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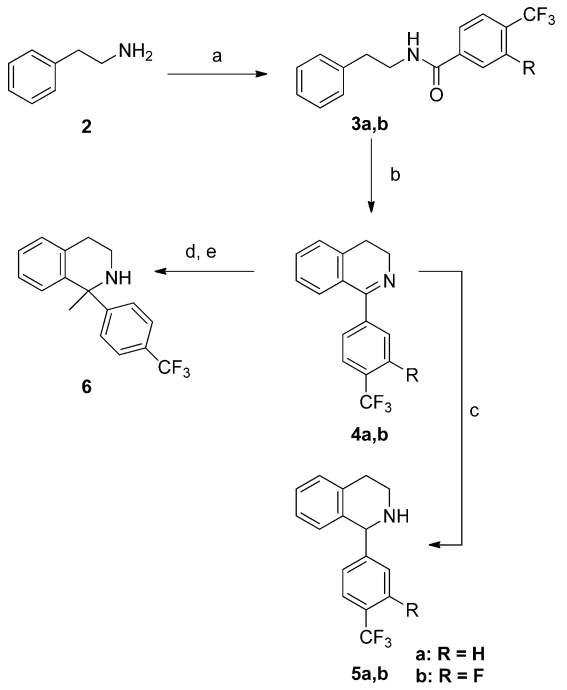
Scheme 1. Synthesis of Tetrahydroisoquinoline Cores 5a,b[a]



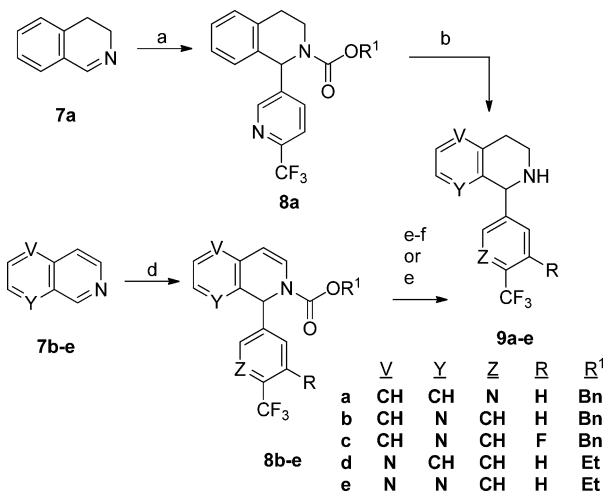
[a]Reagents and conditions: (a) *p*-CF_3PhCOCl, DIPEA, DCM, rt or 4-CF_3-3-F-PhCO_2H, EDCI, HOBt, DIPEA, DMF, rt; (b) P_2O_5, polyphosphoric acid, 165 °C or P_2O_5, $POCl_3$, toluene, reflux; (c) $NaBH_4$, MeOH, rt; (d) **4a**, BnBr, MeCN, reflux, rt; MeMgBr, THF/Et_2O, rt; (e) H_2, Pd/C, AcOH.

borohydride to provide the tetrahydroisoquinolines **5a** and **5b** in good yield. To access the quaternary methyl substituted compound **6**, **4a** was reacted with benzylbromide to provide an intermediate isoquinolium bromide salt, which was alkylated with methylmagnesium bromide to afford a benzyl protected tetrahydroisoquinoline. Deprotection by hydrogenolysis provided the methyl substituted intermediate **6**.

Aza analogues of the tetrahydroisoquinoline core, compounds **9a**–**e**, were prepared as shown in Scheme 2. Starting with 3,4-dihydroisoquinoline **7a**, reaction with benzylchloroformate gave an imminium intermediate which was subsequently reacted with (6-(trifluoromethyl)pyridin-3-yl)-magnesium bromide to form benzylcarbamate **8a**. Hydrogenolysis of the benzylcarbamate provided amine **9a**. Similarly, treatment of naphthyridines **7b**,**d** and pyrido[3,4-*b*]pyrazine **7e** with ethyl or benzylchloroformate gave a transient pyridinium ion which was subsequently reacted with an aryl magnesium halide to form carbamates **8b**–**e**. Hydrogenation of the double bonds and hydrogenolysis of the benzylcarbamates of **8b** and **8c** in a single step using Pd/C under H_2 gave 1,7-tetrahydronapthyridines **9b** and **9c**. In contrast, 1,6-tetrahydronapthyridine **9d** and tetrahydropyridopyrazine **9e** were formed by the stepwise hydrogenation of the double bond of **8d** and **8e**, followed by basic hydrolysis of the ethylcarbamates with K_2CO_3, MeOH.

Tetrahydronaphthyridine analogues **14a** and **14b** and tetrahydropyridopyrimidine **14c** were prepared as shown in Scheme 3. Starting with the appropriately substituted bromoformylpyridines **10a** and **10b** or pyrimidine **10c**, addition of 4-trifluoromethylphenylmagnesium bromide[10] to the aldehydes and Heck coupling[11] of the arylbromides with *N*-vinylphthalimide gave alcohols **11a**–**c**. Hydrogenation of the

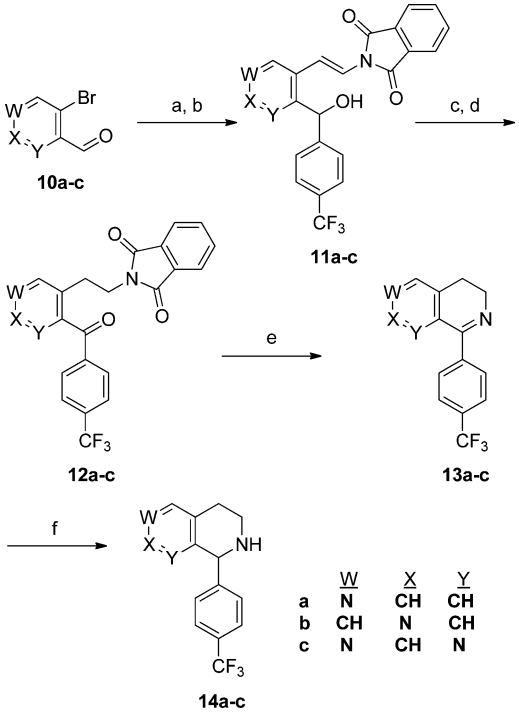
Scheme 2. Synthesis of Aza Containing Cores 8b–e and 9a–e[a]



	V	Y	Z	R	R^1
a	CH	CH	N	H	Bn
b	CH	N	CH	H	Bn
c	CH	N	CH	F	Bn
d	N	CH	CH	H	Et
e	N	N	CH	H	Et

[a]Reagents and conditions: (a) BnOCOCl, THF, (6-(trifluoromethyl)-pyridin-3-yl)magnesium bromide, 0 °C to rt; (b) H_2, Pd/C; (d) BnO_2CCl or EtO_2CCl, THF, rt, 4-CF_3ArMgBr, THF, 0 °C; (e) H_2, Pd/C; (f) K_2CO_3, MeOH, rt.

Scheme 3. Synthesis of Aza Analogues of Tetrahydroisoquinoline Core 14a–c[a]



	W	X	Y
a	N	CH	CH
b	CH	N	CH
c	N	CH	N

[a]Reagents and conditions: (a) 4-CF_3PhMgBr, THF, rt; (b) $Pd(dba)_2$, 2-(dicyclohexylphosphino)biphenyl, *N*-vinylphthalimide, TEA, DMF, 150 °C; (c) H_2, Pd/C, EtOAc, rt; (d) MnO_2, DCM, rt; (e) hydrazine hydrate, EtOH, rt; (f) $NaBH_4$, MeOH, rt.

double bonds followed by oxidation of the alcohols with MnO_2 gave ketones **12a**–**c**. Cleavage of the phthalimides with hydrazine gave transient primary amines, which subsequently cyclized to ketimines **13a**–**c** during the reaction. The ketimines were then reduced with sodium borohydride to give amines **14a**–**c**.

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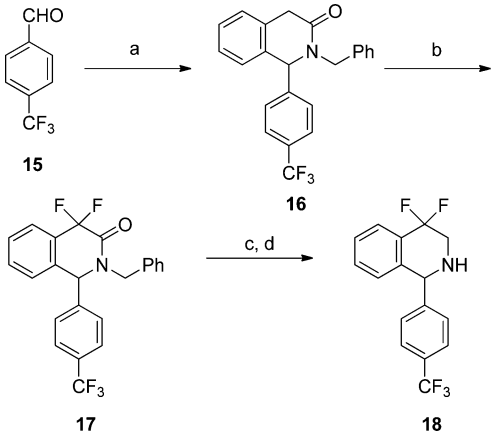
To access the geminal difluoro-substituted tetrahydroisoquinoline analogue **18**, condensation of benzylamine with aldehyde **15** gave an intermediate imine, which was then reacted with phenylacetyl chloride to give lactam **16** (Scheme 4). Base-mediated fluorination of the benzylic position with *N*-fluorobenzenesulfonamide gave difluoro-lactam **17**. Borane mediated reduction of the amide carbonyl and hydrogenolysis of the benzyl protecting group gave amine **18** in good yield.

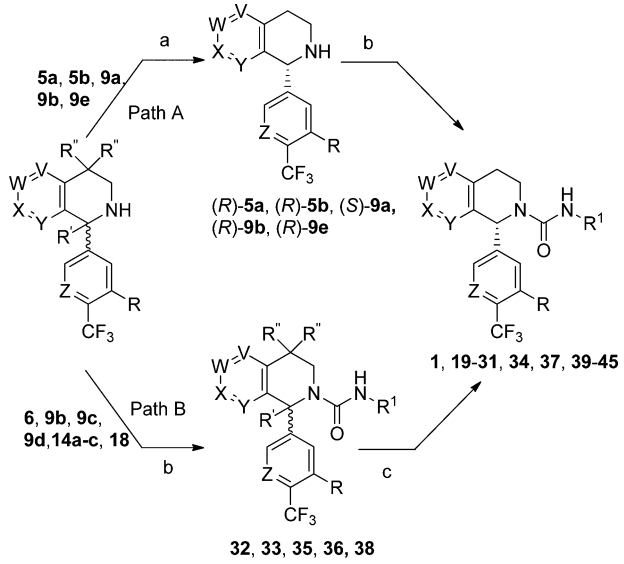
Scheme 4. Synthesis of Difluoro Substituted Tetraisoquinoline Core 18[a]



[a]Reagents and conditions: (a) benzylamine, DCM, 4 Å MS, rt, phenylacetyl chloride, TfOH, rt; (b) LiHMDS, NFSI, THF, 0 °C to rt; (c) BH_3·THF, THF, 0 °C to rt; (d) H_2, Pd/C, EtOH, rt.

The racemic amines **5a**, **5b**, **9a**, **9b**, and **9e** were separated by chiral preparative supercritical fluid chromatography (SFC) to provide enantiomerically pure amines which were subsequently used in coupling reactions to form the final compounds **1**, **19**–**31**, **37**, **39**–**41**, **43**, and **44** (Scheme 5, path A). Ureas were prepared by one of three routes; generally, if isocyanates were commercially available, condensation of tetrahydroisoquinoline intermediates with isocyanates directly gave ureas for in vitro testing. Alternatively, some ureas were made by coupling the tetrahydroisoquinoline intermediates with a reactive intermediate made either from an alkyl amine and CDI or coupling with *p*-nitrophenylcarbamates to provide the targeted ureas. In an alternative path to the final compounds, racemic amines **6**, **9c**, **9d**, **14a**–**c**, and **18** were used in the coupling reactions (Scheme 5, path B). Compounds **32**, **33**, **35**, **36**, and **38** were tested as racemates, and because their potencies on TRPM8 were >200 nM, they were not separated into the respective pure enantiomers. Compound **34** was obtained by chiral preparative SFC of the racemic urea. In contrast, after coupling racemic amines **9b** or **9c** with (*S*)-1,1,1-trifluoropropan-2-amine, the diastereomeric mixtures of ureas containing **42** and **45** and their respective (*S*,*S*)-diastereomers could be separated by silica gel chromatography.

Scheme 5. Synthesis of Urea Final Compounds[a]



[a]Reagents and conditions: (a) SFC chiral separation; (b) (i) R^1-isocyanate, DIPEA, DCM, rt or (ii) carbonyl diimidazole, R^1-amine, DIPEA, DCM, rt or (iii) *p*-nitrophenyl(R^1)carbamate, MeCN, Δ; (c) SFC chiral separation (for **34**) or silica gel chromatography (for **42** and **45**).

RESULTS AND DISCUSSION

Our previous efforts toward the development of TRPM8 inhibitors led to the identification of compound **1**. Although **1** was effective in a rat icilin induced wet dog shake PD model (ED_{50} = 6.2 mg/kg, EC_{50} = 0.60 μM; ED_{90} = 100 mg/kg; EC_{90} =1.68 μM), a major liability with the compound was its marginal pharmacokinetic properties (rat iv CL = 2.9 L/h/kg) and a relatively high oral dose required for full target coverage in vivo. Also, limited increases in exposure were seen at high doses, making the compound unsuitable for advancement to preclinical rodent toxicology studies. Thus, we set out to improve the PK properties of compound **1** while also seeking to increase in vitro potency. Rat and human liver microsomal stabilities (RLM/HLM) were used as an initial screen before compounds were advanced to in vivo pharmacokinetic studies in rats. Antagonist potency was evaluated by the ability of compounds to block Ca^{2+} influx induced by icilin activation in CHO cells stably transfected with h- and rTRPM8.

We began our initial SAR investigations by replacing the 4-fluorophenylurea with various substituted aryl or heteroaryl ureas (Table 1). The ortho fluorine substituted analogue **19** was less potent than **1** and also less stable in microsomes. This result is consistent with what was reported previously in the tetrahydrothienopyridine series.[5] We postulated that addition of a functional group with additional polarity onto the aryl urea of the C-ring might improve the stability of compounds to oxidative metabolism. The 4-benzoic acid urea **20**, although stable in microsomes, lost significant potency. Additional polar functionality was introduced by the addition of a cyano substituent on each position of the phenyl ring (analogues **21**–**23**). The 4-cyanophenyl urea **23** was remarkably stable in both human and rat liver microsomes (CL_{int} <14 μL/mg/min), although it suffered a 3–5-fold loss of potency. Pyridyl ureas **24**–**26** were also made and evaluated. The 3-pyridyl urea **25** was of comparable potency to **1**, and it maintained moderate microsomal stability. In addition to aromatic and hetero-aromatic substitutions, we also explored aliphatic ureas. The initial *tert*-butyl and *iso*-propyl ureas (**27** and **28** respectively) were of comparable potency to **1** but were significantly less stable in microsomes. Substitution of one of the potentially metabolically labile methyl groups on *iso*-propyl urea **28** with a trifluoromethyl group gave diastereomeric compounds **29** and **30** that had improved microsomal stabilities compared to *tert*-butyl and *iso*-propyl ureas **27** and **28**. Addition of a phenyl ring

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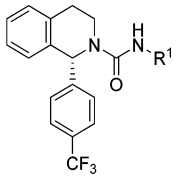
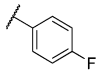
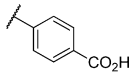
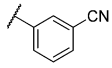
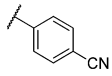
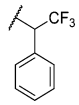
Table 1. SAR of Ureas[a]



Compound	R^1	IC_{50} (nM)[a]		HLM / RLM[b]
		hTRPM8	rTRPM8	CL_{int} [μL/(min•mg)]
1		36	57	17 / 42
19		48	197	33 / 230
20		1383	2053	<14 / <14
21		424	104	90 / 241
22		83	190	15 / 113
23		155	154	<14 / <14
24		171	81	30 / 161
25		28	22	95 / 63
26		398	306	22 / 32
27		37	45	60 / 199
28		44	47	19 / 273
29		14	27	17 / 122
30		52	146	<14 / 38
31		154	168	--

[a]IC_{50} values based on inhibition of icilin (1 μM) induced influx of Ca^{2+} into TRPM8-expressing CHO cells. Each IC_{50} value reported represents an average of at least two independent experiments with three replicates at each concentration, standard deviation values are provided in the Supporting Information. [b]In vitro microsomal stability measured in a high-throughput automated format.[15]

to replace the methyl group of compound **30** gave **31**, which suffered a significant loss of potency. We were encouraged by the results of **29** which showed improved potency vs **1** while maintaining good human microsomal stability. However, rat

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liver microsomal stability was decreased, suggesting further refinements to **29** were worth pursuing.

On the hypothesis that the poor pharmacokinetic properties of the series was at least partially the result of oxidative metabolism by CYP P450s, we evaluated compound **1** in MetaSite,[12] a computational tool used to predict sites prone to metabolism. MetaSite indicated the possibility for multiple sites of metabolism on the tetrahydroisoquinoline core, with the 1, 4, and 6-positions of the core being ranked highest as likely sites of metabolism (Figure 2). We envisioned that blocking potential sites of metabolism with a fluorine or a nitrogen might reduce oxidative metabolism and improve the PK of the series.

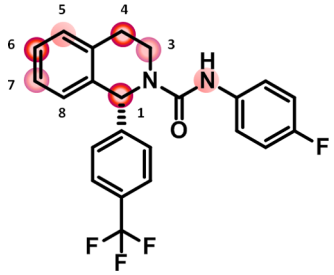
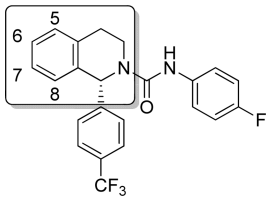


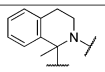
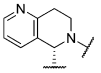
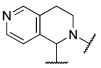
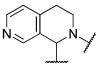
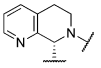
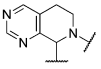
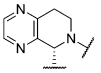
Figure 2. Sites of metabolism calculated using MetaSite prediction software for compound **1** predicting extensive metabolism on the tetrahydroisoquinoline core.

Blocking positions 5−8 of compound **1** by replacement of hydrogen with fluorine was described in our previous manuscript.[5] Although some gains in metabolic stability were achieved, these changes also led to compounds with 2−8-fold loss of potency compared with compound **1**. However, MetaSite also ranked the 1- and 4-positions of **1** as metabolic soft spots. Substitution of **1** with a methyl group at the 1-position gave compound **32**, which resulted in dramatic loss of potency at TRPM8 (>20 μM) (Table 2). Alternatively, substitution of the 4-position of the core with a geminal difluoro group gave compound **33**, which resulted in 10-fold loss of potency on hTRPM8.

By adding additional polarity to the molecule, we theorized that the druglike properties of the series could be improved. An analysis of ureas **1** and **19**−**31** revealed that most compounds with the tetrahydroisoquinoline core fell in a cLogP range of >5, which was outside of the typical range of druglike physicochemical properties.[13] However, calculations indicated that a significant reduction of cLogP to 3−4 would result by the inclusion of 1 or 2 nitrogen atoms in the tetrahydroisoquinoline core. Aza derivatives of compound **1** incorporating heterocyclic replacements are shown in Table 2. Addition of a nitrogen atom at the 5−7 positions to the core was not tolerated in regards to potency with compounds **34**−**36** showing significant decrease in activities compared to compound **1**. Encouragingly, the 8-aza compound **37** was slightly more potent than **1** and also maintained comparable in vitro stability. Unfortunately, evaluation of **37** in rat in vivo PK showed that its parameters were not improved vs compound **1** with high systemic clearance (iv CL = 4.6 L/h/kg) greater than hepatic blood flow. The very high in vivo clearance of **37** was not predicted by its metabolic stability in rat liver microsomes. An exact mechanism of metabolism was not determined, although extrahepatic metabolism is proposed as a likely explanation for the disconnect. Although a nitrogen at the 8-position was

Table 2. Initial SAR of Tetrahydroisoquinoline Core[a]



Compound	Structure	IC_{50} (nM)[a]		HLM / RLM[b]
		hTRPM8	rTRPM8	CL_{int} [μL/(min•mg)]
32[c]		>20000	>20000	--
33[c]		324	548	<14 / 61
34		391	534	32 / <14
35[c]		1262	1157	--
36[c]		463	574	--
37		14	37	<14 / 37
38		786	1168	--
39		200	350	15 / <14

[a]IC_{50} values based on inhibition of icilin (1 μM) induced influx of Ca^{2+} into TRPM8-expressing CHO cells. Each IC_{50} value reported represents an average of at least two independent experiments with three replicates at each concentration; standard deviation values are provided in the Supporting Information. [b]In vitro microsomal stability measured in a high-throughput automated format.[15] [c]These compounds were synthesized and tested as racemates, other compounds are single enantiomers.

tolerated, incorporation of two nitrogen atoms was not tolerated, with pyrimidine **38** and pyrazine **39** resulting in >6-fold decrease in potency.

In the final phase of the investigation, we combined the potent heterocyclic core of tetrahydro-1,7-naphthyridine **37** with various ureas that had proven beneficial to potency and PK from Table 1 (i.e., 3-pyridyl-, 4-cyanophenyl-, and (*S*)-trifluoropropan-2-yl-ureas). The 3-pyridylurea **40** maintained potency but led to decreased microsomal stability compared with **37** (Table 3). Significant improvements in rat microsomal stability were obtained with the 4-cyanophenyl urea **41**, which translated to improvements in the rat PK profile (iv CL = 0.17 L/h/kg, F_{oral} = 70%); however, this change was also accompanied by a 3−5-fold drop in potency compared to the 4-fluorophenyl urea **37**. The combination of the tetrahydro-1,7-naphthyridine core with the (*S*)-1-(1,1,1-trifluoropropan-2-yl)urea, which gave encouraging results with compound **29**, led to compound **42**, which had good microsomal stability in

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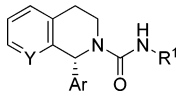
Table 3. SAR Summary for Azatetrahydroquinolines[a]



Compound	Y	Ar	R^1	IC_{50} (nM)[a]		HLM / RLM[b]
				hTRPM8	rTRPM8	CL_{int} [μL/(min•mg)]
40	N			24	48	104 / 59
41	N			79	120	<14 / <14
42	N			25	115	<14 / 16
43	CH			53	127	<14 / 19
44	CH			10	18	<14 / 47
45	N			12	23	<14 / <14

[a]IC_{50} values based on inhibition of icilin (1 μM) induced influx of Ca^{2+} into TRPM8-expressing CHO cells. Each IC_{50} value reported represents an average of at least two independent experiments with three replicates at each concentration; standard deviation values are provided in the Supporting Information. [b]In vitro microsomal stability measured in a high-throughput automated format.[15]

Table 4. Pharmacokinetic Parameters of Selected Compounds in Sprague–Dawley Rats

compd	iv CL[a] (L/h/kg)	V_{ss} (L/kg)	iv $t_{1/2}$ (h)	po dose[b](mg/kg)	C_{max} (μM)	po $AUC_{0-\infty}$ (μM·h)	F_{oral} (%)
1	2.9	15.3	6.7	10	0.64	4.2	57
42	0.48	2.7	3.5	5	1.58	12.0	47
44	0.64	3.0	7.0	5	1.67	12.1	67
45	0.09	1.7	76	5	3.86	71.9	51

[a]Study in fed male Sprague–Dawley rats dosed at 2 mg/kg iv in DMSO with sampling time up to 16 h. n = 3 animals per study. [b]Study in fasted male Sprague–Dawley rats dosed at 5 or 10 mg/kg po as a suspension in 5% Tween 80/Oraplus with sampling time up to 16 h.

both rat and human with minimal loss of potency at hTRPM8. Following iv and po administration in rat, **42** demonstrated an improved PK profile (iv CL = 0.48 L/h/kg, F_{oral} = 47%), with clearance significantly lower than rat liver blood flow.

To test whether the inclusion of a heteroatom at a different location other than within the tetrahydro-1,7-naphthyridine **42** could be tolerated, pyridine **43** was made. The pyridine replacement for a phenyl group achieved a similar level of improvement to microsomal stability, as seen with addition of a nitrogen to the 8 position of the core compound, **42** vs **29**, albeit with some loss of potency. The improved microsomal stability of compound **43** was encouraging, and although computational predictions suggested the 3-position of the B-ring of **42** was not a major contributor to metabolism, we knew from previous SAR that the 3-position was tolerant to the addition of a fluoro substituent with minimal loss of potency. Compound **44**, with a 3-fluoro substituent on the B-ring, was about twice as potent on human and rat TRPM8 as the *des*-fluoro compound **29** and also had significant gains in microsomal stability. Finally, combination of the improved stability and potency achieved by compound **44** with the improved PK properties of the tetrahydro-1,7-naphthyridine **42** gave compound **45**, which attained increased potency while reducing microsomal turnover to <14 μL/min/mg.

Compounds **42**, **44**, and **45**, which had the optimal properties of the molecules described above, were profiled further in iv rat PK studies (Table 4). The pharmacokinetic profile of compound **44** was similar to compound **42**, with slightly longer half-life (iv CL = 0.64 L/h/kg, $t_{1/2}$ = 7.0 h). Compound **45** displayed low systemic clearance (iv CL = 0.09 L/h/kg), a moderate volume of distribution (V_{ss} = 1.7 L/kg), and a long terminal half-life ($t_{1/2}$ = 76 h). Dosed orally in rats

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(5 mg/kg) **45** had good exposure (C_{max} = 3.86 μM, T_{max} = 5.3 h) and bioavailability (F_{oral} = 51%).

The properties of compounds **44** and **45** made them ideal candidates for in vivo evaluation of target coverage in the rat WDS PD model. When dosed orally 1.5 h prior to administration of the TRPM8 agonist icilin (0.5 mg/kg, ip), both compounds significantly inhibited icilin induced rat−wet-dog shakes (Figure 3). Compound **44** (ED_{90} = 4.7 mg/kg, IC_{90} = 0.293 μM, plasma unbound IC_{90} = 0.010 μM) achieved maximal inhibition at 10 mg/kg and required much lower dose and exposure compared to compound **1**.

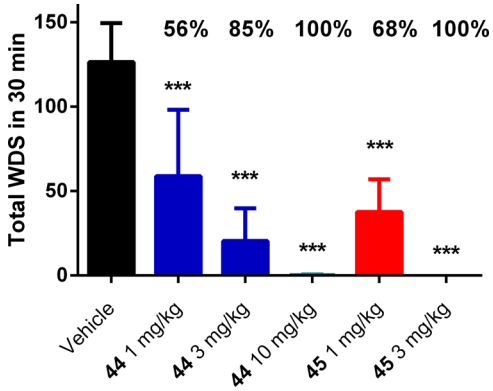

Figure 3. Inhibition of icilin-induced (0.5 mg/kg in 100% PEG400, ip) rat−wet-dog shaking behavior for compounds **44** and **45** dosed 1.5 h prior to icilin (*** $p < 0.05$, one-way ANOVA with Dunnett's test; n = 6 rats/dose).

In the WDS PD model, **45** (ED_{90} = 1.07 mg/kg, IC_{90} = 1.11 μM, plasma unbound IC_{90} = 0.062 μM) resulted in 68% inhibition of WDS at 1 mg/kg and the PD effect was completely blocked at 3 mg/kg. The >50-fold improvement in dose required to achieve >90% target coverage with compound **45** vs **1** can be attributed to increased drug plasma levels due to the improved pharmacokinetic properties of the molecule.

The cold pressor test (CPT) is used as a measure of cold hypersensitivity[14] and as an alternative target coverage model to measure TRPM8 target engagement of an antagonist. Rats with indwelling cartoid artery catheters were anesthetized, connected to a blood pressure analyzer, and immersed in ice water for 5 min, which resulted in an 18% increase in mean arterial blood pressure (vehicle treated animals, Figure 4). Compound **45** dosed orally 2 h prior to the cold challenge significantly inhibited the cold-induced rise in mean arterial blood pressure with 93% inhibition at 3 mg/kg po. The plasma concentration of **45** 2.5 h post dosing was measured at 1.27 ± 0.13 μM. The inhibition of the cold pressor response showed that cold stimulus activation of TRPM8 could be blocked at a similar dose to that of the rat WDS PD study. These results show that **45** is potent and effective against both icilin and cold temperature activation of TRPM8 in vivo.

CONCLUSION

Our efforts to increase the potency and pharmacokinetic properties of the initial lead, compound **1**, led to several compounds that showed significantly improved pharmacokinetic properties. The lead optimization exercise culminated in the identification of compound **45**, which had improved potency in vitro and in vivo and had excellent PK properties suitable for further preclinical advancement. In addition, **45** showed robust inhibition in the cold pressor target engagement model at a level similar to the icilin-induced rat WDS model. The improved potency and pharmacokinetic properties of these antagonists show promise in evaluating efficacy in disorders where TRPM8 contributes significantly. Performance of TRPM8 antagonists in preclinical models of pain toward the goal of identification of a clinical candidate will be reported elsewhere.

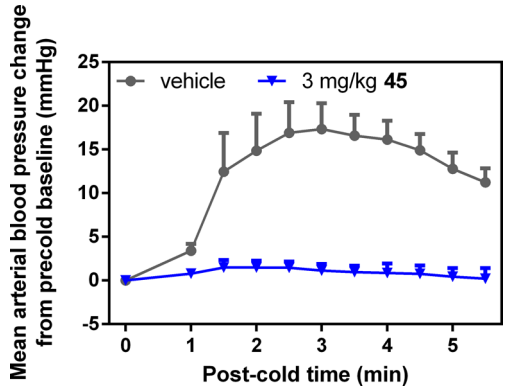

Figure 4. Inhibition of cold-induced mean arterial blood pressure (mmHg) increase in unilateral cartoid artery cannulated rats. Compound **45** dosed 3 mg/kg po, 2 h before recording, resulting in 93% inhibition of the cold pressor response (n = 5 rats).

EXPERIMENTAL SECTION

General. Unless otherwise noted, all materials were obtained from commercial suppliers and used without further purification. Anhydrous solvents were obtained from Aldrich or Fisher Scientific and used directly. All reactions involving air- or moisture-sensitive reagents were performed under a N_2 or Ar atmosphere. All final compounds were purified to >95% purity, as determined by LC/MS obtained on Agilent 1100 and HP 1100 spectrometers. Silica gel chromatography was performed using either glass columns packed with silica gel (100−200 or 200−400 mesh, Aldrich Chemical) or prepacked silica gel cartridges (Biotage or ISCO). ^{1}H NMR spectra were determined with a Bruker 300 MHz or a DRX 400 MHz spectrometer. Chemical shifts are reported in parts per million (ppm, δ units). Chiral purifications by preparative supercritical fluid chromatography (SFC) were performed on an Agilent preparative SFC system using Chiralcel or Chiralpak OD-H, AD, AD-H, or AS columns. Elution was by gradient using 5−55% MeOH with 0.2−1% diethylamine modifier in supercritical carbon dioxide over 2−5 min at 50−70 mL/min. Trifluoromethylphenyl Grignard reagents used in the synthesis of amines **9b−e** and **14a−c** have been reported to undergo highly exothermic decomposition,[10] and appropriate precautions during generation of this reagent should be undertaken.

(*R*)-1-(4-(Trifluoromethyl)phenyl)-1,2,3,4-tetrahydroisoquinoline ((*R*)-5a). Racemic 1-(4-(trifluoromethyl)phenyl)-1,2,3,4-tetrahydroisoquinoline **5a** was prepared as described in our previous article.[5] Separation of the enantiomers was accomplished by preparative chiral SFC purification to give (*R*)-1-(4-(trifluoromethyl)-phenyl)-1,2,3,4-tetrahydroisoquinoline (*R*)-**5a** as a white foam. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.67 (d, J = 8.0 Hz, 2 H), 7.48 (d, J = 8.0 Hz, 2 H), 7.08−7.17 (m, 2 H), 7.02 (t, J = 7.0 Hz, 1 H), 6.62 (d, J = 8.0 Hz, 1 H), 5.09 (s, 1 H), 3.02−3.11 (m, 1 H), 2.83−2.99 (m, 3 H), 2.68−2.78 (m, 1 H). MS (ESI pos ion) m/z: 278.1 (M + 1). $[\alpha]_D^{23}$ −19.4 (c 1.06, $CHCl_3$).

(*R*)-1-(3-Fluoro-4-(trifluoromethyl)phenyl)-1,2,3,4-tetrahydroisoquinoline ((*R*)-5b). Step 1: To a solution of 3-fluoro-4-(trifluoromethyl)benzoic acid (10.00 g, 48.1 mmol), 2-phenylethanamine (**2**, 6.66 mL, 52.9 mmol), and DIPEA (9.87 mL, 57.7 mmol) in DMF (100 mL) was added 1*H*-benzo[*d*][1,2,3]triazol-1-ol (0.649 g, 4.81 mmol) and *N*-(3-dimethylaminopropyl)-*N*-ethylcarbodiimide

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hydrochloride (9.21 g, 48.1 mmol). The solution was stirred at room temperature for 72 h. The reaction mixture was diluted with saturated aqueous $NaHCO_3$ (200 mL) and water (100 mL) and extracted with EtOAc (3 × 100 mL). The combined organic layers were washed with saturated aqueous $NaHCO_3$ (50 mL), water (50 mL), and saturated aqueous NaCl (50 mL). The organic layer was dried ($MgSO_4$) and concentrated to give 3-fluoro-*N*-phenethyl-4-(trifluoromethyl)-benzamide (**3b**, 10.5 g, 70.2%) as a yellow solid, which was used without further purification in the next step. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.60−7.74 (m, 1H), 7.45−7.60 (m, 2H), 7.14−7.42 (m, 5H), 3.66−3.83 (m, 2H), 2.91−3.04 (m, 2H). MS (ESI pos ion) *m*/*z*: 312.0 (M + 1).

Step 2: To a solution of 3-fluoro-*N*-phenethyl-4-(trifluoromethyl)-benzamide (**3b**, 10.5 g, 33.7 mmol) in toluene (300 mL) in a 500 mL one-necked round-bottomed flask fitted with a water jacketed reflux condenser under N_2 was added phosphorus pentoxide (14.36 g, 101 mmol) and phosphoryl trichloride (15.72 mL, 169 mmol). The light-yellow suspension was heated at reflux in a 120 °C oil bath and stirred 18 h. A light-brown solid developed, which adhered to the sides of the flask. The reaction was cooled to room temperature, and the clear supernatant was poured off. Ice water was slowly added to the flask containing the solid residue in (0.5 mL) portions with ice water bath cooling of the reaction mixture, and after 20 mL of water was added, an additional 200 mL of ice water was slowly added. The solution was stirred vigorously, which broke up the solid, yielding a brown suspension. The suspension was extracted with EtOAc (100 mL), and the aqueous layer was separated and basified to pH 9−10 with 5 N NaOH. The aqueous layer was extracted with EtOAc (3 × 100 mL). The organic layers were combined, dried ($MgSO_4$), and concentrated to give 1-(3-fluoro-4-(trifluoromethyl)phenyl)-3,4-dihydroisoquinoline as a brown oil (**4b**, 5.65 g, 57.1%), which was carried on to the next step without further purification. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.60−7.75 (m, 1H), 7.37−7.58 (m, 3H), 7.31 (d, *J* = 7.60 Hz, 3H), 7.13−7.23 (m, 1H), 3.79−3.98 (m, 2H), 2.75−2.89 (m, 2H).

Step 3: To a solution of 1-(3-fluoro-4-(trifluoromethyl)phenyl)-3,4-dihydroisoquinoline (**4b**, 5.63 g, 19.21 mmol) in MeOH (100 mL) in a room temperature water bath, sodium borohydride (1.453 g, 38.4 mmol) was added potionwise. The mixture was stirred at room temperature for 20 h. The MeOH was evaporated, and the residue was partitioned between EtOAc (150 mL) and saturated aqueous $NaHCO_3$ (100 mL), and the organic layer was separated. The aqueous phase was extracted with EtOAc (2 × 100 mL). The combined organic phases were washed with water (100 mL) and saturated aqueous NaCl (100 mL) and then dried ($MgSO_4$) and concentrated to afford the crude product. The product was purified by flash chromatography (120 g SiO_2, 20−50% EtOAc/hexanes) to give 1-(3-fluoro-4-(trifluoromethyl)phenyl)-1,2,3,4-tetrahydroisoquinoline (**5b**, 4.10 g, 72.3%) as a tan solid. The racemate was separated by preparative chiral SFC purification to give (*R*)-1-(3-fluoro-4-(trifluoromethyl)phenyl)-1,2,3,4-tetrahydroisoquinoline ((*R*)-**5b**, 1.87 g, 100% ee) as a white foam. ^{1}H NMR (300 MHz, MeOH-d_4) δ 7.65 (t, *J* = 7.75 Hz, 1H), 7.12−7.31 (m, 4H), 6.99−7.12 (m, 1H), 6.71 (d, *J* = 7.75 Hz, 1H), 5.18 (s, 1H), 3.09−3.24 (m, 1H), 2.93−3.09 (m, 2H), 2.78−2.93 (m, 1H). MS (ESI pos ion) *m*/*z*: 296.0 (M + 1). $[\alpha]_D^{23}$ −20.1 (*c* 1.11, $CHCl_3$).

1-Methyl-1-(4-(trifluoromethyl)phenyl)-1,2,3,4-tetrahydroisoquinoline (6). Step 1: A solution of 1-(4-(trifluoromethyl)phenyl)-3,4-dihydroisoquinoline[5] (**4a**, 0.90 g, 3.3 mmol) in MeCN (15 mL) was treated with 1-(bromomethyl)benzene (0.42 mL, 3.5 mmol), and the solution was heated at reflux for 4 h. The reaction mixture was allowed to cool to room temperature and concentrated to give the crude isoquinolinium bromide as a yellow foamy solid. MS (ESI pos ion) *m*/*z*: 366.2 (M + 1). The crude product was dissolved in THF (10 mL) and to this solution was added methylmagnesium bromide (3.16 M in Et_2O, 1.7 mL, 5.4 mmol) over 5 min, and the mixture was stirred at room temperature for 16 h. The reaction mixture was quenched with saturated aqueous NH_4Cl (25 mL) and extracted with EtOAc (150 mL). The organic layer was washed with saturated aqueous NaCl (35 mL), dried (Na_2SO_4), and concentrated. The crude product was purified by silica gel flash chromatography (0−100% EtOAc/hexanes) to afford 2-benzyl-1-methyl-1-(4-(trifluoromethyl)-phenyl)-1,2,3,4-tetrahydroisoquinoline (1.18 g, 95%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.82 (d, *J* = 8.2 Hz, 2H), 7.68 (d, *J* = 8.4 Hz, 2H), 7.24−7.31 (m, 4H), 7.17−7.23 (m, 1H), 6.96−7.13 (m, 3H), 6.63 (d, *J* = 7.6 Hz, 1H), 3.36 (s, 2H), 2.95−3.08 (m, 1H), 2..67−2.87 (m, 3H), 1.82 (s, 3H). MS (ESI pos ion) *m*/*z*: 382.2 (M + 1).

Step 2: A mixture of 2-benzyl-1-methyl-1-(4-(trifluoromethyl)-phenyl)-1,2,3,4-tetrahydroisoquinoline (1.12 g, 2.94 mmol) and palladium, 10 wt % on activated carbon (0.320 g, 0.857 mmol) in glacial acetic acid (15 mL), was stirred at room temperature for 16 h under H_2 at atmospheric pressure using a balloon. The mixture was filtered through a Celite pad, and the filtrate was concentrated in vacuo to yield a syrupy residue which was taken up in DCM (150 mL) and basified with saturated aqueous $NaHCO_3$ until pH ∼ 8.0 was achieved. The organic layer was separated, washed with brine (50 mL), dried (Na_2SO_4), and concentrated to give the crude product. The crude product was adsorbed on silica gel and purified by silica gel flash chromatography (40 g SiO_2, 0−100% EtOAc/hexanes) to afford 1-methyl-1-(4-(trifluoromethyl)phenyl)-1,2,3,4-tetrahydroisoquinoline (**6**, 0.690 g, 81%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.61 (d, *J* = 8.2 Hz, 2H), 7.47 (d, *J* = 8.2 Hz, 2H), 7.07−7.20 (m, 4H), 2.75−2.97 (m, 3H), 2.55−2.69 (m, 2H), 1.74 (s, 3H). MS (ESI pos ion) *m*/*z*: 292.2 (M + 1).

(*S*)-1-(6-(Trifluoromethyl)pyridin-3-yl)-1,2,3,4-tetrahydroisoquinoline ((*S*)-9a). Step 1: To a solution of 5-bromo-2-(trifluoromethyl)pyridine (5.17 g, 22.9 mmol) in THF (100 mL) at 0 °C was added isopropylmagnesium chloride (2.0 M in THF, 10.3 mL, 20.6 mmol), and the solution was stirred for 2 h under N_2. In a separate flask, to a solution of 3,4-dihydroisoquinoline (**7a**, 1.50 g, 11.4 mmol) in THF (40 mL) at 0 °C was added benzyl chloroformate (1.92 mL, 12.8 mmol), and the reaction was allowed to warm to room temperature and stirred 15 min. The iminium salt solution was recooled to 0 °C, and the solution of aryl magnesium prepared above was added. The reaction allowed to warm to room temperature and stirred for 1 h. The reaction was quenched with saturated aqueous NH_4Cl (200 mL) and extracted with DCM (2 × 100 mL). The combined organic layers were dried ($MgSO_4$) and concentrated. Purification by flash chromatography (12 g SiO_2, 0−50% EtOAc/hexane) gave benzyl 1-(6-(trifluoromethyl)pyridin-3-yl)-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (**8a**, 2.32 g, 49.2% yield) as a yellow oil. MS (ESI pos ion) *m*/*z*: 413.3 (M + 1).

Step 2: To a round-bottomed flask with benzyl 1-(6-(trifluoromethyl)pyridin-3-yl)-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (**8a**, 2.25 g, 5.46 mmol) and palladium on carbon (1.16 g, 1.09 mmol) under N_2 was added MeOH (50 mL). The flask was purged with H_2 (3×), then the reaction was stirred at room temperature under H_2 (1 atm) for 5.5 h. The reaction was filtered through a pad of Celite (EtOAc rinse 3 × 50 mL), and the filtrates were concentrated to give the crude product. The product was purified by flash chromatography (0−100% EtOAc/hexane) to give 1-(6-(trifluoromethyl)pyridin-3-yl)-1,2,3,4-tetrahydroisoquinoline (**9a**, 1.00 g, 65.9% yield) as a white solid. The racemate was separated by preparative chiral SFC purification to give (*S*)-1-(6-(trifluoromethyl)pyridin-3-yl)-1,2,3,4-tetrahydroisoquinoline ((*S*)-**9a**, 471 mg, 47.1%) as a white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.71 (s, 1H), 7.76 (d, *J* = 7.45 Hz, 1H), 7.63 (d, *J* = 8.18 Hz, 1H), 7.20 (d, *J* = 4.09 Hz, 2H), 7.08 (td, *J* = 4.17, 8.04 Hz, 1H), 6.69 (d, *J* = 7.75 Hz, 1H), 5.24 (s, 1H), 3.19−3.30 (m, 1H), 2.98−3.19 (m, 2H), 2.77−2.94 (m, 1H). MS (ESI pos ion) *m*/*z*: 279.0 (M + 1).

(*R*)-8-(4-(Trifluoromethyl)phenyl)-5,6,7,8-tetrahydro-1,7-naphthyridine ((*R*)-9b). Step 1: To a solution of 3-bromo-picoline (25 g, 0.145 mol) in CCl_4 was added *N*-bromosuccinimide (51.7 g, 0.29 mol) and benzoylperoxide (2.5 g, 0.018 mol), then the reaction was gradually heated at reflux for 30 h. The reaction mixture was allowed to cool to room temperature, the succinamide byproduct was filtered off, and the filtrate was concentrated under reduced pressure. The crude product was purified by flash column chromatography using silica (100−200 mesh) with 10% EtOAc in hexane as eluent to furnish 3-bromo-2-(dibromomethyl)pyridine (40.0 g, 83.5%) as a yellow oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.70 (dd, J = 4.5, 1.5 Hz, 1H), 7.88 (dd, J = 8.0, 1.5 Hz, 1H), 7.12−7.23 (m, 2H).

Step 2: A suspension of 3-bromo-2-(dibromomethyl)pyridine (10.0 g, 30.3 mmol) in morpholine (30.0 mL) was stirred at 60 °C for 1 h. The reaction mixture was allowed to cool to room temperature, diluted with EtOAc (200 mL), and the solution adjusted to pH 4 by adding citric acid (40.0 g). The reaction mixture was extracted with EtOAc (3 × 200 mL). The combined organic layers were dried (Na_2SO_4) and concentrated under reduced pressure. The crude product was purified by column chromatography using silica (100−200 mesh) with 3% EtOAc in hexane as eluent to give 3-bromopicolinaldehyde (4.0 g, 71.4%) as a tan solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.27 (s, 1H), 8.78 (dd, J = 4.5, 1.3 Hz, 1H), 8.06 (dd, J = 8.2, 1.3 Hz, 1H), 7.39 (dd, J = 8.1, 4.5 Hz, 1H).

Step 3: A round-bottomed flask was charged with 3-bromopicolinaldehyde (2.0 g, 10.6 mmol), dichlorobis(triphenylphosphine)-palladium(II) (372 mg, 0.53 mmol), copper(I) iodide (101 mg, 0.53 mmol), and DMF (10 mL). The resulting suspension was treated with NEt_3 (1.5 mL, 10.6 mmol), followed by (trimethylsilyl)acetylene (2.6 mL, 19.1 mmol). The reaction mixture was stirred at room temperature for 1.5 h and diluted with EtOAc. The organic layer was washed with water and brine, dried (Na_2SO_4), and concentrated in vacuo. The resulting residue was purified by silica gel chromatography (10−20% EtOAc in hexanes) to give 3-(2-(trimethylsilyl)ethynyl)-picolinaldehyde (1.8 g, 85%) as a colorless oil. MS (ESI pos ion) m/z: 204.0 (M + 1).

Step 4: A solution of 3-(2-(trimethylsilyl)ethynyl)picolinaldehyde (5.0 g, 24.6 mmol) in EtOH (50 mL) was saturated with ammonia. The solution was heated at 80 °C for 2 h in a sealed tube and allowed to cool to room temperature. The solvent was removed in vacuo, and the residue was purified by silica gel chromatography (15% acetone/hexanes) to give 1,7-naphthyridine (**7b**, 1.3 g, 40.6%) as a brownish solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.41 (s, 1H), 9.07 (d, J = 4.0 Hz, 1H), 8.55 (d, J = 5.6 Hz, 1H), 8.45 (d, J = 8.4 Hz, 1H), 7.93 (d, J = 5.6 Hz, 1H), 7.80 (dd, J = 8.4, 4.0 Hz, 1H). MS (ESI pos ion) m/z: 131.0 (M + 1).

Step 5: To a round-bottomed flask was added magnesium turnings (3.41 g, 140 mmol) and THF (75 mL), and the reaction was immersed in a room temperature water bath. 1-Bromo-4-(trifluoromethyl)benzene (12.9 mL, 92 mmol) was added portionwise over 75 min. The solution was allowed to stir for 1 h. To a separate round-bottomed flask containing 1,7-naphthyridine (**7b**, 6.0 g, 46.1 mmol) in THF (60 mL) was added benzyl chloroformate (7.14 mL, 50.7 mmol) dropwise over 4 min. The solution was allowed to stir for 10 min and then cooled to 0 °C as the Grignard solution was added dropwise to the naphthyridine solution over 10 min. The solution was allowed to slowly warm in the ice bath and stirred for 15 h. The reaction was quenched with saturated aqueous NH_4Cl. The aqueous layer was extracted with EtOAc (2 × 20 mL). The combined organic layers were washed with brine and concentrated in vacuo. The crude product was adsorbed onto a plug of silica gel and purified by flash chromatography (120 g SiO_2, 0−15% EtOAc/hexanes) to provide benzyl 8-(4-(trifluoromethyl)phenyl)-1,7-naphthyridine-7(8*H*)-carboxylate (**8b**, 15.3 g, 80.9%) as a light-yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.35 (d, J = 11.4 Hz, 1H), 7.25- 7.56 (m, 10H), 7.03−7.22 (m, 2H), 6.52 (m, 1H), 5.70−5.93 (m, 1H), 5.11−5.38 (m, 2H). MS (ESI pos ion) m/z: 411.0 (M + 1).

Step 6: To a round-bottomed flask containing benzyl 8-(4-(trifluoromethyl)phenyl)-1,7-naphthyridine-7(8*H*)-carboxylate (**8b**, 5.00 g, 12.18 mmol) and Pd/C (1.833 g, 1.722 mmol) under N_2 was added EtOH (25 mL) and 5−6 M HCl in 2-propanol (15 mL, 83 mmol). The solution was allowed to stir for 15 min then H_2 was bubbled through the solution for 15 min and the reaction was stirred under H_2 (1 atm) for 48 h at room temperature. The suspension was filtered through a plug of Celite, and the filtrate was concentrated in vacuo. Purification by flash column chromatography (120 g SiO_2, 0−10% MeOH/DCM) gave 8-(4-(trifluoromethyl)phenyl)-5,6,7,8-tetrahydro-1,7-naphthyridine (**9b**, 2.59 g, 76.4% yield) as a clear, colorless oil. The racemate was separated by preparative chiral SFC purification to give (*R*)-8-(4-(trifluoromethyl)phenyl)-5,6,7,8-tetrahydro-1,7-naphthyridine ((*R*)-**9b**) as a white foam. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.35 (d, J = 4.0 Hz, 2H), 7.70 (d, J = 8.0 Hz, 2H), 7.50 (d, J = 7.5 Hz, 2H), 7.07 (m, 1H), 7.01 (m, 1H), 3.10−3.18 (m, 2H), 2.97−3.06 (m, 2H), 2.18−2.85 (m, 1H). MS (ESI pos ion) m/z: 279.1 (M + 1). $[\alpha]_D^{23}$ −18.1 (c 1.13, $CHCl_3$).

8-(3-Fluoro-4-(trifluoromethyl)phenyl)-5,6,7,8-tetrahydro-1,7-naphthyridine (9c). Step 1: To an oven-dried round-bottomed flask was added magnesium powder (788 mg, 32.4 mmol) and ether (30 mL). The solution was treated with a portion (0.25 mL) of 4-bromo-2-fluoro-1-(trifluoromethyl)benzene (2.75 mL, 19.24 mmol) and 1 M diisobutylaluminum hydride in hexanes (0.1 mL, 0.100 mmol). After stirring for 5 min, the reaction was above room temperature. The solution was immersed in an ice bath and treated with the remaining bromide solution over the next 5 min. The solution went from clear (with Mg particles) to dark brown over the addition and was stirred an additional 1 h. In a separate oven-dried round-bottomed flask was added 1,7-naphthyridine (**7b**, 1.09 g, 8.38 mmol) and THF (20 mL). The solution was treated with 50% benzyl chloroformate solution in toluene (3.50 mL, 10.47 mmol) dropwise over 2 min. After stirring for 10 min, the solution was cooled in an ice bath and the Grignard solution was cannulated over at a fast drip rate. The reaction was allowed to stir in the ice bath as the bath warmed to room temperature over 2 h. The reaction was quenched with saturated aqueous Rochelle's salt (75 mL) and extracted with EtOAc (3 × 40 mL). The combined organic layers were concentrated in vacuo to give an oil. The residue was adsorbed onto a plug of silica gel and purified by flash chromatography (40 g SiO_2, 5−20% EtOAc/hexanes) to provide 8-(3-fluoro-4-(trifluoromethyl)phenyl)-5,6,7,8-tetrahydro-1,7-naphthyridine (**8c**, 1.50 g, 41.8%) as a light-yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.35 (d, J = 11.11 Hz, 1H), 7.14−7.53 (m, 9H), 7.09 (d, J = 7.16 Hz, 1H), 6.69−6.47 (m, 1H), 5.68−5.92 (m, 1H), 5.23 (dd, J = 9.79, 16.08 Hz, 2H), 4.71 (d, J = 5.99 Hz, 1H). MS (ESI pos ion) m/z: 428.9 (M + 1).

Step 2: To a N_2 purged solution of benzyl 8-(3-fluoro-4-(trifluoromethyl)phenyl)-1,7-naphthyridine-7(8*H*)-carboxylate (**8c**, 700 mg, 1.63 mmol) was added 10% Pd/C (200 mg, 0.188 mmol), EtOH (10 mL), and 5N HCl in 2-propanol (3 mL, 15.0 mmol). After 5 min of stirring under N_2, the reaction was capped with a balloon of H_2. The solution was stirred at room temperature under H_2 (1 atm) for 16 h. The reaction was filtered through a pad of Celite, and the Celite pad was rinsed with EtOH. The combined organic layers were washed with brine and concentrated in vacuo. The crude product was adsorbed onto a plug of silica gel and purified by flash chromatography (12 g SiO_2, 0−5% MeOH/EtOAc) to afford 8-(3-fluoro-4-(trifluoromethyl)phenyl)-5,6,7,8-tetrahydro-1,7-naphthyridine (**9c**, 220 mg, 45.4% yield) as a golden oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.39 (d, J = 5.0 Hz, 1H), 7.41−7.64 (m, 2H), 7.21 (d, J = 7.9 Hz, 1H), 7.00−7.17 (m, 2H), 5.24 (s, 1H), 2.98−3.36 (m, 3H), 2.78−2.95 (m, 1H). MS (ESI pos ion) m/z: 297.0 (M + 1).

5-(4-(Trifluoromethyl)phenyl)-5,6,7,8-tetrahydro-1,6-naphthyridine (9d). Step 1: 1-Bromo-4-(trifluoromethyl)benzene (1.5 mL, 10.8 mmol) was added to a suspension of magnesium turnings (261 mg, 10.7 mmol) and a catalytic amount of iodine in THF (10 mL) at room temperature. A different round-bottomed flask containing 1,6-naphthyridine (**7d**, 1.0 g, 7.7 mmol) in anhydrous THF (10 mL) was charged with ethyl chloroformate (0.73 mL, 7.7 mmol) under a stream of N_2, and the mixture was stirred at room temperature for 15 min and then cooled to 0 °C. The previously made Grignard reagent was then cannulated into this solution dropwise, and the reaction mixture was stirred for 1 h at 0 °C followed by 1 h at room temperature. This mixture was quenched with saturated aqueous NH_4Cl and extracted with EtOAc. The organic layer was washed with brine, dried ($MgSO_4$), and concentrated in vacuo. The crude product was purified by silica gel chromatography (20−30% EtOAc in hexanes) to give ethyl 5-(4-(trifluoromethyl)phenyl)-1,6-naphthyridine-6(5*H*)-carboxylate (**8d**, 1.76 g, 65.9%) as an orange oil. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.38 (d, J = 3.7 Hz, 1H), 7.83 (br s, 1H), 7.79 (d, J = 8.2 Hz, 2H), 7.55 (d, J = 7.6 Hz, 2H), 7.35 (br s, 1H), 7.19 (dd, J = 7.2 Hz, 5.1 Hz, 1H), 6.66 (s, 1H), 6.85 (d, J = 6.8 Hz, 2H),

4.18−4.20 (m, 2H), 1.17−1.25 (m, 3H). MS (ESI pos ion) *m/z*: 349.2 (M + 1).

Step 2: A solution of ethyl 5-(4-(trifluoromethyl)phenyl)-1,6-naphthyridine-6(5*H*)-carboxylate (**8d**, 1.68 g, 4.82 mmol) in EtOH (20 mL) was stirred with 10% Pd/C (0.513 g, 4.82 mmol) under H_2 (1 atm) at room temperature for 1 h. The reaction mixture was filtered through a Celite pad, and the filtrate was concentrated in vacuo to provide ethyl 5-(4-(trifluoromethyl)phenyl)-7,8-dihydro-1,6-naphthyridine-6(5*H*)-carboxylate (1.56 g, 92.4%) as yellow oil. The crude product was used in the next step without further purification. MS (ESI pos ion) *m/z*: 351 (M + 1).

Step 3: A round-bottomed flask was charged with potassium hydroxide (10.2 g, 182.4 mmol) and EtOH (100 mL), and the resulting suspension was heated to 80 °C. After the potassium hydroxide was dissolved, crude ethyl 5-(4-(trifluoromethyl)phenyl)-7,8-dihydro-1,6-naphthyridine-6(5*H*)-carboxylate (1.3 g, 3.6 mmol) was added, and the solution was heated at 90 °C for 30 h. The mixture was allowed to cool to room temperature. The solvent was partially removed in vacuo, and the residue was diluted with EtOAc. The organic phase was washed with water and brine, dried ($MgSO_4$), and concentrated in vacuo. The resulting residue was purified by silica gel chromatography (5% MeOH/DCM) to give 5-(4-(trifluoromethyl)-phenyl)-5,6,7,8-tetrahydro-1,6-naphthyridine (**9d**, 624 mg, 61.5%) as an off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.35 (d, J = 4.0 Hz, 2H), 7.70 (d, J = 8.0 Hz, 2H), 7.50 (d, J = 7.5 Hz, 2H), 7.07 (m, 1H), 7.01 (m, 1H), 3.10−3.18 (m, 2H), 2.97−3.06 (m, 2H), 2.18−2.85 (m, 1H). MS (ESI pos ion) *m/z*: 279.2 (M + 1).

(*R*)-5-(4-(Trifluoromethyl)phenyl)-5,6,7,8-tetrahydropyrido-[3,4-b]pyrazine (9e). Step 1: A round-bottomed flask equipped with a reflux condenser was charged with 3,4-diamino pyridine (2.19 g, 20.0 mmol), glyoxal (2.25 mL, 40% aqueous solution, 20.0 mmol), and EtOH (50 mL). The resulting mixture was heated at reflux for 2 h and then allowed to cool to room temperature. The solvent was partially removed in vacuo, and the residue was triturated with ether (20 mL). The resulting precipitate was collected by filtration to provide pyrido[3,4-*b*]pyrazine (**7e**, 1.17 g, 44.5%) as a tan solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.52 (s, 1H), 9.20 (d, J = 1.8 Hz, 1H), 9.11 (d, J = 1.6 Hz, 1H), 8.87 (d, J = 5.7 Hz, 1H), 8.05 (d, J = 5.8 Hz, 1H). MS (ESI pos ion) *m/z*: 132.2 (M + 1).

Step 2: 1-Bromo-4-(trifluoromethyl)benzene (10.5 mL, 76.1 mmol) was added portionwise to a suspension of magnesium turnings (1.86 g, 76.5 mmol) in THF (66 mL). A catalytic amount of iodine was added, and the mixture was refluxed for 2 h. In a different flask, pyrido[3,4-*b*]pyrazine (**7e**, 5.02 g, 7.2 mmol) in THF (60 mL) was treated with ethyl chloroformate (4.0 mL, 41.8 mmol) at room temperature and stirred 20 min then cooled to 0 °C. The previously made Grignard reagent was then cannulated into this solution dropwise, and the reaction mixture was stirred for 1 h at 0 °C followed by 1 h at room temperature. This mixture was quenched with saturated aqueous NH_4Cl and extracted with EtOAc (3 × 100 mL). The organic layer was washed with water (100 mL) and brine (100 mL), dried ($MgSO_4$), and concentrated in vacuo. The crude product was purified by silica gel chromatography (0−50% EtOAc/hexanes) to give ethyl 5-(4-(trifluoromethyl)phenyl)pyrido[3,4-*b*]pyrazine-6(5*H*)-carboxylate (**8e**, 10.7 g, 79.8%) as an orange oil. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.42 (d, J = 2.7 Hz, 1H), 8.35 (d, J = 2.5 Hz, 1H), 7.70 (d, J = 8.2 Hz, 2H), 7.51−7.57 (m, 3H), 6.51 (s, 1H), 6.01 (d, J = 8.0 Hz, 2H), 4.19 (br s, 2H), 1.20−1.25 (m, 2H). MS (ESI pos ion) *m/z*: 350.2 (M + 1).

Step 3: A solution of ethyl 8-(4-(trifluoromethyl)phenyl)-1,7-naphthyridine-7(8*H*)-carboxylate (**8e**, 10.67 g, 30.5 mmol) in EtOH (100 mL) was stirred with 10% Pd/C (1.98 g, 18.6 mmol) under H_2 atmosphere at room temperature for 4 h. Ammonium formate (7.83 g, 124 mmol) was added and the reaction heated to 75 °C without a H_2 balloon. The reaction mixture was allowed to cool to room temperature, filtered through a Celite pad, and the filtrate concentrated in vacuo. The resulting residue was purified by silica gel chromatography (0−80% EtOAc/hexanes) to give ethyl 5-(4-(trifluoromethyl)phenyl)-7,8-dihydropyrido[3,4-*b*]pyrazine-6(5*H*)-carboxylate (8.96 g, 83.5%) as a yellow oil. MS (ESI pos ion) *m/z*: 352.2 (M + 1).

Step 4: To a stirred solution of ethyl 5-(4-(trifluoromethyl)phenyl)-7,8-dihydropyrido[3,4-*b*]pyrazine-6(5*H*)-carboxylate (6.59 g, 18.8 mmol) in $CHCl_3$ (100 mL) was added iodotrimethylsilane (13.3 mL, 93.8 mmol). The dark solution was stirred at 70 °C for 7.5 h. The reaction was allowed to cool to room temperature, and solvent was removed in vacuo. The residue was purified by flash chromatography (40 g SiO_2, 0−10% iPrOH (w/10% NH_4OH) in $CHCl_3$) to afford 5-(4-(trifluoromethyl)phenyl)-5,6,7,8-tetrahydropyrido[3,4-*b*]pyrazine (**9e**, 3.64 g, 69.5%). The racemate was separated by preparative chiral SFC purification to give (*R*)-5-(4-(trifluoromethyl)phenyl)-5,6,7,8-tetrahydropyrido[3,4-*b*]pyrazine ((*R*)-**9e**, 1.10 g, 28.8%) as a white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.53 (d, J = 2.1 Hz, 1H), 8.43 (d, J = 2.3 Hz, 1H), 7.63−7.76 (m, J = 8.2 Hz, 2H), 7.45−7.61 (m, J = 8.2 Hz, 2H), 5.63 (s, 1H), 3.40−3.66 (m, 2H), 3.16−3.40 (m, 2H). MS (ESI pos ion) *m/z*: 280.2 (M + 1).

1-(4-(Trifluoromethyl)phenyl)-1,2,3,4-tetrahydro-2,6-naphthyridine (14a). Step 1: A three-necked, round-bottomed flask equipped with a condenser was charged with magnesium (0.92 g, 37.8 mmol) and 1-bromo-4-(trifluoromethyl)benzene (5.3 mL, 37.9 mmol) in THF (35 mL), and the suspension was stirred under N_2. A catalytic amount of iodine was added, the mixture was heated at reflux for 1.5 h, and the solution was allowed to cool to room temperature. The reaction mixture was treated with 3-bromoisonicotinaldehyde (**10a**, 3.5 g, 18.9 mmol) and stirred at room temperature for 2 h. The mixture was quenched with saturated aqueous NH_4Cl and extracted with EtOAc. The organic layer was washed with water and brine, dried ($MgSO_4$), and concentrated in vacuo. The residue was triturated with DCM, and the product was collected by filtration to give the product (5.55 g) as an ivory-colored solid. The filtrate was concentrated in vacuo and the residue purified by silica gel chromatography (0−100% EtOAc/hexanes) to give (0.37 g) additional product. The solid from the filtration and the solid from chromatography were combined to give (3-bromopyridin-4-yl)-(4-(trifluoromethyl)phenyl)methanol (5.92 g, 94.5%) as an ivory-colored solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.66 (s, 1H), 8.56 (d, J = 5.0 Hz, 1H), 7.53−7.64 (m, 5H), 6.17 (d, J = 3.7 Hz, 1H). MS (ESI pos ion) *m/z*: 331.9, 333.9 (M + 1).

Step 2: A 20 mL, microwave reaction vessel was charged with (3-bromopyridin-4-yl)-(4-(trifluoro methyl)phenyl)methanol (2.0 g, 6.02 mmol), 2-vinylisoindoline-1,3-dione (1.16 g, 6.68 mmol), 2-(dicyclohexylphosphino)biphenyl (0.211 g, 0.60 mmol), $Pd(dba)_2$ (0.176 g, 0.30 mmol), NEt_3 (1.0 mL, 7.23 mmol), and DMF. The mixture was purged with Ar and heated in a microwave synthesizer at 150 °C for 1 h. The reaction mixture was partitioned between water and EtOAc. The EtOAc layer was separated, and the aqueous layer was extracted again with EtOAc. The combined organic layers were washed with saturated aqueous $NaHCO_3$, dried (Na_2SO_4), and concentrated in vacuo. The brown residue was triturated with DCM, and the resulting precipitate was collected by filtration to afford (*E*)-2-(2-(4-(hydroxy(4-(trifluoromethyl)phenyl)methyl)pyridin-3-yl)vinyl)-isoindoline-1,3-dione (**11a**, 1.17 g, 45.8%) as an ivory-colored solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.70 (s, 1H), 8.58 (d, J = 5.1 Hz, 1H), 7.92−7.95 (m, 2H), 7.79−7.7.84 (m, 3H), 7.55−7.64 (m, 5H), 7.24 (s, 0.5H), 7.19 (s, 0.5H), 2.71 (d, J = 3.4 Hz, 1H). MS (ESI pos ion) *m/z*: 425.0 (M + 1).

Step 3: A round-bottomed flask containing a solution of (*E*)-2-(2-(4-(hydroxy(4-(trifluoromethyl)phenyl)methyl)pyridin-3-yl)vinyl)-isoindoline-1,3-dione (**11a**, 1.0 g, 2.4 mmol) in EtOAc (20 mL) was stirred with 10% Pd on carbon (0.41 g, 3.9 mmol) under 1 atm of H_2 at room temperature for 12 h. The catalyst was removed via filtration through a Celite pad. The filtrate was concentrated in vacuo to yield 2-(2-(4-(hydroxy(4-(trifluoromethyl)phenyl)methyl)pyridin-3-yl)ethyl)-isoindoline-1,3-dione (0.925 g, 90.0%) as a yellow solid. The crude product was used in the next step.

Step 4: A round-bottomed flask was charged with 2-(2-(4-(hydroxy(4-(trifluoromethyl)phenyl)methyl)pyridin-3-yl)ethyl)-isoindoline-1,3-dione (0.590 g, 1.38 mmol) and MnO_2 (3.21 g, 36.9 mmol) in DCM (20 mL), and the mixture was stirred at room temperature for 2 h. Additional MnO_2 (1.84, 21.2 mmol) was added,

and the reaction was stirred for an additional 2 h. The MnO_2 was removed via filtration through a Celite pad. The filtrate was concentrated and purified by flash chromatography (50−100% EtOAc in hexanes) to afford 2-(2-(4-(4-(trifluoromethyl)benzoyl)-pyridin-3-yl)ethyl)isoindoline-1,3-dione (**12a**, 0.434 g, 74.0%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.62 (d, J = 5.0 Hz, 1H), 8.59 (s, 1H), 7.98 (d, J = 8.2 Hz, 2H), 7.66−7.79 (m, 6H), 7.19 (d, J = 4.5 Hz, 1H), 3.91 (t, J = 6.7 Hz, 2H), 3.15 (t, J = 6.7 Hz, 2H).

Step 5: A round-bottomed flask was charged with 2-(2-(4-(4-(trifluoromethyl)benzoyl)pyridin-3-yl)ethyl)isoindoline-1,3-dione (**12a**, 98.7 mg, 0.233 mmol) and hydrazine hydrate (30 μL, 0.96 mmol) in EtOH (3 mL). The reaction mixture was stirred at room temperature for 4 h. The solvent was evaporated, and the residue was dissolved in EtOAc. The organic phase was washed with water, dried (Na_2SO_4), and concentrated. The residue was purified by silica gel chromatography (0−5% (iPrOH with 10% NH_4OH) in hexanes)) to give 1-(4-(trifluoromethyl)phenyl)-3,4-dihydro-2,6-naphthyridine (**13a**, 36.3 mg, 56.5%) as a pale-yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.55−8.72 (m, 2H), 7.74 (s, 4H), 7.08 (d, J = 5.12 Hz, 1H), 3.90−4.09 (m, 2H), 2.75−2.91 (m, 2H). MS (ESI pos ion) m/z: 277.2 (M + 1).

Step 6: A solution of 1-(4-(trifluoromethyl)phenyl)-3,4-dihydro-2,6-naphthyridine (**13a**, 36 mg, 0.131 mmol) in MeOH (2.5 mL) was treated with sodium borohydride (17 mg, 0.447 mmol), and the reaction mixture was stirred at room temperature for 30 min. MeOH was removed in vacuo, and the residue was partitioned between EtOAc and water. The EtOAc layer was separated, and the aqueous layer was extracted with EtOAc. The combined organic layers were dried (Na_2SO_4) and concentrated in vacuo to give 1-(4-(trifluoromethyl)-phenyl)-1,2,3,4-tetrahydro-2,6-naphthyridine (**14a**, 30 mg, 82.6%) as a clear oil. The crude product was used in the next step. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.41 (s, 1H), 8.24 (d, J = 5.3 Hz, 1H), 7.50−7.71 (m, J = 8.2 Hz, 2H), 7.33−7.50 (m, J = 8.0 Hz, 2H), 6.60 (d, J = 5.1 Hz, 1H), 5.09 (s, 1H), 3.22−3.48 (m, 1H), 2.95−3.22 (m, 2H), 2.71−2.95 (m, 1H). MS (ESI pos ion) m/z: 279.2 (M + 1).

***N*-(4-Fluorophenyl)-1-(4-(trifluoromethyl)phenyl)-3,4-dihydro-2,7-naphthyridine-2(1*H*)-carboxamide (14b).** Step 1: A three-necked 250 mL, round-bottomed flask equipped with a condenser was charged with magnesium (0.27 g, 11.1 mmol) and 1-bromo-4-(trifluoromethyl)benzene (1.5 mL, 10.9 mmol) in THF (10 mL), and the suspension was stirred under N_2. A catalytic amount of iodine was added, and the mixture was heated at reflux for 1.5 h and then allowed to cool to room temperature. The reaction mixture was treated with 4-bromonicotinaldehyde (**10b**, 1.0 g, 5.4 mmol) and stirred at room temperature for 2 h. The mixture was quenched with saturated aqueous NH_4Cl and extracted with EtOAc. The organic layer was washed with water and brine, dried ($MgSO_4$), and concentrated in vacuo. The residue was triturated with DCM, and the product was collected by filtration to give (0.76 g) product. The filtrate was concentrated in vacuo and purified by silica gel chromatography (30−70% EtOAc/hexanes) to give (0.27 g) additional product. The solid from filtration and the solid from chromatography were combined to give 4-bromopyridin-3-yl)(4-(trifluoromethyl)phenyl)methanol (1.03 g, 57.6%) as a tan solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.75 (s, 1H), 8.36 (d, J = 5.3 Hz, 1H), 7.57−7.65 (m, 4H), 7.52 (d, J = 5.3 Hz, 1H), 6.26 (d, J = 3.8 Hz, 1H), 2.65 (d, J = 3.9 Hz, 1H).

Step 2: A 20 mL vial was charged with (4-bromopyridin-3-yl)-(4-(trifluoro methyl)phenyl)methanol (0.87 g, 2.62 mmol), 2-vinyl-isoindoline-1,3-dione (499 mg, 2.88 mmol), $Pd(dba)_2$ (75.3 g, 0.13 mmol), 2-(dicyclohexylphosphino)biphenyl (91.8 mg, 0.26 mmol), NEt_3 (0.44 mL, 3.14 mmol), and DMF (2 mL). The mixture was purged with Ar and heated in a microwave synthesizer at 150 °C for 1 h. The reaction mixture was partitioned between water and EtOAc. The EtOAc layer was separated, and the aqueous layer was extracted again with EtOAc. The combined organic layers were washed with saturated aqueous $NaHCO_3$, dried (Na_2SO_4), and concentrated in vacuo. The resulting residue was purified by silica gel chromatography (0−10% MeOH/DCM) to give an inseparable mixture of products (0.45 g) consisting of an approximately 1:1 ratio of (*E*)-2-(2-(3-(hydroxy(4-(trifluoromethyl)phenyl)methyl)pyridin-4-yl)vinyl) isoindoline-1,3-dione and 2-(2-(3-(hydroxy(4-(trifluoromethyl)phenyl)-methyl)pyridin-4-yl)ethyl)isoindoline-1,3-dione as a light-yellow semisolid. MS (ESI pos ion) m/z: 425.0 and 427.1 (M + 1).

Step 3: A round-bottomed flask containing a solution of a mixture of products (0.45 g) from above (**14b**, step 2) in MeOH (50 mL) was stirred with 10% Pd on activated carbon (0.2 g, 1.9 mmol) under H_2 (1 atm) at room temperature for 12 h. The catalyst was removed via filtration through a Celite pad. The filtrate was concentrated in vacuo to yield 2-(2-(3-(hydroxy(4-(trifluoromethyl)phenyl)methyl)pyridin-4-yl)ethyl)isoindoline-1,3-dione (0.393 g, 34.2% over 2 steps) as a gray semisolid. The crude product was used in the next step.

Step 4: A round-bottomed flask was charged with 2-(2-(3-(hydroxy(4-(trifluoromethyl)phenyl)methyl)pyridin-4-yl)ethyl)-isoindoline-1,3-dione (393 mg, 0.922 mmol) and MnO_2 (2.40 g, 27.7 mmol) in DCM (20 mL), and the mixture was stirred at room temperature for 12 h. The MnO_2 was removed via filtration through a Celite pad. The filtrate was concentrated in vacuo and purified by silica gel chromatography (0−10% MeOH/DCM) to give 2-(2-(3-(4-(trifluoromethyl)benzoyl)pyridin-4-yl)ethyl)isoindoline-1,3-dione (**12b**, 187 mg, 97.2%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.62 (d, J = 5.1 Hz, 1H), 8.57 (s, 1H), 7.95 (d, J = 8.0 Hz, 2H), 7.68−7.77 (m, 6H), 7.30 (d, J = 5.1 Hz, 1H), 3.98 (t, J = 6.7 Hz, 2H), 3.24 (t, J = 6.7 Hz, 2H). MS (ESI pos ion) m/z: 425.0 (M + 1).

Step 5: A round-bottomed flask was charged with 2-(2-(3-(4-(trifluoromethyl)benzoyl)pyridin-4-yl)ethyl)isoindoline-1,3-dione (**12b**, 130 mg, 0.31 mmol) and hydrazine hydrate (38 μL, 1.2 mmol) in EtOH (50 mL). The reaction mixture was stirred at room temperature for 12 h and concentrated in vacuo. The resulting residue was purified by silica gel chromatography (0−10% MeOH/DCM) to give 1-(4-(trifluoromethyl)phenyl)-3,4-dihydro-2,7-naphthyridine (**13b**, 34 mg, 40%) as a pale-yellow semisolid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.64 (d, J = 4.9 Hz, 1H), 8.47 (s, 1H), 7.88 (dd, J = 3.1, 5.5 Hz, 1H), 7.68−7.82 (m, 4H), 3.87−4.04 (m, 2H), 2.80−2.92 (m, 2H). MS (ESI pos ion) m/z: 277.1 (M + 1).

Step 6: A solution of 1-(4-(trifluoromethyl)phenyl)-3,4-dihydro-2,7-naphthyridine (**13b**, 32 mg, 0.12 mmol) in MeOH (5 mL) was treated with sodium borohydride (15 mg, 0.39 mmol), and the reaction mixture was stirred at room temperature for 30 min. The MeOH was removed in vacuo, and the residue was partitioned between DCM and water. The DCM layer was separated, and the aqueous layer was extracted with DCM. The combined organic layers were dried (Na_2SO_4) and concentrated in vacuo to give 1-(4-(trifluoromethyl)-phenyl)-1,2,3,4-tetrahydro-2,7-naphthyridine (**14b**, 21 mg, 65%) as clear oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.39 (d, J = 4.9 Hz, 1H), 7.98 (s, 1H), 7.57−7.71 (m, J = 8.0 Hz, 2H), 7.40−7.52 (m, J = 8.0 Hz, 2H), 7.12 (d, J = 5.1 Hz, 1H), 5.24 (s, 1H), 3.18−3.34 (m, 1H), 3.01−3.18 (m, 2H), 2.78−2.98 (m, 1H). MS (ESI pos ion) m/z: 279.0 (M + 1).

8-(4-(Trifluoromethyl)phenyl)-5,6,7,8-tetrahydropyrido[3,4-d]pyrimidine (14c). Step 1: A solution of diisopropylamine (2.0 mL, 14.3 mmol) in anhydrous THF (10 mL) was cooled to −78 °C, treated with *n*-BuLi (2.5 M in hexanes, 5.0 mL, 12.5 mmol), and stirred at −78 °C. A different flask containing a solution of 5-bromopyrimidine (1.01g, 6.31 mmol) and 4-(trifluoromethyl)-benzaldehyde (0.843 mL, 6.31 mmol) in THF (16.5 mL) was cooled to −78 °C. The previously made LDA solution (8.5 mL) was added dropwise to this solution. The reaction mixture was stirred for 1.5 h at −78 °C and allowed to warm to 0 °C and stirred for an additional 1 h. The reaction was quenched with ice and extracted with EtOAc. The EtOAc layer was separated, washed with brine, dried (Na_2SO_4), and concentrated in vacuo. The residue was purified by silica gel chromatography (0−60% EtOAc/hexanes) to give (5-bromopyrimidin-4-yl)-(4-(trifluoromethyl)phenyl)methanol (561 mg, 26.7%) as a pale-yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.21 (s, 1H), 8.81 (s, 1H), 7.48−7.62 (m, 4H), 6.01 (d, J = 7.9 Hz, 1H), 4.88 (d, J = 7.9 Hz, 1H).

Step 2: A 20 mL microwave reaction vessel was charged with (5-bromopyrimidin-4-yl)-(4-(trifluoromethyl)phenyl)methanol (560 mg, 1.68 mmol), 2-vinylisoindoline-1,3-dione (327.1 mg, 1.89 mmol), 2-(dicyclohexylphosphino)biphenyl (59.5 mg, 0.17 mmol), $Pd(dba)_2$

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(52.7 mg, 0.092 mmol), NEt_3 (0.3 mL, 2.02 mmol), and DMF (4 mL). The mixture was purged with Ar and heated in a microwave synthesizer at 150 °C for 1 h. The reaction mixture was partitioned between water and EtOAc. The EtOAc layer was separated, and the aqueous layer was extracted again with EtOAc. The combined organic layers were washed with saturated aqueous $NaHCO_3$, dried (Na_2SO_4), and concentrated in vacuo. The resulting residue was purified by silica gel chromatography (0−100% EtOAc/hexanes) to give 2-(2-(4-(4-(trifluoromethyl)benzoyl)pyrimidin-5-yl)ethyl) isoindoline-1,3-dione (**12c**, 90.7 mg, 12.7%) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.20 (s, 1H), 8.79 (s, 1H), 8.03 (d, J = 8.0 Hz, 2H), 7.67−7.74 (m, 6H), 3.98 (t, J = 6.3 Hz, 2H), 3.24 (t, J = 6.5 Hz, 2H). MS (ESI pos ion) m/z: 426.4 (M + 1). (*E*)-2-(2-(4-(hydroxy(4-(trifluoromethyl)phenyl)methyl)pyrimidin-5-yl)vinyl)isoindoline-1,3-dione (58.8 mg, 8.2%) was also collected as a yellow solid. MS (ESI pos ion) m/z: 426.4 (M + 1).

Step 3: A round-bottomed flask was charged with 2-(2-(4-(4-(trifluoromethyl)benzoyl) pyrimidin-5-yl)ethyl)isoindoline-1,3-dione (**12c**, 90.7 mg, 0.21 mmol) and hydrazine hydrate (0.05 mL, 1.59 mmol) in EtOH (3 mL). The reaction mixture was stirred at room temperature for 12 h. The suspension was filtered through a Celite pad, and the filtrate was concentrated in vacuo. The resulting residue was purified by silica gel chromatography (30−100% EtOAc/hexanes) to give 8-(4-(trifluoromethyl)phenyl)-5,6-dihydropyrido[3,4-*d*]-pyrimidine (**13c**, 12.4 mg, 21.0%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.25 (s, 1H), 8.77 (s, 1H), 7.97 (d, J = 8.0 Hz, 2H), 7.72 (d, J = 8.2 Hz, 2H), 4.07−4.12 (m, 2H), 2.92−2.97 (m, 2H).

Step 4: A solution of 8-(4-(trifluoromethyl)phenyl)-5,6-dihydropyrido[3,4-*d*]pyrimidine (**13c**, 12.4 mg, 0.045 mmol) in MeOH (2 mL) was treated with sodium borohydride (11 mg, 0.24 mmol), and the reaction mixture was stirred at room temperature for 2 h. The solvent was removed in vacuo, and the residue was partitioned between EtOAc and water. The EtOAc layer was separated, and the aqueous layer was extracted again with EtOAc. The combined organic layers were washed with brine, dried (Na_2SO_4), and concentrated in vacuo to give 8-(4-(trifluoromethyl)phenyl)-5,6,7,8-tetrahydropyrido-[3,4-*d*]pyrimidine (**14c**, 10.2 mg, 85%). The crude product was used in the next step without further purification. MS (ESI pos ion) m/z: 280.2 (M + 1).

4,4-Difluoro-1-(4-(trifluoromethyl)phenyl)-1,2,3,4-tetrahydroisoquinoline (18). Step 1: 4-(Trifluoromethyl)benzaldehyde (**15**, 1.87 mL, 14.0 mmol) and benzylamine (1.53 mL, 14.0 mmol) were dissolved in DCM (40 mL), and 4 Å molecular sieves were added. The mixture was left under N_2 for 3 days. The sieves were removed via filtration and washed with DCM (25 mL). To the combined filtrates was added phenylacetyl chloride (1.90 mL, 14.4 mmol), and the pale-yellow solution was stirred at room temperature for 2 h. Trifluoromethanesulfonic acid (6.20 mL, 70.1 mmol) was added, and the solution was stirred at room temperature for 1.5 h. The reaction mixture was poured into a mixture of ice (∼100 mL) and 5N NaOH (∼50 mL). The phases were separated, and the aqueous phase was extracted with DCM (3 × 100 mL). The combined organic phases were dried (Na_2SO_4) and concentrated in vacuo. The crude product was purified by silica gel chromatography (0−30% EtOAc/hexanes) to afford 2-benzyl-1-(4-(trifluoromethyl)phenyl)-1,2-dihydroisoquinolin-3(4*H*)-one (**16**, 4.33 g, 81%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.65−7.77 (m, J = 8.3 Hz, 2H), 7.54−7.65 (m, J = 8.2 Hz, 2H), 7.41 (d, J = 6.9 Hz, 1H), 7.08−7.35 (m, 8H), 5.81 (s, 1H), 5.30 (d, J = 15.2 Hz, 1H), 3.96 (d, J = 20.0 Hz, 1H), 3.85 (d, J = 15.4 Hz, 1H), 3.71 (d, J = 19.1 Hz, 1H).

Step 2: To a solution of 2-benzyl-1-(4-(trifluoromethyl)phenyl)-1,2-dihydroisoquinolin-3(4*H*)-one (**16** 1.47 g, 3.86 mmol) in THF (20 mL) at −78 °C was added lithium bis(trimethylsilyl)amide (1.0 M in THF/ethyl benzene, 8.5 mL, 8.5 mmol). The reaction was stirred for 30 min at −78 °C, then *N*-fluorobenzenesulfonimide (2.80 g, 8.88 mmol) in THF (15 mL) was added slowly. The solution was stirred at −78 °C for 1 h, the cold bath was removed, and the reaction mixture was allowed to warm to room temperature. The reaction was diluted with EtOAc and saturated aqueous NH_4Cl (100 mL). The organic layer was separated and the aqueous phase extracted with EtOAc (2 × 60 mL). The combined organic phases were washed with water (100 mL) and saturated aqueous NaCl (100 mL). The combined aqueous washes were combined and extracted with EtOAc (50 mL). The combined organic phases were dried (Na_2SO_4) and concentrated in vacuo to afford the crude compound. The crude product was triturated with DCM and MTBE, and the resulting white solid was removed via filtration. The filtrate was concentrated and the residue purified by silica gel chromatography (0−30% EtOAc/hexanes) to afford 2-benzyl-4,4-difluoro-1-(4-(trifluoromethyl)phenyl)-1,2-dihydroisoquinolin-3(4*H*)-one (**17**, 1.11 g, 69%) as a yellow foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.81−7.93 (m, 1H), 7.65 (d, J = 8.0 Hz, 2H), 7.28−7.52 (m, 7H), 7.16−7.26 (m, 2H), 7.06 (d, J = 7.3 Hz, 1H), 5.65 (d, J = 14.9 Hz, 1H), 5.53 (d, J = 3.5 Hz, 1H), 3.64 (dd, J = 1.6, 14.9 Hz, 1H).

Step 3: To a solution of borane tetrahydrofuran complex (1.0 M in THF, 0.750 mL, 0.750 mmol) in THF (5 mL) was added dropwise 2-benzyl-4,4-difluoro-1-(4-(trifluoromethyl)phenyl)-1,2-dihydroisoquinolin-3(4*H*)-one (**17**, 103 mg, 0.246 mmol) in THF (5 mL). The solution was heated at reflux and stirred for 5 h. The reaction was cooled to 0 °C and 5 N HCl (5 mL) was added, and the solution was stirred and allowed to warm to room temperature over 1 h. Solid $NaHCO_3$ was added to adjust to the reaction to pH ∼8. The solution was extracted with EtOAc (2 × 20 mL). The combined organic phases were washed with water (40 mL) and saturated aqueous NaCl (40 mL). The organic phase was dried (Na_2SO_4) and concentrated in vacuo. The crude product was purified by silica gel chromatography (0−20% EtOAc/hexanes to afford 2-benzyl-4,4-difluoro-1-(4-(trifluoromethyl)phenyl)-1,2,3,4-tetrahydroisoquinoline (41.8 mg, 42%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.70 (d, J = 7.8 Hz, 1H), 7.56 (d, J = 8.2 Hz, 2H), 7.41 (d, J = 8.0 Hz, 2H), 7.16−7.36 (m, 7H), 6.71 (d, J = 7.8 Hz, 1H), 4.73 (d, J = 3.7 Hz, 1H), 3.76 (dd, J = 1.8, 13.6 Hz, 1H), 3.27−3.50 (m, 2H), 2.80−3.02 (m, 1H).

Step 4: To a round-bottomed flask containing 2-benzyl-4,4-difluoro-1-(4-(trifluoromethyl)phenyl)-1,2,3,4-tetrahydroisoquinoline (41.8 mg, 0.10 mmol) and Pd/C (10 wt %, 72.9 mg, 0.69 mmol) under N_2 was added EtOH (5 mL). The reaction mixture was evacuated under vacuum and refilled with H_2 (4×). The mixture was stirred under H_2 (1 atm) at room temperature for 4 h. The catalyst was removed via filtration through a pad of Celite. The filtrate was concentrated to give 4,4-difluoro-1-(4-(trifluoromethyl)phenyl)-1,2,3,4-tetrahydroisoquinoline (**18**, 29.1 mg, 90%) as a clear film. The crude product was used in the next step without further purification. MS (ESI pos ion) m/z: 314.0 (M + 1).

General Procedure for Preparation of Ureas from Isocyanates (19, 21−23, 25, 27, 28, 32−41). To a solution of amine (1.0 equiv) and DIPEA (1.0 equiv) in DCM at room temperature was added the appropriate isocyanate (1.2 equiv). The reaction mixture was stirred at room temperature overnight and then concentrated in vacuo. Purification of the residue by silica flash chromatography or preparative reverse phase HPLC gave the title compounds.

(*R*)-*N*-(2-Fluorophenyl)-1-(4-(trifluoromethyl)phenyl)-3,4-dihydroisoquinoline-2(1*H*)-carboxamide (19). Following the general procedure for preparation of ureas from isocyanates, **19** was obtained from amine (*R*)-**5a** and 2-fluorophenylisocyanate in 57% yield. ^{1}H NMR (300 MHz, MeOH-d_4) δ 7.63 (d, J = 8.0 Hz, 2H), 7.39−7.57 (m, 3H), 7.22−7.37 (m, 4H), 7.16 (d, J = 8.8 Hz, 3H), 6.63 (s, 1H), 3.91 (td, J = 5.9, 12.3 Hz, 1H), 3.57−3.71 (m, 1H), 2.96−3.14 (m, 1H), 2.75−2.92 (m, 1H). MS (ESI pos ion) m/z: calcd for $C_{23}H_{18}F_4N_2O$, 414.1; found, 415.0 (M + 1).

(*R*)-4-(1-(4-(Trifluoromethyl)phenyl)-1,2,3,4-tetrahydroisoquinoline-2-carboxamido)benzoic Acid (20). Step 1: A mixture of (*R*)-1-(4-(trifluoromethyl)phenyl)-1,2,3,4-tetrahydroisoquinoline ((*R*)-**5a**, 0.300 g, 1.08 mmol), DIPEA (0.140 g, 1.08 mmol), and ethyl 4-isocyanatobenzoate (0.207 g, 1.08 mmol) in DCM (10 mL) was stirred at room temperature for 24 h. The mixture was concentrated and purified by silica gel flash chromatography (0−50% EtOAc/hexanes) to give (*R*)-ethyl-4-(1-(4-(trifluoromethyl)phenyl)-1,2,3,4-tetrahydroisoquinoline-2-carboxamido)benzoate (350 mg, 69.1%) as a white solid. ^{1}H NMR (300 MHz, MeOH-d_4) δ 7.85−8.01 (m, 2H), 7.50−7.67 (m, 4H), 7.36−7.49 (m, J = 8.2 Hz, 2H), 7.17−7.33 (m,

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4H), 6.66 (s, 1H), 4.33 (q, J = 7.1 Hz, 2H), 3.86−4.00 (m, 1H), 3.60 (ddd, J = 5.0, 8.3, 13.0 Hz, 1H), 2.95−3.13 (m, 1H), 2.74−2.90 (m, 1H), 1.29−1.43 (m, 3H). MS (ESI pos ion) m/z: calcd for $C_{26}H_{23}F_3N_2O_3$, 468.2; found, 469.0 (M + 1).

Step 2: A mixture of (*R*)-ethyl 4-(1-(4-(trifluoromethyl)phenyl)-1,2,3,4-tetrahydroisoquinoline-2-carboxamido)benzoate (0.210 g, 0.448 mmol) and 5N NaOH (0.4 mL, 2 mmol) in EtOH (5 mL) was stirred at room temperature for 24 h. The mixture was concentrated, taken up in water, and neutralized with 10% aqueous HCl, and the resulting solid was collected by filtration and dried under vacuum to give (*R*)-4-(1-(4-(trifluoromethyl)phenyl)-1,2,3,4-tetrahydroisoquinoline-2-carboxamido)benzoic acid (**20**, 81.4 mg, 41.1%) as a white solid. ^{1}H NMR (300 MHz, MeOH-d_4) δ 7.85−8.02 (m, J = 8.8 Hz, 2H), 7.56 (d, J = 8.8 Hz, 2H), 7.60 (d, J = 8.2 Hz, 2H), 7.37−7.49 (m, J = 8.0 Hz, 2H), 7.15−7.37 (m, 4H), 6.66 (s, 1H), 3.84−4.02 (m, 1H), 3.60 (ddd, J = 5.0, 8.2, 13.0 Hz, 1H), 2.95−3.15 (m, 1H), 2.73−2.92 (m, 1H). MS (ESI pos ion) m/z: calcd for $C_{24}H_{19}F_3N_2O_3$, 440.1; found, 441.0 (M + 1).

(*R*)-*N*-(2-Cyanophenyl)-1-(4-(trifluoromethyl)phenyl)-3,4-dihydroisoquinoline-2(1*H*)-carboxamide (21). Following the general procedure for preparation of ureas from isocyanates, **21** was obtained from amine (*R*)-**5a** and 2-isocyanatobenzonitrile in 37% yield. ^{1}H NMR (400 MHz, MeOH-d_4) δ 7.71 (dd, J = 1.3, 7.7 Hz, 1H), 7.56−7.67 (m, 3H), 7.48 (t, J = 9.2 Hz, 3H), 7.21−7.36 (m, 5H), 6.62 (s, 1H), 3.91 (td, J = 6.0, 12.6 Hz, 1H), 3.63 (ddd, J = 5.1, 8.0, 12.9 Hz, 1H), 3.08 (ddd, J = 5.6, 8.1, 16.0 Hz, 1H), 2.82 (td, J = 5.6, 16.1 Hz, 1H). MS (ESI pos ion) m/z: calcd for $C_{24}H_{18}F_3N_3O$, 421.1; found, 422.1 (M + 1).

(*R*)-*N*-(3-Cyanophenyl)-1-(4-(trifluoromethyl)phenyl)-3,4-dihydroisoquinoline-2(1*H*)-carboxamide (22). Following the general procedure for preparation of ureas from isocyanates, **22** was obtained from amine (*R*)-**5a** and 3-isocyanatobenzonitrile in 37% yield. ^{1}H NMR (400 MHz, MeOH-d_4) δ 7.88 (t, J = 1.7 Hz, 1H), 7.73 (td, J = 1.1, 8.2 Hz, 1H), 7.61 (d, J = 8.2 Hz, 2H), 7.40−7.49 (m, 3H), 7.34−7.39 (m, 1H), 7.19−7.32 (m, 4H), 6.65 (s, 1H), 3.85−3.97 (m, 1H), 3.61 (ddd, J = 5.1, 8.3, 13.1 Hz, 1H), 2.98−3.10 (m, 1H), 2.83 (td, J = 5.4, 16.1 Hz, 1H). MS (ESI pos ion) m/z: calcd for $C_{24}H_{18}F_3N_3O$, 421.1; found, 422.1 (M + 1).

(*R*)-*N*-(4-Cyanophenyl)-1-(4-(trifluoromethyl)phenyl)-3,4-dihydroisoquinoline-2(1*H*)-carboxamide (23). Following the general procedure for preparation of ureas from isocyanates, **23** was obtained from amine (*R*)-**5a** and 4-isocyanatobenzonitrile in 96% yield. ^{1}H NMR (400 MHz, MeOH-d_4) δ 7.57−7.70 (m, 6H), 7.43 (d, J = 8.2 Hz, 2H), 7.20−7.34 (m, 4H), 6.65 (s, 1H), 3.86−3.99 (m, 1H), 3.61 (ddd, J = 5.0, 8.4, 13.1 Hz, 1H), 2.97−3.11 (m, 1H), 2.83 (td, J = 5.4, 16.1 Hz, 1H). MS (ESI pos ion) m/z: calcd for $C_{24}H_{18}F_3N_3O$, 421.1; found, 422.1 (M + 1).

(*R*)-*N*-(Pyridin-2-yl)-1-(4-(trifluoromethyl)phenyl)-3,4-dihydroisoquinoline-2(1*H*)-carboxamide (24). To a solution of (*R*)-1-(4-(trifluoromethyl)phenyl)-1,2,3,4-tetrahydroisoquinoline ((*R*)-**5a**, 100 mg, 361 μmol) in MeCN (2 mL) was added 4-nitrophenyl pyridin-2-ylcarbamate (467 mg, 1803 μmol). The resulting mixture was stirred at 70 °C overnight. The mixture was concentrated, and the residue was purified by silica gel flash column chromatography (solid loading, 0−100% EtOAc/hexane) to give the title compound (**24**, 103 mg, 72%) as a white solid. ^{1}H NMR (400 MHz, MeOH-d_4) δ 8.24 (dd, J = 1.0, 4.9 Hz, 1H), 7.85 (d, J = 8.4 Hz, 1H), 7.70−7.78 (m, 1H), 7.57−7.65 (m, J = 8.4 Hz, 2H), 7.41−7.50 (m, J = 8.4 Hz, 2H), 7.21−7.35 (m, 4H), 7.05 (ddd, J = 0.8, 5.1, 7.2 Hz, 1H), 6.65 (s, 1H), 3.91 (td, J = 6.1, 12.5 Hz, 1H), 3.66 (ddd, J = 5.1, 8.0, 12.9 Hz, 1H), 3.05 (ddd, J = 5.6, 8.1, 16.1 Hz, 1H), 2.84 (td, J = 5.8, 16.1 Hz, 1H). MS (ESI pos ion) m/z: calcd for $C_{22}H_{18}F_3N_3O$, 397.1; found, 398.1 (M + 1).

(*R*)-*N*-(Pyridin-3-yl)-1-(4-(trifluoromethyl)phenyl)-3,4-dihydroisoquinoline-2(1*H*)-carboxamide (25). Following the general procedure for preparation of ureas from isocyanates, **25** was obtained from amine (*R*)-**5a** and 3-isocyanatopyridine in 90% yield. ^{1}H NMR (400 MHz, MeOH-d_4) δ 8.64 (d, J = 2.5 Hz, 1H), 8.16−8.23 (m, 1H), 7.97 (dt, J = 8.4, 1.8 Hz, 1H), 7.56−7.66 (m, J = 8.4 Hz, 2H), 7.40−7.47 (m, J = 8.2 Hz, 2H), 7.36 (dd, J = 8.3, 4.8 Hz, 1H), 7.19−7.32 (m, 4H), 6.65 (s, 1H), 3.85−3.98 (m, 1H), 3.62 (ddd, J = 13.1, 8.3, 5.1 Hz, 1H), 2.98−3.11 (m, 1H), 2.83 (dt, J = 16.1, 5.5 Hz, 1H). MS (ESI pos ion) m/z: calcd for $C_{22}H_{18}F_3N_3O$, 397.1; found, 398.1 (M + 1). $[\alpha]_D^{23}$ −98.4 (c 1.03, $CHCl_3$).

(*R*)-*N*-(Pyridin-4-yl)-1-(4-(trifluoromethyl)phenyl)-3,4-dihydroisoquinoline-2(1*H*)-carboxamide (26). To a solution of (*R*)-1-(4-(trifluoromethyl)phenyl)-1,2,3,4-tetrahydroisoquinoline ((*R*)-**5a**, 200 mg, 721 μmol) in MeCN (3 mL) was added 4-nitrophenyl pyridin-4-ylcarbamate (561 mg, 2164 μmol). The resulting mixture was heated at 55 °C overnight. The mixture was cooled to room temperature, concentrated, and the residue purified by silica gel flash column chromatography (solid loading, 0−100% EtOAc/hexanes) to give the title compound (**26**, 45 mg, 16%) as a white solid. ^{1}H NMR (400 MHz, MeOH-d_4) δ 8.24−8.38 (m, 2H), 7.54−7.65 (m, 4H), 7.43 (d, J = 8.2 Hz, 2H), 7.16−7.35 (m, 4H), 6.66 (s, 1H), 3.87−4.02 (m, 1H), 3.60 (ddd, J = 5.0, 8.5, 13.2 Hz, 1H), 2.97−3.13 (m, 1H), 2.84 (td, J = 5.4, 16.2 Hz, 1H). MS (ESI pos ion) m/z: calcd for $C_{22}H_{18}F_3N_3O$, 397.1; found, 398.1 (M + 1).

(*R*)-*N*-(*tert*-Butyl)-1-(4-(trifluoromethyl)phenyl)-3,4-dihydroisoquinoline-2(1*H*)-carboxamide (27). Following the general procedure for preparation of ureas from isocyanates, **27** was obtained from amine (*R*)-**5a** and 2-isocyanato-2-methylpropane in 74% yield. ^{1}H NMR (400 MHz, MeOH-d_4) δ 7.57 (d, J = 8.2 Hz, 2H), 7.36 (d, J = 8.0 Hz, 2H), 7.16−7.30 (m, 4H), 6.47 (s, 1H), 3.64 (td, J = 6.2, 12.5 Hz, 1H), 3.47 (ddd, J = 5.3, 7.4, 12.5 Hz, 1H), 2.84−2.99 (m, 1H), 2.71 (td, J = 6.1, 16.0 Hz, 1H), 1.36 (s, 9H). MS (ESI pos ion) m/z: calcd for $C_{21}H_{23}F_3N_2O$, 376.2; found, 377.1 (M + 1).

***N*-Isopropyl-1-(4-(trifluoromethyl)phenyl)-3,4-dihydroisoquinoline-2(1*H*)-carboxamide (28).** Following the general procedure for preparation of ureas from isocyanates, **28** was obtained from amine (*R*)-**5a** and 2-isocyanatopropane in 33% yield. ^{1}H NMR (400 MHz, MeOH-d_4) δ 7.57 (d, J = 8.2 Hz, 2H), 7.36 (d, J = 8.2 Hz, 2H), 7.13−7.31 (m, 4H), 6.52 (s, 1H), 3.89−4.05 (m, 1H), 3.67 (td, J = 6.1, 12.6 Hz, 1H), 3.43 (ddd, J = 5.1, 7.8, 12.8 Hz, 1H), 2.92 (ddd, J = 5.6, 7.9, 16.0 Hz, 1H), 2.72 (td, J = 5.8, 16.0 Hz, 1H), 1.16 (d, J = 6.7 Hz, 3H), 1.19 (d, J = 6.5 Hz, 3H). MS (ESI pos ion) m/z: calcd for $C_{20}H_{21}F_3N_2O$, 362.2; found, 363.2 (M + 1).

(*R*)-1-(4-(Trifluoromethyl)phenyl)-*N*-((*R*)-1,1,1-trifluoropropan-2-yl)-3,4-dihydroisoquinoline-2(1*H*)-carboxamide (30). A mixture of (*R*)-1-(4-(trifluoromethyl)phenyl)-1,2,3,4-tetrahydroisoquinoline ((*R*)-**5a**, 0.161 g, 0.582 mmol) and (*R*)-4-nitrophenyl (1,1,1-trifluoropropan-2-yl)carbamate (0.155 g, 0.563 mmol) in 1,4-dioxane (4 mL) was heated by a microwave reactor for 30 min at 160 °C. The reaction was allowed to cool, concentrated, and purified by preparative HPLC to give the title compound (**30**, 110 mg, 45.4%) as a white solid. ^{1}H NMR (300 MHz, MeOH-d_4) δ 7.58 (d, J = 8.2 Hz, 2H), 7.35 (d, J = 8.2 Hz, 2H), 7.15−7.31 (m, 4H), 6.53 (s, 1H), 4.65 (td, J = 7.4, 14.9 Hz, 1H), 3.62−3.82 (m, 1H), 3.47 (ddd, J = 5.2, 7.8, 12.8 Hz, 1H), 2.87−3.03 (m, 1H), 2.73 (td, J = 5.9, 16.1 Hz, 1H), 1.35 (d, J = 7.0 Hz, 3H). MS (ESI pos ion) m/z: calcd for $C_{20}H_{18}F_6N_2O$, 416.1; found, 417.2 (M + 1).

***N*-(4-Fluorophenyl)-1-methyl-1-(4-(trifluoromethyl)phenyl)-3,4-dihydroisoquinoline-2(1*H*)-carboxamide (32).** Following the general procedure for preparation of ureas from isocyanates, **32** was obtained from amine **6** and 4-fluorophenyl isocyanate in 79% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.73 (s, 1H), 7.51−7.59 (m, 4H), 7.29 (dd, J = 5.0, 9.0 Hz, 2 H), 7.20 (d, J = 7.0 Hz 1 H), 6.96−7.10 (m, 4H), 6.73 (d, J = 7.5 Hz, 1 H), 4.11−4.19 (m, 1H), 3.61 (t, J = 10.3 Hz, 1H), 3.23−3.29 (m, 1H), 3.92−3.02 (m, 1H), 2.13 (s, 3H). MS (ESI pos ion) m/z: calcd for $C_{24}H_{20}F_4N_2O$, 428.2; found, 429.1 (M + 1).

4,4,4-Trifluoro-*N*-(4-fluorophenyl)-1-(4-(trifluoromethyl)-phenyl)-3,4-dihydroisoquinoline-2(1*H*)-carboxamide (33). Following the general procedure for preparation of ureas from isocyanates, **33** was obtained from amine **18** and 4-fluorophenyl isocyanate in 23% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.82 (d, J = 4.7 Hz, 1H), 7.55−7.64 (m, J = 8.2 Hz, 2H), 7.45−7.55 (m, 2H), 7.36−7.45 (m, J = 8.2 Hz, 2H), 7.27−7.33 (m, 2H), 7.17 (d, J = 5.1 Hz, 1H), 6.93−7.08 (m, 2H), 6.83 (s, 1H), 6.53 (s, 1H), 4.04−4.23

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(m, 1H), 3.52−3.76 (m, 1H). MS (ESI pos ion) m/z: calcd for $C_{23}H_{16}F_6N_2O$, 450.1; found, 451.1 (M + 1).

(*R*)-*N*-(4-Fluorophenyl)-5-(4-(trifluoromethyl)phenyl)-7,8-dihydro-1,6-naphthyridine-6(5*H*)-carboxamide (34). Following the general procedure for preparation of ureas from isocyanates, **34** was obtained from amine **9d** and 4-fluorophenyl isocyanate in 36% yield after separation of the enantiomers by chiral preparative SFC. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.78 (s, 1H), 8.49 (d, J = 4.5 Hz, 1H), 7.71 (d, J = 8.5 Hz, 2H), 7.53 (d, J = 7.5 Hz, 1H), 7.50 (dd, J = 8.8 Hz, 4.8 Hz, 2H), 7.44 (d, J = 8.0 Hz, 2H), 7.29 (dd, J = 7.5 Hz, 4.5 Hz, 1H), 7.09 (t, J = 8.8 Hz, 2H), 6.71 (s, 1H), 4.14 (dd, J = 8.8 Hz, 4.3 Hz, 1H), 3.36−3.43 (m, 1H), 3.07−3.14 (m, 1H), 2.92−2.96 (m, 1H). MS (ESI pos ion) m/z: calcd for $C_{22}H_{17}F_4N_3O$, 415.1; found, 416.2 (M + 1).

***N*-(4-Fluorophenyl)-1-(4-(trifluoromethyl)phenyl)-3,4-dihydro-2,6-naphthyridine-2(1*H*)-carboxamide (35).** Following the general procedure for preparation of ureas from isocyanates, **35** was obtained from amine **14a** and 4-fluorophenyl isocyanate in 35.5% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.54 (s, 1H), 8.49 (d, J = 5.1 Hz, 1H), 7.59 (d, J = 8.2 Hz, 2H), 7.40 (d, J = 8.2 Hz, 2H), 7.27−7.33 (m, 2H), 7.08 (d, J = 5.0 Hz, 1H), 6.98−7.04 (m, 2H), 6.68 (s, 1H), 6.46 (s, 1H), 3.78−3.86 (m, 1H), 3.56−3.65 (m, 1H), 3.03−3.13 (m, 1H), 2.87−2.95 (m, 1H). MS (ESI pos ion) m/z: calcd for $C_{22}H_{17}F_4N_3O$, 415.1; found, 416.1 (M + 1).

***N*-(4-Fluorophenyl)-1-(4-(trifluoromethyl)phenyl)-3,4-dihydro-2,7-naphthyridine-2(1*H*)-carboxamide (36).** Following the general procedure for preparation of ureas from isocyanates, **36** was obtained from amine **14b** and 4-fluorophenyl isocyanate in 71% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.75 (s, 1H), 8.46 (s, 1H), 8.42 (d, J = 5.0 Hz, 1H), 7.71 (d, J = 8.2 Hz, 2H), 7.41−7.51 (m, 4H), 7.32 (d, J = 5.0 Hz, 1H), 7.04−7.13 (m, 2H), 6.72 (s, 1H), 3.97−4.02 (m, 1H), 3.35−3.40 (m, 1H), 2.97−3.05 (m, 1H), 2.79−2.86 (m, 1H). MS (ESI pos ion) m/z: calcd for $C_{22}H_{17}F_4N_3O$, 415.1; found, 416.1 (M + 1).

(*R*)-*N*-(4-Fluorophenyl)-8-(4-(trifluoromethyl)phenyl)-5,6-dihydro-1,7-naphthyridine-7(8*H*)-carboxamide (37). Following the general procedure for preparation of ureas from isocyanates, **37** was obtained from amine (*R*)-**9b** and 4-fluorophenyl isocyanate in 99% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.81 (s, 1H), 8.47 (dd, J = 4.6 Hz, 1.5 Hz, 1H),7.68−7.73 (m, 3H), 7.45−7.51 (m, 4H), 7.33 (dd, J = 7.6 Hz, 4.7 Hz, 1H), 7.05−7.12 (m, 2H), 6.59 (s, 1H), 4.10−4.15 (m, 1H), 3.34−3.37 (m, 1H), 3.01−3.09 (m,1H), 2.81−2.87 (m, 1H). MS (ESI pos ion) m/z: calcd for $C_{22}H_{17}F_4N_3O$, 415.1; found, 416.1 (M + 1). $[\alpha]_D^{23}$ −95.2 (c 1.46, $CHCl_3$).

***N*-(4-Fluorophenyl)-8-(4-(trifluoromethyl)phenyl)-5,6-dihydropyrido[3,4-d]pyrimidine-7(8*H*)-carboxamide (38).** Following the general procedure for preparation of ureas from isocyanates, **38** was obtained from amine **14c** and 4-fluorophenyl isocyanate in 58% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.11 (s, 1H), 8.65 (s, 1H), 7.63 (d, J = 8.5 Hz, 2H), 7.55 (d, J = 8.5 Hz, 2H), 7.20−7.31 (m, 4H), 6.93−7.04 (m, 2H), 6.48 (s, 1H), 6.40 (s, 1H), 4.09 (td, J = 4.7, 13.5 Hz, 1H), 3.60 (ddd, J = 4.4, 9.8, 13.7 Hz, 1H), 3.03−3.25 (m, 1H), 2.79−3.03 (m, 1H). MS (ESI pos ion) m/z: calcd for $C_{21}H_{16}F_4N_4O$, 416.1; found, 417.0 (M + 1).

(*R*)-*N*-(4-Fluorophenyl)-5-(4-(trifluoromethyl)phenyl)-7,8-dihydropyrido[3,4-*b*]pyrazine-6(5*H*)-carboxamide (39). Following the general procedure for preparation of ureas from isocyanates, **39** was obtained from amine (*R*)-**9e** and 4-fluorophenyl isocyanate in 91% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.51 (s, 2H), 7.62 (d, J = 8.4 Hz, 2H), 7.53 (d, J = 8.4 Hz, 2H), 7.27−7.34 (m, 2H), 6.95−7.06 (m, 2H), 6.64 (s, 1H), 6.43 (s, 1H), 4.10 (td, J = 4.6, 13.6 Hz, 1H), 3.61−3.76 (m, 1H), 3.33 (ddd, J = 5.8, 10.6, 16.9 Hz, 1H), 3.14 (td, J = 4.3, 17.1 Hz, 1H). MS (ESI pos ion) m/z: calcd for $C_{21}H_{16}F_4N_4O$, 416.1; found, 417.1 (M + 1).

(*R*)-*N*-(Pyridin-3-yl)-8-(4-(trifluoromethyl)phenyl)-5,6-dihydro-1,7-naphthyridine-7(8*H*)-carboxamide (40). Following the general procedure for preparation of ureas from isocyanates, **40** was obtained from amine (*R*)-**9b** and 3-isocyanatopyridine in 50% yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.99 (s, 1H), 8.69 (d, J = 2.2 Hz, 1H), 8.48 (dd, J = 1.5, 4.7 Hz, 1H), 8.19 (dd, J = 1.5, 4.7 Hz, 1H), 7.85−8.02 (m, 1H), 7.59−7.79 (m, 3H), 7.48 (d, J = 8.2 Hz, 2H), 7.24−7.41 (m, 2H), 6.61 (s, 1H), 4.16 (td, J = 4.5, 13.3 Hz, 1H), 3.34−3.45 (m, 1H), 3.00−3.17 (m, 1H), 2.77−2.94 (m, 1H). MS (ESI pos ion) m/z: calcd for $C_{21}H_{17}F_3N_4O$, 398.1; found, 399.1 (M + 1).

(*R*)-*N*-(4-Cyanophenyl)-8-(4-(trifluoromethyl)phenyl)-5,6-dihydro-1,7-naphthyridine-7(8*H*)-carboxamide (41). Following the general procedure for preparation of ureas from isocyanates, **41** was obtained from amine (*R*)-**9b** and 4-cyanophenyl isocyanate in 85% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.24 (s, 1H), 8.48 (dd, J = 4.7 Hz, 1.6 Hz, 1H), 1.69−7.14 (m, 7H), 7.71 (d, J = 8.2 Hz, 2H), 7.34 (dd, J = 7.7 Hz, 4.8 Hz, 1H), 6.60 (s, 1H), 4.17 (dt, J = 13.5, 4.4 Hz, 1H, 3.36−3.41 (m, 1H), 3.03−3.11 (m, 1H), 2.85 (dt, J = 16.6, 3.8 Hz, 1H). MS (ESI pos ion) m/z: calcd for $C_{23}H_{17}F_3N_4O$, 422.1; found, 423.0 (M + 1).

General Procedure for Preparation of Ureas from CDI Coupling (29, 31, 42, 43−45). To a solution of amine (1.0 equiv) in 4:1 DCM:THF (5 mL) was added 1,1′-carbonyldiimidazole (1.5 equiv), and the reaction was stirred at room temperature for 1−2 h. 1,2,3,4-Tetrahydroisoquinoline ((*R*)-**5a**, (*R*)-**5b**, or **9a**−**c**, 1.25 equiv) in DCM was added, and the reactions were stirred at room temperature 2−18 h. The reactions were either filtered and purified by preparative reverse phase HPLC or diluted with $NaHCO_3$, extracted with DCM (3×), the organic layers combined, dried ($MgSO_4$), and concentrated, and the residue purified by silica gel flash chromatography to afford the title compounds.

(*R*)-1-(4-(Trifluoromethyl)phenyl)-*N*-((*S*)-1,1,1-trifluoropropan-2-yl)-3,4-dihydroisoquinoline-2(1*H*)-carboxamide (29). Following the general procedure for preparation of ureas from CDI coupling, **29** was obtained from amine (*R*)-**5a** and (*S*)-1,1,1-trifluoropropan-2-amine in 64.2% yield. ^{1}H NMR (300 MHz, MeOH-d_4) δ 7.58 (d, J = 8.0 Hz, 2H), 7.36 (m, J = 8.0 Hz, 2H), 7.12−7.31 (m, 4H), 6.55 (s, 1H), 4.64 (td, J = 7.4, 14.9 Hz, 1H), 3.70−3.87 (m, 1H), 3.38−3.53 (m, 1H), 2.84−3.04 (m, 1H), 2.65−2.84 (m, 1H), 1.37 (d, J = 7.2 Hz, 3H). MS (ESI pos ion) m/z: calcd for $C_{20}H_{18}F_6N_2O$, 416.1; found, 416.9 (M + 1).

(*R*)-*N*-(2,2,2-Trifluoro-1-phenylethyl)-1-(4-(trifluoromethyl)-phenyl)-3,4-dihydroisoquinoline-2(1*H*)-carboxamide (31). Following the general procedure for preparation of ureas from CDI coupling, **31** was obtained from amine (*R*)-**5a** and 2,2,2-trifluoro-1-phenethanamine in 69% yield. ^{1}H NMR (300 MHz, MeOH-d_4) δ 7.51−7.59 (m, 3H), 7.18−7.50 (m, 10H), 6.59 (s, 0.5H), 6.53 (s, 0.5H), 5.61−5.83 (m, 1H), 3.74−3.99 (m, 1H), 3.39−3.63 (m, 1H), 2.85−3.04 (m, 1H), 2.67−2.85 (m, 1H). MS (ESI pos ion) m/z: calcd for $C_{25}H_{20}F_6N_2O$, 478.1; found, 479.0 (M + 1).

(*R*)-8-(4-(Trifluoromethyl)phenyl)-*N*-((*S*)-1,1,1-trifluoropropan-2-yl)-5,6-dihydro-1,7-naphthyridine-7(8*H*)-carboxamide (42). Following the general procedure for preparation of ureas from CDI coupling, **42** was obtained from racemic amine **9b** and (*S*)-1,1,1-trifluoropropan-2-amine. The (*R*,*S*) and (*S*,*S*) diastereomers were separated by flash column chromatography (20−50% EtOAc/hexanes) to provide **42** in 37.5% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.39−8.57 (m, 1H), 7.57 (d, J = 8.2 Hz, 2H), 7.54 (d, J = 8.0 Hz, 1H), 7.43 (d, J = 8.2 Hz, 2H), 7.21 (dd, J = 4.8, 7.7 Hz, 1H), 6.40 (s, 1H), 4.59−4.80 (m, 2H), 3.83 (td, J = 5.5, 13.0 Hz, 1H), 3.56 (ddd, J = 4.7, 8.9, 13.3 Hz, 1H), 2.95−3.10 (m, 1H), 2.85 (td, J = 5.0, 16.2 Hz, 1H), 1.33 (d, J = 6.7 Hz, 3H). MS (ESI pos ion) m/z: calcd for $C_{19}H_{17}F_6N_3O$, 417.1; found, 418.1 (M + 1).

(*S*)-1-(6-(Trifluoromethyl)pyridin-3-yl)-*N*-((*S*)-1,1,1-trifluoropropan-2-yl)-3,4-dihydroisoquinoline-2(1*H*)-carboxamide (43). Following the general procedure for preparation of ureas from CDI coupling, **43** was obtained from amine (*S*)-**9a** and (*S*)-1,1,1-trifluoropropan-2-amine in 61.4% yield. ^{1}H NMR (300 MHz, MeOH-d_4) δ 8.56 (s, 1H), 7.66−7.86 (m, 2H), 6.61 (s, 1H), 4.63 (td, J = 7.3, 14.6 Hz, 1H), 3.69−3.90 (m, 1H), 3.47 (ddd, J = 5.0, 8.3, 13.1 Hz, 1H), 2.88−3.07 (m, 1H), 2.68−2.87 (m, 1H), 1.37 (d, J = 7.2 Hz, 3H). MS (ESI pos ion) m/z: calcd for $C_{19}H_{17}F_6N_3O$, 417.1; found, 418.0 (M + 1).

(*R*)-1-(3-Fluoro-4-(trifluoromethyl)phenyl)-*N*-((*S*)-1,1,1-trifluoropropan-2-yl)-3,4-dihydroisoquinoline-2(1*H*)-carboxamide (44). Following the general procedure for preparation of ureas from CDI coupling, **44** was obtained from amine (*R*)-**5b** and (*S*)-1,1,1-trifluoropropan-2-amine in 61.2% yield. ^{1}H NMR (300 MHz,

MeOH-d_4) δ 7.59 (t, J = 7.5 Hz, 1H), 7.18−7.36 (m, 4H), 6.95−7.18 (m, 2H), 6.52 (br. s., 1H), 4.50−4.74 (m, 1H), 3.63−3.88 (m, 1H), 3.48 (d, J = 4.8 Hz, 1H), 2.84−3.02 (m, 1H), 2.61−2.84 (m, 1H), 1.37 (d, J = 6.6 Hz, 3H). MS (ESI pos ion) m/z: calcd for $C_{20}H_{17}F_7N_2O$, 434.1; found, 435.0 (M + 1).

(*R*)-1-(3-Fluoro-4-(trifluoromethyl)phenyl)-*N*-((*S*)-1,1,1-trifluoropropan-2-yl)-3,4-dihydroisoquinoline-2(1*H*)-carboxamide (45). To a solution of (*S*)-1,1,1-trifluoropropan-2-amine (1.190 g, 10.52 mmol) in DCM (20 mL) was added 1,1′-carbonyldiimidazole (1.71 g, 10.5 mmol), and the reaction was stirred for 1.15 h at room temperature under N_2. A solution of 8-(3-fluoro-4-(trifluoromethyl)-phenyl)-5,6,7,8-tetrahydro-1,7-naphthyridine hydrochloride (**9c**, 2.00 g, 6.01 mmol) and DIPEA (2.30 mL, 13.2 mmol) in DCM (10 mL) was added, and the reaction was stirred for 22 h at room temperature. The reaction was poured into saturated aqueous $NaHCO_3$ (50 mL), the organic layer was separated, and the aqueous layer was extracted with DCM (50 mL). The combined organic layers were washed with brine, dried ($MgSO_4$), and concentrated. The residue was purified by flash column chromatography (80 g SiO_2, 0−50% EtOAc/hexanes). Purification by flash chromatography gave (*R*)-1-(3-fluoro-4-(trifluoromethyl)phenyl)-*N*-((*S*)-1,1,1-trifluoropropan-2-yl)-3,4-dihydroisoquinoline-2(1*H*)-carboxamide (**45**, 950 mg, 2.18 mmol, 36.3%) as a white foam. Also isolated from flash chromatography was the more polar, undesired diastereomer (*S*)-8-(3-fluoro-4-(trifluoromethyl)phenyl)-*N*-((*S*)-1,1,1-trifluoropropan-2-yl)-5,6-dihydro-1,7-naphthyridine-7(8*H*)-carboxamide (945 mg, 2.171 mmol, 36.1% yield) isolated as a white foam. Spectral data for **45**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.52 (dd, J = 1.5, 4.8 Hz, 1H), 7.47−7.64 (m, 2H), 7.11−7.27 (m, 3H), 6.43 (s, 1H), 4.57−4.83 (m, 2H), 3.81 (td, J = 5.6, 12.9 Hz, 1H), 3.54 (ddd, J = 4.7, 8.8, 13.2 Hz, 1H), 2.94−3.12 (m, 1H), 2.85 (td, J = 5.0, 16.3 Hz, 1H), 1.36 (d, J = 6.7 Hz, 3H). MS (ESI pos ion) m/z: calcd for $C_{19}H_{16}F_7N_3O$, 435.1; found, 436.0 (M + 1).

Biological Assays. *In Vitro Human and Rat TRPM8 Functional Assay.* Chinese hamster ovary (CHO) cells stably expressing rat TRPM8 were generated using tetracycline inducible T-REx expression system from Invitrogen, Inc. (Carlsbad, CA). To enable a luminescence readout based on intracellular increase in calcium, each cell line was also cotransfected with pcDNA3.1 plasmid containing jellyfish aequorin cDNA.[16] Cells were seeded in 96-well plates 24 h before the assay, and TRPM8 channel expression was induced with 0.5 μg/mL of tetracycline. On assay day, the growth media was removed and cells were incubated with assay buffer for 2 h. Cells were then exposed to test compounds (at varying concentrations) and incubated for 2.5 min prior to adding the agonist, 1 μM icilin (1-(2-hydroxyphenyl)-4-(3-nitrophenyl)-3,6-dihydropyrimidin-2-one).[17] The luminescence was measured by a charged-couple device (CCD) camera-based FLASH-luminometer built by Amgen, Inc. Compound activity was calculated using GraphPad Prism 4.01 (GraphPad Software Inc., San Diego, CA).

Human and Rat Liver Microsomal Stability. Liver microsomal stability was measured at 37 °C in phosphate buffer (66.7 mM, pH 7.4). Test compounds (1 μM) were incubated with pooled human or rat liver microsomes at 0.25 mg/mL of protein, with or without NADPH (1 mM). After 30 min, the reaction was stopped by the addition of acetonitrile containing 0.5% formic acid and internal standard. The quenched samples were centrifuged at 1650g for 20 min. The supernatants were analyzed directly for unchanged test compound using liquid chromatography and tandem mass spectrometric detection (LC-MS/MS). Intrinsic clearance was calculated based on substrate disappearance rate assuming first-order elimination of compound over the 30 min incubation.

Rat Pharmacokinetics. Male Sprague−Dawley rats (n = 3 per group) were dosed to fed rats intravenously, with test article formulated in dimethylsulfoxide (DMSO) or to fasted rats by oral gavage with test compound formulated as a suspension in 5% Tween 80/Oraplus. Blood samples were taken over 16 h post dose, with plasma prepared by centrifugation and analyzed by LC-MS/MS. Total plasma concentration time-course data were analyzed by non-compartmental pharmacokinetic methods.

In Vivo Assays. Icilin-Induced "Wet-Dog" Shaking in Rats. Male Sprague−Dawley rats (220−300 g, Harlan, n = 6/treatment) were first habituated to the testing room for 30 min and then to a transparent Plexiglas observation cylinder for 20 min. The cylinders were placed on a custom, opaque, plastic apparatus such that one rat could not view any other rats. Antagonists **44** or **45** (2% HPMC 1% Tween 80 pH 2.2 with MSA) or vehicle control (2% HPMC 1% Tween 80 pH 2.2 with MSA) were administered po 90 min prior to administration of icilin (0.5 mg/kg, ip, 100% PEG 400), and WDS were counted for a duration of 30 min post icilin administration.

Cold Pressor Test (CPT) in Rats. TRPM8 antagonists were evaluated in rat CPT to determine whether TRPM8 antagonists would attenuate the increase in blood pressure resulting from exposure to cold stimulation of the paws and ventral half of the body. Male Sprague−Dawley rats weighing 350−450 g were instrumented with a unilateral carotid artery cannula connected to a transducer for measuring blood pressure using a Digi-Med blood pressure analyzer, model 400. Animals were orally administrated with vehicle (2% HPMC 1% Tween 80 pH 2.2 with MSA) or compound **45** 120 min prior to the cold challenge and anesthetized with sodium pentobarbital at 60 mg/kg ip 20 min prior to cold. Blood pressure was recorded for 4 min for precold baseline and additional 5 min during immersion of the paws and ventral half of body in ice water (approximately 0 °C). Percent inhibition attributed to treatment with test compound was then determined using the following formula: [1 − (cold evoked change in MBP/cold evoked change in MBP postvehicle)] × 100. Plasma was collected through artery catheter immediately after CPT for PK analysis.

Absolute Stereochemical Determination. Absolute stereochemistry of tetrahydroisoquinoline **1** was determined by vibrational circular dichroism (VCD) and optical roatation as described in our previous article.[5] Enantiomerically pure amine (*R*)-**5a** was then used to remake **1** and to prepare compounds **19**−**31** in Table 1. Absolute stereochemistries for selected compounds were determined by comparison of quantum mechanically predicted optical rotation values[18] to those measured experimentally providing confirmation of stereochemistry. Optical rotations were measured in $CHCl_3$ at room temperature using a Perkin-Elmer digital polarimeter at 589 nm (sodium D line) in a 1.0 dm cell. Please see ref 18 and Supporting Information for full details.

■ ASSOCIATED CONTENT

Supporting Information

MetaSite metabolite software prediction for compound **1**, computed and observed optical rotation data used for absolute stereochemical determination, and TRPM8 IC_{50}s including standard deviations. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

$AUC_{0-\infty}$, area under the plasma concentration time curve from time 0 to infinity; CL, total body clearance; DRG, dorsal root ganglia; CDI, 1,1′-carbonyldiimidazole; CPT, cold-pressor test; DIPEA, *N*,*N*-diisopropylethylamine; F_{oral}, oral bioavailability; HLM, human liver microsomes; LPC, lysophosphatidylcholine; NFSI, *N*-fluorobenzensulfonimide; PIP_2, phosphatidylinositol 4,5-bisphosphate; RLM, rat liver microsomes; $t_{1/2}$, terminal half-life; TfOH, trifluoromethanesulfonic acid; TRPM8, transient receptor potential melastatin type 8; TG, trigeminal ganglia; V_{ss}, volume of distribution; WDS, wet-dog shake

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