

Discovery of a Potent (4R,5S)-4-Fluoro-5-Methylproline Sulfonamide Transient Receptor Potential Ankyrin 1 (TRPA1) Antagonist and its Methylene Phosphate Prodrug Guided By Molecular Modeling

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J. Med. Chem., **Just Accepted Manuscript** • DOI: 10.1021/acs.jmedchem.8b00117 • Publication Date (Web): 29 Mar 2018

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Discovery of a Potent (4*R*,5*S*)-4-Fluoro-5-Methylproline Sulfonamide Transient Receptor Potential Ankyrin 1 (TRPA1) Antagonist and its Methylene Phosphate Prodrug Guided By Molecular Modeling

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ABSTRACT

TRPA1 is a non-selective cation channel expressed in sensory neurons where it functions as an irritant sensor for a plethora of electrophilic compounds and is implicated in pain, itch, and respiratory disease. To study its function in various disease contexts, we sought to identify novel, potent and selective small molecule TRPA1 antagonists. Herein we describe the evolution of an *N*-isopropyl glycine sulfonamide lead (**1**) to a novel and potent (*4R,5S*)-4-fluoro-5-methylproline sulfonamide series of inhibitors. Molecular modeling was utilized to derive low energy three-dimensional conformations to guide ligand design. This effort led to compound **20**, which possessed a balanced combination of potency and metabolic stability, but poor solubility that ultimately limited *in vivo* exposure. To improve solubility and *in vivo* exposure, we developed methylene phosphate prodrug **22**, which demonstrated superior oral exposure and robust *in vivo* target engagement in a rat model of AITC-induced pain.

INTRODUCTION

Transient receptor potential ankyrin 1 (TRPA1) is one of the 28 members of the transient receptor potential (TRP) channel family and the sole member of the TRPA subfamily in mammals.¹ Like all TRP channels, TRPA1 possesses a tetrameric structure where each subunit is comprised of six transmembrane helices and 14 *N*-terminal ankyrin repeats. TRPA1 acts as a polymodal sensor for noxious external stimuli and is activated by a wide variety of small molecule agonists including allylisothiocyanate (AITC), cinnamaldehyde, and acrolein via covalent, cysteine-reactive mechanisms.^{2,3,4}

There has been strong interest in TRPA1 as a drug target following several rodent knockout studies that suggested TRPA1 may play a central role in pain.^{5,6,7,8,9} This is further supported by human genetic evidence demonstrating that a gain-of-function mutation in TRPA1 causes

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3 familial episodic pain syndrome.¹⁰ Based on these data, several groups have reported potent and
4 orally bioavailable small molecule antagonists that target TRPA1.^{11,12,13,14,15} Unfortunately,
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6 conflicting reports in the literature have called into question the efficacy of TRPA1 antagonists
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8 in rodent models of inflammatory and neuropathic pain.^{11,14,16} Most notably, the antagonist
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10 AMG0902 achieved limited analgesic efficacy in several rat *in vivo* pain models despite systemic
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12 free concentrations reaching 4-fold coverage of the *in vitro* rat IC₉₀, suggesting that TRPA1
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14 activation may not significantly contribute to neural signaling in the relevant underlying pain
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16 pathways.¹⁶

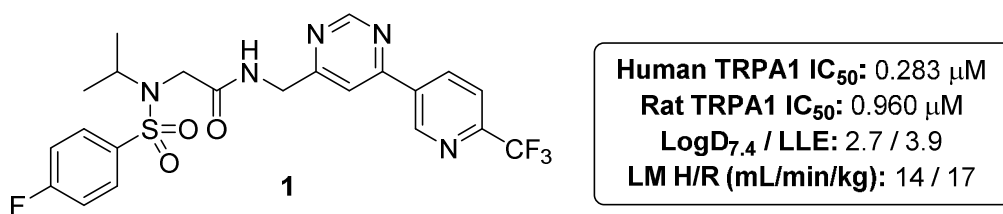
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19 More recently, a growing body of literature supports the investigation of TRPA1 in
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21 neuroinflammatory pathways involved in asthma.^{17,18} TRPA1 expression in the primary afferent
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23 sensory neurons that innervate the airways suggests that TRPA1 may act as a sensor for inhaled
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25 irritants.^{19,20} In support of this hypothesis, both genetic and pharmacological studies in rodents
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27 show that interfering with TRPA1 function results in a decrease in the asthmatic response in an
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29 ovalbumin-sensitized asthma model.^{21,22} Despite these data, the exact role of TRPA1 in the
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31 pathophysiology of asthma remains unclear.

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34 While many series of TRPA1 inhibitors have now been reported,^{11,12,13,14,15,23} at the time we
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36 began our own work very few *in vivo* tool compounds were available in the literature. In
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38 addition, it is likely that many of the reported antagonists bind at different receptor sites and thus
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40 it is possible that the reported efficacy of each inhibitor class could vary accordingly. Based on
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42 the literature evidence supporting TRPA1 as a target for both pain and asthma indications, we
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44 initiated a program centered on the identification of novel small molecule inhibitors with good
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46 oral bioavailability and the ability to demonstrate on-target activity in a rat model of AITC-
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3 induced pain. Herein we describe our efforts towards the identification, optimization and *in vivo*
4 characterization of a novel series of proline sulfonamide-based TRPA1 antagonists.
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8 9 RESULTS AND DISCUSSION

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11 Compound **1** is an *N*-isopropyl glycine sulfonamide-based reversible TRPA1 inhibitor (Figure
12 1) with moderate liver microsome stability in human and rat (LM H/R), and potency (IC_{50})
13 against the human and rat TRPA1 channels of 0.283 μ M and 0.960 μ M, respectively. Due to the
14 desirable drug-like properties of **1** ($LogD_{7.4} = 2.7$, lipophilic ligand efficiency^{24,25} (LLE) = 3.9)
15 and related compounds, we decided to further explore this series of TRPA1 inhibitors with the
16 objective of improving potency and metabolic stability to enable *in vivo* characterization of
17 TRPA1 antagonists in an AITC-evoked rodent pain model.
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37 **Figure 1.** Profile of *N*-isopropyl glycine sulfonamide TRPA1 inhibitor **1**.

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39 Recently a cryo-EM structure of the full-length human TRPA1 channel has been published,²⁶
40 however, the resolution was not sufficient to enable structure-based design or prediction of
41 binding mode for novel ligands. In the absence of structure-based drug design, we used ligand-
42 based modeling techniques to derive low energy conformations to guide structure-activity
43 relationship (SAR) analysis and the lead optimization process. To sufficiently sample the
44 conformational landscape, we employed the mixed torsional/large-scale low mode sampling
45 method in MacroModel™ as implemented in Maestro for conformational search.²⁷ A diverse set
46 of 125 conformations with pairwise heavy atom root-mean-square deviation (RMSD) ranging
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3 from 0.3 to 5.4 Å were generated, followed by quantum mechanics (QM) optimizations of each
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5 conformer using Jaguar™ with the Poisson-Boltzmann solvation model for water.²⁷
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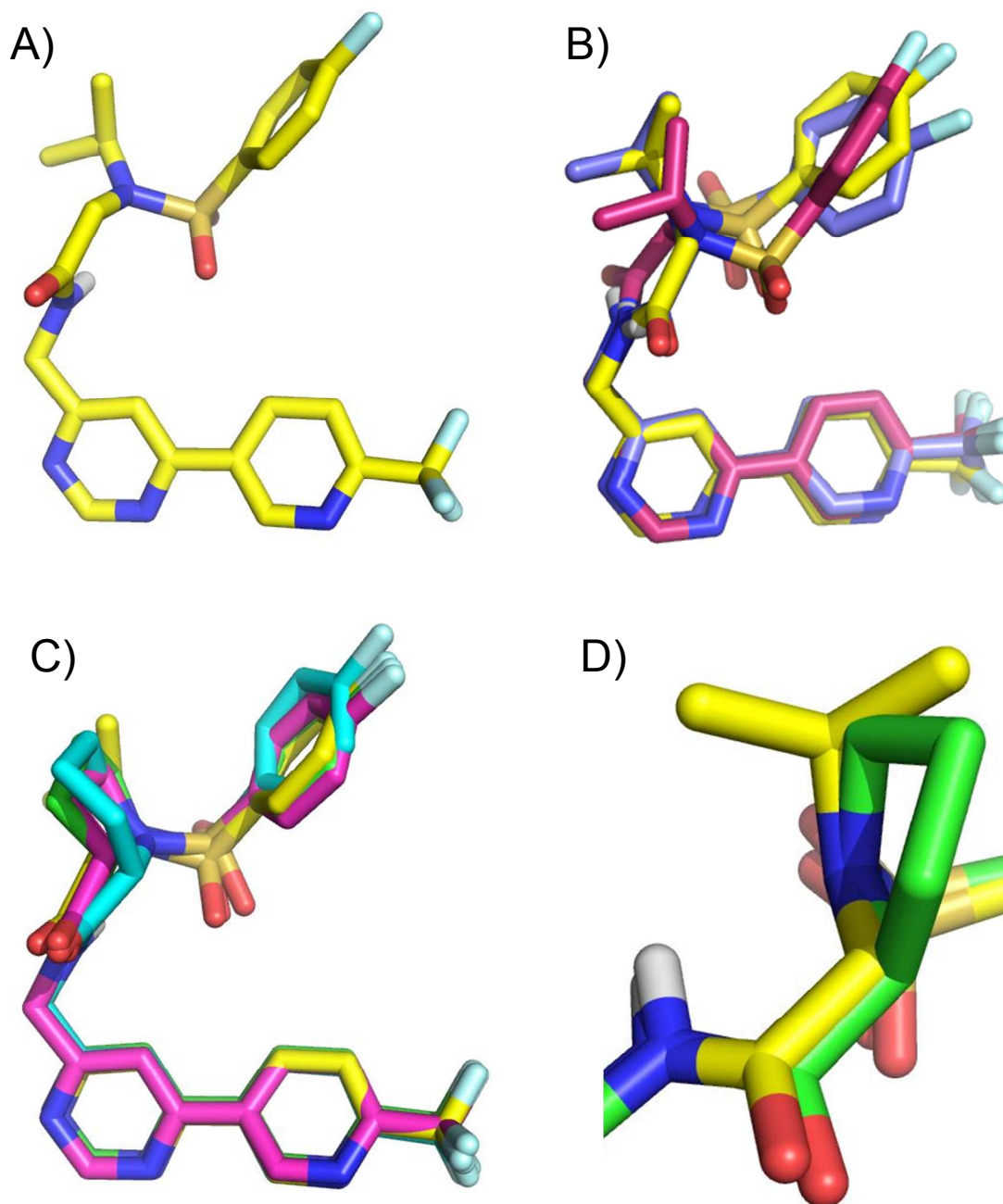


Figure 2. Lowest energy quantum mechanical (QM) three-dimensional models of representative compounds. (A) QM model of compound **1** colored in yellow. (B) Overlay of two

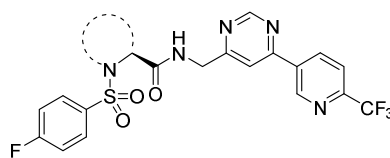
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3 distinct small molecule X-ray conformations of compound **1** (purple and red) to its lowest energy
4 QM model (yellow). (C) Overlay of QM models of compounds **1** (yellow), **2** (magenta), **3**
5 (green), and **4** (cyan). (D) Close-up view of the compound **1** (yellow) overlaid with compound
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10 **3** (green).

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13 After comparing the solution phase energies of the optimized conformations for compound **1**,
14 we identified a U-shape conformation as the lowest energy (Figure 2A), limiting the total and
15 polar surface area of the ligand. Existing structure-activity relationships in the Gly sulfonamide
16 series were consistent with the U-shape QM model (data not shown), giving us confidence in
17 using the model to guide lead optimization.
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20 A small molecule X-ray structure of compound **1** lent further support to the U-shape QM
21 model of **1**. There are two independent conformations in the asymmetric unit cell; one highly
22 similar to our global minimum QM model with a heavy atom RMSD of 0.7 Å, and a second one
23 with a heavy atom RMSD of 1.5 Å (Figure 2B). Based on the U-shape model, it was
24 hypothesized that cyclic amino acids would restrict the conformational flexibility to reinforce our
25 proposed U-shape conformation.
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28 Comparison of the four-, five-, and six-membered ring analogs **2-4** (Table 1) showed a
29 dramatic preference for proline **3**, with a nearly two-fold improvement in potency (0.154 μM)
30 relative to **1** (0.283 μM). Notably, the four- and six-membered ring analogs **2** and **4** showed a 5-
31 and 10-fold loss in potency, respectively. An overlay of all four models of compounds **1-4**
32 (Figure 2C) suggested the 5-membered proline most effectively mimics the *N*-isopropyl group of
33 glycine sulfonamide **1**. The moderate potency and LLE improvement of compound **3** encouraged
34 us to further explore this series.
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55 **Table 1.** Representative examples of cyclized sulfonamide SAR
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Ex	Heterocycle	Human TRPA1 IC ₅₀ ^a (μM)	LLE ^b	LM ^c H/R ^d (mL/min/kg)	LogD _{7.4}	Ex	Heterocycle	Human TRPA1 IC ₅₀ ^a (μM)	LLE ^b	LM ^c H/R ^d (mL/min/kg)	LogD _{7.4}
2		1.5	3.3	10 / 27	2.4	6		4.4	3.6	7 / 40	1.8
3		0.154	4.1	16 / 43	2.7	7		0.013	4.7	19 / 47	2.9
4		2.3	2.2	18 / 46	3.4	8		0.239	3.5	19 / 30	4.3
5		1.2	3.4	5 / 10	2.4	9		0.179	3.8	10 / 19	2.7

^aAntagonist IC₅₀, FLIPR assay. All IC₅₀ values represent geometric means of at least two determinations.

^bLLE was calculated using LogD_{7.4} (LLE = pIC₅₀ – LogD_{7.4}).

^cLiver microsome-predicted hepatic clearance.

^dH/R = human/rat.

Metabolite identification studies suggested oxidation of the pyrrolidine moiety of the proline as the primary metabolic pathway for this class of molecules (data not shown). To block metabolism, we explored both (4*R*)- and (4*S*)-stereoisomers of 4-fluoroproline (compounds **5** and **6**) to introduce polarity as well as potentially provide steric shielding from CYP-mediated oxidative metabolism. While both molecules possessed significant loss in human TRPA1

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3 potency relative to **3** (1.2 and 4.4 μM for **5** and **6**, respectively), liver microsome data suggested
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5 that the fluorination strategy was effective in mitigating some of the metabolic instability of the
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7 unsubstituted proline. The potency loss of compound **5** could be the result of an unfavorable
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9 intramolecular electronic repulsion between the 4-fluoro and the phenyl ring of the sulfonamide,
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11 while the loss in potency of compound **6** could not be rationalized based on the conformation
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13 alone. Despite the potency loss in **5**, we were delighted to see very low clearance *in vivo* (rat iv
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15 $\text{CLp} = 15 \text{ mL/min/kg}$) in agreement with the *in vitro* liver microsome data ($\text{RLM} = 10$
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17 mL/min/kg).

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23 SAR from the *N*-isopropyl glycine sulfonamide series suggested that both methyl groups of the
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25 isopropyl were critical to potency (data not shown). Based on the overlay of models for
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27 compounds **1** and **3** (Figure 2D), we hypothesized that addition of a (5*S*)-methyl at the C5
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29 position of the proline might effectively mimic the *N*-isopropyl of compound **1** and could be a
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31 source of additional TRPA1 potency. Gratifyingly, the (5*S*)-5-methylproline **7** brought a greater
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33 than 10-fold increase in human TRPA1 potency relative to unsubstituted proline **3** (0.013 μM vs.
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35 0.154 μM); the (5*R*)-5-methylproline stereoisomer **8**, however, did not provide a potency benefit
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37 (human TRPA1 $\text{IC}_{50} = 0.239 \mu\text{M}$). This result suggests that the (5*S*)-methyl may effectively fill a
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39 hydrophobic pocket within the TRPA1 binding site and that the resulting van der Waals contacts
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41 improve overall ligand binding and potency. Despite this gain in potency, the added methyl of **7**
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43 did not improve liver microsome stability relative to proline **3**.

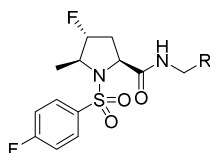
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48 We next hypothesized that the combination of the metabolic stability of (4*R*)-fluoro **5** and the
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50 potency of (5*S*)-methyl **7** might balance competing potency/stability trends if employed in the
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52 same compound. Consistent with this hypothesis, (4*R*,5*S*)-4-fluoro-5-methylproline **9** achieved a
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54 balance of potency and stability with a human TRPA1 IC_{50} of 0.179 μM and moderate liver
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3 microsome stability (HLM = 10 mL/min/kg, Table 1). We were pleased that optimization of the
4 substituents around the proline ring of **9** maintained acceptable lipophilic ligand efficiency (LLE
5 = 3.8) relative to prototype proline **3** (LLE = 4.1), while also incorporating metabolic stability.
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8 One of the drawbacks of the proline decoration was the addition of three stereogenic centers
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10 relative to the original *N*-isopropyl glycine sulfonamide **1**. Despite this additional synthetic
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12 complexity, we chose the more potent (4*R*,5*S*)-4-fluoro-5-methylproline sulfonamide core as the
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14 basis of our continued lead discovery efforts en route to a compound that could achieve *in vivo*
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16 target engagement.
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22 Optimization efforts were next focused on the right-hand side (RHS) biaryl portion of the
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24 molecule where we hoped to find additional potency while continuing to improve metabolic
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26 stability, using the U-shape conformational model as a guide. We also began to closely track
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28 compound solubility during this phase of the discovery effort as compound **9** possessed poor
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30 kinetic solubility (2 μ M) despite a reasonably low LogD_{7.4} (2.7) and acceptable solubility index
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32 (5.7).²⁸ As we began to rapidly assess oral absorption profiles of the (4*R*,5*S*)-4-fluoro-5-
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34 methylproline series, it became clear that the poor solubility of the series was contributing to
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36 poor absorption when dosed orally. To address both metabolic stability and solubility concerns,
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38 we first sought to lower the LogD_{7.4} of the molecule through the addition of nitrogen atoms to
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40 the RHS. To our satisfaction, 2-trifluoromethylpyrimidine **10** (Table 2) possessed a human
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42 TRPA1 IC₅₀ of 0.064 μ M, a nearly 3-fold improvement relative to the parent **9**, while
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44 maintaining good *in vitro* stability. In addition, **10** also showed improved kinetic solubility (53
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46 μ M) and LLE (4.5 versus 3.8). Conversely, the regioisomeric 2-trifluoromethylpyrazine **11** did
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48 not improve TRPA1 potency (0.184 μ M) and provided only a modest improvement in kinetic
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50 solubility (18 μ M). We believe that QM modeling provides at least some explanation for the
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potency difference between the regioisomers, as pyrimidine **10** was predicted to have a roughly two-fold enrichment in the lowest energy conformation relative to pyridine **9** due to the added symmetry of the pyrimidine ring, while pyrazine **11** was predicted to have no such advantage as a result of the added asymmetric nitrogen. Due to the clear benefit of the 2-trifluoromethylpyrimidine, this moiety was employed in all subsequent analogs within the series.

Table 2. Representative examples of biaryl SAR



Ex	R	Human TRPA1 IC ₅₀ ^a (μM)	LLE ^b	LM ^c H/R ^d (mL/min/kg)	Kinetic Solubility (μM)	LogD _{7.4}	Ex	R	Human TRPA1 IC ₅₀ ^a (μM)	LLE ^b	LM ^c H/R ^d (mL/min/kg)	Kinetic Solubility (μM)	LogD _{7.4}
10		0.064	4.5	11 / 18	53	2.5	16		0.013	5.4	15 / 21	8	2.5
11		0.184	4.4	14 / 18	18	2.3	17		0.033	4.8	13 / 18	84	2.6
12		0.005	4.5	10 / 22	<1	3.8	18		0.010	5.0	9 / 16	14	3
13		0.113	3.6	16 / 18	132	3.3	19		0.005	4.7	11 / 20	<1	3.6
14		0.069	4.1	12 / 25	<1	3	20		0.008	5.4	10 / 17	<1	2.6
15		0.096	4.7	17 / 25	123	2.3	21		0.328	4.0	14 / 36	8	2.5

^aAntagonist IC₅₀, FLIPR assay. All IC₅₀ values represent geometric means of at least two determinations.

^bLLE was calculated using LogD_{7.4} (LLE = pIC₅₀ – LogD_{7.4}).

^cLiver microsome-predicted hepatic clearance.

^dH/R = human/rat.

We next explored different substitutions at the 2-position of the central pyrimidine ring and found that a wide range of substitutions were tolerated (data not shown). Cyclopropyl **12** was among the most potent (human TRPA1 IC₅₀ = 0.005 μM, Table 2) while maintaining an equivalent LLE. Although this compound was metabolically stable *in vitro*, it was significantly less soluble (kinetic solubility < 1 μM) than the unsubstituted pyrimidine **10**.

We then studied the contributions of the pyrimidine nitrogens through the synthesis of pyridine regioisomers **13** and **14**. Both matched pair pyridines **13** and **14** were less efficient (LLE = 3.6 and 4.1, respectively) relative to pyrimidine **10**, without the benefit of improved liver microsome stability. Several electron-withdrawing substituents of different polarity and sizes (cyano, chloro and fluoro) were then installed at the newly available carbon atom on the pyridine ring in both regioisomeric series; in all cases the substituents increased both TRPA1 potency and LLE. Within the regioisomeric series of pyridine **13**, nitrile **15** had the highest kinetic solubility (123 μM) although without a significant increase in TRPA1 potency (human TRPA1 IC₅₀ = 0.096 μM, Table 2). However, chloropyridine **16** and fluoropyridine **17** provided efficient increases in TRPA1 activity with IC₅₀ values of 0.013 and 0.033 μM, respectively.

Regioisomeric pyridines **18-20** were then synthesized, each displaying even larger increases in potency than the matched pairs **15-17**, with IC₅₀ values from 0.005 to 0.010 μM, and LLE values ranging from 4.7 to 5.4 (Table 2). Compounds **18-20** possessed equal or better liver microsome stabilities, yet lower kinetic solubilities relative to the benchmark pyrimidine **10**. Notably, fluoropyridine **20** was the most efficient molecule within the series (LLE = 5.4) with moderate predicted metabolic stability (HLM = 10 mL/min/kg, Table 2) and a 35-fold increase in potency

relative to *N*-isopropyl glycine sulfonamide **1**, without the addition of lipophilicity or metabolic liabilities.

To validate our U-shape QM model for ligand conformation, we synthesized difluoropyridine **21**. We hypothesized that fluorine substitution towards the concave edge of the ligand might destabilize the preferred ligand conformation and reduce TRPA1 potency. As expected, we observed a 41-fold loss in potency from **20** to **21**. This result further strengthened our U-shape hypothesis for future lead optimization efforts.

Fluoropyridine **20** was chosen for additional potency and DMPK profiling (Table 3) to enable rat *in vivo* characterization. In line with other sulfonamide-based TRPA1 inhibitors within the series, potency against the rat TRPA1 receptor (0.036 μM) was weaker than the human isoform (0.008 μM), with no measurable inhibition of other human TRP channels such as TRPV1 ($\text{IC}_{50} > 20 \mu\text{M}$) and TRPM8 ($\text{IC}_{50} > 20 \mu\text{M}$). Similarly, **20** did not show significant activity against the human Ether-à-go-go-Related Gene (hERG) potassium channel (14.3% inhibition at 10 μM) in an automated patch clamp assay conducted at Chantest (Cleveland, OH). Despite high MDCK permeability ($A:B = 11.2 \times 10^{-6} \text{ cm s}^{-1}$), poor measured kinetic solubility ($< 1 \mu\text{M}$) suggested that **20** may require formulation optimization to achieve sufficient oral exposure. Fluoropyridine **20** demonstrated good hepatocyte stability and reasonable free fraction in plasma (Table 3). Consistent with *in vitro* liver microsome and hepatocyte data, **20** displayed low hepatic clearance in rat ($\text{CL}_p = 4.4 \text{ mL/min/kg}$) with a moderate volume of distribution ($V_{ss} = 7.5 \text{ L kg}^{-1}$), translating into an *in vivo* half-life of 7.5 hours.

Table 3. Rat potency, TRP channel selectivity, physicochemical properties and DMPK summary of **20**

Rat TRPA1 IC_{50}^a (μM)	Human TRPM8 IC_{50}^a (μM)	Human TRPV1 IC_{50}^a (μM)	MDCK ^b Permeability (A:B) ^c ($\times 10^{-6} \text{ cm/s}$)	Hep ^d H/R ^e (mL/min/kg)	% PPB H/R	LogD _{7.4}	Rat iv PK (1 mg/kg)		
							CL _p (mL/min/kg)	t _{1/2} (h)	V _{ss} (L/kg)
0.036	>20	>20	11.2	6.6 / 19.9	98.0 / 97.2	2.6	4.4	7.5	7.5

^aAntagonist IC₅₀, FLIPR assay. All IC₅₀ values represent geometric means of at least two determinations.

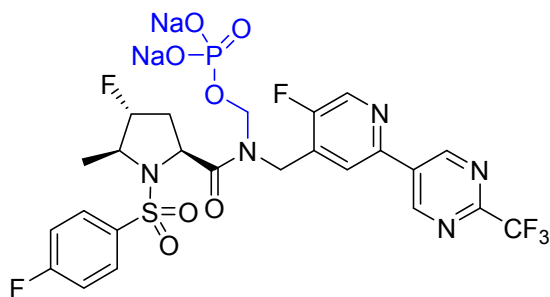
^bMadin-Darby Canine Kidney cells.

^cApical-to-basolateral.

^dCryopreserved hepatocyte-predicted hepatic clearance.

^eH/R = human/rat.

Oral rat PK of **20** was first investigated using crystalline material in a low-dose (1 mg kg⁻¹) MCT (0.5% methylcellulose with 0.2% Tween 80 in water) suspension (Table 4). Oral bioavailability was low (12%), suggestive of solubility-limited drug absorption and poor fraction absorbed, while observed C_{Max} and AUC₀₋₂₄ exposure provided poor coverage of the rat IC₅₀ *in vivo* (C_{Max,unbound} / rat TRPA1 IC₅₀ = 0.02). Higher doses (30 and 100 mg kg⁻¹) were explored using an amorphous suspension for oral dosing by dissolving the crystalline solid in DMSO, then precipitating in an aqueous MCT vehicle to achieve a final composition of 15% DMSO and 85% MCT. Oral exposure in the 30 mg kg⁻¹ experiment (AUC₀₋₂₄ = 32.8 μM*h) was greater than dose-proportional compared to the 1 mg kg⁻¹ MCT dosing, achieving an improved free concentration multiple relative to the rat TRPA1 IC₅₀ (1.8-fold coverage of rat IC₅₀). Unfortunately, efforts to increase the exposure of **20** at 100 mg kg⁻¹ were less than dose proportional, suggesting solubility-limiting absorption despite the use of an amorphous formulation. It became clear that the poor kinetic solubility (<1 μM) was hindering the *in vivo* characterization of **20** and other sulfonamide-based TRPA1 inhibitors and that a new approach would be needed to assess the pharmacology of TRPA1 *in vivo*.

Table 4. TRPA1 potency, physicochemical properties and rat oral PK summary of **20** and prodrug **22**

Prodrug 22:
Human TRPA1 IC₅₀^a: >20 μM
Kinetic Solubility: 133 μM

Ex.	po Dose (mg/kg)	Vehicle	AUC ₀₋₂₄ (μM·h)	C _{Max} (μM)	<i>In vivo</i> Target Multiple $\left(\frac{C_{Max, unbound}}{Rat IC_{50}^d} \right)$
20	1	MCT ^b	0.56	0.03	0.02
	30	DMSO/MCT (15/85) ^c	32.8	2.33	1.81
	100	DMSO/MCT (15/85)	39.6	2.51	1.95
22	1	MCT	0.88	0.07	0.05
	50	MCT	78.7	6.24	3.85

^aAntagonist IC₅₀, FLIPR assay. All IC₅₀ values represent geometric means of at least two determinations.

^bCompound dissolved in 0.5% methylcellulose with 0.2% Tween 80 in water.

^cCompound was formulated as amorphous suspension for oral dosing by dissolving the solid in DMSO then precipitating in aqueous MCT vehicle. The final composition is 15% DMSO and 85% MCT.

^dRat TRPA1 IC₅₀ of active compound **20** (0.036 μM).

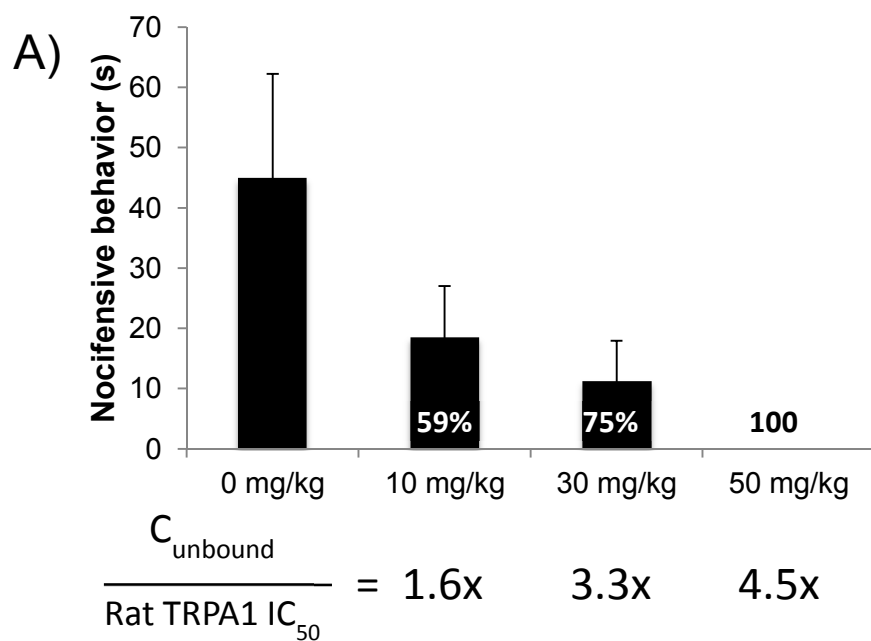
Prodrugs are often employed to enhance aqueous solubility and oral absorption of poorly soluble small molecule drugs. Many diverse solubilizing groups have been developed, including

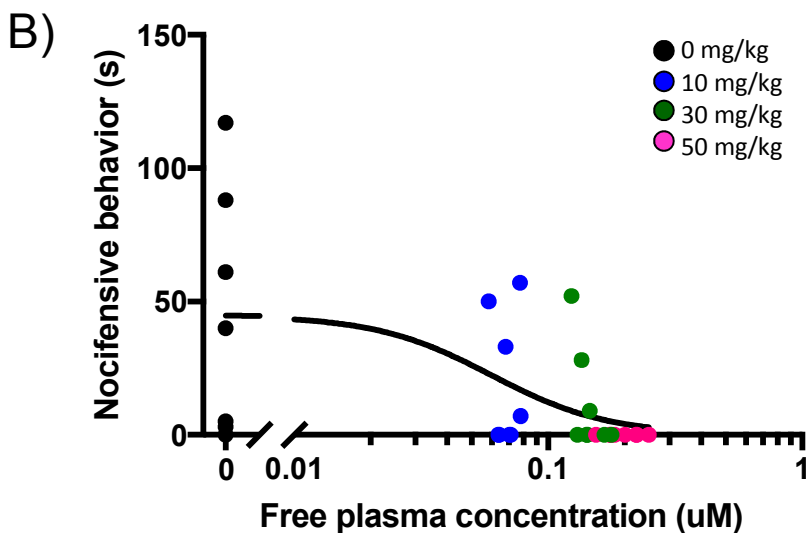
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3 phosphates and carboxylic acids, to modify a variety of common functional handles present in
4 small molecule drugs.^{29,30} In the case of **20**, a highly soluble methylenephosphate group was
5 appended to the secondary amide moiety to yield prodrug **22** as the disodium salt (Table 4). If
6 successful, we believed this could be a broadly applicable strategy to the proline sulfonamide-
7 based inhibitor class to improve both solubility and oral exposure.
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10 Prodrug **22** lacked measurable human TRPA1 potency (Table 4) as a result of capping the
11 secondary amide with a methylene phosphate moiety. Measured kinetic solubility was 133 μM ,
12 greater than 100-fold more soluble than the unfunctionalized matched pair **20** (kinetic solubility
13 $<1 \mu\text{M}$). Low dose (1 mg kg^{-1}) oral rat PK in MCT with prodrug **22** provided only modest
14 increases in observed C_{Max} and $\text{AUC}_{0-24\text{h}}$ exposure of **20** when compared to an equivalent dose of
15 the parent drug **20**. Meanwhile, prodrug **22** was not detected in any of the plasma samples, as the
16 methylene phosphate is cleaved prior to absorption at the brush border of the enterocytes by
17 alkaline phosphatases.²⁹ Gratifyingly, a 50 mg kg^{-1} dose of prodrug **22** demonstrated a greater
18 than dose-proportional increase in oral exposure of **20**. This resulted in higher exposures ($C_{\text{Max}} =$
19 $2.33 \mu\text{M}$ and $\text{AUC}_{0-24\text{h}} = 78.7 \mu\text{M}\cdot\text{h}$) and free drug target multiples ($C_{\text{Max,unbound}} / \text{rat TRPA1}$
20 $\text{IC}_{50} = 3.9$) than the parent drug **20** at 100 mg kg^{-1} (Table 4).
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40 Prodrug **22** was selected for further *in vivo* benchmarking in a rat AITC target engagement
41 assay at doses of 10, 30, and 50 mg kg^{-1} (Figure 3). Behavioral responses evoked by
42 administration of the TRPA1 agonist AITC to rodents has previously been shown to be inhibited
43 by prior dosing of a TRPA1 antagonist.^{11,12,13,14,15} Following an oral dose of **22**, a solution of
44 0.1% AITC is injected into the hind paw at the projected T_{Max} (3 h). The time spent engaging in
45 nocifensive behavior (i.e. lifting, licking, shaking, or guarding the injected paw) was then
46 measured over a 5 minute period. Rats dosed with prodrug **22** displayed a statistically significant
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3 dose-dependent reduction in the amount of AITC-induced nocifensive behavior relative to
4 control, with complete suppression at 50 mg kg⁻¹ (Figure 3A). The rat target multiples achieved
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6 (C_{3h,unbound} / rat TRPA1 IC₅₀) for the 10, 30, and 50 mg kg⁻¹ doses were 1.6, 3.3, and 4.5 fold,
7
8 respectively. Using the individual exposure-response relationships (Figure 3B) an unbound *in*
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10 *vivo* EC₅₀ of 0.060 μM was calculated, which is within two-fold of the rat TRPA1 FLIPR assay
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12 (0.036 μM). This work suggests that the prolyl sulfonamide-based TRPA1 inhibitors are suitable
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17 *in vivo* tools for the study of TRPA1.
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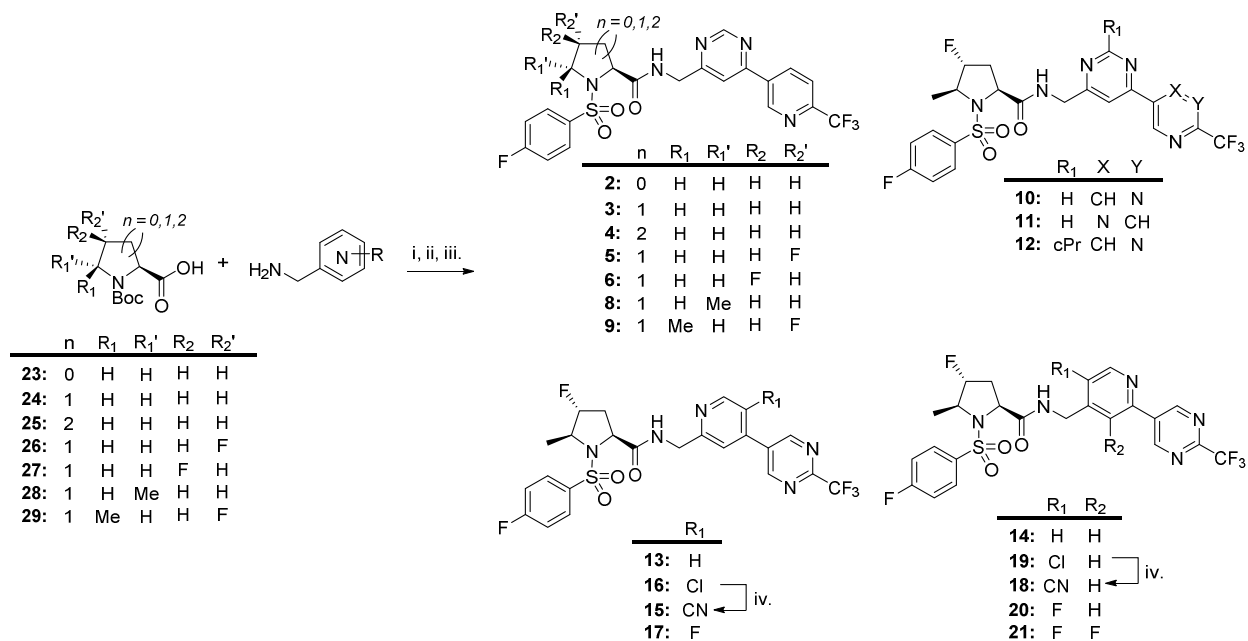




22 **Figure 3.** Summary of rat AITC target engagement upon oral dosing of prodrug **22**. (A) Dose-
23 dependent reduction in time spent engaging in nocifensive behaviors. (B) Exposure-response
24 relationship by individual animals.
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29 Chemistry

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31 The sulfonamide-based TRPA1 inhibitors presented in Table 1 and 2 share a final three-step
32 sequence, as depicted in Scheme 1, from three component parts: a cyclic amino acid, a benzylic
33 biaryl amine, and 4-fluorobenzenesulfonyl chloride. The amide bond is first formed between the
34 *N*-Boc-protected cyclic amino acid (**23-29**) and the appropriate benzylic amine via HATU,
35 followed by TFA-deprotection of the *N*-Boc protecting group and final sulfonamide formation
36 between the free secondary amine and the sulfonyl chloride. Cyanopyridines **15** and **18** were
37 further modified from the corresponding chloropyridines **16** and **19**, respectively, via palladium-
38 catalyzed cyanation.
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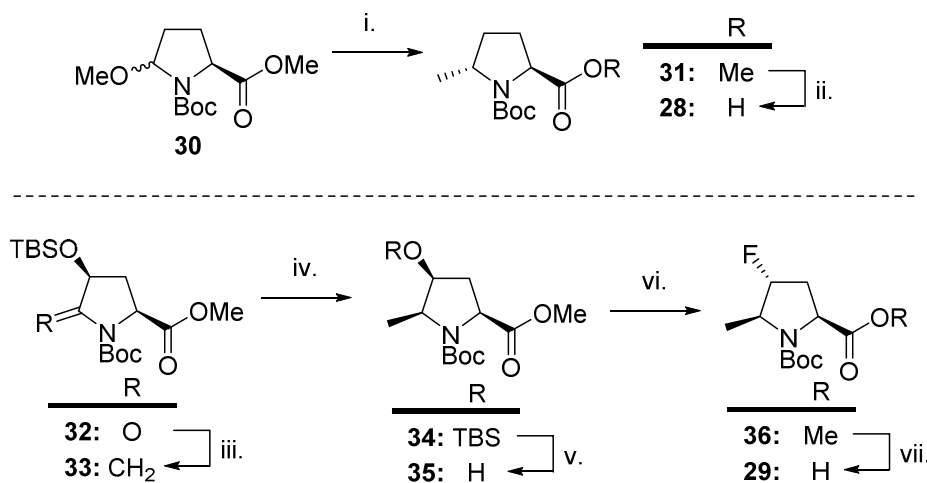


Scheme 1. General scheme for the synthesis of sulfonamide-based TRPA1 inhibitors from amino acids, biaryl amines, and 4-fluorobenzenesulfonyl chloride. (i) HATU, DIPEA, DMF, rt; (ii) TFA, DCM; (iii) 4-fluorobenzenesulfonyl chloride, Et₃N, DCM; (iv) Zn(CN)₂, Pd₂(dba)₃, dppf, DMF, microwave, 100-150 °C, 1 h.

The various cyclic *N*-Boc amino acids used in Scheme 1 were obtained commercially (**23**, **24**, **25**, **26**), synthesized via literature methods (**27**),³¹ or synthesized as described below (**28**, **29**) (Scheme 2). (5*R*)-substituted methylproline **28** was accessed from methoxypyrrolidine **30**³² via Lewis acid-catalyzed iminium formation and stereoselective methyl cuprate addition to afford the methyl ester **31**, which was converted to the acid **28** upon saponification with lithium hydroxide.

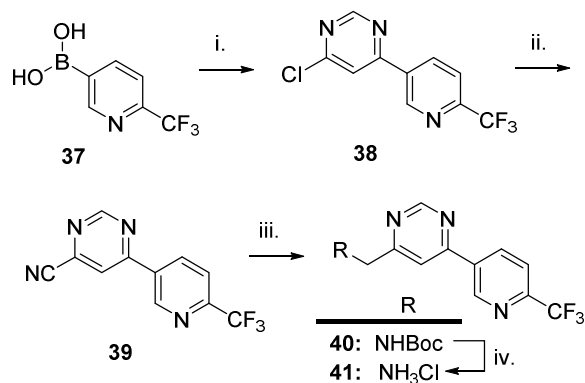
(4*S*,5*R*)-Substituted 4-fluoro-5-methylproline **29** could be synthesized from commercially available (2*S*,4*R*)-4-hydroxyproline methyl ester via the substituted pyroglutamate **32**³³ (Scheme 2). Tebbe olefination provided *N*-Boc enamine **33**, which could be diastereoselectively reduced

to the (5*S*)-methyl **34**. Deprotection (\rightarrow **35**), fluorination (\rightarrow **36**), and saponification provided key proline **29** that forms the basis of this work.



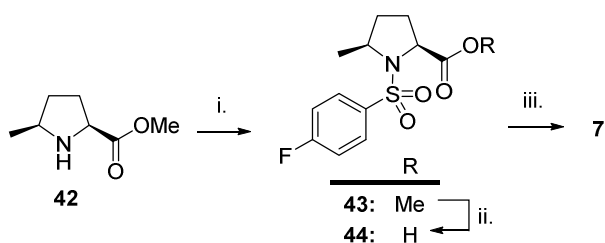
Scheme 2. Synthesis of substituted prolines **28** and **29**. (i) $\text{BF}_3 \cdot \text{Et}_2\text{O}$, CuBr , MeMgBr , Et_2O , -40 to -78 °C to rt; (ii) LiOH , $\text{MeOH}/\text{H}_2\text{O}$; (iii) $\text{Cp}_2\text{Ti}(\text{CH}_3)_2$, pyridine, $(\eta^5\text{-C}_5\text{H}_5)_2\text{TiCl}_2$, toluene, 67 °C, 2 h; (iv) H_2 , Pd/C , MeOH ; (v) TBAF , THF ; (vi) BAST , DCM , 0 °C to rt, 64 h; (vii) NaOH , MeOH/THF , 0 °C to rt, 16 h.

Synthesis of the benzylic amine **41** begins via Suzuki cross-coupling of 4,6-dichloropyrimidine and boronic acid **37** (Scheme 3). Cyanation (\rightarrow **39**), a one-pot reduction/Boc-protection sequence (\rightarrow **40**), followed by acid deprotection then afforded the key biaryl amine **41**. Together with the various *N*-Boc amino acids shown in Scheme 1 (**23-29**), amine **41** affords final compounds **2-6** and **8-9** via HATU coupling, Boc-deprotection, and sulfonylation.



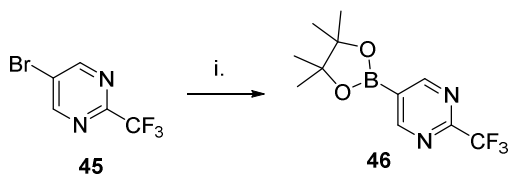
Scheme 3. Synthesis of amine **41**. (i) 4,6-dichloropyrimidine, $\text{Pd}(\text{PPh}_3)_2\text{Cl}_2$, Na_2CO_3 , 1,4-dioxane, H_2O , 50°C , 48 h; (ii) NaCN , DBU , DMSO , H_2O ; (iii) $(\text{Boc})_2\text{O}$, H_2 , Pd/C , MeOH ; (iv) HCl (gas), EtOAc .

Compound **7** was synthesized by a reverse sulfonylation / amide bond coupling sequence described in Scheme 4. (5*S*)-Methylproline ester **42**³⁴ was first sulfonylated to afford sulfonamide **43**, which was then treated with lithium hydroxide to provide the carboxylic acid **44**. HATU coupling with amine **41**, then provided the final target **7**.



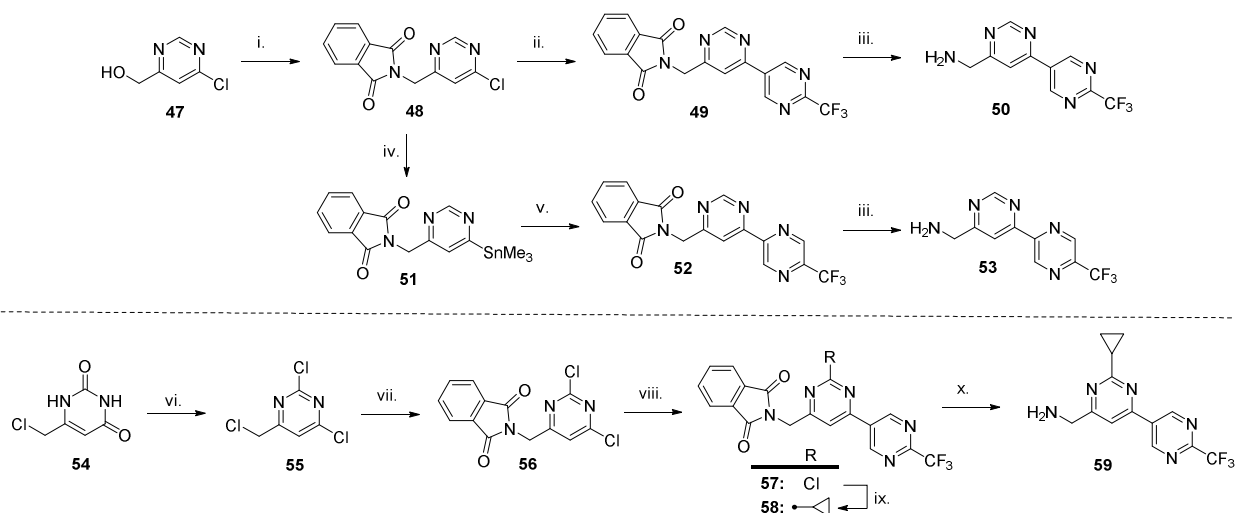
Scheme 4. Synthesis of proline sulfonamide **7**. (i) 4-fluorobenzenesulfonyl chloride, Et_3N , DMAP , DCM ; (ii) LiOH , $\text{MeOH}/\text{H}_2\text{O}$; (iii) **41**, HATU , DIPEA , DMF , rt .

Discovery of the trifluoromethylpyrimidine present in final compounds **10** and **12-21** was critical to the potency and efficiency of this class of TRPA1 inhibitors. As such, boronic ester **46** was a critical synthetic intermediate that was prepared on large scale to support this work (>150 g). Towards this end, previously described bromopyrimidine **45**³⁵ could be treated with palladium acetate and bis(pinacolato)diboron to provide boronate **46** in 50% yield (Scheme 5).



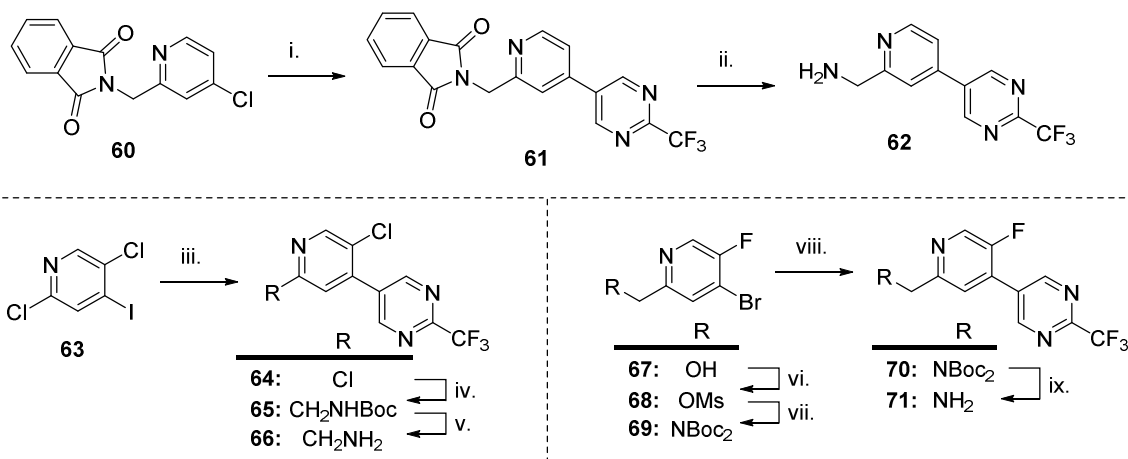
Scheme 5. Synthesis of boronate ester **46**. (i) (PinB)₂, Pd(OAc)₂, DMF, 80 °C, 3 h.

Synthesis of the various biaryl amines used in Table 2 began with the synthesis of bis-pyrimidine **50** (Scheme 6), en route to final compound **10**. Alcohol **47**³⁶ was converted to phthalimide **48** via Mitsunobu reaction, followed by Suzuki cross-coupling between the chloropyrimidine moiety in **48** and boronate **46** to provide protected bis-pyrimidine **49**. Final deprotection with hydrazine hydrate afforded the desired biaryl amine **50**. Chloropyrimidine **48** could also be converted to the trimethylstannane **51** under palladium-catalyzed conditions. Stannane **51** could then be cross-coupled with 2-chloro-5-(trifluoromethyl)pyrazine to provide access to biaryl phthalimide **52**, which was then also deprotected with hydrazine to yield biaryl amine intermediate **53** used in the synthesis of final compound **11**.



Scheme 6. Synthesis of amines **50**, **53**, and **59**. (i) Phthalimide, DIAD, PPh₃, THF; (ii) **46**, Pd(dppf)Cl₂, K₂CO₃, 1,4-dioxane, H₂O, 70 °C, 3 h; (iii) hydrazine hydrate, MeOH, 50 °C; (iv) (Me₃Sn)₂, Pd(PPh₃)₄, toluene, 110 °C, 45 min; (v) 2-chloro-5-(trifluoromethyl)pyrazine, Pd(PPh₃)₄, toluene, 110 °C; (vi) POCl₃, 100 °C, 12 h; (vii) phthalimide, DMF; (viii) **46**, Pd(dppf)Cl₂, K₂CO₃, DMF, 70 °C, 12 h; (ix) cyclopropylboronic acid, Pd(dppf)Cl₂, K₂CO₃, 1,4-dioxane, 90 °C, 12 h; (x) hydrazine hydrate, MeOH, 50 °C, 12 h.

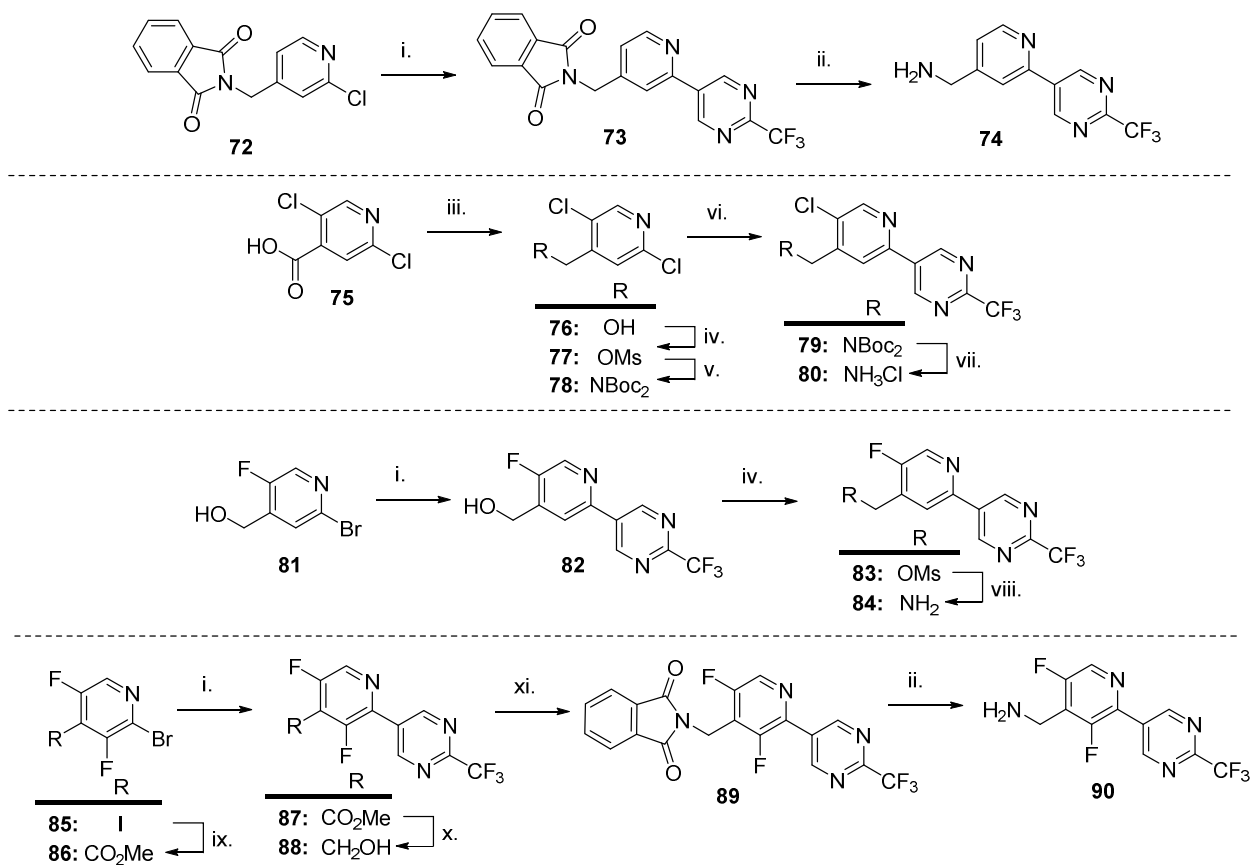
Synthesis of 2-substituted pyrimidines required access to 2,4-dichloropyrimidine intermediate **55**, which could be synthesized via treatment of pyrimidinedione **54** with phosphoryl chloride (Scheme 6). Phthalimide alkylation to afford **56** then set the stage for difunctionalization of the dichloropyrimidine group. The chlorine at the 4-position reacted first under palladium-catalyzed conditions with boronate **46** to yield 2-chloropyrimidine **57**, which was again subjected to Suzuki cross-coupling conditions, now with cyclopropylboronic acid, to yield fully functionalized bispyrimidine **58**. Phthalimide deprotection then afforded the final biaryl amine intermediate **59** to be used towards the synthesis of final compound **12**.



Scheme 7. Synthesis of amines **62**, **66**, and **71**. (i) **46**, Pd(dppf)Cl₂, K₂CO₃, 1,4-dioxane, 110 °C, 12 h; (ii) hydrazine hydrate, EtOH, 80 °C, 12 h; (iii) **46**, Pd(dppf)Cl₂, K₂CO₃, 1,4-dioxane, H₂O, 60 °C, 3 h; (iv) *N*-Boc-aminomethyltrifluoroborate, Pd(PPh₃)₂Cl₂, Na₂CO₃, EtOH, H₂O, 85 °C; (v) HCl (gas), DCM; (vi) MsCl, Et₃N, DCM, 0 °C; (vii) Boc₂NH, NaH, DMF, 0 to 60 °C; (viii) **46**, Pd(dppf)Cl₂, K₂CO₃, 1,4-dioxane, H₂O, 90 °C, 1 h; (ix) HCl (gas), DCM, MeOH, 0 °C.

Scheme 7 describes the synthesis of biaryl amines **62**, **66**, and **71** used in the synthesis of final compounds **13** and **15-17**. Chloropyridine **60**³⁷ was cross-coupled with boronate **46** and the phthalimide was deprotected under standard hydrazine conditions to provide **62**. Towards **66**, 2,5-dichloro-4-iodopyridine **63** was selectively functionalized at the 4-position using Suzuki cross-coupling conditions, again with boronate **46** as the coupling partner, to yield biaryl **64**. *N*-Boc-aminomethyltrifluoroborate was then used in a site-selective Suzuki cross-coupling at the 2-position of dichloropyridine **64** to provide *N*-Boc amine **65**, which was deprotected under acidic conditions to provide the final amine intermediate **66**. Lastly, primary alcohol **67** was converted to bis-Boc-amine **69** via a two-step protocol via mesylate **68** using Boc₂NH as a nucleophile

following deprotonation with sodium hydride. Following cross-coupling with **46**, *N*-Boc-biaryl **70** was then deprotected under standard conditions to provide amine **71**.

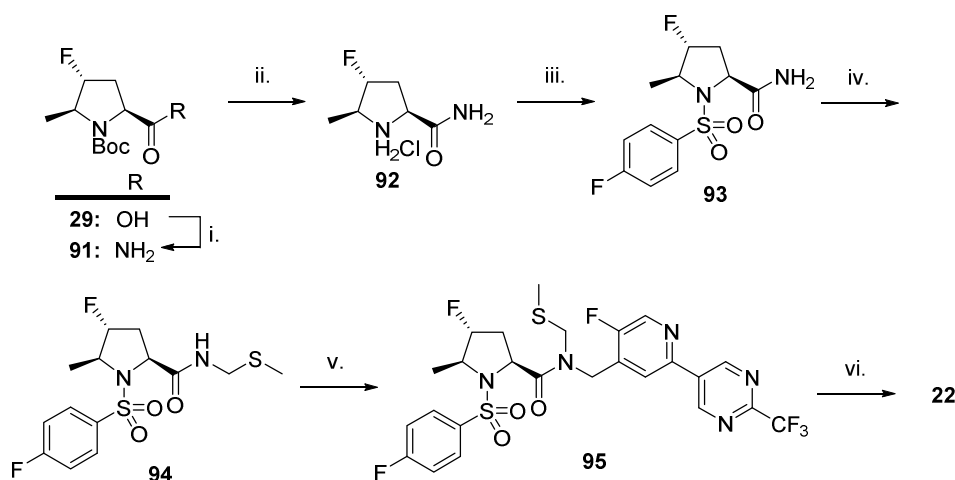


Scheme 8. Synthesis of amines **74**, **80**, **84**, and **90**. (i) **46**, Pd(dppf)Cl₂, K₂CO₃, 1,4-dioxane, H₂O, 80 °C, 12 h; (ii) hydrazine hydrate, MeOH; (iii) BH₃-THF, THF, 0 °C to rt; (iv) MsCl, Et₃N, DCM, 0 °C to rt; (v) Boc₂NH, NaH, DMF; (vi) **46**, Pd(dppf)Cl₂, Cs₂CO₃, 1,4-dioxane, H₂O, 110 °C, 5 h; (vii) HCl (gas), MeOH; (viii) NH₃ in MeOH, 40 °C, 6 h; (ix) CO (gas), Pd(dppf)Cl₂, Et₃N, MeOH, 50 °C; (x) DIBAL-H, hexane, -78 °C; (xi) phthalimide, DIAD, PPh₃, THF.

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3 The synthesis of regioisomeric pyridine intermediates **74**, **80**, **84**, and **90** used in the
4 preparation of **14**, **19**, **20**, and **21**, respectively, are described in Scheme 8. Following similar
5 protocols as previously described, 2-chloropyridine **72** could be coupled with boronic ester **46**
6 (\rightarrow **73**) and the phthalimide could then be deprotected to afford the final biaryl amine **74**. Access
7 to bis-Boc amine **78** began with borane reduction of carboxylic acid **75** (\rightarrow **76**), followed by
8 mesylation (\rightarrow **77**) and nucleophilic displacement by Boc_2NH . Standard Suzuki cross-coupling
9 with boronate ester **46** selectively functionalized the chlorine at the 2-position of the pyridine
10 (\rightarrow **79**), which could then be deprotected under acidic conditions to yield the final amine
11 intermediate **80**. A similar but reversed sequence was used in the preparation of fluoropyridine
12 intermediate **84**. 2-Bromopyridine **81** was first coupled with pyrimidine **46** to yield biaryl alcohol
13 **82** which could then be mesylated (\rightarrow **83**) and converted directly to the primary amine **84** via
14 treatment with ammonia in methanol at 40 °C. Difluoropyridine intermediate **90** required a
15 slightly longer sequence to install the additional fluorine substituent. The process began with the
16 selective esterification of the iodine in 2-bromo-3,5-difluoro-4-iodopyridine **85** to afford methyl
17 ester **86**, which was subsequently cross-coupled with pyrimidine **46** to provide biaryl **87**.
18 Reduction (\rightarrow **88**), installation of the phthalimide protecting group via Mitsunobu (\rightarrow **89**), and
19 final hydrazine deprotection, provided the biaryl amine intermediate **90**.
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42 Prodrug **22** is a synthetically modified form of fluoropyridine antagonist **20**, yet initial attempts
43 to synthesize **22** directly from **20** proved unsuccessful due to difficulties alkylating the sterically
44 congested secondary amide. To circumvent this issue, a synthetic plan was developed that
45 allowed installation of the RHS biaryl at a late stage and conversion of the thiomethyl to the final
46 phosphate (Scheme 9). Towards this end, substituted proline carboxylic acid **29** was converted to
47 primary amide **91** via the mixed anhydride. The *N*-Boc protecting group was removed under
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standard acidic deprotection conditions (\rightarrow **92**), followed by installation of the 4-fluorophenylsulfonamide (\rightarrow **93**) by treatment with the corresponding sulfonyl chloride reagent. (Chloromethyl)(methyl)sulfide alkylation provided the secondary amide **94** which was then treated with biaryl mesylate **83** (see Scheme 8) with sodium hydride to afford penultimate intermediate **95**. Oxidation of the thiomethyl group with *N*-iodosuccinimide followed by displacement with phosphoric acid provided the desired prodrug **22**. As the salt form of the phosphate was found to be critical to characterization and stability, the di-sodium salt was prepared by addition of sodium carbonate prior to reversed-phase column chromatography.



Scheme 9. Synthesis of prodrug **22**. (i) $(\text{NH}_4)_2\text{CO}_3$, $(t\text{-BuO})_2\text{CO}$, pyridine, 1,4-dioxane; (ii) HCl in EtOAc; (iii) 4-fluorobenzenesulfonyl chloride, Et_3N , DCM; (iv) $\text{ClCH}_2\text{SCH}_3$, TFA, DCM; (v) **83**, NaH, NaI, THF, -5°C to rt; (vi) $\text{H}_3\text{O}_4\text{P}$, NIS, 3 Å mol. sieves; THF.

CONCLUSION

N-Isopropyl glycine sulfonamide TRPA1 antagonist (**1**) with moderate potency against the human and rat TRPA1 channels (IC_{50} values of 0.283 and 0.960 μM , respectively), provided the

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3 starting point for a lead optimization campaign aimed at establishing *in vivo* target engagement
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5 in a rat model for pain. QM modeling provided low energy conformations that supported a U-
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7 shape binding model, which ultimately resulted in the discovery of a (4*R*,5*S*)-4-fluoro-5-
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9 methylproline core with a balance of potency ($IC_{50} = 0.008 \mu\text{M}$) and *in vitro* stability. Careful
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11 modifications to the RHS biaryl aimed at increasing potency without eroding metabolic stability,
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13 resulted in fluoropyridine **20**, however solubility-limiting absorption prevented the use of **20** at
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15 doses necessary to achieve target coverage *in vivo*. Ultimately, the highly soluble prodrug **22**, a
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17 methylene phosphate-derived version of **20**, achieved dose-proportional exposures up to 50 mg
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19 kg^{-1} and excellent target coverage in rat. Prodrug **22** was used in a rat AITC target engagement
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21 study to demonstrate that the sulfonamide class of TRPA1 inhibitors is capable of reversing
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23 behavioral responses evoked by AITC in rodents at *in vivo* concentrations similar to measured rat
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25 TRPA1 FLIPR potencies. Further investigation of TRPA1 pharmacology *in vivo* using efficacy
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27 models of acute pain and asthma will be reported in due course.
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34 EXPERIMENTAL SECTION

35 Chemistry

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39 *General Methods.* Unless otherwise indicated, all commercial reagents and anhydrous solvents
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41 were used without additional purification. Intermediates **23-26**, **37**, **54**, **63**, and **75** were obtained
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43 via commercial sources. $^1\text{H-NMR}$ spectra were measured on Bruker Avance III 300, 400, or 500
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45 MHz spectrometers. $^{13}\text{C-NMR}$ spectra were measured on a Bruker Avance III 125.80 MHz
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47 spectrometer. Chemical shifts (in ppm) were referenced to internal standard tetramethylsilane (δ
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49 = 0 ppm). The reported carbon multiplicities and coupling constants are from C-F coupling.
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51 High-resolution mass spectrometry of final compounds were performed on a Thermo
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53 UHPLC/QE with a Thermo-Q Exactive mass spectrometry detector using ESI ionization, after
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3 elution on a Acquity BEH C18 (2.1 mm × 50 mm; 1.7 μm particle size) stationary phase using a
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5 gradient of water/acetonitrile (3–97% over 7 min; 0.1% formic acid in both phases). Reactions
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7 were monitored by walkup Shimadzu LCMS/UV system with LC-30AD solvent pump, 2020
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9 MS, Sil-30AC autosampler, SPD-M30A UV detector, CTO-20A column oven, using 2-98%
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11 acetonitrile/0.1% formic acid (or 0.01% Ammonia) over 2.5 min OR Waters Acquity LCMS
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13 system using 2-98% acetonitrile/0.1% formic acid (or 0.1% Ammonia) over 2 min. Flash column
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15 chromatography purifications were done on a Teledyne Isco Combiflash Rf utilizing Silicycle
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17 HP columns. Reverse-phase purification was carried out on a Phenomenex Gemini-NX C18 (30
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19 x 100mm,5um) with a gradient of 5–95% acetonitrile/water (with 0.1% Formic Acid or 0.1%
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21 NH₄OH) over 10 min at 60 mL/min. Preparative SFC separations were performed on a PIC
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23 Solutions instrument, with conditions indicated in the Experimental Section. Analytical purity
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25 was greater than 95% as determined by LCMS using UV 254 nM detection unless stated
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27 otherwise. The melting point was determined by Differential Scanning Calorimetry (DSC) (TA
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29 Instruments-Waters L.L.C.) by using 5mg of solid sample and measuring the onset melting
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31 temperature.
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38 **(S)-1-((4-fluorophenyl)sulfonyl)-N-((6-(6-(trifluoromethyl)pyridin-3-yl)pyrimidin-4-**
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40 **yl)methyl)azetidione-2-carboxamide (2)**. The title compound was prepared from carboxylic acid
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42 **23** and amine **41** in a manner analogous to **20**. LCMS, $m/z = 496.1$ [M+H]⁺. ¹H NMR (400 MHz,
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44 DMSO-*d*₆) δ 9.49 (d, $J = 2.1$ Hz, 1H), 9.30 (d, $J = 1.3$ Hz, 1H), 8.96 (t, $J = 5.9$ Hz, 1H), 8.80 (dd,
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46 $J = 8.3, 2.2$ Hz, 1H), 8.16 (d, $J = 1.2$ Hz, 1H), 8.09 (d, $J = 8.3$ Hz, 1H), 8.05 – 7.97 (m, 2H), 7.56
47
48 (t, $J = 8.8$ Hz, 2H), 4.61 (dd, $J = 17.3, 6.2$ Hz, 1H), 4.51 (dd, $J = 17.3, 5.7$ Hz, 1H), 4.38 (dd, $J =$
49
50 9.1, 7.7 Hz, 1H), 3.77 (td, $J = 8.5, 4.2$ Hz, 1H), 3.59 (q, $J = 8.4$ Hz, 1H), 2.36 – 2.13 (m, 2H).
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(S)-1-((4-fluorophenyl)sulfonyl)-N-(((6-(6-(trifluoromethyl)pyridin-3-yl)pyrimidin-4-yl)methyl)pyrrolidine-2-carboxamide (3). The title compound was prepared from carboxylic acid **24** and amine **41** in a manner analogous to **20**. HRMS (ESI) Calc for C₂₂H₂₀F₄N₅O₃S (M+H)⁺: 510.1217. Found: 510.1210. ¹H NMR (500 MHz, CDCl₃) δ 9.51 (s, 1H), 9.27 (s, 1H), 8.70 (d, *J* = 8.0 Hz, 1H), 8.08 (s, 1H), 7.93 (m, 2H), 7.81 (d, *J* = 8.5 Hz, 1H), 7.62 (m, 1H), 7.30 (t, *J* = 8.5 Hz, 2H), 5.02 (dd, *J* = 8.0, 17.5 Hz, 1H), 4.49 (dd, *J* = 4.5, 17.5 Hz, 1H), 4.17 (m, 1H), 3.70 (m, 1H), 3.18 (m, 1H), 2.19 (m, 1H), 1.83 (m, 2H), 1.70 (m, 1H).

(S)-1-((4-fluorophenyl)sulfonyl)-N-(((6-(6-(trifluoromethyl)pyridin-3-yl)pyrimidin-4-yl)methyl)piperidine-2-carboxamide (4). The title compound was prepared from carboxylic acid **25** and amine **41** in a manner analogous to **20**. HRMS (ESI) Calc for C₂₃H₂₂F₄N₅O₃S (M+H)⁺: 524.1374. Found: 524.1360. ¹H NMR (400 MHz, DMSO-*d*₆) δ 9.47 (d, *J* = 2.0 Hz, 1H), 9.29 (d, *J* = 1.3 Hz, 1H), 8.78 (dd, *J* = 8.1, 2.1 Hz, 1H), 8.69 (t, *J* = 5.8 Hz, 1H), 8.17 – 8.10 (m, 1H), 8.08 (d, *J* = 1.4 Hz, 1H), 7.91 – 7.82 (m, 2H), 7.34 (t, *J* = 8.8 Hz, 2H), 4.66 – 4.59 (m, 1H), 4.42 (d, *J* = 5.9 Hz, 2H), 3.75 – 3.66 (m, 1H), 3.49 – 3.38 (m, 1H), 2.04 (d, *J* = 13.8 Hz, 1H), 1.57 (d, *J* = 12.4 Hz, 1H), 1.53 – 1.40 (m, 2H), 1.38 – 1.17 (m, 2H).

(2S,4R)-4-fluoro-1-((4-fluorophenyl)sulfonyl)-N-(((6-(6-(trifluoromethyl)pyridin-3-yl)pyrimidin-4-yl)methyl)pyrrolidine-2-carboxamide (5). The title compound was prepared from carboxylic acid **26** and amine **41** in a manner analogous to **20**. HRMS (ESI) Calc for C₂₂H₁₉F₅N₅O₃S (M+H)⁺: 528.1123. Found: 528.1114. ¹H-NMR (400 MHz, DMSO-*d*₆) δ 9.48 (d, *J* = 1.9 Hz, 1H), 9.30 (d, *J* = 1.2 Hz, 1H), 9.11 (t, *J* = 5.9 Hz, 1H), 8.79 (dd, *J* = 8.3, 1.7 Hz, 1H), 8.23 (s, 1H), 8.08 (d, *J* = 8.3 Hz, 1H), 8.05 – 7.98 (m, 2H), 7.47 (t, *J* = 8.8 Hz, 2H), 5.21 (d, *J* = 52.4 Hz, 1H), 4.53 (d, *J* = 5.9 Hz, 2H), 4.25 (dd, *J* = 9.8, 7.2 Hz, 1H), 3.79 – 3.60 (m, 2H), 2.46 – 2.37 (m, 1H), 2.25 – 2.04 (m, 1H).

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3 **(2S,4S)-4-fluoro-1-((4-fluorophenyl)sulfonyl)-N-((6-(6-(trifluoromethyl)pyridin-3-**
4 **yl)pyrimidin-4-yl)methyl)pyrrolidine-2-carboxamide (6).** The title compound was prepared
5
6 from carboxylic acid **27** and amine **41** in a manner analogous to **20**. Calc for C₂₂H₁₉F₅N₅O₃S
7
8 (M+H)⁺: 528.1123. Found: 528.1110. ¹H-NMR (400 MHz, DMSO-*d*₆) δ 9.43 (d, *J* = 1.9 Hz,
9
10 1H), 9.28 (d, *J* = 1.2 Hz, 1H), 8.89 (t, *J* = 6.1 Hz, 1H), 8.75 (dd, *J* = 8.1, 1.8 Hz, 1H), 8.14 – 8.01
11
12 (m, 4H), 7.55 – 7.43 (m, 2H), 5.26 (dt, *J* = 53.3, 3.7 Hz, 1H), 4.61 – 4.44 (m, 2H), 4.39 (d, *J* =
13
14 9.5 Hz, 1H), 3.81 (dd, *J* = 22.4, 12.2 Hz, 1H), 3.45 (ddd, *J* = 36.0, 12.3, 3.8 Hz, 1H), 2.37 – 2.24
15
16 (m, 1H), 2.07 – 1.85 (m, 1H).

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19 **(2S,5S)-1-((4-fluorophenyl)sulfonyl)-5-methyl-N-((6-(6-(trifluoromethyl)pyridin-3-**
20 **yl)pyrimidin-4-yl)methyl)pyrrolidine-2-carboxamide (7).** A mixture of carboxylic acid **44**
21
22 (0.150 g, 0.52 mmol), HATU (285 mg, 0.750 mmol, 1.50 equiv), diisopropylethyl amine (0.190
23
24 g, 1.47 mmol), and amine **41** (182 mg, 0.626 mmol, 1.20 equiv) in *N,N*-dimethylformamide (4
25
26 mL) was stirred for 1 h at room temperature. The resulting solution was diluted with ethyl
27
28 acetate, washed with brine, dried over anhydrous sodium sulfate, and concentrated under
29
30 vacuum. The residue was purified by Prep-HPLC to afford 99.7 mg as a white solid (36% yield,
31
32 over 2 steps). HRMS (ESI) Calc for C₂₃H₂₂F₄N₅O₃S (M+H)⁺: 524.1374. Found: 524.1364. ¹H
33
34 NMR (300 MHz, CDCl₃) δ 9.49 (s, 1H), 9.27 (s, 1H), 8.71 - 8.70 (d, *J* = 1.5 Hz, 1H), 8.04 (s,
35
36 1H), 7.95 - 7.90 (m, 2H), 7.82 - 7.80 (m, 1H), 7.69 - 7.67 (m, 1H), 7.31 - 7.26 (m, 2H), 5.01 -
37
38 4.92 (m, 1H), 4.60 - 4.53 (m, 1H), 4.22 - 4.17 (m, 1H), 3.73 - 3.71 (m, 1H), 2.19 - 2.16 (m, 1H),
39
40 1.77 - 1.68 (m, 3H), 1.60 - 1.51 (m, 3H).

41
42 **(2S,5R)-1-((4-fluorophenyl)sulfonyl)-5-methyl-N-((6-(6-(trifluoromethyl)pyridin-3-**
43 **yl)pyrimidin-4-yl)methyl)pyrrolidine-2-carboxamide (8).** The title compound was prepared
44
45 from carboxylic acid **28** and amine **41** in a manner analogous to **20**. HRMS (ESI) Calc for

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2
3 $C_{23}H_{22}F_4N_5O_3S$ (M+H)⁺: 524.1374. Found: 524.1370. ¹H NMR (300 MHz, CDCl₃) δ 9.49 (s,
4 1H), 9.26 (s, 1H), 8.68 - 8.65 (d, *J* = 8.4 Hz, 1H), 8.15 (s, 1H), 7.97 - 7.93 (m, 2H), 7.77 - 7.16
5 (m, 1H), 7.26 - 7.16 (m, 3H), 4.94 - 4.82 (m, 1H), 4.65 - 4.57 (m, 1H), 4.43 - 4.41 (m, 1H), 4.28 -
6 (m, 1H), 2.29 - 2.15 (m, 3H), 1.65 - 1.59 (m, 1H), 1.14 - 1.12 (m, 3H).
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12 **(2S,4R,5S)-4-fluoro-1-((4-fluorophenyl)sulfonyl)-5-methyl-N-((6-(6-**
13 **(trifluoromethyl)pyridin-3-yl)pyrimidin-4-yl)methyl)pyrrolidine-2-carboxamide (9)**. The
14 title compound was prepared from carboxylic acid **29** and amine **41** in a manner analogous to **20**.
15
16 LCMS, *m/z* = 542.2 [M+H]⁺. ¹H NMR (400 MHz, CDCl₃) δ 9.55 (s, 1H), 9.31 (m, 1H), 8.75 -
17 8.73 (s, 1H), 8.20 - 8.16 (s, 1H), 7.94 - 7.89 (m, 2H), 7.84 - 7.83 (m, 1H), 7.61 - 7.51 (m, 1H),
18 7.28 - 7.18 (m, 2H), 5.05 - 4.90 (m, 1H), 4.82 - 4.69 (m, 1H), 4.70 - 4.58 (m, 1H), 4.31 - 4.22 (m,
19 1H), 4.15 - 4.10 (m, 1H), 2.60 - 2.57 (m, 1H), 2.57 - 2.27 (m, 1H), 1.44 - 1.35 (m, 3H).
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28 **(2S,4R,5S)-4-fluoro-1-((4-fluorophenyl)sulfonyl)-5-methyl-N-((2'-(trifluoromethyl)-[4,5'-**
29 **bipyrimidin]-6-yl)methyl)pyrrolidine-2-carboxamide (10)**. The title compound was prepared
30 from carboxylic acid **29** and amine **50** in a manner analogous to **20**. mp 182 °C. HRMS (ESI)
31 Calc for $C_{22}H_{20}F_5N_5O_3S$ (M+H)⁺: 543.1232. Found: 543.1221. ¹H NMR (400 MHz, CDCl₃)
32 δ 9.70 (s, 2H), 9.32 (m, 1H), 8.26 (s, 1H), 7.95 - 7.92 (s, 2H), 7.47 (s, 1H), 7.30 - 7.28 (m, 2H),
33 5.07 (s, 1H), 4.82 - 4.69 (d, *J* = 52 Hz, 1H), 4.55 - 4.40 (m, 1H), 4.31 (s, 1H), 4.14 - 4.07 (m,
34 1H), 2.63 - 2.62 (m, 1H), 2.40 - 2.15 (m, 1H), 1.44 - 1.43 (m, 3H).
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44 **(2S,4R,5S)-4-fluoro-1-((4-fluorophenyl)sulfonyl)-5-methyl-N-((6-(5-**
45 **(trifluoromethyl)pyrazin-2-yl)pyrimidin-4-yl)methyl)pyrrolidine-2-carboxamide (11)**. The
46 title compound was prepared from carboxylic acid **29** and amine **53** in a manner analogous to **20**.
47
48 HRMS (ESI) Calc for $C_{22}H_{20}F_5N_5O_3S$ (M+H)⁺: 543.1232. Found: 543.1227. ¹H NMR (400
49
50
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56
57
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MHz, CDCl₃) δ 9.85 (s, 1H), 9.36 (s, 1H), 9.05 (s, 1H), 8.52 (s, 1H), 7.97 - 7.91 (m, 2H), 7.28 - 7.24 (m, 3H), 4.88 - 4.68 (m, 3H), 4.29 - 4.11 (m, 2H), 2.58 - 2.34 (m, 2H), 1.44 - 1.43 (m, 3H).

(2S,4R,5S)-N-((2-cyclopropyl-2'-(trifluoromethyl)-[4,5'-bipyrimidin]-6-yl)methyl)-4-fluoro-1-((4-fluorophenyl)sulfonyl)-5-methylpyrrolidine-2-carboxamide (12). The title compound was prepared from carboxylic acid **29** and amine **59** in a manner analogous to **20**. HRMS (ESI) Calc for C₂₅H₂₄F₅N₆O₃S (M+H)⁺: 583.1545. Found: 583.1533. ¹H NMR (300 MHz, CD₃OD) δ 9.69 (s, 2H), 8.11 (s, 1H), 8.05 - 8.00 (m, 2H), 7.36 - 7.31 (t, *J* = 8.7 Hz, 2H), 4.91 - 4.73 (m, 1H), 4.65 - 4.49 (m, 2H), 4.30 - 4.25 (m, 1H), 4.11 - 4.04 (m, 1H), 2.47 - 2.32 (m, 3H), 1.37 - 1.35 (d, *J* = 6.9 Hz, 3H), 1.27 - 1.12 (m, 4H).

(2S,4R,5S)-4-fluoro-1-((4-fluorophenyl)sulfonyl)-5-methyl-N-((2-(trifluoromethyl)pyrimidin-5-yl)pyridin-2-yl)methyl)pyrrolidine-2-carboxamide (13). The title compound was prepared from carboxylic acid **29** and amine **62** in a manner analogous to **20**. HRMS (ESI) Calc for C₂₃H₂₁F₅N₅O₃S (M+H)⁺: 542.1280. Found: 542.1269. ¹H NMR (400 MHz, CDCl₃) δ 9.65 (s, 2H), 8.80 (s, 1H), 8.13 (s, 1H), 7.93 - 7.90 (m, 2H), 7.54 - 7.51 (m, 2H), 7.28 - 7.25 (m, 1H), 5.03 - 4.99 (m, 1H), 4.82 - 4.68 (d, *J* = 51.6 Hz, 1H), 4.50 - 4.32 (m, 1H), 4.30 - 4.27 (m, 1H), 4.15 - 4.08 (m, 1H), 2.65 - 2.55 (m, 1H), 2.39 - 2.23 (m, 1H), 1.48 - 1.38 (m, 3H).

(2S,4R,5S)-4-fluoro-1-((4-fluorophenyl)sulfonyl)-5-methyl-N-((2-(trifluoromethyl)pyrimidin-5-yl)pyridin-4-yl)methyl)pyrrolidine-2-carboxamide (14). The title compound was prepared from carboxylic acid **29** and amine **74** in a manner analogous to **20**. HRMS (ESI) Calc for C₂₃H₂₁F₅N₅O₃S (M+H)⁺: 542.1280. Found: 542.1273. ¹H NMR (400 MHz, CDCl₃) δ 9.65 (s, 2H), 8.80 (s, 1H), 8.13 (s, 1H), 7.93 - 7.90 (m, 2H), 7.54 - 7.51 (m, 2H), 7.28 - 7.25 (m, 1H), 5.03 - 4.99 (m, 1H), 4.82 - 4.68 (d, *J* = 51.6 Hz, 1H), 4.50 - 4.32 (m, 1H),

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2
3 4.30 - 4.27 (m, 1H), 4.15 - 4.08 (m, 1H), 2.65 - 2.55 (m, 1H), 2.39 - 2.23 (m, 1H), 1.48 - 1.38 (m,
4
5 3H).

6
7 **(2S,4R,5S)-N-((5-cyano-4-(2-(trifluoromethyl)pyrimidin-5-yl)pyridin-2-yl)methyl)-4-**
8 **fluoro-1-((4-fluorophenyl)sulfonyl)-5-methylpyrrolidine-2-carboxamide (15).** A mixture of
9
10 **16** (60 mg, 0.10 mmol), Zn(CN)₂ (13 mg, 0.11 mmol), Pd₂(dba)₃.CHCl₃ (11 mg, 0.01 mmol),
11
12 dppf (17 mg, 0.03 mmol), and *N,N*-dimethylformamide was irradiated with microwave radiation
13
14 for 1 h at 150 °C under nitrogen. The resulting solution was diluted with 80 mL of water,
15
16 extracted with ethyl acetate, dried over anhydrous sodium sulfate, and concentrated under
17
18 vacuum. The residue was purified on a C18 silica gel column eluting with CH₃CN/H₂O (10
19
20 mmol/L NH₄HCO₃, 5% to 95%, over 30 min) to provide the title compound (30.5 mg, 52%) as a
21
22 white solid. mp 176 °C. HRMS (ESI) Calc for C₂₄H₂₀F₅N₆O₃S (M+H)⁺: 567.1232 Found:
23
24 567.1223. ¹H NMR (400 MHz, CDCl₃) δ 9.27 (s, 2H), 9.04 (s, 1H), 7.89 - 7.86 (m, 3H), 7.54 -
25
26 7.52 (m, 1H), 7.28 - 7.24 (m, 2H), 5.13 - 5.07 (m, 1H), 4.81 - 4.60 (m, 2H), 4.26 (t, *J* = 9.2Hz,
27
28 1H), 4.13 - 4.04 (m, 1H), 2.64 - 2.53 (m, 1H), 2.39 - 2.21 (m, 1H), 1.41 (d, *J* = 7.2Hz, 3H).

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33 **(2S,4R,5S)-N-((5-chloro-4-(2-(trifluoromethyl)pyrimidin-5-yl)pyridin-2-yl)methyl)-4-**
34 **fluoro-1-((4-fluorophenyl)sulfonyl)-5-methylpyrrolidine-2-carboxamide (16).** The title
35
36 compound was prepared from carboxylic acid **29** and amine **66** in a manner analogous to **20**.
37
38 HRMS (ESI) Calc for C₂₃H₂₀F₅N₅O₃S (M+H)⁺: 576.0890. Found: 576.0883. ¹H NMR(400 MHz,
39
40 CDCl₃) δ 9.15 (s, 2H), 8.75(s, 1H), 7.89 - 7.86 (m, 2H), 7.59 (m, 2H), 7.28 - 7.22 (m, 2H), 4.98 -
41
42 4.92 (m, 1H), 4.80 - 4.58 (m, 2H), 4.26 - 4.21 (m, 1H), 4.13 - 4.06 (m, 1H), 2.61 - 2.50 (m, 1H),
43
44 2.40 - 2.23 (m, 1H), 1.39 (d, *J* = 7.2 Hz, 3H).

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51 **(2S,4R,5S)-4-fluoro-N-((5-fluoro-4-(2-(trifluoromethyl)pyrimidin-5-yl)pyridin-2-**
52 **yl)methyl)-1-((4-fluorophenyl)sulfonyl)-5-methylpyrrolidine-2-carboxamide (17).** The title
53
54

1
2
3 compound was prepared from carboxylic acid **29** and amine **71** in a manner analogous to **20**.
4
5 HRMS (ESI) Calc for $C_{23}H_{20}F_6N_5O_3S$ (M+H)⁺: 560.1186. Found: 560.1182. ¹H NMR (300
6
7 MHz, CDCl₃) δ 9.24 (s, 2H), 8.62 (s, 1H), 7.91 - 7.86 (m, 2H), 7.76 - 7.74 (d, *J* = 5.7 Hz, 1H),
8
9 7.55 (m, 1H), 7.24 - 7.21 (m, 2H), 5.01 - 4.95 (m, 1H), 4.80 - 4.56 (m, 2H), 4.27 - 4.21 (t, *J* = 9.6
10
11 Hz, 1H), 4.14 - 4.00 (m, 1H), 2.62 - 2.18 (m, 2H), 1.39 - 1.37 (d, *J* = 7.2 Hz, 3H).
12
13

14
15 **(2S,4R,5S)-N-((5-cyano-2-(2-(trifluoromethyl)pyrimidin-5-yl)pyridin-4-yl)methyl)-4-**
16
17 **fluoro-1-((4-fluorophenyl)sulfonyl)-5-methylpyrrolidine-2-carboxamide (18)**. A mixture of
18
19 **19** (0.750 g, 1.20 mmol), Zn(CN)₂ (0.170 g, 1.45 mmol), Pd₂(dba)₃ (110 mg, 0.12 mmol), dppf
20
21 (0.20 g, 0.36 mmol), and *N,N*-dimethylformamide was irradiated with microwave radiation for 1
22
23 h at 100°C under nitrogen. The resulting solution was diluted with ethyl acetate, washed with
24
25 brine, dried over anhydrous sodium sulfate, and concentrated under vacuum. The residue was
26
27 purified by a silica gel column eluting with ethyl acetate/petroleum ether (1:1) to afford the title
28
29 compound (510 mg, 75% yield) as a white solid. mp 192 °C. HRMS (ESI) Calc for
30
31 $C_{24}H_{20}F_5N_6O_3S$ (M+H)⁺: 567.1232. Found: 567.1224. ¹H NMR (400 MHz, DMSO) δ 9.70 (s,
32
33 2H), 9.23 - 9.17 (m, 2H), 8.35 (s, 1H), 8.08 - 8.04 (m, 2H), 7.49 (t, *J* = 8.8 Hz, 2H), 4.96 (d, *J* =
34
35 51.6 Hz, 1H), 4.72 - 4.59 (m, 2H), 4.25 - 4.21 (m, 1H), 4.01 - 3.92 (m, 1H), 2.43 - 2.08 (m, 2H),
36
37 1.21 (d, *J* = 6.8 Hz, 3H).
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43 **(2S,4R,5S)-N-((5-chloro-2-(2-(trifluoromethyl)pyrimidin-5-yl)pyridin-4-yl)methyl)-4-**
44
45 **fluoro-1-((4-fluorophenyl)sulfonyl)-5-methylpyrrolidine-2-carboxamide (19)**. The title
46
47 compound was prepared from carboxylic acid **29** and amine **80** in a manner analogous to **20**.
48
49 HRMS (ESI) Calc for $C_{23}H_{20}F_5N_5O_3SCl$ (M+H)⁺: 576.0890. Found: 576.0878. ¹H NMR (400
50
51 MHz, CDCl₃) δ 9.62 (s, 2H), 8.71 (s, 1H), 8.21 (s, 1H), 7.91-7.94 (m, 2H), 7.38 (s, 1H), 7.30-7.26
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(m, 2H), 5.06-5.00 (m, 1H), 4.75 (d, $J = 52.8$ Hz, 1H), 4.49-4.43 (m, 1H), 4.32 (t, $J = 8.8$ Hz, 1H), 4.17-4.07 (m, 1H), 2.67-2.57 (m, 1H), 2.37-2.20 (m, 1H), 1.27 (s, 3H).

(2S,4R,5S)-4-fluoro-N-((5-fluoro-2-(2-(trifluoromethyl)pyrimidin-5-yl)pyridin-4-yl)methyl)-1-((4-fluorophenyl)sulfonyl)-5-methylpyrrolidine-2-carboxamide (20). A mixture of carboxylic acid **29** (1.60 g, 6.47 mmol), diisopropylethyl amine (2.39 g, 18.5 mmol), HATU (2.82 g, 7.42 mmol), and amine **84** (1.68 g, 6.17 mmol) in *N,N*-dimethylformamide (60 mL) was stirred for 30 min at room temperature. The reaction was diluted with water, extracted with ethyl acetate, washed with brine, dried over anhydrous sodium sulfate, and concentrated under vacuum. The residue was purified by silica gel chromatography eluting with ethyl acetate/petroleum ether (1/5) to afford 1.95 g of 3-fluoro-5-(((5-fluoro-2-(2-(trifluoromethyl)pyrimidin-5-yl)pyridin-4-yl)methyl)carbamoyl)-2-methylpyrrolidine-1-carboxylate as a yellow oil (60% yield). LCMS, $m/z = 502$ $[M+H]^+$. 1H NMR (400 MHz, $CDCl_3$) δ 9.52 (s, 2H), 8.54 (s, 1H), 8.05 (s, 1H), 7.13 (s, 1H), 4.98 – 4.78 (m, 1H), 4.79 – 4.67 (m, 1H), 4.59 – 4.44 (m, 2H), 4.25 – 4.14 (m, 1H), 2.53 – 2.37 (m, 2H), 1.40 (s, 9H), 1.19 (d, $J = 6.9$ Hz, 3H).

A mixture of 3-fluoro-5-(((5-fluoro-2-(2-(trifluoromethyl)pyrimidin-5-yl)pyridin-4-yl)methyl)carbamoyl)-2-methylpyrrolidine-1-carboxylate from the previous step (1.95 g, 3.89 mmol) in dichloromethane (80 mL) and trifluoroacetic acid (20 mL) was stirred for 12 h at room temperature. The resulting mixture was then concentrated under vacuum. The residue was diluted with water and the resulting solution was adjusted to pH 8 with a saturated solution of sodium bicarbonate. The resulting mixture was extracted with ethyl acetate, washed with brine, dried over anhydrous sodium sulfate, and concentrated under vacuum. This resulted in 900 mg (crude) of (2S,4R,5S)-4-fluoro-N-((5-fluoro-2-(2-(trifluoromethyl)pyrimidin-5-yl)pyridin-4-

1
2
3 yl)methyl)-5-methylpyrrolidine-2-carboxamide as a yellow oil which was used for the next step
4
5 without further purification. LCMS, $m/z = 402$ $[M+H]^+$.
6

7
8 A mixture of crude (2S,4R,5S)-4-fluoro-N-((5-fluoro-2-(2-(trifluoromethyl)pyrimidin-5-
9
10 yl)pyridin-4-yl)methyl)-5-methylpyrrolidine-2-carboxamide (900 mg) from the previous step,
11
12 triethylamine (0.680 g, 6.72 mmol), and 4-fluorobenzene-1-sulfonyl chloride (656 mg, 3.37
13
14 mmol) in dichloromethane (50 mL) was stirred for 12 h at room temperature. The mixture was
15
16 diluted with water, extracted with ethyl acetate, washed with brine, dried over anhydrous sodium
17
18 sulfate, and concentrated under vacuum. The residue was purified by silica gel chromatography
19
20 eluting with dichloromethane/ethyl acetate (1/5) to provide 650 mg of **20** as a white solid (30%
21
22 yield, over 2 steps). mp 118 °C. HRMS (ESI) Calc for $C_{23}H_{20}F_6N_5O_3S$ (M+H)⁺: 560.1186.
23
24 Found: 560.1178. ¹H NMR (500 MHz, DMSO-*d*₆) δ 9.60 (s, 2H), 9.07 (t, *J* = 5.9 Hz, 1H), 8.79
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26 (d, *J* = 1.0 Hz, 1H), 8.25 (d, *J* = 5.7 Hz, 1H), 8.09 – 8.03 (m, 2H), 7.49 (t, *J* = 8.8 Hz, 2H), 4.90
27
28 (dd, *J* = 3.0, 51.4 Hz, 1H), 4.61 (dd, *J* = 6.2, 17.0 Hz, 1H), 4.49 (dd, *J* = 5.6, 17.0 Hz, 1H), 4.23
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30 (dd, *J* = 7.1, 10.2 Hz, 1H), 3.95 (dq, *J* = 1.1, 6.9, 21.3 Hz, 1H), 2.37 (dddd, *J* = 1.1, 7.1, 13.7,
31
32 17.3 Hz, 1H), 2.20 (dddd, *J* = 3.0, 10.2, 13.7, 45.5 Hz, 1H), 1.21 (d, *J* = 6.9 Hz, 3H). ¹³C NMR
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34 (126 MHz, DMSO-*d*₆) δ 171.13, 164.75 (d, *J* = 251.8 Hz), 157.48 (d, *J* = 256.5 Hz), 155.86,
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36 154.45 (q, *J* = 36.3 Hz), 146.34 (d, *J* = 3.4 Hz), 138.02 (d, *J* = 25.1 Hz), 136.77 (d, *J* = 12.9 Hz),
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38 132.92, 132.79, 130.74 (d, *J* = 9.2 Hz), 120.83, 119.55 (q, *J* = 274.6 Hz), 116.29 (d, *J* = 22.2
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40 Hz), 96.11 (d, *J* = 176.9 Hz), 63.09 (d, *J* = 21.3 Hz), 60.55, 35.85 (d, *J* = 2.5 Hz), 35.24 (d, *J* =
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42 21.0 Hz), 19.29 (d, *J* = 10.6 Hz).
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49 **(2S,4R,5S)-N-((3,5-difluoro-2-(2-(trifluoromethyl)pyrimidin-5-yl)pyridin-4-yl)methyl)-4-**
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51 **fluoro-1-((4-fluorophenyl)sulfonyl)-5-methylpyrrolidine-2-carboxamide (21)**. The title
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53 compound was prepared from carboxylic acid **29** and amine **90** in a manner analogous to **20**.
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3 HRMS (ESI) Calc for $C_{23}H_{19}F_7N_5O_3S$ (M+H)⁺: 578.1091. Found: 578.1086. ¹H NMR (400
4 MHz, DMSO-d₆) δ 9.70 (s, 2H), 9.23 - 9.19 (m, 2H), 8.35 (s, 1H), 8.08 - 8.04 (m, 1H), 7.49 (t, *J*
5 = 8.8 Hz, 2H), 4.97 - 4.84 (m, 1H), 4.72 - 4.59 (m, 2H), 4.23 - 4.21 (m, 1H), 4.01 - 3.92 (m, 1H),
6 2.43 - 2.13 (m, 2H), 1.22 (d, *J* = 6.8 Hz, 3H).

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12 **Sodium ((2*S*,4*R*,5*S*)-4-fluoro-*N*-((5-fluoro-2-(2-(trifluoromethyl)pyrimidin-5-yl)pyridin-4-
13 yl)methyl)-1-((4-fluorophenyl)sulfonyl)-5-methylpyrrolidine-2-carboxamido)methyl**

14
15 **phosphate (22)**. A mixture of sulfide **95** (330 mg, 0.53 mmol), tetrahydrofuran (20 mL),
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17 phosphoric acid (1.60 g, 16.3 mmol) and 3 Å sieves was stirred for 15 min at room temperature.
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19 *N*-Iodosuccinimide (0.240 g, 1.06 mmol) was added in one portion at 0 °C. The resulting
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21 solution was stirred for 1 h at room temperature and then diluted with 20 mL of methanol. The
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23 solid was filtered off and the filtrate was quenched by 1 M aqueous sodium thiosulfate. The
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25 solution was adjusted to pH 10 with solid sodium carbonate and the resulting solid was filtered
26
27 off and the liquid was concentrated under vacuum. The residue was purified by Prep-HPLC
28
29 using the following conditions: C18 silica gel column; mobile phase: methanol/H₂O gradient
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31 from 100/0 to 0/100 over 30 min; UV 254 nm detection. The fractions were concentrated and
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33 lyophilized to dryness to provide the 300 mg of **22** as an off-white solid (84% yield). HRMS
34
35 (ESI) Calc for $C_{24}H_{23}F_6N_5O_7SP$ (M+H)⁺: 670.0955. Found: 670.0941. ¹H NMR (500 MHz,
36
37 Methanol-d₄) δ 9.58 (s, 2H), 8.56 (d, *J* = 1.3 Hz, 1H), 8.15 (d, *J* = 5.8 Hz, 1H), 8.08 - 8.02 (m,
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39 2H), 7.34 - 7.25 (m, 2H), 5.70 (dd, *J* = 7.5, 11.3 Hz, 1H), 5.25 - 5.15 (m, 3H), 4.91 (d, *J* = 17.5
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41 Hz, 1H), 4.83 (dd, *J* = 3.0, 51.5 Hz, 1H), 4.08 - 3.85 (m, 1H), 2.88 - 2.76 (m, 1H), 2.28 (dddd, *J*
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43 = 3.4, 9.7, 14.4, 43.3 Hz, 1H), 1.28 (d, *J* = 7.0 Hz, 3H). ¹³C NMR (126 MHz, Methanol-d₄) δ
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45 176.05, 166.93 (d, *J* = 252.5 Hz), 159.69 (d, *J* = 257.9 Hz), 157.52, 156.83 (q, *J* = 36.7 Hz),
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47 148.44 (d, *J* = 3.7 Hz), 139.04 (d, *J* = 25.2 Hz), 137.76 (d, *J* = 12.8 Hz), 135.34, 134.82, 132.03
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(d, $J = 9.2$ Hz), 121.95, 121.20 (q, $J = 274.3$ Hz), 117.50 (d, $J = 23.1$ Hz), 98.19 (d, $J = 179.2$ Hz), 75.30 (d, $J = 2.7$ Hz), 64.74 (d, $J = 21.7$ Hz), 59.38, 46.13 (d, $J = 4.4$ Hz), 37.50 (d, $J = 21.8$ Hz), 19.70 (d, $J = 9.4$ Hz).

(2S,5R)-1-(tert-butoxycarbonyl)-5-methylpyrrolidine-2-carboxylic acid (28). A mixture of pyrrolidine **31** (180 mg, 0.74 mmol) and lithium hydroxide (1.6 mg, 0.07 mmol) in 10:1 methanol/water (2.2 mL) was stirred overnight at room temperature and then concentrated under vacuum. The residue was diluted with 10 mL of water and the solution was adjusted to pH 3 with diluted hydrochloric acid. The resulting solution was extracted with dichloromethane, dried over sodium sulfate, and concentrated under vacuum. This resulted in 110 mg of **28** as a light yellow oil (65% yield). LCMS, $m/z = 230.0$ $[M+H]^+$.

(2S,4R,5S)-1-(tert-butoxycarbonyl)-4-fluoro-5-methylpyrrolidine-2-carboxylic acid (29). To a solution of methyl ester **36** (120 g, 460 mmol) in MeOH (600 mL) and THF (600 mL) were added NaOH (27.4 g in 600 mL H₂O, 685 mmol) dropwise at 0-5 °C. The mixture was then stirred at 25 °C for 16 h. One additional reaction was set up as described above. Both reaction mixtures were combined for workup. The organic solvent was removed under vacuum, and the residue was washed with MTBE (3 x 1 L), and was acidified to pH 4-5 with 1N HCl. The mixture was extracted with dichloromethane (3 x 1 L). The combined organic layers were dried over Na₂SO₄ and concentrated under reduced pressure to provide 105 g of **29** (46% yield). LCMS, $m/z = 246.2$ $[M-H]^-$. ¹H NMR (400 MHz, DMSO-d₆) δ 10.8 (s, 1 H), 4.72-4.85 (m, 1 H), 4.34-4.47 (m, 1 H), 4.13-4.15 (m, 1 H), 2.41-2.49 (m, 2 H), 1.36-1.42 (m, 9 H), 1.09-1.19 (m, 3 H).

(2S,5R)-1-tert-butyl 2-methyl 5-methylpyrrolidine-1,2-dicarboxylate (31). To a mixture of copper(I) bromide-dimethyl sulfide (1.05 g, 5.11 mmol) and diethyl ether (13 mL) was added

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3 methylmagnesium bromide (1.7 mL, 3M in Et₂O) dropwise at -40 °C under nitrogen. After 45
4 min at -40 °C the mixture was cooled to -78 °C and BF₃·Et₂O (0.62 mL, 5.2 mmol, 4.0 equiv)
5 was added dropwise at -78 °C. The reaction was stirred for 30 min and pyrrolidine **30**³² (320 mg,
6 1.2 mmol) in diethyl ether (17 mL) was added at -78 °C. The resulting solution was stirred for 30
7 min at -78 °C and then warmed to room temperature for 1 h. The reaction mixture was then
8 stirred with aqueous ammonium chloride for 1 h at room temperature. The resulting solution was
9 extracted with ether, washed with sodium bicarbonate and brine, dried over anhydrous sodium
10 sulfate, and concentrated under vacuum. The residue was purified by silica gel chromatography
11 eluting with ethyl acetate/petroleum ether (1:4) to provide 180 mg as a colorless oil (60% yield).
12 LCMS, *m/z* = 244 [M+H]⁺.
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26 **(2S,4S)-1-tert-butyl 2-methyl 4-((tert-butyldimethylsilyl)oxy)-5-methylenepyrrolidine-1,2-**
27 **dicarboxylate (33).** To a solution of dimethyltitanocene (2.1 kg, assay 28.9%) in anhydrous
28 toluene (1 L) was added pyridine (233 g, 2.94 mol), titanocene dichloride (18 g, 72 mmol) and
29 pyroglutamate **32**³³ (0.550 kg, 1.47 mol). The atmosphere was then purged with nitrogen. The
30 mixture was stirred at 67 °C for 18 h. Two additional reactions were set up as described above.
31 All three reaction mixtures were combined for workup. The resulting solution was cooled to 25
32 °C and concentrated under vacuum below 35 °C. Petroleum ether (6 L) was added to the mixture
33 and the solids were filtered. The filtrate was concentrated under vacuum below 35 °C. The
34 residue was applied onto a silica gel column eluted with ethyl acetate/petroleum ether (1:100 to
35 1:50) to give 1.05 kg of **33** as a yellow oil (64% yield). LCMS, *m/z* = 372 [M+H]⁺. ¹H NMR
36 (300 MHz, CDCl₃) δ 4.44 (t, *J* = 7.2 Hz, 1H), 4.26 (t, *J* = 7.2 Hz, 1H), 3.74 (s, 3H), 3.73 - 3.66
37 (m, 1H), 2.60 - 2.50 (m, 1H), 2.02 - 1.91 (m, 2H), 1.47 (s, 9H), 0.89 (s, 9H), 0.14 - 0.09 (m, 6H).
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3 **(2S,4S,5S)-1-tert-butyl 2-methyl 4-((tert-butyldimethylsilyl)oxy)-5-methylpyrrolidine-1,2-**
4 **dicarboxylate (34).** Olefin **33** (0.120 kg, 323 mmol), Pd/C (15.0 g, wet) and MeOH (1200 mL)
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6 were added to a reaction vessel, where the atmosphere was purged with H₂ three times. The
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8 mixture was then stirred at 25 °C under H₂ (50 Psi) for 18 h. Eight additional reactions were set
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10 up as described above. All nine reaction mixtures were combined for workup. The mixture was
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12 filtered, and the filtrate was concentrated under vacuum to provide 1.08 kg of **34** (100% yield).
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14 LCMS, *m/z* = 374 [M+H]⁺. ¹H NMR (400 MHz, CDCl₃) δ 4.27 - 4.15 (m, 2H), 4.00 - 3.82 (m,
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16 1H), 3.74 (s, 3H), 2.34 - 2.31 (m, 1H), 2.00 - 1.97 (m, 1H), 1.48 - 1.42 (m, 9H), 1.24 - 1.20 (m,
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18 3H), 0.90 (s, 9H), 0.12 (s, 6H).

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23 **(2S,4S,5S)-1-tert-butyl 2-methyl 4-hydroxy-5-methylpyrrolidine-1,2-dicarboxylate (35).**
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25 To a solution of silyl ether **34** (0.270 kg, 723 mmol) in anhydrous THF (1.35 L) was added
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27 TBAF (1 M in THF, 795 mL). The mixture was then stirred at 25 °C for 1 h. Three additional
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29 reactions were set up as described above. All four reaction mixtures were combined for workup.
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31 The solvent was removed under vacuum below 35 °C. The resulting residue was washed with
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33 petroleum ether (3 x 1 L), the organic layers were then removed and the residue was purified by
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35 silica gel column eluting with ethyl acetate/petroleum ether (1:3 to 1:1) to provide 640 g of **35** as
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37 yellow oil (85% yield). LCMS, *m/z* = 260 [M+H]⁺. ¹H NMR (300 MHz, CDCl₃) δ 4.50 - 4.23
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39 (m, 2H), 4.00 - 3.85 (m, 1H), 3.75 (s, 3H), 3.07 - 2.97 (m, 1H), 2.41 - 2.32 (m, 1H), 1.43 (m,
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41 9H), 1.35 - 1.30 (m, 3H).

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46 **(2S,4R,5S)-1-tert-butyl 2-methyl 4-fluoro-5-methylpyrrolidine-1,2-dicarboxylate (36).** To
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48 a solution of alcohol **35** (0.320 kg, 1.23 mol) in dichloromethane (3.2 L) was added bis(2-
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50 methoxyethyl)aminosulfur trifluoride (0.490 kg, 2.21 mol) dropwise at 0-5 °C under nitrogen.
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52 The resulting solution was stirred at 25 °C for 64 h. One additional reaction was set up as
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described above. Both reaction mixtures were combined for workup. The mixture was quenched by 10% Na₂CO₃ (12 L) at 0-10 °C and the pH value was adjusted to 9. The organic layer was then washed with 1N HCl (4 L) and dried over Na₂SO₄ and filtered. The solvent was removed under vacuum below 35 °C. The residue was purified by silica-gel chromatography eluting with an ethyl acetate/petroleum ether gradient (1:100 → 1:5) to provide 240 g of **36** as a yellow oil (37% yield). LCMS, *m/z* = 262 [M+H]⁺. ¹H NMR (300 MHz, CDCl₃) δ 4.97 - 4.75 (m, 1H), 4.48 - 4.36 (m, 1H), 4.30 - 4.05 (m, 1H), 3.75 (s, 3H), 2.41 - 2.32 (m, 1H), 2.26 - 2.04 (m, 1H), 1.58 - 1.41(m, 9H), 1.28 - 1.22 (m, 3H).

4-chloro-6-(6-(trifluoromethyl)pyridin-3-yl)pyrimidine (38). A 5 L 4-necked round-bottom flask under nitrogen was charged with a solution of 4,6-dichloropyrimidine (936 g, 6.16 mol) in 1,4-dioxane (3 L), [6-(trifluoromethyl)pyridin-3-yl]boronic acid **37** (0.600 kg, 3.08 mol), Pd(PPh₃)₂Cl₂ (110.3 g, 157.1 mmol), sodium carbonate (635 g, 5.99 mol), and water (400 mL). The resulting solution was stirred at 50 °C for 48 h and then diluted with 6 L of ethyl acetate. The organic layer was then washed with water (3 L) and brine (4 x 1 L), dried over anhydrous sodium sulfate and concentrated under vacuum. The residue was purified on a silica gel column eluting with ethyl acetate/petroleum ether (0:100 → 1:20) to afford 410 g of **38** as a white solid (50% yield). LCMS, *m/z* = 260 [M+H]⁺. ¹H NMR (400 MHz, DMSO-d₆) δ 9.56 (s, 1H), 9.23(s, 1H), 8.89-8.88 (d, *J* = 2.0Hz, 1H), 8.58 (s, 1H), 8.15-8.13 (d, *J* = 8.0Hz, 1H).

6-(6-(trifluoromethyl)pyridin-3-yl)pyrimidine-4-carbonitrile (39). A 5 L 4-necked round-bottom flask was charged with a solution of chloropyrimidine **38** (0.280 kg, 1.06 mol) in DMSO (2800 mL), NaCN (116 g, 2.33 mol), 1,4-diazabicyclo[2.2.2]octane (60.51 g, 528.6 mmol), and water (700 mL). The resulting solution was stirred at room temperature for 4 h and then diluted with 5 L of ethyl acetate. The organic layer was washed with brine (6 x 1 L), dried over

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3 anhydrous sodium sulfate and concentrated under vacuum. The residue was purified on a silica
4 gel column eluting with ethyl acetate/petroleum ether (1:20 → 1:3) to afford 190 g of **39** as a
5 light yellow solid (68% yield). LCMS, $m/z = 251$ $[M+H]^+$.
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10 **tert-butyl ((6-(6-(trifluoromethyl)pyridin-3-yl)pyrimidin-4-yl)methyl)carbamate (40)**. A 2
11 L round-bottom flask purged and maintained with an inert atmosphere of nitrogen was charged
12 with a solution of nitrile **39** (0.190 kg, 744 mmol) in methanol (1.5 L), (Boc)₂O (215.24 g,
13 967.59 mmol), followed by the addition of Pd/C (30 g, 10%) in several batches. H₂ gas was
14 introduced to the mixture, and the resulting solution was stirred at room temperature for 12 h.
15 The solids were filtered, and the filtrate was concentrated under vacuum. The residue was then
16 purified on a silica gel column eluting with ethyl acetate/petroleum ether (1:5 → 1:3) to afford
17 211 g of **40** as an off-white solid (76% yield). LCMS, $m/z = 355$ $[M+H]^+$.
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22 **(6-(6-(trifluoromethyl)pyridin-3-yl)pyrimidin-4-yl)methanamine hydrochloride salt (41)**.
23 A 5 L 3-necked round-bottom flask was charged with a solution of *N*-Boc amine **40** (211 g, 584
24 mmol) in ethyl acetate (3 L). HCl (gas) was introduced to the mixture at 0°C and the resulting
25 solution was stirred at room temperature for 4 h. The solids were collected by filtration and
26 rinsed with ethyl acetate (3 x 200 mL) followed by ether (3 x 150 mL) to afford 140 g of **41** as a
27 light red solid (92% yield). LCMS, $m/z = 255$ $[M+H]^+$. ¹H NMR (300 MHz, D₂O) δ 9.15 (s, 1H),
28 9.03 (s, 1H), 8.48 (d, $J = 8.1$ Hz, 1H), 7.98 (s, 1H), 7.87 (d, $J = 8.1$ Hz, 1H), 4.43 (2H, s).
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33 **(2S,5S)-methyl 1-((4-fluorophenyl)sulfonyl)-5-methylpyrrolidine-2-carboxylate (43)**. A
34 mixture of pyrrolidine **42**³⁴ (0.350 g, 2.44 mmol), triethylamine (987 mg, 9.75 mmol), 4-
35 dimethylaminopyridine (28 mg, 0.23 mmol), and 4-fluorobenzene-1-sulfonyl chloride (567 mg,
36 2.91 mmol) in dichloromethane (30 mL) was stirred for 8 h at room temperature. The resulting
37 solution was diluted with ethyl acetate, washed with brine, and dried over anhydrous sodium
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3 sulfate. The residue was purified by silica gel chromatography eluting with ethyl
4 acetate/petroleum ether (1:3) to afford 190 mg of **43** as light yellow oil (26% yield). LCMS, m/z
5 = 302 $[M+H]^+$. 1H NMR (300 MHz, $CDCl_3$) δ 8.08 – 7.74 (m, 2H), 7.38 – 7.07 (m, 2H), 4.30
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7 (dd, $J = 7.9, 5.6$ Hz, 1H), 3.92 – 3.80 (m, 1H), 3.74 (s, 3H), 2.03 – 1.78 (m, 3H), 1.72 – 1.51 (m,
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9 1H), 1.31 (d, $J = 6.3$ Hz, 3H).

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14 **(2S,5S)-1-((4-fluorophenyl)sulfonyl)-5-methylpyrrolidine-2-carboxylic acid (44).** A
15 mixture of pyrrolidine sulfonamide **43** (160 mg, 0.53 mmol) and lithium hydroxide (25.5 mg,
16 1.06 mmol) in methanol (4 mL)/water(0.5 mL) was stirred overnight at room temperature. The
17 resulting solution was diluted with 20 mL of water and the resulting solution was adjusted to pH
18 9 with sodium carbonate. The resulting solution was extracted with dichloromethane, dried over
19 anhydrous sodium sulfate, and concentrated under vacuum 180 mg (crude) of **44** as a white solid,
20 which was used in the next reaction without further purification. LCMS, $m/z = 286 [M-H]^-$. 1H
21 NMR (400 MHz, $CDCl_3$) δ 8.11 – 7.78 (m, 2H), 7.25 (t, $J = 8.5$ Hz, 2H), 4.30 (dd, $J = 8.3, 5.3$
22 Hz, 1H), 3.84 (h, $J = 6.3$ Hz, 1H), 2.28 – 2.09 (m, 1H), 2.07 – 1.54 (m, 3H), 1.37 (d, $J = 6.3$ Hz,
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37 **5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-2-(trifluoromethyl)pyrimidine (46).** A 5 L
38 4-necked round-bottom flask purged and maintained with an inert atmosphere of nitrogen was
39 charged with 5-bromo-2-(trifluoromethyl)pyrimidine **45**³⁵ (250 g, 1.1 mol), *N,N*-
40 dimethylformamide (2.5 L), potassium acetate (325 g, 3.31 mol), 4,4,5,5-tetramethyl-2-
41 (tetramethyl-1,3,2-dioxaborolan-2-yl)-1,3,2-dioxaborolane (282 g, 1.11 mol) and $Pd(OAc)_2$ (5.0
42 g, 22 mmol). The resulting mixture was stirred for 3 h at 80 °C and then cooled to room
43 temperature, quenched by the addition of 6 L of water/ice, and extracted with ethyl acetate (3 x 2
44 L). The combined organic layer was washed with water (2 x 2 L) and brine (2 L), dried over
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3 anhydrous sodium sulfate and concentrated under vacuum. The residue was then purified on a
4 silica gel column eluting with ethyl acetate/petroleum ether (1:100) to afford 151 g of **46** as a
5 white solid (50% yield). LCMS, $m/z = 193$ $[M+H]^+$ (boronic acid). 1H NMR (DMSO- d_6 ,
6 300MHz): δ 9.15 (2H, s), 1.34 (12H, s).
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12 **2-((6-chloropyrimidin-4-yl)methyl)isoindoline-1,3-dione (48)**. Into a 3 L 4-necked round-
13 bottom flask was placed alcohol **47**³⁶ (50.0 g, 346 mmol), tetrahydrofuran (1.5 L), 2,3-dihydro-
14 1H-isoindole-1,3-dione (76.4 g, 519 mmol), and PPh_3 (136 g, 518 mmol) followed by the
15 addition of DIAD (105 g, 519 mmol) dropwise with stirring at 0 °C. The resulting solution was
16 stirred at room temperature overnight, concentrated under vacuum, diluted with 1 L of ethyl
17 acetate, stirred for an additional 30 min, and filtered. The filter cake was washed with ethyl
18 acetate (300 mL) to afford 65 g of **48** as an off-white solid (69% yield). LCMS, $m/z = 274$
19 $[M+H]^+$. 1H NMR (300 MHz, $CDCl_3$) δ 8.91 (d, $J = 1.1$ Hz, 1H), 7.94 (dd, $J = 5.4, 3.1$ Hz, 2H),
20 7.92 – 7.84 (m, 2H), 7.33 (d, $J = 1.1$ Hz, 1H), 4.99 (s, 2H).
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33 **2-((2'-(trifluoromethyl)-[4,5'-bipyrimidin]-6-yl)methyl)isoindoline-1,3-dione (49)**. Into a 3
34 L 4-necked round-bottom flask purged and maintained with an inert atmosphere of nitrogen was
35 placed chloropyrimidine **48** (50.0 g, 183 mmol), 1,4-dioxane (1.5 L), water (70 mL), potassium
36 carbonate (50.0 g, 362 mmol), boronic ester **46** (130.0 g, 474.2 mmol), and $Pd(dppf)Cl_2$ (4.0 g,
37 5.5 mmol). The resulting solution was stirred at 70 °C for 3 h, cooled to room temperature,
38 quenched by the addition of 3 L of water, and extracted with ethyl acetate (1 L). The combined
39 organic layers were dried over anhydrous sodium sulfate and concentrated under vacuum. The
40 residue was applied onto a silica gel column eluted with ethyl acetate/petroleum ether (1:10) to
41 afford 55 g of **49** as a white solid (78% yield). LCMS, $m/z = 386$ $[M+H]^+$. 1H NMR (400 MHz,
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3 CDCl₃) δ 9.54 (s, 2H), 9.29 (d, *J* = 1.3 Hz, 1H), 7.96 (dd, *J* = 5.5, 3.1 Hz, 2H), 7.89 – 7.65 (m,
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5 3H), 5.13 (s, 2H).
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8 **(2'-(trifluoromethyl)-[4,5'-bipyrimidin]-6-yl)methanamine (50)**. Into a 3 L 4-necked round-
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10 bottom flask was placed bis-pyrimidine **49** (55.0 g, 143 mmol), methanol (1.5 L), and
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12 NH₂NH₂·H₂O (110 g). The resulting solution was stirred at 50 °C overnight, cooled to room
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14 temperature, and filtered. The filtrate was diluted with H₂O (1 L) and extracted with ethyl acetate
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16 (3 x 500 mL). The combined organic layers were dried over anhydrous sodium sulfate and
17
18 concentrated under vacuum to afford 35 g of **50** as a brown solid (96% yield). LCMS, *m/z* = 256
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20 [M+H]⁺. ¹H NMR (300MHz, DMSO-d₆): δ 9.78 (s, 2H), 9.45 (s, 1H), 8.67-8.76 (m, 3H), 8.62 (s,
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22 1H), 4.33-4.37 (t, *J* = 4.8 Hz, 2H).
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27 **2-((6-(trimethylstannyl)pyrimidin-4-yl)methyl)isoindoline-1,3-dione (51)**. A mixture of
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29 pyrimidine **48** (0.340 g, 1.24 mmol), Pd(PPh₃)₄ (67.8 mg, 0.06 mmol), toluene (10 mL), and
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31 hexamethyldistannane (453 mg, 1.38 mmol) was stirred for 45 min at 110 °C under nitrogen. The
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33 resulting mixture was concentrated under vacuum to afford the title compound (475 mg, crude)
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35 as a yellow solid, which was carried to the next step without further purification. LCMS, *m/z* =
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37 404 [M+H]⁺.
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41 **2-((6-(5-(trifluoromethyl)pyrazin-2-yl)pyrimidin-4-yl)methyl)isoindoline-1,3-dione (52)**. A
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43 mixture of crude stannane **51** (475 mg), Pd(PPh₃)₄ (68 mg, 0.06 mmol), toluene (11 mL), and 2-
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45 chloro-5-(trifluoromethyl)pyrazine (345 mg, 1.89 mmol) was stirred overnight at 110 °C under
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47 nitrogen. The resulting mixture was concentrated under vacuum and purified by a silica gel
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49 column eluting with ethyl acetate/petroleum ether (1:3) to afford the title compound (350 mg,
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51 77%) as a yellow solid. LCMS, *m/z* = 386 [M+H]⁺. ¹H NMR (400 MHz, CDCl₃) δ 9.80 (s, 1H),
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53 9.24 (s, 1H), 9.17 (s, 1H), 8.48 (s, 1H), 7.97 - 7.93 (m, 2H), 7.92 - 7.85 (m, 2H), 5.15 (s, 2H).
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3 **(6-(5-(trifluoromethyl)pyrazin-2-yl)pyrimidin-4-yl)methanamine (53)**. A mixture of
4 pyrazine **52** (330 mg, 0.85 mmol), methanol (10.0 L, 415 mmol), and hydrazine hydrate (80%)
5 (3.0 mL, 62 mmol) was stirred overnight at room temperature. The solids were filtered, and the
6 filtrate was concentrated under vacuum to afford the title compound (217 mg, 100% yield) as a
7 brown solid. LCMS, $m/z = 256$ [M+H]⁺.
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12 **2,4-dichloro-6-(chloromethyl)pyrimidine (55)**. A mixture of tetrahydropyrimidinedione **54**
13 (9.2 g, 57 mmol) and POCl₃ (50 mL) was stirred for 12 h at 100 °C. The reaction was then
14 poured into water/ice, extracted with ethyl acetate, washed with brine, dried over anhydrous
15 sodium sulfate, and concentrated under vacuum. The residue was purified by silica gel
16 chromatography eluting with petroleum ether to afford the 9.5 g of **55** as a light yellow solid
17 (84% yield). LCMS, $m/z = 197$ [M+H]⁺.
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22 **2-((2,6-dichloropyrimidin-4-yl)methyl)isoindoline-1,3-dione (56)**. A mixture of 2-potassio-
23 2,3-dihydro-1*H*-isoindole-1,3-dione (13.0 g, 70.0 mmol), dichloropyrimidine **55** (9.2 g, 47
24 mmol), and *N,N*-dimethylformamide (100 mL) was stirred for 3 h at room temperature. The
25 resulting solution was diluted with water. The solids were then collected by filtration and dried
26 under vacuum to afford the title compound 11.5 g of **56** as a yellow solid (80% yield). LCMS,
27 $m/z = 308$ [M+H]⁺. ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.01 (s, 1H), 7.98 – 7.86 (m, 4H), 4.93 (s,
28 2H).
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33 **2-((2-chloro-2'-(trifluoromethyl)-[4,5'-bipyrimidin]-6-yl)methyl)isoindoline-1,3-dione**
34 **(57)**. A mixture of dichloropyrimidine **56** (3.98 g, 12.9 mmol), boronate ester **46** (2.81 g, 10.2
35 mmol), Pd(dppf)Cl₂ (945 mg, 1.29 mmol), potassium carbonate (5.477 g, 39.63 mmol), and *N,N*-
36 dimethylformamide (150 mL) was stirred for 12 h at 70 °C under nitrogen. The solids were then
37 filtered. The resulting solution was diluted with brine, extracted with ethyl acetate, washed with
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3 brine, dried over anhydrous sodium sulfate, and concentrated under vacuum. The residue was
4 purified by silica gel chromatography eluting with dichloromethane/ethyl acetate (50/1) to afford
5 1.75 g of **57** as a yellow solid (32% yield). LCMS, $m/z = 420$ $[M+H]^+$. 1H NMR (300 MHz,
6 $CDCl_3$) δ 9.20(s, 2H), 7.98 – 7.90 (m, 2H), 7.86 – 7.77 (m, 2H), 7.20 (d, $J = 0.8$ Hz, 1H), 4.96 (d,
7 $J = 0.8$ Hz, 2H).
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15 **2-((2-cyclopropyl-2'-(trifluoromethyl)-[4,5'-bipyrimidin]-6-yl)methyl)isoindoline-1,3-**
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17 **dione (58)**. A mixture of chloropyrimidine **57** (190 mg, 0.45 mmol), cyclopropylboronic acid
18 (195 mg, 2.27 mmol), Pd(dppf)Cl₂ (33 mg, 0.045 mmol) and potassium carbonate (188 mg, 1.36
19 mmol) in 1,4-dioxane (10 mL) was stirred for 12 hours at 90°C under nitrogen. The solids were
20 then filtered off. The resulting solution was diluted with brine, extracted with ethyl acetate,
21 washed with brine, dried over anhydrous sodium sulfate, and concentrated under vacuum. The
22 residue was purified by silica gel chromatography eluting with dichloromethane/ethyl acetate
23 (50/1) to afford 175 mg of **58** as yellow solid (91% yield). LCMS, $m/z = 426$ $[M+H]^+$.
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33 **(2-cyclopropyl-2'-(trifluoromethyl)-[4,5'-bipyrimidin]-6-yl)methanamine (59)**. A mixture
34 of cyclopropyl pyrimidine **58** (175 mg, 0.41 mmol) and hydrazine hydrate (206 mg, 41.1 mmol)
35 in methanol (20 mL) was heated to reflux for 12 hours in an oil bath. The resulting mixture was
36 concentrated under vacuum and dissolved in ethyl acetate. The precipitated solids were filtered
37 off. The resulting solution was concentrated under vacuum to afford the title compound (121
38 mg, 100% yield) as a yellow oil, which was used in the next step without further purification.
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47 LCMS, $m/z = 296$ $[M+H]^+$.
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49 **2-((4-(2-(trifluoromethyl)pyrimidin-5-yl)pyridin-2-yl)methyl)isoindoline-1,3-dione (61)**. A
50 mixture of chloropyridine **60**³⁷ (2.0 g, 7.3 mmol), boronate ester **46** (3.0 g, 11 mmol), potassium
51 carbonate (3.1 g, 22 mmol), Pd(dppf)Cl₂ (534 mg, 0.65 mmol), and 1,4-dioxane (100 mL) was
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3 stirred for 12 h at 110 °C under nitrogen. The solids were then filtered off. The resulting mixture
4 was concentrated under vacuum and the residue was purified by a silica gel column eluting with
5 ethyl acetate/petroleum ether (1:5) to afford the title compound (1.5 g, 53% yield) as a white
6 solid. LCMS, $m/z = 385$ $[M+H]^+$.
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12 **(4-(2-(trifluoromethyl)pyrimidin-5-yl)pyridin-2-yl)methanamine (62)**. A mixture of biaryl
13 **61** (1.50 g, 3.90 mmol), ethanol (100 mL), and hydrazine hydrate (3.91 g, 78.1 mmol) was stirred
14 for 12 h at 80 °C. The resulting solution was diluted with 100 mL of ethyl acetate and the solids
15 were then filtered off. The filtrate was concentrated under vacuum to afford the title compound
16 (850 mg, 86% yield) as a brown solid. LCMS, $m/z = 255$ $[M+H]^+$.
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22 **5-(2,5-dichloropyridin-4-yl)-2-(trifluoromethyl)pyrimidine (64)**. Into a 3 L 4-necked round-
23 bottom flask purged and maintained with an inert atmosphere of nitrogen was placed 2,5-
24 dichloro-4-iodopyridine **63** (120.0 g, 438.1 mmol), 1,4-dioxane (1.8 L), water (180 mL),
25 potassium carbonate (182 g, 1.32 mol), boronate ester **46** (132.6 g, 483.9 mmol), and
26 Pd(dppf)Cl₂ (6.0 g, 8.2 mmol). The resulting solution was stirred at 60 °C for 3 h, cooled to room
27 temperature, quenched by the addition of 4 L of water/ice, and extracted with ethyl acetate (2 x 2
28 L). The combined organic layers were washed with H₂O (1 L) and brine (1 L), dried over
29 anhydrous sodium sulfate and concentrated under vacuum. The residue was purified by silica gel
30 chromatography with ethyl acetate/petroleum ether (1:100) to afford 78 g of **64** as a white solid
31 (61% yield). LCMS, $m/z = 294$ $[M+H]^+$. ¹H NMR (400 MHz, CDCl₃) δ 9.05 (s, 2H), 8.59 (s,
32 1H), 7.40 (s, 1H).
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49 **tert-butyl ((5-chloro-4-(2-(trifluoromethyl)pyrimidin-5-yl)pyridin-2-yl)methyl)carbamate**
50 **(65)**. Into a 5 L 4-necked round-bottom flask purged and maintained with an inert atmosphere of
51 nitrogen was placed dichloropyridine **64** (75.0 g, 255 mmol), ethanol (2.25 L), water (450 mL),
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3 sodium carbonate (82.5 g, 778 mmol), potassium *N*-Boc-aminomethyltrifluoroborate (90.0 g,
4 0.380 mol), and Pd(PPh₃)₂Cl₂ (5.0 g, 7.1 mmol). The resulting solution was stirred at 85°C
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6 overnight, cooled to room temperature, concentrated under vacuum, and diluted with ethyl
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8 acetate (2 L). The organic layer was washed sequentially with water (1 L) and brine (1 L), dried
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10 over anhydrous sodium sulfate, and concentrated under vacuum. The residue was purified by
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12 silica gel chromatography using ethyl acetate/petroleum ether (1:10). The crude product was re-
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14 crystallized from 10:1 petroleum ether / ethyl acetate to afford 36 g of **65** as a yellow solid (36%
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16 yield). LCMS, *m/z* = 389 [M+H]⁺.
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21 **(5-chloro-4-(2-(trifluoromethyl)pyrimidin-5-yl)pyridin-2-yl)methanamine hydrochloride**
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23 **(66)**. A solution of biaryl **65** (35.0 g, 90.0 mmol) and dichloromethane (600 mL) was saturated
24
25 with hydrogen chloride (gas). The resulting solution was stirred at room temperature overnight.
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27 The resulting solid was collected by filtration and washed with DCM (2 L) to afford 25 g of **66**
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29 as an off-white solid (85% yield). LCMS, *m/z* = 289 [M+H]⁺. ¹H NMR (300MHz, DMSO-*d*₆) δ
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31 9.33-9.41 (s, 2H), 8.88-8.91 (s, 1H), 8.79 (s, 3H), 7.97-7.98 (s, 1H), 4.23-4.29 (m, 2H).
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36 **(4-bromo-5-fluoropyridin-2-yl)methyl methanesulfonate (68)**. To a 1 L 4-necked round-
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38 bottom flask purged and maintained with an inert atmosphere of nitrogen was placed (4-bromo-
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40 5-fluoropyridin-2-yl)methanol (**67**) (50.0 g, 243 mmol), dichloromethane (500 mL) and
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42 triethylamine (49.0 g, 484 mmol) followed by the addition of methanesulfonyl chloride (33.4 g,
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44 292 mmol) dropwise with stirring at 0 °C. The resulting solution was stirred at room temperature
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46 for 30 min, quenched by the addition of water/ice, and extracted with dichloromethane (2 x 500
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48 mL). The combined organic layers were washed with brine (500 mL), dried over anhydrous
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50 sodium sulfate and concentrated under vacuum to afford 65 g of **68** as a yellow solid (94%
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52 yield). LCMS, *m/z* = 284 [M+H]⁺.
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tert-butyl N-[(4-bromo-5-fluoropyridin-2-yl)methyl]-N-[(tert-butoxy)carbonyl]carbamate (69). To a 3 L 4-necked round-bottom flask purged and maintained with an inert atmosphere of nitrogen was placed tert-butyl N-[(tert-butoxy)carbonyl]carbamate (59.7 g, 275 mmol) and *N,N*-dimethylformamide (350 mL) followed by the addition of NaH (11 g, 60% in mineral oil) portionwise at 0 °C. The resulting solution was stirred at 0 °C for 2 h. To this was added a solution of mesylate **68** (65.0 g, 229 mmol) in *N,N*-dimethylformamide (300 mL) dropwise with stirring at 0 °C. The resulting solution was stirred at 60 °C for an additional 2 h, cooled to room temperature, quenched by the addition of 1.2 L of water/ice, and extracted with ethyl acetate (3 x 500 mL). The combined organic layers were washed with water (2 x 400 mL), brine (400 mL), dried over anhydrous sodium sulfate and concentrated under vacuum. The residue was purified by silica gel chromatography eluting with ethyl acetate/petroleum ether (1/19) to afford 60 g of **69** as a yellow solid (65% yield). LCMS, $m/z = 405$ [M+H]⁺.

tert-butyl N-[(tert-butoxy)carbonyl]-N-([5-fluoro-4-[2-(trifluoromethyl)pyrimidin-5-yl]pyridin-2-yl)methyl]carbamate (70). A 2 L 4-necked round-bottom flask under nitrogen was charged with fluoropyridine **69** (60.0 g, 148 mmol), 1,4-dioxane (900 mL), water (90 mL), potassium carbonate (61.3 g, 444 mmol), boronate ester **46** (60.8 g, 222 mmol) and Pd(dppf)Cl₂ (5.4 g, 7.4 mmol, 0.05 equiv). The resulting solution was stirred at 90 °C for 1 h, cooled to room temperature, and filtered. The filtrate was extracted with ethyl acetate (3 x 500 mL). The combined organic layers were washed with brine (500 mL), dried over anhydrous sodium sulfate and concentrated under vacuum. The residue was purified by silica gel chromatography eluting with ethyl acetate/petroleum ether (1/9) to afford 60 g of **70** as a white solid (86% yield). LCMS, $m/z = 473$ [M+H]⁺. ¹H NMR (300 MHz, DMSO-₆) δ 9.37 (2H, d, $J = 0.6$ Hz), 8.73 (1H, d, $J = 1.8$ Hz), 8.73 (1H, d, $J = 6.0$ Hz), 4.89 (s, 2H), 1.43 - 1.36 (m, 9H).

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3 **(5-fluoro-4-(2-(trifluoromethyl)pyrimidin-5-yl)pyridin-2-yl)methanamine hydrochloride**
4 **(71)**. Hydrogen chloride (gas) was bubbled into an ice-cooled solution of biaryl **70** (60 g, 127.00
5 mmol), methanol (600 mL), and dichloromethane (60 mL). The resulting saturated solution was
6 stirred at 0 °C for 2 h and concentrated under vacuum. The residue was rinsed with DCM and
7 Et₂O and the solids were collected by filtration. The filter cake was washed with Et₂O and dried
8 in an oven under reduced pressure to afford 24 g of **71** as a white solid (61% yield). LCMS, *m/z*
9 = 273 [M+H]⁺. ¹H NMR (300MHz, DMSO-*d*₆): δ 9.44 (s, 2H), 8.89 (s, 1H), 8.51~8.39 (s, 3H),
10 8.07~8.05 (d, *J* = 6.9Hz, 1H), 4.29~4.27 (d, *J* = 6 Hz, 2H).
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22 **2-((2-(2-(trifluoromethyl)pyrimidin-5-yl)pyridin-4-yl)methyl)isoindoline-1,3-dione (73)**. A
23 mixture of chloropyridine **72** (7.84 g, 28.8 mmol), boronate ester **46** (16.8 g, 61.3 mmol),
24 Pd(dppf)Cl₂ (1.00 g, 1.36 mmol), potassium carbonate (9.94 g, 72.0 mmol), 1,4-dioxane (350
25 mL), and water (20 mL) was stirred for 12 h at 80 °C under nitrogen. The solids were then
26 filtered off, diluted with ethyl acetate, washed with brine, and concentrated under vacuum. The
27 residue was purified by a silica gel column eluting with ethyl acetate/hexane (1/5) to afford the
28 title compound (6 g, 54% yield) as a gray solid. LCMS, *m/z* = 385 [M+H]⁺.
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38 **(2-(2-(trifluoromethyl)pyrimidin-5-yl)pyridin-4-yl)methanamine hydrochloride (74)**. A
39 mixture of biaryl **73** (3.2 g, 8.3 mmol), methanol (110 mL), and hydrazine hydrate (4.2 g, 84
40 mmol) was stirred for 12 h at 50 °C. The resulting mixture was concentrated under vacuum and
41 the residue was dissolved in 500 mL of ethyl acetate. The solids were filtered off and the filtrate
42 was concentrated under vacuum to afford the title compound (2.4 g) as a brown solid that was
43 used without further purification. LCMS, *m/z* = 255 [M+H]⁺.
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52 **(2,5-dichloropyridin-4-yl)methanol (76)**. To an ice-cooled solution of carboxylic acid **75**
53 (0.200 kg, 1.04 mol) and tetrahydrofuran (2 L) was added 1 M BH₃-THF (3.14 L, 3.14 mol)
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3 dropwise. The resulting solution was stirred at room temperature for 2 h, quenched by the
4 addition of water (2 L), extracted with ethyl acetate (3 x 1.5 L). The combined organic layers
5 were washed with water (2 x 1.5 L), brine (3 x 1.5 L), dried over anhydrous sodium sulfate and
6 concentrated under vacuum to afford 140 g of **76** as a white solid (75% yield). LCMS, $m/z = 178$
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12 $[M+H]^+$. 1H NMR (300 MHz, $CDCl_3$) δ 8.30 (s, 1H), 7.60 (t, $J = 1.0$ Hz, 1H), 4.81 (d, $J = 1.1$
13 Hz, 2H).
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17 **(2,5-dichloropyridin-4-yl)methyl methanesulfonate (77)**. To a 3 L 4-necked round-bottom
18 flask charged with alcohol **76** (0.100 kg, 562 mmol), dichloromethane (1.4 L) and triethylamine
19 (172 g, 1.70 mol) was added methanesulfonyl chloride (77.3 g, 678 mmol) dropwise with stirring
20 at 0 °C. The resulting solution was stirred at room temperature for 1 h and then quenched by the
21 addition of water (500 mL). The organic layer was dried over anhydrous sodium sulfate and
22 concentrated under vacuum to afford 129 g of **77** as a yellow oil (90% yield). LCMS, $m/z = 256$
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31 $[M+H]^+$.
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33 ***N*-[(*tert*-butoxy)carbonyl]-*N*-[(2,5-dichloropyridin-4-yl)methyl]carbamate (78)**. To a 5 L
34 4-necked round-bottom flask under nitrogen charged with *tert*-butyl *N*-[(*tert*-
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tert-butoxy)carbonyl]carbamate (104.3 g, 480.07 mmol) and *N,N*-dimethylformamide (1.2 L) was
added NaH (21.3 g, 576.87 mmol, 65% in mineral oil) in several batches at room temperature.
The mixture was then stirred at room temperature for 4 h. To this mixture was added a solution
of mesylate **77** (129 g, 504 mmol) in *N,N*-dimethylformamide (200 mL) dropwise with stirring.
The resulting solution was stirred at 50 °C for 2 h, quenched by the addition of 2 kg of ice, and
extracted with ethyl acetate (2 x 1.5 L). The combined organic layers were washed with brine (5
x 800 mL), dried over anhydrous sodium sulfate and concentrated under vacuum. The residue
was purified by silica gel chromatography eluting with ethyl acetate/petroleum ether (1:20). The

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3 crude product was re-crystallized from petroleum ether to afford 80 g of **78** as a white solid (42%
4 yield). LCMS, $m/z = 377$ $[M+H]^+$. 1H NMR (300 MHz, DMSO- d_6) δ 8.52 (s, 1H), 7.15 (s, 1H),
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6 4.79 (s, 2H), 1.40 (s, 18H).
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10 **tert-butyl N-[(tert-butoxy) carbonyl]-N-([5-chloro-2-[2-(trifluoromethyl)pyrimidin-5-**
11 **yl]pyridin-4-yl)methyl]carbamate (79)**. A 2 L 4-necked round-bottom flask under nitrogen was
12 charged with dichloropyridine **78** (70.0 g, 186 mmol), boronate ester **46** (76.5 g, 279 mmol), 1,4-
13 dioxane (700 mL), CS_2CO_3 (71.6 g, 2.00 equiv), water (200 mL) and $(PPh_3)_4Pd$ (5.4 g). The
14 resulting solution was refluxed for 5 h, cooled to room temperature, diluted with H_2O (500 mL),
15 and extracted with ethyl acetate (3 x 500 mL). The combined organic layer was dried over
16 anhydrous sodium sulfate and concentrated under vacuum. The residue was purified by silica gel
17 chromatography eluting with ethyl acetate/petroleum ether (1:10) to afford 45 g of **79** as a white
18 solid (50% yield). LCMS, $m/z = 489$ $[M+H+CH_3CN]^+$. 1H NMR (300 MHz, DMSO- d_6) δ 9.56
19 (s, 2H), 8.84 (s, 1H), 7.96 (s, 1H), 4.90(s, 2H), 1.40 (m, 18H).
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33 **(5-chloro-2-(2-(trifluoromethyl)pyrimidin-5-yl)pyridin-4-yl)methanamine hydrochloride**
34 **(80)**. Hydrogen chloride (gas) was bubbled into a solution of biaryl **79** (42.0 g, 85.9 mmol) in
35 methanol (600 mL). The resulting saturated solution was stirred at room temperature for 3 h,
36 concentrated under vacuum, and diluted with ether (300 mL). The solids were collected by
37 filtration and rinsed with ether (200 mL) to afford 22.4 g of **80** as a white solid (80%yield).
38 LCMS, $m/z = 330$ $[M+H]^+$. 1H NMR (300 MHz, DMSO- d_6): δ 9.73 (s, 2H), 8.93 (s, 3H), 8.91 (s,
39 1H), 8.81-8.78 (d, $J = 9.0Hz$, 1H), 4.30 (s, 2H).
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49 **(5-fluoro-2-(2-(trifluoromethyl)pyrimidin-5-yl)pyridin-4-yl)methanol (82)**. A 2 L 4-necked
50 round-bottom flask under nitrogen was charged with alcohol **81** (40.0 g, 194 mmol), 1,4-dioxane
51 (600 mL), water (60 mL), K_2CO_3 (80.4 g, 578 mmol), boronate ester **46** (64.0 g, 233 mmol), and
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3 Pd(dppf)Cl₂ (14.2 g, 19.4 mmol). The resulting solution was stirred at 80 °C overnight and then
4 concentrated under vacuum. The residue was purified by silica gel chromatography with ethyl
5 acetate/petroleum ether (0:100-40:60). The resulting product was washed with hexane (200 mL)
6 and dried to afford 50 g of **82** as a white solid (94% yield). LCMS, *m/z* = 274 [M+H]⁺.
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12 **2-Fluoro-3-((3-(2-(hydroxymethyl)cyclopropyl)-2-methyl-5-oxo-5H-thiazolo[3,2-**
13 **a]pyrimidin-7-yl)methyl)benzotrile (83)**. To an ice-cooled solution of alcohol **82** (50.0 g, 183
14 mmol), dichloromethane (500 mL), and triethylamine (55.5 g, 548 mmol) was added
15 methanesulfonyl chloride (31.4 g, 274.11 mmol) dropwise. The resulting solution was stirred at
16 room temperature for 1 h, quenched by the addition of water (500 mL), and extracted with
17 dichloromethane (3 x 170 mL). The combined organic layers were washed with brine (3 x 300
18 mL), dried over anhydrous sodium sulfate, and concentrated under vacuum. The resulting
19 residue was washed with methanol (1 L) and dried to afford 35 g of **83** as a white solid (54%
20 yield). LCMS, *m/z* = 352 [M+H]⁺.
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33 **(5-fluoro-2-(2-(trifluoromethyl)pyrimidin-5-yl)pyridin-4-yl)methanamine (84)**. Into a 2 L
34 pressure tank reactor was placed mesylate **83** (35 g, 0.10 mol) and 7 M ammonia in methanol
35 (700 mL, 4.9 mol). The resulting solution was stirred at 40 °C for 6 h, concentrated under
36 vacuum, washed with ethyl acetate (400 mL), and filtered. The filter cake was dried in an oven
37 under reduced pressure to afford 20.1 g of **84** as a white solid (74% yield). LCMS, *m/z* = 273
38 [M+H]⁺. ¹H NMR (300MHz, CD₃OD): δ 9.59 (s, 2H), 8.78-8.77 (d, 1H, *J* = 1.2 Hz), 8.33-8.32
39 (d, 1H, *J* = 5.7 Hz), 4.39 (s, 2H).
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49 **methyl 2-bromo-3,5-difluoroisonicotinate (86)**. A mixture of 2-bromo-3,5-difluoro-4-
50 iodopyridine (**85**) (1.8 g, 5.6 mmol), Pd(dppf)Cl₂ (0.40 g, 0.54 mmol), MeOH (40 mL), and
51 triethylamine (1.8 g, 18 mmol) was stirred overnight at 50°C under carbon monoxide. The solids
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3 were filtered off and the filtrate was concentrated under vacuum. The residue was purified by a
4 silica gel column eluting with ethyl acetate/petroleum ether (1:20) to afford the title compound
5 (700 mg, 49% yield) as green oil. LCMS, $m/z = 252$ $[M+H]^+$.
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9
10 **methyl 3,5-difluoro-2-(2-(trifluoromethyl)pyrimidin-5-yl)isonicotinate (87)**. A mixture of
11 pyridine **86** (0.750 g, 2.97 mmol), Pd(dppf)Cl₂ (218 mg, 0.29 mmol), boronate ester **46** (1.24 g,
12 4.52 mmol), potassium carbonate (1.24 g, 8.97 mmol), and 1,4-dioxane (30 mL) was stirred
13 overnight at 90 °C under nitrogen. The solids were filtered off and the filtrate was concentrated
14 under vacuum. The residue was purified by a silica gel column eluting with ethyl
15 acetate/petroleum ether (1:10) to afford the title compound (725 mg, 76% yield) as a white solid.
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17 LCMS, $m/z = 320$ $[M+H]^+$.
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22 **(3,5-difluoro-2-(2-(trifluoromethyl)pyrimidin-5-yl)pyridin-4-yl)methanol (88)**. DIBAL
23 (440 mg, 3.1 mmol) in hexane (3 mL) was added dropwise to a solution of biaryl **87** (331 mg,
24 1.03 mmol) in dichloromethane (10 mL) at -78 °C under nitrogen. The resulting solution was
25 stirred for 1 h, quenched with methanol, and concentrated under vacuum. The residue was
26 purified by a silica gel column eluting with ethyl acetate/petroleum ether (1:3) to afford the title
27 compound (140 mg, 46% yield) as green oil. LCMS, $m/z = 292$ $[M+H]^+$.
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32 **2-((3,5-difluoro-2-(2-(trifluoromethyl)pyrimidin-5-yl)pyridin-4-yl)methyl)isoindoline-1,3-**
33 **dione (89)**. DIAD (521 mg, 2.57 mmol) was added dropwise to a solution of alcohol **88** (250 mg,
34 0.85 mmol), 2,3-dihydro-1H-isoindole-1,3-dione (253 mg, 1.72 mmol), PPh₃ (450 mg, 1.7
35 mmol), and tetrahydrofuran (20 mL) at room temperature. The resulting solution was stirred for
36 overnight at room temperature. The resulting mixture was concentrated under vacuum. The
37 residue was purified by a silica gel column eluting with ethyl acetate/petroleum ether (1:2) to
38 afford the title compound (224 mg, 62% yield) as a white solid. LCMS, $m/z = 421$ $[M+H]^+$.
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3 **(3,5-difluoro-2-(2-(trifluoromethyl)pyrimidin-5-yl)pyridin-4-yl)methanamine (90).** A
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5 mixture of phthalimide **89** (224 mg, 0.53 mmol), methanol (5 mL), and hydrazine hydrate (332
6
7 mg, 6.63 mmol) was stirred overnight at room temperature. The resulting mixture was
8
9 concentrated under vacuum and the resulting residue was purified by a silica gel column eluting
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11 with dichloromethane/methanol (20:1) to afford the title compound (150 mg, 97% yield) as green
12
13 oil. LCMS, $m/z = 291$ $[M+H]^+$.
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17 **(2S,3R,5S)-tert-butyl 5-carbamoyl-3-fluoro-2-methylpyrrolidine-1-carboxylate (91).**
18
19 Ammonium carbonate (6.1 g, 63 mmol) was added portionwise into a mixture of carboxylic acid
20
21 **29** (12.0 g, 48.5 mmol), dioxane (240 mL), pyridine (2.4 mL), and di-tert-butyl dicarbonate (13.8
22
23 g, 63.2 mmol) at 0°C under nitrogen. The resulting solution was then stirred for 12 h at room
24
25 temperature. The resulting mixture was concentrated under vacuum, diluted with ethyl acetate,
26
27 washed with citric acid (20% aqueous) and brine, dried over anhydrous sodium sulfate, and
28
29 concentrated under vacuum. This provided 13 g (crude) of **91** as a light yellow solid, which was
30
31 carried forward without further purification. LCMS, $m/z = 247$ $[M+H]^+$.
32
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35 **(2S,4R,5S)-4-fluoro-5-methylpyrrolidine-2-carboxamide (92).** A mixture of crude amide **91**
36
37 from the previous step (13 g) and 1.9 M HCl in ethyl acetate (100 mL) was stirred for 12 h at
38
39 room temperature. The resulting mixture was concentrated under vacuum to afford 10 g (crude)
40
41 of **92** as a light yellow solid, which was carried forward without further purification. LCMS, m/z
42
43 = 147 $[M+H]^+$.
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47 **(2S,4R,5S)-4-fluoro-1-((4-fluorophenyl)sulfonyl)-5-methylpyrrolidine-2-carboxamide**
48
49 **(93).** A mixture of pyrrolidine amide **92** (10.1 g, 55.3 mmol), dichloromethane (100 mL),
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51 triethylamine (16.7 g, 165 mmol), and 4-fluorobenzene-1-sulfonyl chloride (16.1 g, 82.7 mmol)
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53 was stirred for 12 h at room temperature. The resulting solution was diluted with water,
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3 extracted with ethyl acetate, washed with saturated solution of ammonium chloride and brine,
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5 dried over sodium sulfate, and concentrated under vacuum. The residue was purified by silica
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7 gel chromatography eluting with ethyl acetate/petroleum ether (99/1) to afford 10 g of **93** as a
8
9 white solid (68% yield, over 3 steps). LCMS, $m/z = 305$ $[M+H]^+$. 1H NMR (300 MHz, $CDCl_3$) δ
10
11 7.99 – 7.82 (m, 2H), 7.32 – 7.15 (m, 2H), 6.89 – 6.84 (m, 1H), 5.81 (s, 1H), 4.79–4.58 (m, $J = 63$
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13 Hz, 1H), 4.22 – 4.01 (m, 2H), 2.56 – 2.23 (m, 2H), 1.33 (d, $J = 7.0$ Hz, 3H).
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17 **(2S,4R,5S)-4-fluoro-1-((4-fluorophenyl)sulfonyl)-5-methyl-N-**
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19 **((methylthio)methyl)pyrrolidine-2-carboxamide (94)**. A mixture of pyrrolidine sulfonamide
20
21 **93** (10.0 g, 32.9 mmol), dichloromethane (10 mL), trifluoroacetic acid (10 mL), and
22
23 chloro(methylsulfonyl)methane (10.0 mL, 119 mmol) was stirred for 2 days at room
24
25 temperature. The resulting solution was diluted with water, extracted with ethyl acetate, dried
26
27 over anhydrous sodium sulfate, and concentrated under vacuum. The residue was purified by
28
29 silica gel chromatography eluting with ethyl acetate/petroleum ether (1/2) to afford 5.6 g of **94** as
30
31 a light yellow oil (47% yield). LCMS, $m/z = 365$ $[M+H]^+$. 1H NMR (300 MHz, $DMSO-d_6$) δ 8.76
32
33 (t, $J = 6.2$ Hz, 1H), 7.97 (ddd, $J = 8.9, 5.2, 2.6$ Hz, 2H), 7.63 – 7.26 (m, 2H), 4.85 (dd, $J = 51.6,$
34
35 2.9 Hz, 1H), 4.38 – 4.08 (m, 4H), 2.38 – 2.17 (m, 2H), 2.10 (s, 3H), 1.17 (dd, $J = 9.1, 7.1$
36
37 Hz, 3H).
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42 **(2S,4R,5S)-4-fluoro-N-((5-fluoro-2-(2-(trifluoromethyl)pyrimidin-5-yl)pyridin-4-**
43
44 **yl)methyl)-1-((4-fluorophenyl)sulfonyl)-5-methyl-N-((methylthio)methyl)pyrrolidine-2-**
45
46 **carboxamide (95)**. Sodium hydride (1.02 g, 60% in mineral oil) was added portionwise into a
47
48 mixture of pyrrolidine sulfonamide **94** (3.1 g, 8.5 mmol) and tetrahydrofuran (600 mL) at -5 °C
49
50 under nitrogen. The resulting mixture was stirred for 1 hour at -5 °C. Sodium iodide (1.7 g, 11
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52 mmol) was added portionwise at -5 °C followed by mesylate **83** (3.9 g, 11 mmol) in 100 mL of
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3 THF was added dropwise with stirring at -5 °C over 90 min. The resulting solution was allowed
4
5 to stir for an additional 12 h at room temperature. The reaction was quenched by water,
6
7 extracted with ethyl acetate, washed with brine, dried over anhydrous sodium sulfate, and
8
9 concentrated under vacuum. The residue was purified by silica gel chromatography eluting with
10
11 ethyl acetate/petroleum ether (1/4). The product was purified further by Prep-HPLC with the
12
13 following conditions: Column, C18 silica gel; mobile phase, CH₃CN/water=5% increasing to
14
15 CH₃CN/water=95% within 30 min; Detector, UV 254 nm. This provided 300 mg of **95** as a
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17 white solid (6% yield). LCMS, $m/z = 620 [M+H]^+$.
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22 **Molecular Modeling**

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25 *Conformation generation.* The starting conformations for all modeled compounds were
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27 generated from 2D sdf file using Ligprep followed by minimization using MacroModel with
28
29 OPLS_2005 forcefield.²⁷ The lowest energy conformation of each compound was used as a
30
31 starting point for conformational search using MacroModel's large-scale low-mode sampling
32
33 and/or mixed torsional/large-scale low-mode sampling to generate a diverse set of
34
35 conformations. The energy window for saved conformations is 5.02 kcal/mol and conformers
36
37 with maximum atom deviation less than 0.5 Å were considered redundant and eliminated. The
38
39 maximum number of structures to be saved was set to 125 and all conformations were minimized
40
41 for a maximum of 2000 steps using Truncated Newton Conjugate Gradient (TNCG) method with
42
43 implicit water solvent model.
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48 *Quantum mechanical calculations.* All minimized conformations from the prior
49
50 conformational search were optimized using Jaguar (Version 7.8) where DFT theory with
51
52 B3LYP functional and 6-31 G**+ basis set was used.²⁷ The optimizations were carried out using
53
54 the Poisson-Boltzmann Solvation model (PBF) for water solvent. Relative solution- and gas-
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3 phase energies were calculated for the optimized conformations using Jaguar energy converter
4 script. The lowest energy conformation for each compound was selected to be the global
5
6 minimum conformation.
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11 **Pharmaceutical and Biological Assay Protocols:**

12
13 **Calcium influx dose-response assays.** The calcium influx assay was performed as reported
14 previously.³⁸ In the assay, a 384 -well format assay was developed using a fluorometric imaging
15
16 plate reader (FLIPR) and Calcium assay kit (BD#640178). Cells stably expressing human
17
18 TRPA1 or rat TRPA1 were loaded with calcium dye, then incubated with varying concentration
19
20 of compounds for 20 min. EC80 concentration of Cinnamaldehyde (75 mM for human and 45
21
22 mM for at TRPA1) was used to activate the channels and induced Ca²⁺ influx, as reflected by
23
24 increase in fluorescence signals. The peak responses were used to derive concentration-
25
26 dependent block.
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32 **Rat AITC target engagement assay.** Experimental procedures involving animals were
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34 approved by Genentech's Institutional Animal Care and Use Committee and conducted in
35
36 accordance with the National Institutes of Health Guide for the Care and Use of Laboratory
37
38 Animals and the recommendations of the International Association for the Study of Pain. Adult
39
40 male Sprague-Dawley rats (300-350 g; Charles River, Hollister) were pair-housed and
41
42 maintained on a 14 h / 10 h light/dark cycle in a temperature-controlled environment with free
43
44 access to food and water. Rats were dosed by oral gavage with vehicle (15% dimethylsulfoxide
45
46 (DMSO), 85% methylcellulose/Tween (MCT); 5 ml kg⁻¹) or compound and returned to their
47
48 home cages. Three hours after dosing, each rat was lightly restrained and an intraplantar injection
49
50 of 25 µl of a 0.1% solution of allylisothiocyanate (AITC) in mineral oil was made to one hind
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55 paw using a 30-gauge disposable needle attached to a luer-tipped Hamilton syringe. The rat was
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2
3 then immediately placed into a plexiglass test chamber on a room temperature glass surface and
4
5 its behavior videotaped for 5 minutes. The responses of up to 6 rats at a time were recorded for
6
7 offline scoring of nocifensive behavior, defined as licking, lifting, shaking, or guarding the
8
9 injected paw. Video scoring was performed by an experimenter who was blind to dose group.
10
11 Immediately after the video session, the rats were removed from the test chambers and
12
13 euthanized by CO₂ inhalation followed by blood collection via cardiac puncture. Whole blood
14
15 samples were collected into ethylenediaminetetraacetic acid (EDTA)-containing test tubes and
16
17 centrifuged at 20,000 x g for 2 minutes, and the plasma supernatant was isolated for analysis of
18
19 test compound concentration.
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24 25 ASSOCIATED CONTENT

26
27 **Supporting Information** X-ray crystal structure report for compound **1** and **20** are provided as
28
29 supporting information. Molecular formula strings are also available. This material is available
30
31 free of charge via the Internet at <http://pubs.acs.org>.
32
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44

45 46 ACKNOWLEDGMENTS

47
48 We thank the Genentech Analytical, Purification, DMPK, *In vivo* Studies Group, and Safety
49
50 Assessment colleagues for their contributions. Special thanks to Antonio DePasquale (UC
51
52 Berkeley, X-Ray Crystallographic Facility) for help determining X-ray structures of **1** and **20**.
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ABBREVIATIONS USED

TRP, Transient receptor potential; TRPA1, Transient receptor potential ankyrin 1; TRPV1, Transient receptor potential vanilloid subfamily, member 1; TRPM8, Transient receptor potential cation channel subfamily M member 8; AITC, allylisothiocyanate; SAR, structure-activity relationship; FLIPR, fluorometric imaging plate reader; LogD_{7.4}, measured distribution coefficient between octanol and water at pH 7.4; QM, Quantum Mechanics; PBF, Poisson Boltzmann Finite; RHS, right hand side; LLE, lipophilic ligand efficiency; LM, liver microsome; hERG, human Ether-à-go-go-Related Gene; HATU, 1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium3-oxid hexafluorophosphate; THF, tetrahydrofuran; MeOH, methanol; DMSO, dimethylsulfoxide; MDCK, Madin-Darby canine kidney cells; PPB, plasma protein binding; AUC, area under the curve; MCT, methylcellulose/tween; TPSA, topographical polar surface area; CHO, Chinese hamster ovary.

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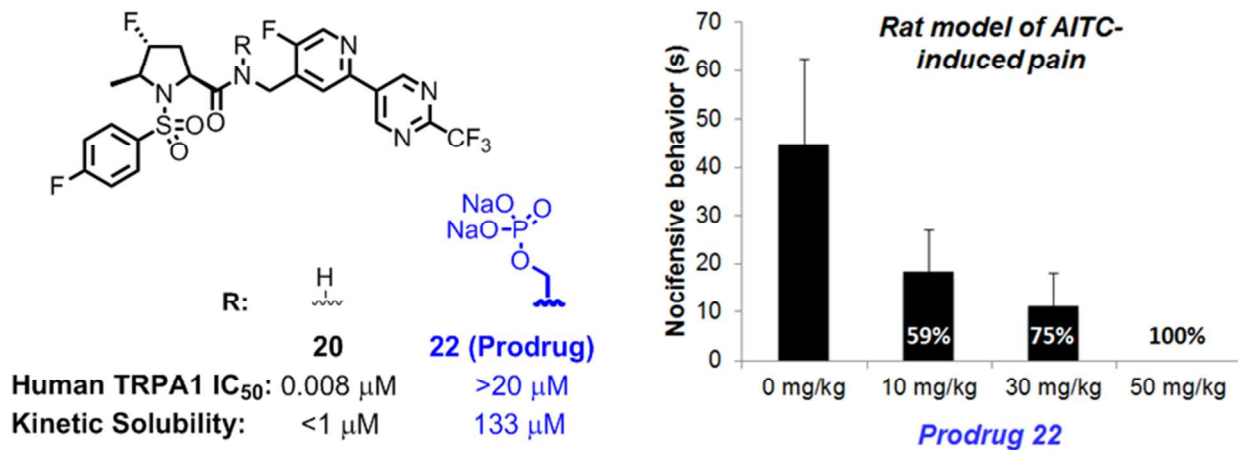
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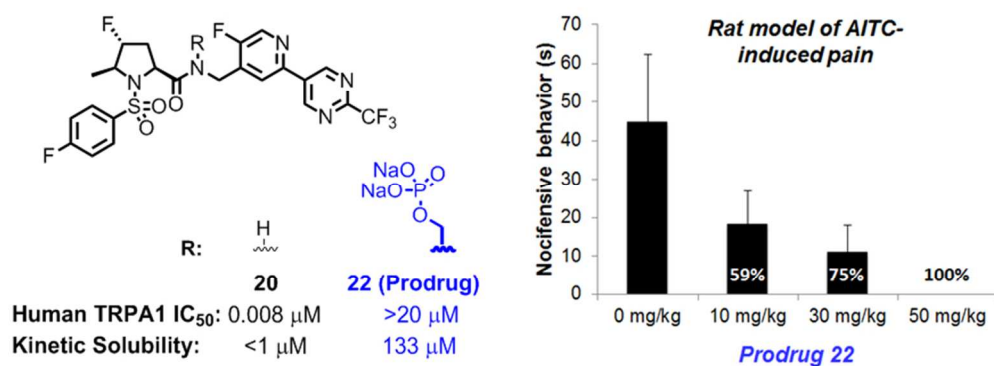
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