

Discovery of a Potent and Selective CCR4 Antagonist that Inhibits Treg Trafficking into the Tumor Microenvironment

Jeffrey James Jackson, John Michael Ketcham, Ashkaan Younai, Betty Abraham, Berenger Biannic, Hilary Plake Beck, Minna Hue Thanh Bui, David Chian, Gene Cutler, Raymond Diokno, Dennis X Hu, Scott Jacobson, Emily Karbarz, Paul D Kassner, Lisa Marshall, Jenny McKinnell, Cesar Meleza, Abood Okal, Deepa Pookot, Maureen Kay Reilly, Omar Robles, Hunter Paul Shunatona, Oezcan Talay, James Ross Walker, Angela Wadsworth, David J. Wustrow, and Mikhail Zibinsky

J. Med. Chem., **Just Accepted Manuscript** • DOI: 10.1021/acs.jmedchem.9b00506 • Publication Date (Web): 17 Jun 2019

Downloaded from <http://pubs.acs.org> on June 17, 2019

Just Accepted

“Just Accepted” manuscripts have been peer-reviewed and accepted for publication. They are posted online prior to technical editing, formatting for publication and author proofing. The American Chemical Society provides “Just Accepted” as a service to the research community to expedite the dissemination of scientific material as soon as possible after acceptance. “Just Accepted” manuscripts appear in full in PDF format accompanied by an HTML abstract. “Just Accepted” manuscripts have been fully peer reviewed, but should not be considered the official version of record. They are citable by the Digital Object Identifier (DOI®). “Just Accepted” is an optional service offered to authors. Therefore, the “Just Accepted” Web site may not include all articles that will be published in the journal. After a manuscript is technically edited and formatted, it will be removed from the “Just Accepted” Web site and published as an ASAP article. Note that technical editing may introduce minor changes to the manuscript text and/or graphics which could affect content, and all legal disclaimers and ethical guidelines that apply to the journal pertain. ACS cannot be held responsible for errors or consequences arising from the use of information contained in these “Just Accepted” manuscripts.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46

Discovery of a Potent and Selective CCR4 Antagonist that Inhibits T_{reg} Trafficking into the Tumor Microenvironment

47
48
49
50
51
52
53
54
55
56
57
58
59
60

*Jeffrey J. Jackson[†], John M. Ketcham^{†§}, Ashkaan Younai, Betty Abraham, Berenger Biannic[‡],
Hilary P. Beck[⊥], Minna H. T. Bui[#], David Chian, Gene Cutler, Raymond Diokno, Dennis X. Hu,
Scott Jacobson, Emily Karbarz, Paul D. Kassner, Lisa Marshall, Jenny McKinnell^{||}, Cesar
Meleza[∇], Abood Okal[∩], Deepa Pookot, Maureen K. Reilly[≡], Omar Robles, Hunter P. Shunatona^{||},
Oezcan Talay, James R. Walker^Δ, Angela Wadsworth, David J. Wustrow^{*}, Mikhail Zibinsky^{*}*

RAPT Therapeutics, 561 Eccles Avenue, South San Francisco, CA 94080

ABSTRACT

Recruitment of suppressive CD4⁺ FOXP3⁺ regulatory T cells (T_{reg}) to the tumor
microenvironment (TME) has the potential to weaken the anti-tumor response in

1
2
3 patients receiving treatment with immuno-oncology (IO) agents. Human T_{reg} express
4
5
6
7 CCR4 and can be recruited to the TME through the CC chemokine ligands CCL17 and
8
9
10 CCL22. In some cancers, T_{reg} accumulation correlates with poor patient prognosis.
11
12
13
14 Preclinical data suggests that preventing the recruitment of T_{reg} and increasing the
15
16
17 population of activated effector T cells (T_{eff}) in the TME can potentiate anti-tumor
18
19
20 immune responses. We have developed a novel series of potent, orally bioavailable
21
22
23 small molecule antagonists of CCR4. From this series, several compounds exhibited
24
25
26
27 high potency in distinct functional assays in addition to good in vitro and in vivo ADME
28
29
30 properties. The design, synthesis, and SAR of this series and confirmation of its in vivo
31
32
33 activity is reported.
34
35
36
37
38
39

40 INTRODUCTION

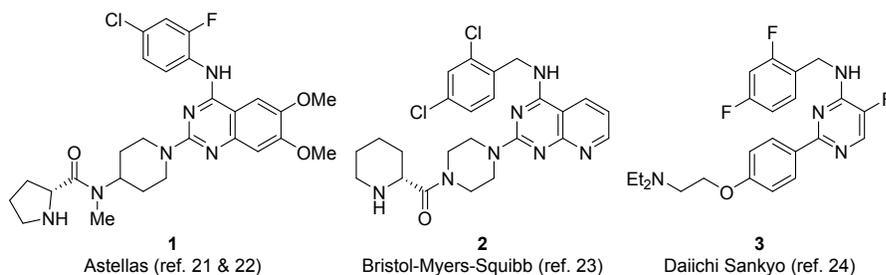
41
42
43
44 CC chemokine receptor 4 (CCR4) is a seven-transmembrane G protein-coupled
45
46
47 receptor (GPCR) that is known to play a dominant role in the recruitment of highly
48
49
50 immunosuppressive $CD4^+$, $CD25^+$, and $FOXP3^+$ regulatory T cells (T_{reg}) into the tumor
51
52
53 microenvironment (TME).¹⁻² Within the TME, dendritic cells and macrophages produce
54
55
56
57
58
59
60

1
2
3 the cognate ligands for CCR4, CCL17 and CCL22.³⁻⁴ This chemokine gradient attracts
4
5
6
7 T_{reg} into the TME,⁵ where they accumulate and suppress the function of CD8⁺ effector T
8
9
10 cells (T_{eff}), which would otherwise act to fight the tumor.⁶ Several studies have shown
11
12
13 that accumulation of T_{reg} in the TME and increased levels of CCL22 can lead to poor
14
15
16
17 patient prognosis.⁷⁻¹¹ Thus, the antagonism of CCR4 leading to the inhibition of this T_{reg}
18
19
20 trafficking pathway makes CCR4 an attractive target for the development of an immuno-
21
22
23
24 oncology (IO) therapy.¹²
25
26
27
28

29 The CCR4 receptor also plays a role in the recruitment of T helper type 2 cell
30
31
32 (Th2) subsets for autoimmune disorders such as asthma, allergic rhinitis, and atopic
33
34
35 dermatitis.^{13,14} To probe the role of CCR4 receptor antagonists in inflammatory
36
37
38
39 disorders, several attempts have been made to design potent small molecule
40
41
42
43 antagonists of CCR4,^{15,16} leading to two series differing in their proposed binding
44
45
46 mode.¹⁷⁻²⁰ Class I antagonists have been shown to bind to an extracellular portion of the
47
48
49 GPCR (1-3,²¹⁻²⁴ Figure 1), while Class II antagonists (4-6,²⁵⁻²⁷ Figure 1) bind
50
51
52
53 intracellularly.¹⁹ In general, Class I antagonists contain a heteroaromatic ring with a
54
55
56
57
58
59
60

lipophilic arene, and a side chain containing a basic amine. Class II antagonists tend to be sulfonamides that are flanked by a heteroaromatic ring and a more lipophilic arene.

Class I Antagonists



Class II Antagonists

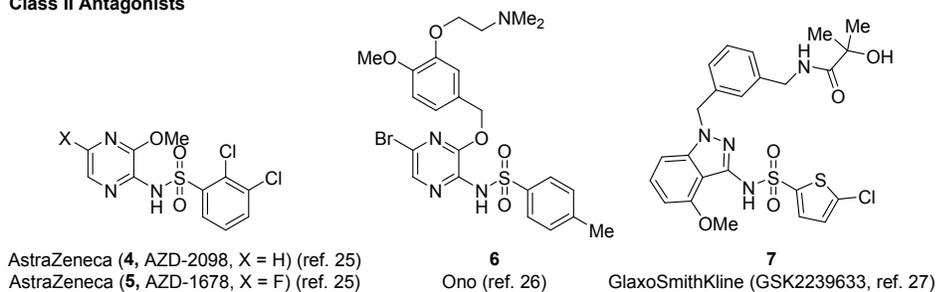


Figure 1. Representative CCR4 Antagonists

The most prominent of these antagonists is a Class II antagonist GSK2239633 (**6**), developed by GlaxoSmithKline.²⁷ Mentioning asthma as a possible therapeutic indication, GSK2239633 was evaluated in a Phase I clinical trial in healthy volunteers that ended in 2011.²⁸ In this study, the compound suffered low target engagement and low exposure in the blood which prevented further development. AstraZeneca has also reported two Class II antagonists from their CCR4 program, identified as preclinical

1
2
3 candidates AZD-2098 and AZD-1678 (**4** and **5**).²⁵ While AZD-2098 was licensed to
4
5
6
7 Cancer Research UK as a potential therapy for kidney cancer in 2013,²⁹ no further
8
9
10 development of this compound has been disclosed.

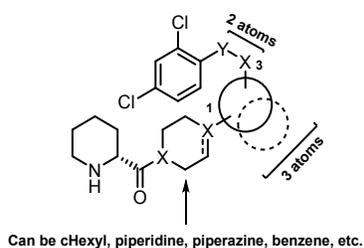
11
12
13
14 More recently, a CCR4-targeted cell-depleting monoclonal antibody,
15
16
17 mogamulizumab (KY-0761, Kyowa Hakko Kirin Co., Ltd.) has received approval from
18
19
20 the FDA to treat patients with two subtypes of relapsed Cutaneous T-Cell Lymphoma
21
22
23 (CTCL).³⁰ However, treatment has shown a risk of serious side effects such as
24
25
26
27 dermatologic toxicity, autoimmune issues, and complications from stem cell treatment
28
29
30 prior to mogamulizumab administration.³¹

31
32
33
34
35 While targeting CCR4 via an antibody-dependent cell mediated cytotoxicity
36
37
38 (ADCC) mechanism has shown clinical efficacy, the mechanism of action is much
39
40
41 different compared to targeting CCR4 with a small molecule antagonist. Depletion of
42
43
44 T_{reg} that serve other essential functions could cause a severe autoimmune reaction
45
46
47
48 leading to further complications.³²⁻³³ However, simply inhibiting T_{reg} trafficking into the
49
50
51
52 TME via a small molecule antagonist may lead to tumor killing without affecting other
53
54
55
56 vital immune cell functions.

1
2
3
4 To this end, we have designed novel CCR4 antagonists and evaluated their
5
6
7 ability to inhibit T_{reg} migration into the tumor microenvironment. Herein, we highlight the
8
9
10 SAR of a series of novel CCR4 antagonists that demonstrate best-in-class potency and
11
12
13
14 pharmacokinetic properties.
15

16 17 RESULTS AND DISCUSSION 18

19
20
21 Structure-Activity Relationships. Upon reviewing the literature of known Class I
22
23
24 antagonists, a pharmacophore map was built to aide in the design of a new class of
25
26
27
28 CCR4 antagonists (Figure 2).¹⁷ In general, these antagonists are comprised of a
29
30
31 heteroarene core structure with a side chain containing a basic nitrogen on the left-hand
32
33
34
35 portion and a lipophilic arene on the northern end. The lipophilic arene and basic amine
36
37
38 side chain are attached to the core via a 1,3-relationship.
39
40



50
51 **Figure 2.** Pharmacophore Map of Class I CCR4 Antagonists
52
53
54
55
56
57
58
59
60

1
2
3
4 To assess potency of the compounds, they were first put into a calcium flux
5
6
7 assay. Calcium flux is a robust, high-throughput functional assay that we utilized to
8
9
10 rapidly triage compounds for in vitro cellular migration mediated by chemokine
11
12
13 receptors.^{34,35} Based on this pharmacophore model, a series of amides closely related
14
15
16 to **8** were made which were found to inhibit calcium flux in the double-digit nM range,
17
18
19 however these compounds suffered from low bioavailability in rats (Figure 3).³⁶ Upon
20
21
22 surveying several different cores, a novel pyrazolopyrazine, compound **9**, was
23
24
25
26 discovered that was within 5-fold potency of the parent compound **8**, as a racemic
27
28
29
30
31 mixture. Additionally, this core increased the bioavailability in rats by more than 3-fold.
32
33
34 To broaden the SAR of these antagonists, various piperidinyll-containing side chains
35
36
37 were appended onto the left-hand side of the molecule. Replacing the amide side chain
38
39
40
41 with a piperidinyll-piperidine gave **10**, which resulted in a 10-fold shift in potency. To
42
43
44 create more conformational restriction about the 4-piperidinyll-piperidine side chain, a
45
46
47 methyl group was installed in the 3-position of the piperidine to afford **11**. Fortunately,
48
49
50
51
52 incorporation of the 3'-methyl-1,4'-bipiperidine was equipotent to the amide linkage
53
54
55
56
57
58
59
60

found in compound to **9**. From this result, we began focusing our early stage efforts on evaluating the SAR of these substituted piperidinyl-piperidine side chains.

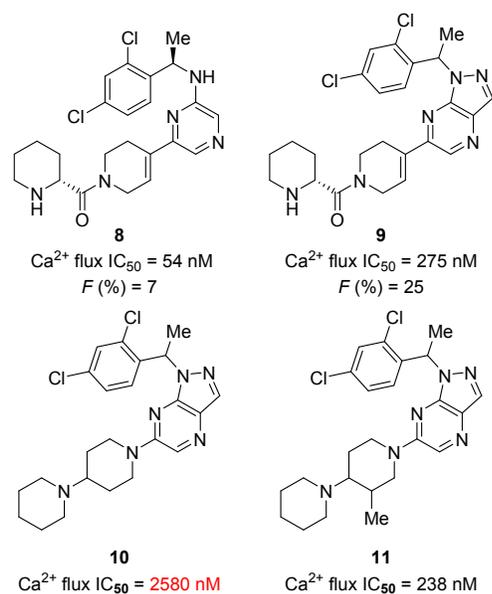
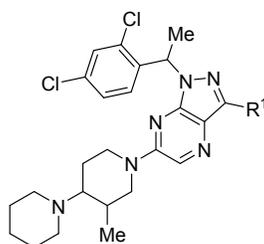


Figure 3. Discovery of a Pyrazolopyrazine Core and 3'-Methyl-1,4'-Bipiperidine Side Chain

Substitution on the C3 position of the pyrazolopyrazine core was carefully examined while leaving the left hand 3'-methyl-1,4'-bipiperidine intact. Replacing the C3-H substituent in **11** with more polar functional groups to give amide **12** and nitrile **13** resulted in compounds that were equipotent, but also suffered from extremely high hepatic clearance and low AUC_∞ when dosed IV in rats. Installation of the more lipophilic Me (**14**) and CF₃ (**15**) groups dramatically improved the IV clearance in rats, however

the C3-CF₃ was 8-fold less potent. Consequently, our SAR focused on developing new side chains to append onto the C3-methyl pyrazolopyrazine core.

Table 1. Evaluation of the C3 Position of the Pyrazolopyrazine Core



Compound d	R ¹	Ca ²⁺ flux IC ₅₀ (nM) ^a	rat IV CL (mL/min/kg) ^b	rat IV AUC _∞ (hr*ng/mL)
11	H	238	121.7	68
12	CONH 2	228	101.7	82
13	CN	251	101.7	82
14	Me	126	38.3	217
15	CF ₃	837	56.7	148

^aAssay run in the absence of serum. ^bDose of 0.5 mg/kg IV

Rapid evaluation of the C3-position helped to focus our efforts on confirmation of the stereochemistry required for potent CCR4 antagonism in this series. From previous

1
2
3 literature and our extensive in-house SAR, we knew the benzylic methyl position on the
4
5
6 northern fragment required the *R*-stereochemistry. With an enantioselective route to the
7
8
9
10 C3-methyl core in hand (see experimental section for details), determination of the
11
12
13 stereochemistry about the piperidinyl- piperidine side chain was undertaken. Chiral
14
15
16
17 resolution of *trans*-3-methylpiperidin-4-yl pivalate enabled a stereoselective route for the
18
19
20
21 *cis*-enantiomers of 3'-methyl-1,4'-bipiperidine (see experimental section for details).
22
23
24 Appending these side chains onto the C3-Me-core revealed that the (*R*, *S*)-
25
26
27
28 diastereomer **16** was 19-fold more potent than the (*S*, *R*)-diastereomer **17** toward
29
30
31 inhibiting calcium flux (Figure 4).
32
33

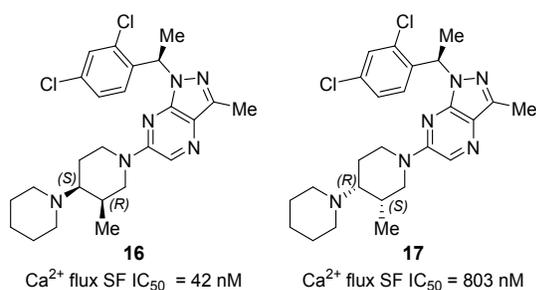


Figure 4. Stereochemical Analysis

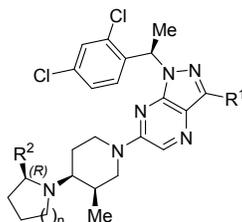
After completing the stereochemical analysis of this series, the path was cleared to evaluate the SAR of the basic amine containing side chain. Antagonists that inhibited

1
2
3 calcium flux in the double-digit nanomolar range were also run in a physiologically more
4
5
6 relevant chemotaxis (CTX) assay to evaluate their ability to inhibit CCL22-mediated
7
8
9 migration of CCR4 expressing CEM cells. This 96-well migration platform is considered
10
11 the gold standard assay for interrogating in vitro cellular migration.³⁵ Importantly, this
12
13
14 medium-throughput assay can be adapted for use with relevant primary immune cells
15
16
17 (i.e. T_{reg}). Since the assay is run in 100% human serum (HS), the reported potencies
18
19
20 reflect the protein binding of the compounds. Although calcium flux allowed us to triage
21
22
23 compounds based on their CCR4 affinity, a significant shift in CTX potency was often
24
25
26 observed, likely due to plasma protein binding.
27
28
29
30
31
32
33

34
35 Expansion or contraction of the distal piperidine ring to afford the 4-,5-,6-, and 7-
36
37 membered rings (**16**, **18-20**) resulted in antagonists that inhibit calcium flux with similar
38
39 potencies (Table 2). Unfortunately, these compounds suffered a severe shift in potency
40
41 when evaluated in the CTX assay. While the size of the outermost ring had little effect
42
43 on the potency of these compounds, focusing on the 5-membered pyrrolidines allowed
44
45
46 for the installation of a variety of commercially available, enantiopure substituted
47
48
49 heterocycles. To this end, both enantiomers of 2-methylpyrrolidine were explored to
50
51
52
53
54
55
56
57
58
59
60

1
2
3
4 replace the simple pyrrolidine ring (**18**). While installation of the 2-methyl substituent did
5
6
7 not suppress the shift in potency between calcium flux and CTX, the (*R*)-2-methyl-
8
9
10 pyrrolidine used in **21** provided a 4-fold more potent compound than the (*S*)-enantiomer
11
12
13
14 **22**. To probe what effect the cLogP would have on the CTX shift, several polar
15
16
17 functionalities were evaluated including amides (**23**, **24**), sulfone (**25**), sulfonamide (**26**),
18
19
20 nitrile (**27**), and carboxylic acid (**28**). Unfortunately, lowering the cLogP by introduction
21
22
23
24 of these polar groups had little influence on the CTX shift. Surprisingly, attenuating the
25
26
27 oxidation state of the carboxylic acid **28** to the prolinol derived **29** produced a compound
28
29
30
31 with a CTX IC_{50} = 151 nM. Further, replacement of the C3-Me core in **29** with a C3-CN
32
33
34
35 core to afford **30** resulted in one of the first compounds to exhibit an extremely low CTX
36
37
38 shift with a CTX IC_{50} = 58 nM.

41
42 **Table 2.** SAR of the 3,4-Substituted Piperidinyl Side Chain
43
44

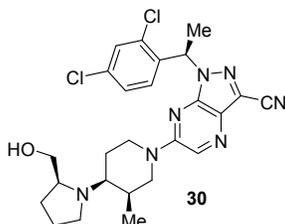


Compound	R ¹	R ²	n	Ca ²⁺ flux IC ₅₀ (nM) ^a	CTX IC ₅₀ (nM) ^b
16	Me	H	2	42	361
18	Me	H	1	69	N.D.
19	Me	H	0	58	218
20	Me	H	3	23	195
21	Me	Me	1	47	381
22	Me	Me (<i>S</i>) ^c	1	172	N.D.
23	Me	C(O)NH ₂	1	170	N.D.
24	Me	CH ₂ CH ₂ C(O)NH ₂	1	37	355
25	Me	CH ₂ CH ₂ SO ₂ Me	1	104	N.D.
26	Me	CH ₂ CH ₂ SO ₂ NH ₂	1	32	259
27	Me	CH ₂ CH ₂ CN	1	41	865
28	Me	COOH	1	670	N.D.
29	Me	CH ₂ OH	1	32	151
30	CN	CH ₂ OH	1	27	58

^aAssay run in absence of serum. ^bAssay run in 100% human serum (HS). ^cOpposite enantiomer used.

Encouraged by these results, we evaluated **30** in several in vitro and in vivo assays (Table 3). While it did not display any inhibition of CYP enzymes, when exposing compound **30** to human and rat hepatocytes, a substantial amount remained in human (74%) while rat showed a significant decrease in recovery (19%). The low in vitro metabolic stability in rat hepatocytes correlated well with the superhepatic clearance that was observed in vivo. In addition to extremely high clearance (114.6 mL/min/kg), **30** suffered from low bioavailability (%F = 7) and a high volume of distribution (13.4 L/kg).

Table 3. Profile of Piperidine **30**



Assays	Compound 30
Ca ²⁺ flux IC ₅₀ (nM)	27
CTX IC ₅₀ (nM) ^a	58
CYP Inhibition IC ₅₀ (μM) ^b	>10
% remaining hHep/rHep	74/19
rat in vivo PK ^c	

Cl (mL/min/kg)	114.6
V _{ss} (L/kg)	13.4
T _{1/2} (h)	2.1
PO AUC _{0-∞} (hr*ng/mL)	23.2
F (%)	7

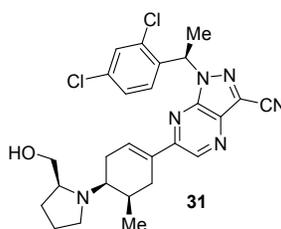
^aAssay run in 100% human serum (HS). ^bEnzymes tested: 1A2, 2C9, 2C19, 2D6, 3A4.

^cDose of 0.5 mg/kg IV or 2 mg/kg PO

To address these issues, we hypothesized that by replacing the piperidine nitrogen attached to the core in compound **30** with a carbon, the shift in cLogP may bring about more favorable pharmacokinetics for these antagonists. Up to this point, none of the nitrogen-linked antagonists displayed acceptable pharmacokinetic properties, therefore, we integrated the carbon-carbon link similar to our earlier antagonists **8** and **9**. The requisite carbon-carbon bond to achieve this goal brought about a significant synthetic challenge, and thus we envisioned first replacing the 3-Me-piperidine ring with a 3-Me-cyclohexene ring (*vide infra*).

1
2
3
4 Given the properties of prolinol-piperidine **30**, the cyclohexenyl compound **31**
5
6
7 was also evaluated in several in vitro and in vivo studies (Table 4). Replacement of the
8
9
10 anilinic nitrogen connection to the core with a styrenyl bond gave a compound that was
11
12
13
14 equipotent in the CTX assay, no inhibition of CYP enzymes, and similar in vitro hepatic
15
16
17 stability in human and rat hepatocytes, 81% and 32% remaining, respectively.
18
19
20
21 Interestingly, the in vivo pharmacokinetic properties of **31** were far superior to that of
22
23
24 compound **30**, displaying a longer terminal half-life ($T_{1/2}$) and a 4-fold higher
25
26
27
28 bioavailability (% F = 28).
29
30

31 **Table 4.** Profile of Cyclohexene **31**
32
33



43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Assays	Compound 31
Ca ²⁺ flux IC ₅₀ (nM)	40
CTX IC ₅₀ (nM) ^a	70
CYP Inhibition IC ₅₀ (μM) ^b	>10

% remaining hHep/rHep	81/32
rat in vivo PK^c	
Cl (mL/min/kg)	74.7
V _{ss} (L/kg)	9.8
T _{1/2} (h)	4.1
PO AUC _{0-∞} (hr*ng/mL)	125
F (%)	28

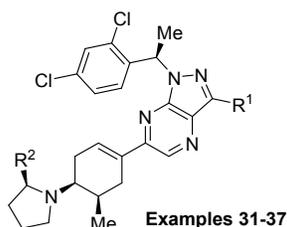
^aAssay run in 100% human serum (HS). ^bEnzymes tested: 1A2, 2C9, 2C19, 2D6, 3A4.

^cDose of 0.5 mg/kg IV or 2 mg/kg PO

Encouraged by the in vivo pharmacokinetics of compound **31**, the SAR of these cyclohexenyl antagonists was further assessed (Table 5). To begin, the C3-position of the core (R¹) was again interrogated. Replacing the C3-nitrile in **31** with a C3-methyl substituent (**32**) resulted in an almost 3-fold drop in potency within the CTX assay and little change in the in vitro hepatic stability. Given these data, the C3-nitrile was conserved throughout to focus our efforts on the R² substituents on the pyrrolidine ring. Building steric hindrance around the primary alcohol in **31** to give the tertiary alcohol in **33** resulted in a significant loss of potency in the CTX assay. Replacement of prolinol

with prolinamine (**34**) or prolinamide (**35**) increased the stability of these compounds toward human and rat hepatocytes. However, the increase in stability was accompanied by a significant decrease in potency in the CTX assay. Interestingly, installation of a 2-*(R)*-Me-pyrrolidine (**36**) resulted in a compound that was equipotent to **31** and displayed higher in vitro hepatic stability in rats. Replacement of the R² methyl with a more lipophilic trifluoromethyl substituent (**37**), rendered the compound completely inactive.

Table 5. Primary SAR of Cyclohexenyl Antagonists

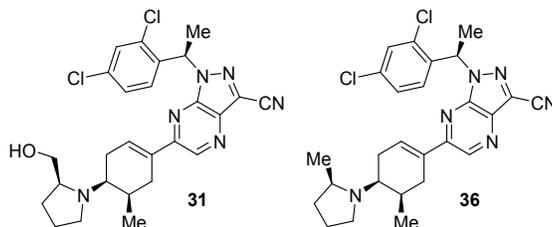


Example	R ¹	R ²	Ca ²⁺ flux IC ₅₀ (nM) ^a	CTX IC ₅₀ (nM) ^b	% remaining hHep/rHep
31	CN	CH ₂ OH	40	70	81/32
32	Me	CH ₂ OH	36	179	99/35
33	CN	C(Me) ₂ OH	384	1200	N.D.

34	CN	CH ₂ NH ₂	229	638	100/100
35	CN	C(O)NH ₂	54	1380	97/71
36	CN	Me	34	44	86/72
37	CN	CF ₃	>5000	N.D.	N.D.

^aAssay run in absence of serum. ^bAssay run in 100% human serum (HS).

Compounds **31** and **36** were the most potent analogs in their ability to inhibit CCL22-mediated CTX of CCR4 expressing CEM cells in 100% human serum. These compounds were further assessed for their ability to inhibit CTX in mouse induced T_{reg} (miT_{reg}) and for their PK characteristics in mice. Although the in vivo clearance in mice was lower for **36** (1.17 vs 12.0 mL/min/kg), leading to a substantially longer $T_{1/2}$ (30 hours vs 7.6, Table 6), the superior potency, synthetic availability, and lower lipophilicity of compound **31** ultimately led us to advance this compound into an in vivo mouse model to determine its ability to suppress T_{reg} trafficking into the tumor microenvironment.

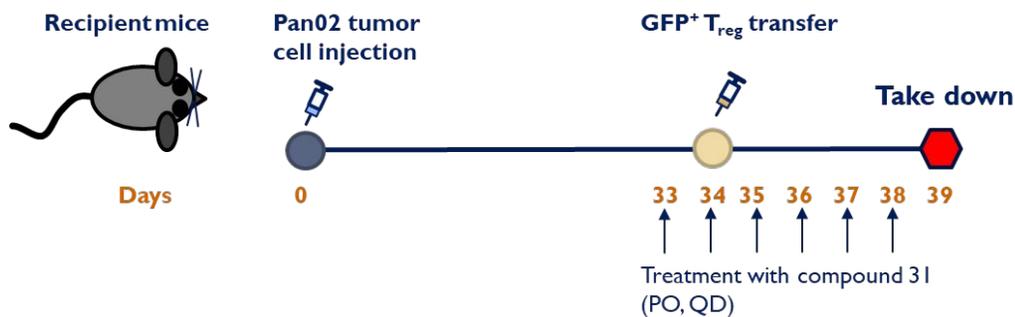
Table 6. Comparison of Compounds **31** and **36** in iT_{reg} CTX and Mouse In Vivo PK

Example	cLogP/tPSA	miT_{reg} CTX IC_{50} (nM)	mouse in vivo PK: Cl / V_{ss} / $T_{1/2}$ / %F
31	4.5/87.6	165	12.0 / 5.7 / 7.6 / 26
36	5.6/67.4	325	1.17 / 4.9 / 30.3 / 27

In Vivo Pharmacology in Mouse. Various murine tumor models were examined to assess their levels of CCR4 ligand (CCL17 and CCL22) expression, and it was found that the pancreatic ductal adenocarcinoma (Pan02) model displayed a high expression of these ligands.³⁷ Compound **31** was dosed QD for 6 days at 10 and 30 mg/kg in mice

1
2
3 bearing Pan02 tumors (Figure 5A). Mouse T_{reg} were generated in vitro from naive CD4⁺
4
5
6
7 T cells isolated from Foxp3⁺GFP⁺ transgenic mice (GFP⁺ miT_{reg})^{38,39} as described in the
8
9
10 supporting information. These GFP⁺ miT_{reg} were injected into the animals 24 h after the
11
12
13 first dose. Inhibition of the migration of GFP⁺ miT_{reg} into tumor and other tissues was
14
15
16 compared to vehicle treated animals (Figure 5B). A significant dose-dependent
17
18
19 decrease in the number of GFP⁺ miT_{reg} was found in the tumor tissue when compared
20
21
22 to the vehicle group. The 30 mg/kg dose showed nearly complete suppression of T_{reg}
23
24
25 migration into the tumor. Plasma levels at 24-hr post dose for **31** showed that 30 mg/kg
26
27
28 migration into the tumor. Plasma levels at 24-hr post dose for **31** showed that 30 mg/kg
29
30
31 QD covered the approximate IC₉₀ of the miT_{reg} CTX. Importantly, compound **31** was
32
33
34 able to selectively inhibit T_{reg} trafficking into the tumor without affecting the migration of
35
36
37 these cells into the spleen (Figure 5), further demonstrating that these antagonists may
38
39
40 not disrupt the functions of T_{reg} in other healthy tissues.⁴⁰
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

A)



B)

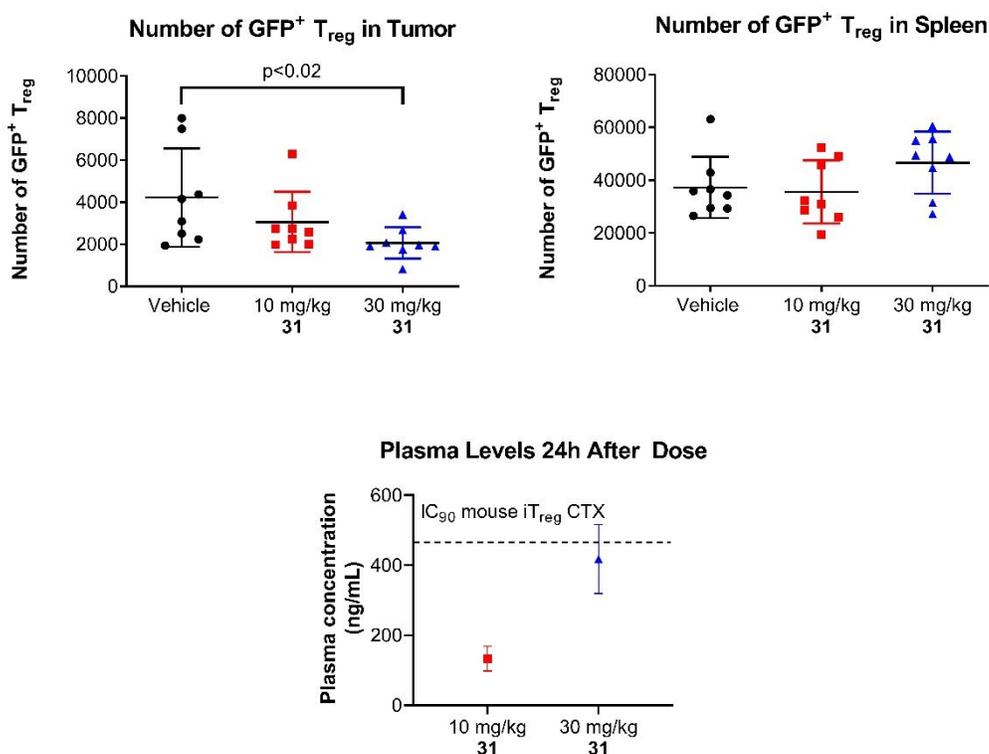


Figure 5. Treatment with Compound 31 selectively reduces T_{reg} trafficking into the

tumor. A) Schematic outline of treatment and tumor model that is used in this study. B)

Number of GFP⁺ T_{reg} in tumor (left) and spleen (right). Plasma concentration of compound 31 is

measured 24 hours after last treatment (bottom). For statistical analysis, the one-way ANOVA was used.

Pharmacokinetic Profiling of 31. Results from the T_{reg} migration study were encouraging and led to further profiling the in vivo pharmacokinetics of compound **31** in rat, dog, and cyno (Table 7). Additionally, the predicted hepatic clearance of **31** was found across species by monitoring the in vitro metabolic stability of **31** in a time-course assay with hepatocytes. The in vivo CL of all species were within 2-fold of their predicted hepatic CL, demonstrating good in vivo/in vitro correlation. When comparing the in vivo hepatic clearance across species, **31** suffered from very high clearance in rats while displaying lower clearance in dog and cynomolgus monkeys.

Table 7. PK Profile of **31** Across Species^a

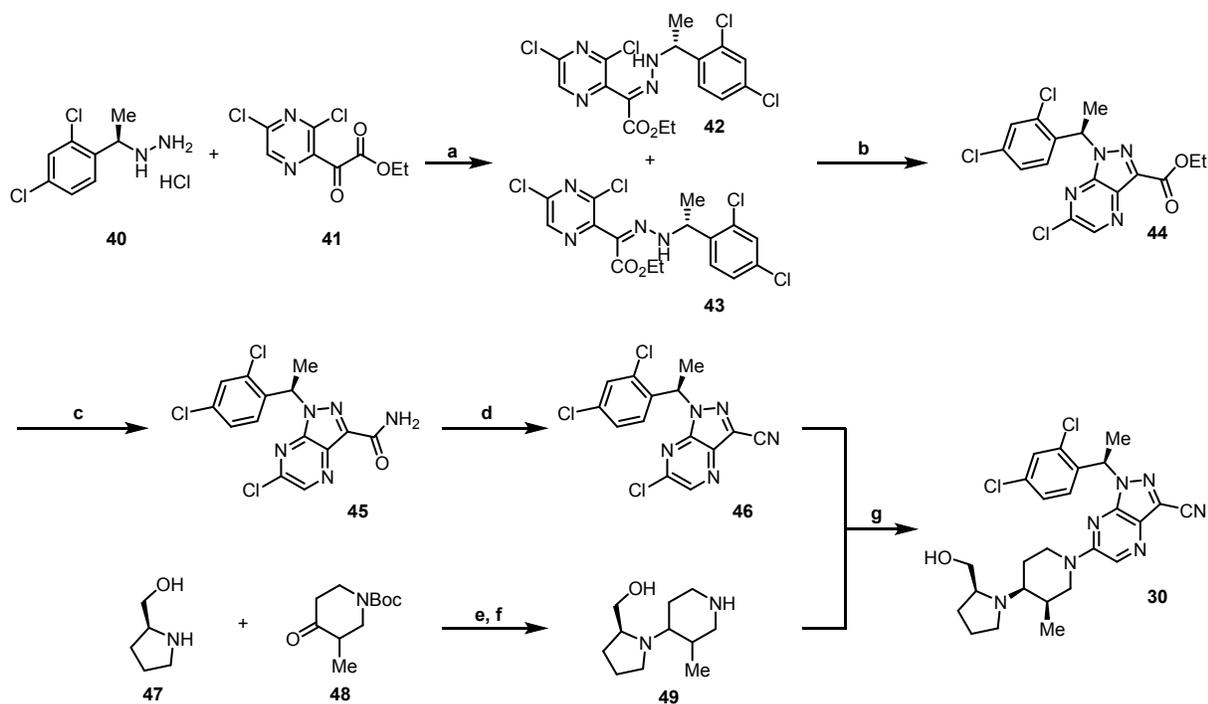
	rat ^b	dog ^b	cyno ^b	human
Pred. Hepatic CL (mL/min/kg)	45.0	18.3	12.5	7.2
in vivo CL (mL/min/kg)	74.7	10.7	11.3	---
IV Terminal Half Life (hr)	2.2	16.4	9.9	---
<i>F</i> (%)	28	75	30	---

1
2
3
4 ^aReagents and conditions: (a) *tert*-butyl 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-
5
6
7 3,6-dihydropyridine-1(2*H*)-carboxylate, 1M Na₂CO₃ (aq.), Pd(dppf)Cl₂, THF, 80-100 °C;
8
9
10
11 (b) 4N HCl (in dioxane), MeOH, rt; (c) HATU, DIPEA, DMF, rt; (d) 4N HCl (in dioxane),
12
13
14 MeOH, rt.
15
16
17

18
19 As a representative example of the piperidyl-pyrrolidine series, the synthesis of
20
21 compound **30** is shown (Scheme 2). The core structure was prepared by first heating
22
23 hydrazine **40** (see experimental section for synthesis) with glyoxylate **41** in THF to form
24
25 a mixture of hydrazones **42** and **43**. Treating these hydrazones with NaH in THF
26
27
28 initiated an intramolecular S_NAr to form the pyrazolo portion of the core structure in **44**.
29
30
31
32 Conversion of ester **44** to the final nitrile core **46** proceeded cleanly upon stirring with
33
34
35
36 NH₄OH to form **45**, followed by dehydration with Burgess reagent. Reductive amination
37
38
39
40 of prolinol **47** with ketone **48** followed by treatment with TFA to remove the Boc-
41
42
43
44 protecting group provided the requisite amino side chain **49** as a mixture of
45
46
47
48
49 stereoisomers. Completion of compound **30** was achieved via simple S_NAr of **49** with
50
51
52
53 the chloropyrazine core **46** under thermal conditions to afford the desired antagonist **30**
54
55
56
57
58
59
60

after chiral separation. Stereochemical confirmation for these antagonists was confirmed via an enantioselective synthesis of a similar analogue (see experimental section for details).

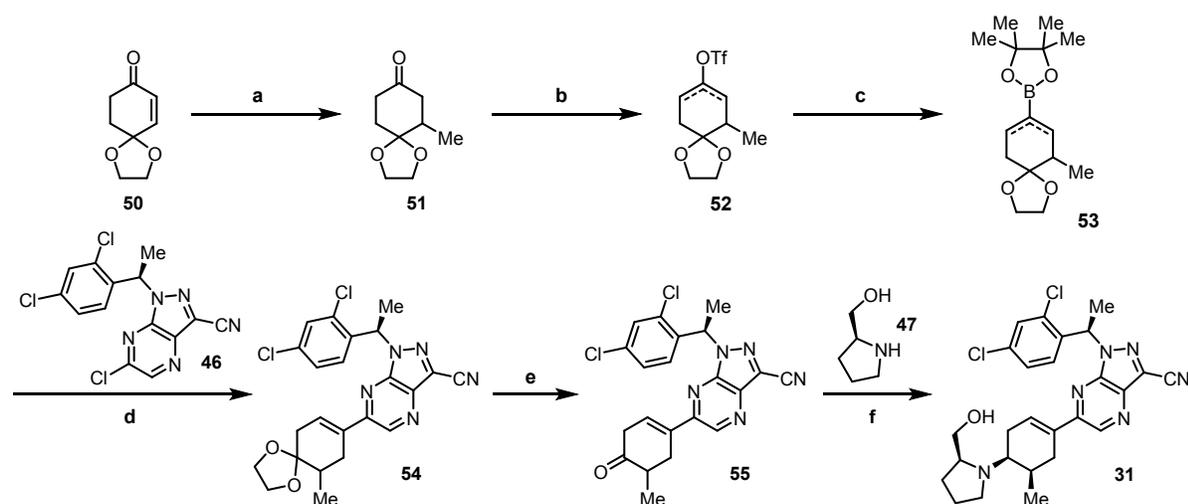
Scheme 2. Synthesis of Compound 30^a



^aReagents and conditions: (a) THF, 80 °C; (b) NaH, THF, 0 °C to rt, 50% yield over two steps; (c) NH₄OH (29% in water), dioxane, rt, 95% yield; (d) Burgess Reagent, CH₂Cl₂, rt, 88%; (e) Na(OAc)₃BH, AcOH, 1,2-DCE, rt; (f) TFA, CH₂Cl₂, 40% over two steps (g) DIPEA, DMF, 100 °C, 10%.

1
2
3
4 Synthesis of the cyclohexenyl CCR4 antagonists created a significant challenge
5
6
7 and is represented in the synthesis of compound **31** (Scheme 3). Gilman addition of
8
9
10 methyl cuprate to the α, β -unsaturated ketone **50** was accomplished to form **51**.
11
12
13
14 Deprotonation of **51** with LDA and treatment with phenyl triflimide gave triflate **52** as a
15
16
17 mixture of olefin regio-isomers, which underwent a palladium catalyzed formation of the
18
19
20 Bpin-**53**. Separation of the desired olefin isomer followed by Suzuki-Miyaura coupling
21
22
23 with chloride **46** gave ketal **54**, followed by treatment with TFA to unmask ketone **55**.
24
25
26
27
28 Formation of the final analogue was completed via a simple reductive amination with
29
30
31 (*R*)-prolinol (**47**) and ketone **55**, followed by chiral separation to afford compound **31**.
32
33
34

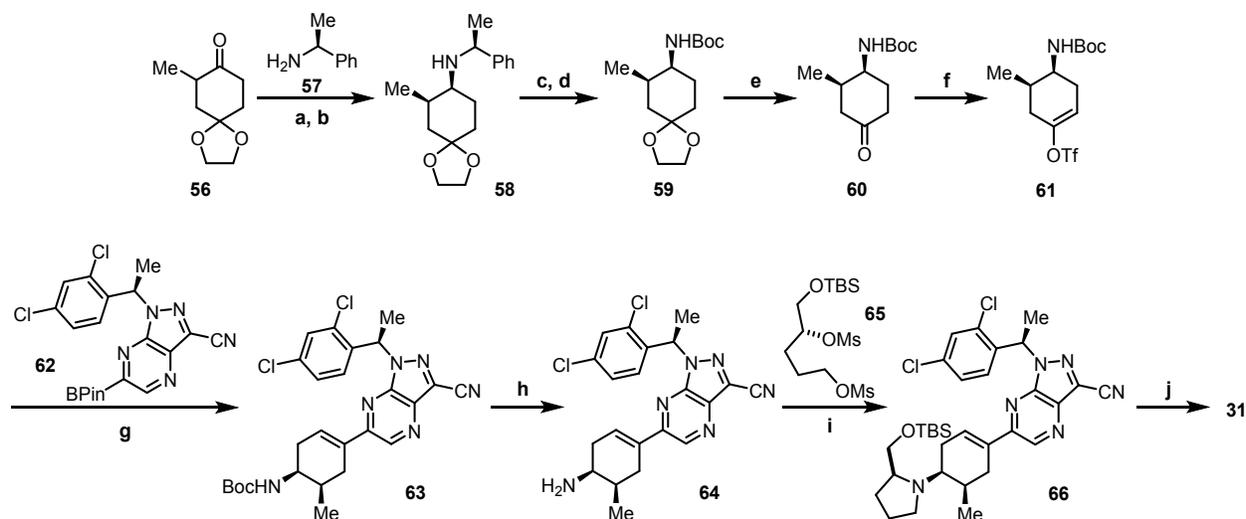
35 **Scheme 3. Synthesis of Cyclohexenyl CCR4 Antagonist **31**^a**



1
2
3
4 ^aReagents and conditions: (a) MeLi, CuCN, TMSCl, Et₂O:THF, -40 °C; (b) LDA, then
5
6
7 PhNTf₂, THF, -78 °C, 53% over two steps; (c) Bpin₂, Pd(dppf)Cl₂, KOAc, KBr, dioxane,
8
9
10 95 °C; (d) Pd(dppf)Cl₂, Na₂CO₃, THF:H₂O, 90 °C; (e) TFA, CH₂Cl₂, rt; (f) NaBH₃CN,
11
12
13
14 AcOH, 1,2-DCE, rt.
15
16
17

18 For larger scale synthesis of **31**, a more selective route was needed that avoided
19
20
21 chiral separations in the final step (Scheme 4). Reductive amination between ketone **56**
22
23
24 and (*R*)- α -methylbenzylamine (**57**) set the stereocenters on the cyclohexane ring in
25
26
27
28
29 >10:1 selectivity, with the desired isomer being easily isolated by silica gel
30
31
32 chromatography. Subsequent protecting group manipulation and formation of the vinyl
33
34
35 triflate proceeded in >30:1 regioselectivity of olefin **61**. Suzuki coupling with Bpin-**62**
36
37
38 (obtained from **46**) provided compound **63** in good yield. Removal of the Boc group and
39
40
41
42 bisalkylation with **65** afforded the desired protected prolinol **66**, which after a final TBS-
43
44
45
46 deprotection yielded compound **31** as a single isomer.
47
48
49

50 **Scheme 4.** 2nd Generation Synthesis of Cyclohexenyl CCR4 Antagonist **31**^a
51
52
53
54
55
56
57
58
59
60



^aReagents and conditions: (a) benzene, 95 °C, quant. yield; (b) NaBH(OAc)₃, CH₂Cl₂:AcOH (10:1), rt, 65%; (c) Pd(OH)₂, H₂, MeOH, rt, 97%; (d) Boc₂O, Et₃N, CH₂Cl₂, rt, 98%; (e) TsOH, THF:H₂O, rt, 70%; (f) LiHMDS, PhNTf₂, THF, -78 °C, 97%; (g) Pd(PPh₃)₄, Na₂CO₃, toluene:EtOH:H₂O, 90 °C, 76%; (h) 4 N HCl in dioxane, CH₂Cl₂, rt, quant. yield; (i) DIPEA, MeCN, rt, 75%; (j) 4 N HCl in dioxane, CH₂Cl₂, rt, 82%.

CONCLUSIONS

A novel, orally bioavailable cyclohexenyl series of CCR4 antagonists has been discovered that is potent and selective (see Supporting Information for data) against CCR4. Compound 31 exhibits good in vitro and in vivo ADME properties, but importantly, it inhibits T_{reg} trafficking into the tumor microenvironment without

1
2
3 suppressing the number of T_{reg} in healthy tissues. In order to achieve near complete
4
5
6
7 blockade of this migration, we targeted trough concentrations that approximated the IC_{90}
8
9
10 in a mouse chemotaxis assay. This is consistent with other PK/PD relationships
11
12
13
14 observed for other chemokine receptor antagonists.⁴¹ These studies helped lead to the
15
16
17 design and discovery of FLX475, which is currently in human clinical trials.
18
19
20

21 EXPERIMENTAL METHODS

22
23
24 **General Methods for Chemistry.** All commercial reagents and solvents were used
25
26
27 as received unless otherwise noted. An inert atmosphere of nitrogen was used for
28
29
30
31 reactions involving air or moisture sensitive reagents. Analytical thin layer
32
33
34 chromatography (TLC) was performed using 2.5 x 7.5 cm Merck silica gel 60 F_{254} thin
35
36
37 layer plates (EMD Millipore 1.15341.0001) visualized using combinations of UV
38
39
40
41 visualization, p-anisaldehyde, potassium permanganate, and iodine staining. Silica gel
42
43
44 column chromatography was performed using Teledyne ISCO RediSep Rf normal
45
46
47 phase (35–70 μ m) silica gel columns on a Teledyne ISCO CombiFlash Rf or
48
49
50
51 CombiFlash Rf+ purification system (detection at 254 nm). Reverse phase preparative
52
53
54
55 HPLC was carried out using a Gemini-NX-C18 column (10 μ m, 250 x 30 mm,
56
57
58
59
60

1
2
3 Phenomenex, Torrance, CA) eluting with a linear gradient from 5 to 100% acetonitrile in
4
5
6
7 water containing 0.1% trifluoroacetic acid over 30 minutes on a Teledyne ISCO EZ
8
9
10 Prep, Teledyne ISCO ACCQPrep HP125, or Agilent 1200 Series purification system.
11
12
13 Analytical reverse phase HPLC was performed using a Gemini-NX-C18 column (5 μm ,
14
15
16
17 250 x 4.6 mm, Phenomenex, Torrance, CA) eluting with MeCN in water with 0.1% TFA
18
19
20 on an Agilent 1200 Series purification system (detection at 254 nm). Proton NMR
21
22
23 spectra were recorded on a Varian Oxford 400 MHz spectrometer and carbon NMR
24
25
26
27 spectra were recorded at 101 MHz. Chemical shifts are expressed in δ ppm referenced
28
29
30 to tetramethylsilane ($\delta = 0$ ppm). Abbreviations used in describing peak signal
31
32
33
34 multiplicity are as follows: s = singlet, d = doublet, dd = double doublets, t = triplet, q =
35
36
37
38 quartet, m = multiplet, br = broad peak. Analytical LC-MS was performed using a
39
40
41 ZORBAX SB-C18 column (1.8 μm , 2.1 x 50 mm, 600 bar, Agilent, Santa Clara, CA)
42
43
44
45 eluting with a linear gradient from 0% to 100% B over 2 min and then 100% B for 3 min
46
47
48 (A = 5% MeCN in H₂O with 0.1% formic acid, B = MeCN + 0.1% formic acid, flow rate
49
50
51 0.4 mL/min) using an Agilent 1260 Infinity II LC System (detection at 254 nm) equipped
52
53
54
55 with an Agilent 6120 Quadrupole LC/MS in electrospray ionization mode (ESI+). The
56
57
58
59
60

1
2
3
4 purity of all compounds used in bioassays was determined by this method to be >95%
5
6
7 pure.
8
9

10 **General Procedure A for S_nAr.** The appropriate heteroaryl chloride and amine
11
12 (1.0 to 2.0 equiv) were dissolved in DMF, DMSO, or CH₂Cl₂ and *N,N*-
13
14 diisopropylethylamine (2.0 to 3.0 equiv) was added. The reaction was heated to 40 –
15
16
17
18 100 °C until the complete consumption of starting material was observed by either LC-
19
20
21 MS or TLC. After cooling, the mixture was diluted with water and ethyl acetate and the
22
23
24 layers were separated. The organic layer was washed with brine solution, dried over
25
26
27
28 sodium sulfate, concentrated via rotary evaporation, and the residue was purified by
29
30
31 silica gel chromatography or reverse phase preparative HPLC.
32
33
34
35
36
37

38 **General Procedure B for Suzuki Coupling.** The appropriate aryl halide and aryl or
39
40
41 alkenyl pinacol boronate (1.0 to 1.5 equiv) were dissolved in THF (0.2 M) and 1 M
42
43
44 aqueous Na₂CO₃ (2.0 to 5.0 equiv) was added. [1,1'-
45
46
47
48 bis(diphenylphosphino)ferrocene]dichloropalladium (II) (15 mol%) was added and the
49
50
51
52 reaction was heated to 80 – 100 °C until the complete consumption of starting material
53
54
55
56 was observed by LC-MS or TLC. After cooling, the mixture was diluted with water and
57
58
59
60

1
2
3 ethyl acetate and the layers were separated. The organic layer was washed with brine
4
5
6
7 solution, dried over sodium sulfate, concentrated via rotary evaporation, and the residue
8
9
10 was purified by silica gel chromatography unless otherwise noted.

11
12
13
14 **General Procedure C for HATU Coupling.** The appropriate carboxylic acid and
15
16
17 amine (1.0 to 2.0 equiv) were dissolved in DMF (0.2 M) and *N,N*-diisopropylethylamine
18
19
20 (3.0 to 5.0 equiv) was added. HATU (1.1 equiv) was added and the reaction was stirred
21
22
23
24 at room temperature until complete consumption of the starting material was observed
25
26
27
28 by LC-MS or TLC. The mixture was diluted with ethyl acetate, washed with water,
29
30
31 washed with brine solution, dried over sodium sulfate, and concentrated by rotary
32
33
34 evaporation. The residue was purified by silica gel chromatography or reverse phase preparative
35
36 HPLC.
37
38

39 **General Procedure D for reductive amination.** The appropriate ketone and amine (1.2
40
41 equiv) were dissolved in 1,2-dichloroethane (0.3 M) then acetic acid (1.5 equiv) and sodium
42
43 triacetoxyborohydride or sodium cyanoborohydride (1.5 eq) were added. The mixture was stirred
44
45
46 at room temperature until the desired product was formed, monitoring by LC-MS. The reaction
47
48 was quenched with saturated aqueous sodium bicarbonate solution and extracted with
49
50 dichloromethane. The combined organic layers were dried over sodium sulfate and concentrated
51
52 via rotary evaporation to afford the desired product, which was purified by silica gel
53
54 chromatography, preparative reverse phase HPLC, or used without further purification.
55
56
57
58
59
60

1
2
3 **(4-(6-(((*R*)-1-(2,4-dichlorophenyl)ethyl)amino)pyrazin-2-yl)-3,6-dihydropyridin-**
4
5
6 **1(2*H*)-yl)((*R*)-piperidin-2-yl)methanone hydrochloride (8).** Step 1. The Suzuki coupling
7
8
9 was performed according to general procedure B using (*R*)-6-chloro-*N*-(1-(2,4-
10
11
12 dichlorophenyl)ethyl)pyrazin-2-amine (1.0 g, 3.30 mmol), and *tert*-butyl 4-(4,4,5,5-
13
14
15 tetramethyl-1,3,2-dioxaborolan-2-yl)-3,6-dihydropyridine-1(2*H*)-carboxylate (1.0 equiv)
16
17
18 at 100 °C for 18 h. The residue was purified by silica gel chromatography (50% ethyl
19
20
21 acetate in hexanes) to afford *tert*-butyl (*R*)-4-(6-((1-(2,4-
22
23
24 dichlorophenyl)ethyl)amino)pyrazin-2-yl)-3,6-dihydropyridine-1(2*H*)-carboxylate (67%
25
26
27
28
29
30 yield). LRMS-ESI⁺: *m/z* calcd for C₂₂H₂₆Cl₂N₄O₂ [M+H]⁺ = 449.2; found, 449.
31
32

33 Step 2. *Tert*-butyl (*R*)-4-(6-((1-(2,4-dichlorophenyl)ethyl)amino)pyrazin-2-yl)-3,6-
34
35
36 dihydropyridine-1(2*H*)-carboxylate (414 mg, 0.92 mmol) was dissolved in MeOH (15
37
38
39 mL) and HCl (4N in 1,4-dioxane, 5 mL) was added. The reaction was stirred at 23 °C for
40
41
42
43 2 h. The mixture was basified with 1 N NaOH, diluted with CH₂Cl₂, and the layers were
44
45
46 separated. The organic layer was washed with brine solution, dried over sodium sulfate,
47
48
49 concentrated via rotary evaporation, and used in the next step without purification. The
50
51
52
53 HATU coupling was performed according to general procedure C using the deprotected
54
55
56
57
58
59
60

1
2
3 amine (275 mg, 0.79 mmol), (*R*)-1-(*tert*-butoxycarbonyl)piperidine-2-carboxylic acid (1.0
4
5
6
7 equiv), *N,N*-diisopropylethylamine (2.0 equiv), and HATU (1.1 equiv) in DMF (2.5 mL) at
8
9
10 23 °C for 14 h. The residue was purified by silica gel chromatography (0 to 100% ethyl
11
12
13 acetate in hexanes) to afford *tert*-butyl (*R*)-2-(4-(6-(((*R*)-1-(2,4-
14
15
16
17 dichlorophenyl)ethyl)amino)pyrazin-2-yl)-1,2,3,6-tetrahydropyridine-1-
18
19
20
21 carbonyl)piperidine-1-carboxylate (69% yield). LRMS-ESI⁺: *m/z* calcd for C₂₈H₃₅Cl₂N₅O₃
22
23
24 [M+H]⁺ = 560.2; found, 560.
25
26
27

28 Step 3. *Tert*-butyl (*R*)-2-(4-(6-(((*R*)-1-(2,4-dichlorophenyl)ethyl)amino)pyrazin-2-
29
30
31 yl)-1,2,3,6-tetrahydropyridine-1-carbonyl)piperidine-1-carboxylate (305 mg, 0.54 mmol)
32
33
34 was dissolved in MeOH (10 mL) and HCl (4N in 1,4-dioxane, 5 mL) was added. The
35
36
37 mixture was stirred at 23 °C for 5 h. The solution was basified with 1 N NaOH and
38
39
40 extracted with CH₂Cl₂. The organic layer was washed with brine solution, dried over
41
42
43 sodium sulfate, and concentrated via rotary evaporation and used in the next step
44
45
46 without further purification. The deprotected amine (250 mg, 0.543 mmol) was dissolved
47
48
49 in CH₂Cl₂ (10 mL) and HCl (4N in 1,4-dioxane, 0.136 mL, 0.543 mmol) was added
50
51
52
53
54
55
56 dropwise. The reaction was stirred for 10 minutes and concentrated via rotary
57
58
59
60

1
2
3 evaporation to afford (4-(6-(((*R*)-1-(2,4-dichlorophenyl)ethyl)amino)pyrazin-2-yl)-3,6-
4
5
6
7 dihydropyridin-1(2*H*)-yl)((*R*)-piperidin-2-yl)methanone hydrochloride (**8**, 100% yield). ¹H
8
9
10 NMR (400 MHz, CDCl₃; HCl Salt) δ 7.89 (d, J = 3.2 Hz, 1H), 7.66 (d, J = 3.3 Hz, 1H),
11
12
13 7.38 (d, J = 2.1 Hz, 1H), 7.31 (d, J = 8.4 Hz, 1H), 6.69 – 6.38 (m, 1H), 5.37 – 5.17 (m,
14
15
16 1H), 5.08 (d, J = 5.6 Hz, 1H), 3.87 – 3.54 (m, 2H), 3.28 – 2.96 (m, 3H), 2.70 (q, J = 11.6,
17
18
19 11.1 Hz, 1H), 2.53 (s, 2H), 1.92 (d, J = 9.8 Hz, 1H), 1.76 (d, J = 12.6 Hz, 1H), 1.60 (t, J
20
21
22 = 13.7 Hz, 1H), 1.53 (d, J = 6.8 Hz, 3H), 1.41 (dd, J = 26.8, 12.7 Hz, 1H), 1.31 – 1.19
23
24
25 = 13.7 Hz, 1H), 1.53 (d, J = 6.8 Hz, 3H), 1.41 (dd, J = 26.8, 12.7 Hz, 1H), 1.31 – 1.19
26
27
28 (m, 3H). LRMS-ESI⁺: m/z calcd for C₂₃H₂₇Cl₂N₅O [M+H]⁺ = 460.2; found, 460.
29
30

31
32 **(4-(1-(1-(2,4-dichlorophenyl)ethyl)-1*H*pyrazolo[3,4-*b*]pyrazin-6-yl)-3,6-**
33
34
35 **dihydropyridin-1(2*H*)-yl)((*R*)-piperidin-2-yl)methanone 2,2,2-trifluoroacetate (**9**). Step 1.**
36
37
38 The Suzuki coupling was performed according to general procedure B using 6-chloro-1-
39
40
41 (1-(2,4-dichlorophenyl)ethyl)-1*H*pyrazolo[3,4-*b*]pyrazine (0.275 mmol), and *tert*-butyl 4-
42
43
44 (4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3,6-dihydropyridine-1(2*H*)-carboxylate (1.0
45
46
47 equiv) at 100 °C for 18 h. The residue was purified by silica gel chromatography (0 to
48
49
50
51 100% ethyl acetate in hexanes) to afford *tert*-butyl 4-(1-(1-(2,4-dichlorophenyl)ethyl)-1*H*-
52
53
54
55
56
57
58
59
60

1
2
3
4 pyrazolo[3,4-*b*]pyrazin-6-yl)-3,6-dihydropyridine-1(2*H*)-carboxylate (60% yield). LRMS-

5
6
7 ESI⁺: *m/z* calcd for C₂₃H₂₅Cl₂N₅O₂ [M+H]⁺ = 474.2; found, 474.
8
9

10
11 Step 2. *Tert*-butyl 4-(1-(1-(2,4-dichlorophenyl)ethyl)-1*H*-pyrazolo[3,4-*b*]pyrazin-6-
12
13
14 yl)-3,6-dihydropyridine-1(2*H*)-carboxylate (0.120 mmol) was dissolved in CH₂Cl₂ (2 mL)
15
16
17 and TFA (0.5 mL) was added. The reaction was stirred at 23 °C for 2 h. The mixture
18
19
20
21 was basified with 1 N NaOH, diluted with CH₂Cl₂, and the layers were separated. The
22
23
24 organic layer was washed with brine solution, dried over sodium sulfate, concentrated
25
26
27
28 via rotary evaporation, and used in the next step without purification. The HATU
29
30
31 coupling was performed according to general procedure C using the deprotected amine
32
33
34 (275 mg, 0.79 mmol), (*R*)-1-(*tert*-butoxycarbonyl)piperidine-2-carboxylic acid (180 mg,
35
36
37 0.79 mmol), *N,N*-diisopropylethylamine (0.14 mL, 1.58 mmol), and HATU (1.1 equiv) in
38
39
40
41 DMF (2.5 mL). The residue was purified by silica gel chromatography (0 to 100% ethyl
42
43
44 acetate in hexanes) to afford *tert*-butyl (*R*)-2-(4-(6-(((*R*)-1-(2,4-
45
46
47
48 dichlorophenyl)ethyl)amino)pyrazin-2-yl)-1,2,3,6-tetrahydropyridine-1-
49
50
51
52 carbonyl)piperidine-1-carboxylate. The residue was dissolved in CH₂Cl₂ (2 mL) and TFA
53
54
55
56 (0.5 mL) was added. The reaction was stirred 1 h at room temperature and
57
58
59
60

1
2
3 concentrated to afford (4-(1-(1-(2,4-dichlorophenyl)ethyl)-1*H*-pyrazolo[3,4-*b*]pyrazin-6-
4
5
6
7 yl)-3,6-dihydropyridin-1(2*H*)-yl)((*R*)-piperidin-2-yl)methanone 2,2,2-trifluoroacetate (**9**,
8
9
10 69% yield). ¹H-NMR (400 MHz; CDCl₃; TFA Salt) δ 8.78 (br s, 2H), 8.30 (s, 1H), 7.43 –
11
12
13 7.41 (m, 2H), 7.20 – 7.17 (m, 1H), 6.76 – 6.63 (m, 2H), 5.49 (br s, 1H), 3.80 – 3.72 (m,
14
15
16 4H), 3.14 – 2.79 (m, 2H), 2.05 – 1.97 (m, 11 H), 1.26 – 1.19 (m, 3 H). LRMS-ESI⁺: m/z
17
18
19
20
21 calcd for C₂₄H₂₇Cl₂N₆O [M+H]⁺ = 485.2; found, 485.
22
23

24
25 **6-([1,4'-bipiperidin]-1'-yl)-1-(1-(2,4-dichlorophenyl)ethyl)-1*H*-pyrazolo[3,4-**
26
27
28 ***b*]pyrazine 2,2,2-trifluoroacetate (**10**)**. The SnAr was performed according to general
29
30
31 procedure A using 1,4'-bipiperidine (2.0 equiv), 6-chloro-1-(1-(2,4-dichlorophenyl)ethyl)-
32
33
34 1*H*-pyrazolo[3,4-*b*]pyrazine (**39**, 90 mg, 0.275 mmol), and *N,N*-diisopropylethylamine
35
36
37 (2.0 equiv) in DMF (5 mL) at 100 °C for 2 h. Purified by reverse phase preparative
38
39
40
41 HPLC (Gemini-NX, 10 μm, 250 x 30 mm, C18 column, Phenomenex, Torrance, CA),
42
43
44
45 eluent: 0 to 100% acetonitrile in water, both eluents containing 0.1% TFA, gradient
46
47
48
49 elution over 30 minutes to afford 6-([1,4'-bipiperidin]-1'-yl)-1-(1-(2,4-
50
51
52 dichlorophenyl)ethyl)-1*H*-pyrazolo[3,4-*b*]pyrazine 2,2,2-trifluoroacetate (**10**) as a mixture
53
54
55
56 of diastereomers (7% yield). ¹H NMR (400 MHz, CDCl₃; TFA Salt) δ 11.21 (bs, 1H),
57
58
59
60

1
2
3
4 8.21 (s, 1H), 8.10 (s, 1H), 7.38 (d, $J = 2.2$ Hz, 1H), 7.37 (d, $J = 4.1$ Hz, 1H), 7.17 (dd, $J =$
5
6
7 8.5, 2.1 Hz, 1H), 6.41 (q, $J = 7.1$ Hz, 1H), 4.63 (t, $J = 12.3$ Hz, 2H), 3.62 – 3.42 (m, 3H),
8
9
10 3.09 – 2.98 (m, 2H), 2.82 – 2.65 (m, 2H), 2.20 (t, $J = 12.4$ Hz, 2H), 2.12 – 1.98 (m, 2H),
11
12
13 1.94 (d, $J = 7.1$ Hz, 3H), 1.92 – 1.87 (m, 2H), 1.83 – 1.66 (m, 2H), 1.48 – 1.22 (m, 2H).
14
15
16

17 LRMS-ESI⁺: m/z calcd for C₂₃H₂₈Cl₂N₆ [M+H]⁺ = 459.2; found, 459.
18

19 **1-(1-(2,4-dichlorophenyl)ethyl)-6-(3'-methyl-[1,4'-bipiperidin]-1'-yl)-1H-**
20 **pyrazolo[3,4-*b*]pyrazine hydrochloride (11).** The SnAr was performed according to general
21 procedure A using 3'-methyl-1,4'-bipiperidine (2.0 equiv), 6-chloro-1-(1-(2,4-
22 dichlorophenyl)ethyl)-1H-pyrazolo[3,4-*b*]pyrazine (**39**, 90 mg, 0.275 mmol), and *N,N*-
23 diisopropylethylamine (2.0 equiv) in DMF (5 mL) at 100 °C for 2 h. Purified by reverse phase
24 preparative HPLC (Gemini-NX, 10 μm, 250 x 30 mm, C18 column, Phenomenex, Torrance,
25 CA), eluent: 0 to 100% acetonitrile in water, both eluents containing 0.1% TFA, gradient elution
26 over 30 minutes. The TFA salt was neutralized by passing it through a basification column (PL-
27 HCO₃ MP SPE, 500 mg per 5 mL tube, Agilent) using dichloromethane and methanol (4:1) as
28 the eluent and concentrated under reduced pressure. HCl (1 N in diethyl ether, 1 mL) was added
29 and stirred 10 minutes. The mixture was concentrated under reduced pressure to afford 1-(1-(2,4-
30 dichlorophenyl)ethyl)-6-(3'-methyl-[1,4'-bipiperidin]-1'-yl)-1H-pyrazolo[3,4-*b*]pyrazine
31 hydrochloride (**11**) as a mixture of diastereomers (6% yield). ¹H NMR (400 MHz, CDCl₃; HCl
32 Salt) δ 11.22 (bs, 1H), 8.21 (s, 0.66H), 8.20 (s, 0.34H), 8.09 (s, 0.66H), 8.09 (s, 0.34H), 7.40 –
33 7.35 (m, 2H), 7.17 (dd, $J = 8.5, 2.0$ Hz, 1H), 6.39 (q, $J = 7.2$ Hz, 1H), 4.86 – 4.63 (m, 3H), 4.57 –
34 4.47 (m, 1H), 3.88 – 3.78 (m, 1H), 3.73 – 3.64 (m, 1H), 3.34 – 3.23 (m, 1H), 3.13 – 3.04 (m,
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 1H), 2.98 – 2.88 (m, 1H), 2.83 – 2.69 (m, 2H), 2.64 – 2.54 (m, 1H), 2.26 – 2.00 (m, 3H), 1.93 (d,
4
5 $J = 7.0$ Hz, 3H), 1.91 – 1.88 (m, 2H), 1.52 – 1.40 (m, 1H), 1.21 (d, $J = 6.6$ Hz, 2H), 1.13 (d, $J =$
6
7 6.6 Hz, 1H). LRMS-ESI⁺: m/z calcd for C₂₄H₃₀Cl₂N₆ [M+H]⁺ = 473.2; found, 473.

8
9
10
11 **1-(1-(2,4-dichlorophenyl)ethyl)-6-((3'*R*,4'*S*)-3'-methyl-[1,4'-bipiperidin]-1'-yl)-1*H*-**
12 **pyrazolo[3,4-*b*]pyrazine-3-carbonitrile hydrochloride (12).** Step 1. To a solution of *n*-BuLi
13 (2.5 M in hexanes, 132 mL, 0.329 mol) was added 2,2,6,6-tetramethylpiperidine (56 mL, 0.329
14 mol) in THF (200 mL) at -78 °C over 15 min. The yellow slurry was stirred at -78 °C for 15 min.
15
16 In a separate flask, diethyl oxalate (41 mL, 0.302 mol) and 2,6-dichloropyrazine (40 g, 0.274
17 mol) were dissolved in THF (685 mL) and cooled to -78 °C. The lithium 2,2,6,6-
18 tetramethylpiperidine solution was added to the 2,6-dichloropyrazine solution via cannula over
19 15 min at -78 °C. The reaction was stirred at -78 °C for 30 min before the addition of AcOH (20
20 mL). The mixture was warmed to room temperature and quenched with saturated aqueous
21 NH₄Cl. The mixture was extracted with EtOAc and combined organic layers were washed with
22 saturated aqueous NH₄Cl, dried over MgSO₄ and concentrated under reduced pressure. The
23 residue was dissolved in EtOH (50 mL) and (1-(2,4-dichlorophenyl)ethyl)hydrazine
24 hydrochloride (9.83 g, 40.7 mmol) was added. The reaction was stirred at room temperature for
25 16 h and saturated aqueous NaHCO₃ was added. The mixture was concentrated under reduced
26 pressure to remove the EtOH and the aqueous layer was extracted with dichloromethane. The
27 combined organic layers were dried over Na₂SO₄ and concentrated under reduced pressure. The
28 residue was purified by silica gel chromatography (0 to 20% ethyl acetate in hexanes) to afford
29 ethyl (*E*)-2-(2-(1-(2,4-dichlorophenyl)ethyl)hydrazineylidene)-2-(3,5-dichloropyrazin-2-
30 yl)acetate and ethyl (*Z*)-2-(2-(1-(2,4-dichlorophenyl)ethyl)hydrazineylidene)-2-(3,5-
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 dichloropyrazin-2-yl)acetate as a mixture of isomers (57% yield). LRMS-ESI⁺: *m/z* calcd for
4
5 C₁₆H₁₄Cl₄N₄O₂ [M+H]⁺ = 435.0; found, 435.
6
7

8
9 Step 2. A mixture of ethyl (*E*)-2-(2-(1-(2,4-dichlorophenyl)ethyl)hydrazono)-2-(3,5-
10
11 dichloropyrazin-2-yl)acetate and ethyl (*Z*)-2-(2-(1-(2,4-dichlorophenyl)ethyl)hydrazono)-2-(3,5-
12
13 dichloropyrazin-2-yl)acetate (54 g, 126 mmol) was dissolved in THF (500 mL) and cooled to 5
14
15 °C. NaH (60% dispersion in oil, 5.56 g, 139 mmol) was added portion wise and the mixture was
16
17 warmed to room temperature then stirred for 1 h. An additional portion of NaH (60% dispersion
18
19 in oil, 5.0 g, 126 mmol) was added portion-wise and the mixture was stirred at room temperature
20
21 for 2 h. *Tert*-BuOH (5 drops) was added and the mixture was stirred at room temperature for 10
22
23 d. The mixture was diluted with saturated aqueous NH₄Cl and extracted with ethyl acetate. The
24
25 organic layer was dried over MgSO₄ and concentrated under reduced pressure. The residue was
26
27 recrystallized from dichloromethane and hexanes to afford ethyl 6-chloro-1-(1-(2,4-
28
29 dichlorophenyl)ethyl)-1*H*-pyrazolo[3,4-*b*]pyrazine-3-carboxylate (60% yield). LRMS-ESI⁺: *m/z*
30
31 calcd for C₁₆H₁₃Cl₃N₄O₂ [M+H]⁺ = 399.0; found, 399.
32
33
34
35
36

37 Step 3. The SnAr was performed following general procedure A using ethyl 6-chloro-1-
38
39 (1-(2,4-dichlorophenyl)ethyl)-1*H*-pyrazolo[3,4-*b*]pyrazine-3-carboxylate (0.388 mmol), 3'-
40
41 methyl-1,4'-bipiperidine 2,2,2-trifluoroacetate (0.388 mmol), *N,N*-diisopropylethylamine (0.776
42
43 mmol) in DMSO (1.0 mL) at room temperature for 16 h. The residue was purified by silica gel
44
45 chromatography (10% MeOH in CH₂Cl₂) to afford ethyl 1-(1-(2,4-dichlorophenyl)ethyl)-6-(3'-
46
47 methyl-[1,4'-bipiperidin]-1'-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine-3-carboxylate (50% yield). LRMS-
48
49 ESI⁺: *m/z* calcd for C₂₇H₃₄Cl₂N₆O₂ [M+H]⁺ = 545.2; found, 545.
50
51
52
53
54
55
56
57
58
59
60

1
2
3
4 Step 4. Ethyl 1-(1-(2,4-dichlorophenyl)ethyl)-6-(3'-methyl-[1,4'-bipiperidin]-1'-yl)-1*H*-
5
6
7 pyrazolo[3,4-*b*]pyrazine-3-carboxylate (35 mg, 0.064 mmol) was suspended in a mixture
8
9
10 of EtOH (0.26 mL), THF (0.26 mL), and water (0.13 mL). Lithium hydroxide (12 mg, 0.52
11
12
13
14 mmol) was added and the mixture was heated to 60 °C for 16 h. The reaction was
15
16
17 cooled to room temperature and the solvent was removed under reduced pressure to
18
19
20
21 afford 1-(1-(2,4-dichlorophenyl)ethyl)-6-(3'-methyl-[1,4'-bipiperidin]-1'-yl)-1*H*-
22
23
24 pyrazolo[3,4-*b*]pyrazine-3-carboxylic acid which was used without further purification. To
25
26
27
28 a solution of the carboxylic acid (13 mg, 0.025 mmol) in DMF (0.15 mL) was added
29
30
31 HATU (12 mg, 0.030 mmol) and *N,N*-diisopropylethylamine (9 μ L, 0.050 mmol).
32
33
34
35 Ammonia gas was bubbled through the solution for 2 min, then the solution was stirred
36
37
38 under ammonia atmosphere for 10 min. 0.5 M aqueous HCl solution (10 mL) was
39
40
41
42 added, the solid was collected by filtration and washed with additional 0.5 M aqueous
43
44
45 HCl solution. The solid was dried under reduced pressure to afford 1-(1-(2,4-
46
47
48 dichlorophenyl)ethyl)-6-(3'-methyl-[1,4'-bipiperidin]-1'-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine-3-
49
50
51
52 carboxamide hydrochloride (**12**) as a mixture of diastereomers (98% yield). ¹H NMR
53
54
55
56 (400 MHz, CD₃OD, HCl salt; 1:1 mix of isomers) δ 8.47 (s, 0.5H), 8.46 (s, 0.5H), 7.50 –
57
58
59
60

1
2
3 7.38 (m, 2H), 7.30 – 7.24 (m, 1H), 6.51 – 6.44 (m, 1H), 3.79 – 3.61 (m, 2H), 3.54 – 3.46
4
5
6
7 (m, 1H), 3.20 – 3.12 (m, 1H), 3.11 – 2.88 (m, 4H), 2.68 – 2.58 (m, 1H), 2.24 (d, J = 12.6
8
9
10 Hz, 1H), 2.01 (s, 2H), 1.95 (dd, J = 7.0, 1.1 Hz, 3H), 1.91 – 1.65 (m, 4H), 1.58 (s, 1H),
11
12
13 1.28 (s, 1H), 1.03 (d, J = 6.9 Hz, 1.5H), 0.94 (d, J = 7.0 Hz, 1.5H). LRMS-ESI⁺: *m/z*
14
15
16
17 calcd for C₂₅H₃₂Cl₂N₇O [M+H]⁺ = 516.2; found, 516.
18
19
20
21

22 **1-(1-(2,4-dichlorophenyl)ethyl)-6-(3'-methyl-[1,4'-bipiperidin]-1'-yl)-1*H***

23
24
25 **pyrazolo[3,4-*b*]pyrazine-3-carbonitrile 2,2,2-trifluoroacetate (13).** Step 1. To a solution of

26
27
28
29 1-(1-(2,4-dichlorophenyl)ethyl)-6-(3'-methyl-[1,4'-bipiperidin]-1'-yl)-1*H*-pyrazolo[3,4-
30
31
32 *b*]pyrazine-3-carboxamide hydrochloride (12, 12 mg, 0.023 mmol) was added *N,N*-
33
34
35 diisopropylethylamine (6 μL, 0.035 mmol) and methyl *N*-
36
37
38
39 (triethylammoniosulfonyl)carbamate (36 mg, 0.15 mmol). The reaction mixture was
40
41
42

43 stirred at room temperature for 16 h before and concentrated under reduced pressure.
44
45

46 The residue was purified by reverse phase preparative HPLC (Gemini-NX, 10 μm, 250 x
47
48
49 30 mm, C18 column, Phenomenex, Torrance, CA), eluent: 0 to 100% acetonitrile in
50
51
52
53 water, both eluents containing 0.1% TFA, gradient elution over 30 minutes to afford 1-
54
55
56
57
58
59
60

1
2
3
4 (1-(2,4-dichlorophenyl)ethyl)-6-(3'-methyl-[1,4'-bipiperidin]-1'-yl)-1*H*-pyrazolo[3,4-
5
6
7 *b*]pyrazine-3-carbonitrile 2,2,2-trifluoroacetate (**13**) as a mixture of diastereomers (30%
8
9
10 yield). ¹H NMR (400 MHz, CD₃OD, TFA salt; 1:1 mix of isomers) δ 8.52 (d, J = 5.3 Hz,
11
12
13 1H), 7.49 (td, J = 1.4, 0.7 Hz, 1H), 7.42 – 7.31 (m, 2H), 6.48 (q, J = 7.1 Hz, 1H), 3.79 – 3.62
14
15
16 (m, 2H), 3.55 – 3.47 (m, 1H), 3.24 – 2.89 (m, 4H), 2.68 – 2.58 (m, 1H), 2.25 (d, J = 12.8 Hz,
17
18
19 1H), 2.07 – 1.96 (m, 2H), 1.93 (dd, J = 7.1, 0.8 Hz, 3H), 1.90 – 1.51 (m, 4H), 1.31 – 1.26 (m,
20
21
22 1H), 1.02 (d, J = 6.9 Hz, 1.5H), 0.92 (d, J = 6.9 Hz, 1.5H). LRMS-ESI⁺: *m/z* calcd for
23
24 C₂₅H₂₉Cl₂N₇ [M+H]⁺ = 498.2; found, 498.
25
26

27 **1-(1-(2,4-dichlorophenyl)ethyl)-3-methyl-6-(3'-methyl-[1,4'-bipiperidin]-1'-yl)-1*H*-**
28 **pyrazolo[3,4-*b*]pyrazine 2,2,2-trifluoroacetate (**14**).** Step 1. (1-(2,4-
29
30 dichlorophenyl)ethyl)hydrazine hydrochloride (5.0 g, 20.7 mmol) was dissolved in ethanol (35
31
32 mL) at room temperature and 1-(3,5-dichloropyrazin-2-yl)ethan-1-one (3.6 g, 18.8 mmol) was
33
34 added. The mixture was stirred at room temperature for 8 h and then concentrated under reduced
35
36 pressure. The residue was suspended in 20% ethyl acetate in hexanes (20 mL) and filtered
37
38 through a silica gel plug, eluting with 20% ethyl acetate in hexanes. The filtrate was concentrated
39
40 under reduced pressure to give (*Z*)-3,5-Dichloro-2-(1-(2-(1-(2,4-
41
42 dichlorophenyl)ethyl)hydrazono)ethyl)pyrazine and (*E*)-3,5-dichloro-2-(1-(2-(1-(2,4-
43
44 dichlorophenyl)ethyl)hydrazono)ethyl)pyrazine (9:1) as a viscous orange oil which were used in
45
46 the next step without further purification. LRMS-ESI⁺: *m/z* calcd for C₁₄H₁₂Cl₄N₄ [M+H]⁺ =
47
48 377.0; found, 377.
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 Step 2. A mixture of (*Z*)-3,5-dichloro-2-(1-(2-(1-(2,4-
4 dichlorophenyl)ethyl)hydrazono)ethyl)pyrazine and (*E*)-3,5-dichloro-2-(1-(2-(1-(2,4-
5 dichlorophenyl)ethyl)hydrazono)ethyl)pyrazine (9:1) (3.4 g, 9.2 mmol) was dissolved in *N*-
6 methyl-2-pyrrolidone (20 mL) at room temperature and 2,6-lutidine (3.2 mL, 27.6 mmol) was
7 added. The mixture was degassed with nitrogen and then heated to 100 °C under nitrogen for 8 h.
8 The reaction mixture was cooled to room temperature and poured into a separatory funnel
9 containing 50 mL of 1 M HCl in water and 50 mL of ethyl acetate. The layers were separated,
10 and the organic layer was washed with 50 mL of 1M HCl in water, dried over sodium sulfate,
11 and concentrated under reduced pressure. The residue was purified by silica gel chromatography
12 (0 to 20% (1:1 MTBE:CH₂Cl₂) in hexanes) to provide 6-chloro-1-(1-(2,4-dichlorophenyl)ethyl)-
13 3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazine as off-white solid (65% yield). LRMS-ESI⁺: *m/z* calcd for
14 C₁₄H₁₁Cl₃N₄ [M+H]⁺ = 341.0; found, 341.
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30

31 Step 3. The SnAr was performed following general procedure A using 6-chloro-1-(1-
32 (2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazine (181 mg, 0.53 mmol), 3'-
33 methyl-1,4'-bipiperidine (146 mg, 0.80 mmol), and *N,N*-diisopropylethylamine (0.50 mL, 3.18
34 mmol) in DMSO (2.0 mL) at 100 °C for 2 h. The residue was purified by reverse phase
35 preparative HPLC (Gemini-NX, 10 μm, 250 x 30 mm, C18 column, Phenomenex, Torrance,
36 CA), eluent: 0 to 100% acetonitrile in water, both eluents containing 0.1% TFA, gradient elution
37 over 30 minutes, to afford 1-(1-(2,4-dichlorophenyl)ethyl)-3-methyl-6-(3'-methyl-[1,4'-
38 bipiperidin]-1'-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine 2,2,2-trifluoroacetate (**14**) as a 1.6:1 mixture of
39 diastereomers (10% yield). ¹H NMR (400 MHz, CD₃OD; TFA Salt) δ 8.27 (s, 0.6H), 8.25 (s,
40 0.4H), 7.45 – 7.43 (m, 1H), 7.40 (d, *J* = 8.5 Hz, 0.6H), 7.34 (d, *J* = 8.5 Hz, 0.4H), 7.28 – 7.23 (m,
41 1H), 6.30 (q, *J* = 7.1 Hz, 1H), 4.82 – 4.70 (m, 1H), 4.70 – 4.55 (m, 1H), 3.75 (d, *J* = 12.4 Hz,
42 1H), 3.50 (s, 3H), 3.45 (s, 3H), 3.30 (s, 3H), 3.25 (s, 3H), 3.15 (s, 3H), 3.10 (s, 3H), 3.05 (s, 3H),
43 3.00 (s, 3H), 2.95 (s, 3H), 2.90 (s, 3H), 2.85 (s, 3H), 2.80 (s, 3H), 2.75 (s, 3H), 2.70 (s, 3H),
44 2.65 (s, 3H), 2.60 (s, 3H), 2.55 (s, 3H), 2.50 (s, 3H), 2.45 (s, 3H), 2.40 (s, 3H), 2.35 (s, 3H),
45 2.30 (s, 3H), 2.25 (s, 3H), 2.20 (s, 3H), 2.15 (s, 3H), 2.10 (s, 3H), 2.05 (s, 3H), 2.00 (s, 3H),
46 1.95 (s, 3H), 1.90 (s, 3H), 1.85 (s, 3H), 1.80 (s, 3H), 1.75 (s, 3H), 1.70 (s, 3H), 1.65 (s, 3H),
47 1.60 (s, 3H), 1.55 (s, 3H), 1.50 (s, 3H), 1.45 (s, 3H), 1.40 (s, 3H), 1.35 (s, 3H), 1.30 (s, 3H),
48 1.25 (s, 3H), 1.20 (s, 3H), 1.15 (s, 3H), 1.10 (s, 3H), 1.05 (s, 3H), 1.00 (s, 3H), 0.95 (s, 3H),
49 0.90 (s, 3H), 0.85 (s, 3H), 0.80 (s, 3H), 0.75 (s, 3H), 0.70 (s, 3H), 0.65 (s, 3H), 0.60 (s, 3H),
50 0.55 (s, 3H), 0.50 (s, 3H), 0.45 (s, 3H), 0.40 (s, 3H), 0.35 (s, 3H), 0.30 (s, 3H), 0.25 (s, 3H),
51 0.20 (s, 3H), 0.15 (s, 3H), 0.10 (s, 3H), 0.05 (s, 3H), 0.00 (s, 3H).
52
53
54
55
56
57
58
59
60

1
2
3 1H), 3.65 (m, $J = 12.6$ Hz, 1H), 3.49 (dt, $J = 12.4, 4.0$ Hz, 1H), 3.17 – 3.08 (m, 1H), 3.05 – 2.88
4 (m, 3H), 2.64 – 2.55 (m, 1H), 2.50 (s, 3H), 2.22 (d, $J = 12.4$ Hz, 1H), 2.06 – 1.94 (m, 2H), 1.90 –
5
6 1.68 (m, 7H), 1.62 – 1.48 (m, 1H), 1.02 (d, $J = 6.8$ Hz, 1.8H), 0.94 (d, $J = 6.9$ Hz, 1.2H). LRMS-
7
8 ESI⁺: m/z calcd for C₂₅H₃₂Cl₂N₆ [M+H]⁺ = 487.2; found, 487.
9

10
11
12
13 **1-(1-(2,4-dichlorophenyl)ethyl)-3-methyl-6-(3'-methyl-[1,4'-bipiperidin]-1'-yl)-1H-**
14 **pyrazolo[3,4-*b*]pyrazine hydrochloride (15).** Step 1. To a solution of 2,2,6,6-
15
16 tetramethylpiperidine (13.7 mL, 80.6 mmol) in THF (200 mL) at -40 °C was added *n*-BuLi (2.5
17
18 M in hexanes, 34.9 mL, 87.3 mmol). The mixture was stirred at -40 °C for 30 min. In a separate
19
20 flask, ethyl 2,2,2-trifluoroacetate (10.4 mL, 87.3 mmol) and 2,6-dichloropyrazine (10.0 g, 67.1
21
22 mmol) were dissolved in THF (200 mL) and cooled to -90 °C. The lithium 2,2,6,6-
23
24 tetramethylpiperidine solution was added to the 2,6-dichloropyrazine solution via cannula over
25
26 30 min at -90 °C. The mixture was stirred at -90 °C for 30 min and then (1-(2,4-
27
28 dichlorophenyl)ethyl)hydrazine hydrochloride (9.73 g, 40.3 mmol) was added, and the mixture
29
30 was warmed to room temperature. The mixture was concentrated under reduced pressure, then
31
32 ethanol (200 mL) was added and the mixture was stirred at room temperature for 24 h. The
33
34 mixture was concentrated under reduced pressure and the residue was purified by silica gel
35
36 chromatography (0 to 100% ethyl acetate in hexanes) to provide (*E*)-3,5-Dichloro-2-(1-(2-(1-
37
38 (2,4-dichlorophenyl)ethyl)hydrazono)-2,2,2-trifluoroethyl)pyrazine and (*Z*)-3,5-dichloro-2-(1-(2-
39
40 (1-(2,4-dichlorophenyl)ethyl) hydrazono)-2,2,2-trifluoroethyl)pyrazine (21% yield) as a viscous
41
42 orange oil. LRMS-ESI⁺: m/z calcd for C₁₄H₉Cl₄F₃N₄ [M+H]⁺ = 431.0; found, 431.
43
44
45
46
47
48
49

50
51 Step 2. (*E*)-3,5-dichloro-2-(1-(2-(1-(2,4-dichlorophenyl)ethyl)hydrazono)-2,2,2-
52
53 trifluoroethyl)pyrazine and (*Z*)-3,5-dichloro-2-(1-(2-(1-(2,4-dichlorophenyl)ethyl) hydrazono)-
54
55 2,2,2-trifluoroethyl)pyrazine (2.5 g, 5.79 mmol) were dissolved in THF (58 mL) and the solution
56
57
58
59
60

1
2
3 was cooled to 0 °C. 1,8-Diazabicyclo[5.4.0]undec-7-ene (1.73 mL, 11.6 mmol) was added
4
5 dropwise. After the addition was complete, the mixture warmed to room temperature and stirred
6
7 for 10 h. The mixture was concentrated under reduced pressure and the residue was purified by
8
9 silica gel chromatography (0 to 20% ethyl acetate in hexanes) to provide 6-chloro-1-(1-(2,4-
10
11 dichlorophenyl)ethyl)-3-(trifluoromethyl)-1*H*-pyrazolo[3,4-*b*]pyrazine (79% yield) as a light
12
13 orange oil. LRMS-ESI⁺: *m/z* calcd for C₁₄H₈Cl₂F₃N₄ [M+H]⁺ = 395.0; found, 395.
14
15
16
17

18 Step 3. The SnAr was performed following general procedure A using 6-chloro-1-(1-
19
20 (2,4-dichlorophenyl)ethyl)-3-(trifluoromethyl)-1*H*-pyrazolo[3,4-*b*]pyrazine (0.253 mmol), 3'-
21
22 methyl-1,4'-bipiperidine (51 mg, 0.278 mmol), and *N,N*-diisopropylethylamine (88 μL, 0.506
23
24 mmol) in dichloromethane (5 mL) at room temperature for 8 h. The residue was purified by
25
26 silica gel chromatography (0 to 20% methanol in dichloromethane). After concentrating the
27
28 fractions containing the desired product, HCl (1 N in diethyl ether, 1 mL) was added and stirred
29
30 1 minute. The mixture was concentrated under reduced pressure to provide 1-(1-(2,4-
31
32 dichlorophenyl)ethyl)-3-methyl-6-(3'-methyl-[1,4'-bipiperidin]-1'-yl)-1*H*-pyrazolo[3,4-
33
34 *b*]pyrazine hydrochloride (**15**) as a mixture of diastereomers (78% yield) as a yellow solid. ¹H
35
36 NMR (400 MHz, CDCl₃; HCl Salt) δ 8.28 (s, 0.6H), 8.28 (s, 0.4H), 7.41 – 7.33 (m, 2H), 7.22 –
37
38 7.15 (m, 1H), 6.49 – 6.40 (m, 1H), 4.59 (d, *J* = 13.2 Hz, 1H), 4.40 (t, *J* = 13.1 Hz, 1H), 3.06 (d, *J* =
39
40 = 12.9 Hz, 1H), 2.88 (t, *J* = 13.1 Hz, 1H), 2.55 – 2.38 (m, 4H), 2.36 – 2.19 (m, 2H), 1.93 (d, *J* =
41
42 7.1 Hz, 3H), 1.60 (m, 6H), 1.50 – 1.41 (m, 2H), 0.91 (d, *J* = 6.9 Hz, 1.8H), 0.84 (d, *J* = 6.8 Hz,
43
44 1.2H). LRMS-ESI⁺: *m/z* calcd for C₂₅H₂₉Cl₂F₃N₆ [M+H]⁺ = 541.2; found, 541.
45
46
47
48
49
50

51 **1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-6-((3'*R*,4'*S*)-3'-methyl-[1,4'-**
52
53 **bipiperidin]-1'-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine hydrochloride (**16**). Step 1. To a solution of**
54
55 *trans*-methylpiperidin-4-yl pivalate (1.93 g, 9.65 mmol) in EtOAc (8 mL) at room temperature
56
57
58
59
60

1
2
3 was added a solution of (+)-*O,O'*-dibenzoyl-*D*-tartaric acid (1.73 g, 4.8 mmol) in EtOAc (13
4 mL). A precipitate formed immediately and the solution was then cooled to 0 °C then filtered
5
6 through a Buchner funnel. The crystals were washed with cold EtOAc and dried in vacuo. The
7
8 crystals were recrystallized in refluxing MeOH (14 mL), slowly cooled to room temperature, and
9
10 filtered with a Buchner funnel to afford a white solid which was washed with minimal cold
11
12 MeOH. The product was dried in vacuo, taken up in diethyl ether, and washed with 1N NaOH
13
14 three times. The organic layer was dried over sodium sulfate, filtered, and concentrated in vacuo
15
16 to afford (3*R*,4*R*)-3-methylpiperidin-4-yl pivalate (38% yield). ¹HNMR and LCMS matched
17
18 literature values previously reported.⁴² Stereochemistry was confirmed by obtained a single
19
20 crystal structure of the tartrate salt (Cambridge Crystallographic Data Centre Deposition Number
21
22 1915201, authors will release the atomic coordinates upon article publication).
23
24
25
26
27
28
29

30 Step 2. To a solution of (3*R*,4*R*)-3-methylpiperidin-4-yl pivalate (0.73 g, 3.65 mmol) and
31
32 benzaldehyde (0.46 g, 4.4 mmol) in dichloromethane (10 mL) at room temperature was added
33
34 sodium triacetoxyborohydride. The solution was stirred overnight. The reaction was quenched
35
36 with sat. aq. NaHCO₃, extracted with EtOAc, dried over sodium sulfate, filtered, and
37
38 concentrated via rotary evaporation to afford the desired product which was used without further
39
40 purification. This crude oil was then taken up in MeOH (20 mL) and treated with NaOMe (25%
41
42 in MeOH, 4 mL). The solution was refluxed overnight, cooled to room temperature, and
43
44 concentrated. The crude material was diluted with diethyl ether, washed with 1N NaOH (x2),
45
46 dried over sodium sulfate, filtered, and concentrated in vacuo. The residue was purified via silica
47
48 gel chromatography (0 to 20% MeOH in dichloromethane) to afford (3*R*,4*R*)-1-benzyl-3-
49
50 methylpiperidin-4-ol (33% yield). Ee: 83% Chiralpak® IF-3; 250mmx4.6mm, 10% EtOH in
51
52
53
54
55
56
57
58
59
60

1
2
3 heptanes (with 0.1% Et₂NH); flow rate 1 mL/min; detection at 254 nm; t₁ = 4.3 min (minor) t₂ =
4
5 6.2 min (major). LRMS-ESI⁺: *m/z* calcd for C₁₃H₁₉NO [M+H]⁺ = 206.2; found, 206.
6
7

8
9 Step 3. To a solution of (3*R*,4*R*)-1-benzyl-3-methylpiperidin-4-ol (690 mg, 3.2 mmol) in
10
11 dichloromethane (10 mL) at 0 °C was added triethylamine (0.49 mL, 3.5 mmol) and
12
13 methanesulfonyl chloride (0.25 mL, 3.2 mmol) in that order. After 1 hr the solution was treated
14
15 with sat. aq. NaHCO₃, extracted with dichloromethane, dried over sodium sulfate, filtered, and
16
17 concentrated in vacuo. The crude product was purified via silica gel chromatography (0 to 100%
18
19 EtOAc in hexanes) to afford (3*R*,4*R*)-1-benzyl-3-methylpiperidin-4-yl methanesulfonate (70%
20
21 yield). To a solution of (3*R*,4*R*)-1-benzyl-3-methylpiperidin-4-yl methanesulfonate (636 mg,
22
23 2.24 mmol) in DMF (10 mL) was added sodium azide (291 mg, 4.48 mmol) at room temperature
24
25 and the reaction was heated to 60 °C for 18 h. The solution was cooled to room temperature,
26
27 diluted with water, and extracted with diethyl ether (x3). The combined organic phases were
28
29 dried over sodium sulfate, filtered, and concentrated in vacuo. The residue was purified via silica
30
31 gel chromatography (0 to 100% EtOAc in hexanes) to afford (3*R*,4*S*)-4-azido-1-benzyl-3-
32
33 methylpiperidine (39% yield). LRMS-ESI⁺: *m/z* calcd for C₁₃H₁₈N₄ [M+H]⁺ = 231.2; found, 231.
34
35
36
37
38

39 Step 4. To a solution of (3*R*,4*S*)-4-azido-1-benzyl-3-methylpiperidine (200 mg, 0.87
40
41 mmol) in diethyl ether (16 mL) at 0 °C was added lithium aluminum hydride (1.0 mL, 4M in
42
43 diethyl ether) and the solution was left to slowly warm to room temperature overnight. The
44
45 reaction mixture was quenched using the standard Fieser workup, filtered through a plug of celite
46
47 with EtOAc and concentrated in vacuo to afford (3*R*,4*S*)-1-benzyl-3-methylpiperidin-4-amine
48
49 which was used without further purification. To a solution of the crude amine (30 mg, 0.15
50
51 mmol) in acetonitrile (2 mL) was added 1,5-dibromopentane (18 μL, 0.14 mmol) and potassium
52
53 carbonate (37 mg, 0.27 mmol) at room temperature. The solution was heated to 60 °C for 18 h,
54
55
56
57
58
59
60

1
2
3 cooled to room temperature, and filtered through a pad of celite with MeOH. The mixture was
4 concentrated in vacuo and purified via reversed phase preparative HPLC (Gemini-NX, 10 μ m,
5 250 x 30 mm, C18 column, Phenomenex, Torrance, CA), eluent: 0 to 100% acetonitrile in water,
6 both eluents containing 0.1% TFA, gradient elution over 30 minutes, to afford (3'*R*,4'*S*)-1'-
7 benzyl-3'-methyl-1,4'-bipiperidine (22% yield). LRMS-ESI⁺: *m/z* calcd for C₁₈H₂₈N₂ [M+H]⁺ =
8 273.2; found, 273.
9
10
11
12
13
14
15
16
17

18 Step 5. A solution of (3'*R*,4'*S*)-1'-benzyl-3'-methyl-1,4'-bipiperidine (50 mg, 0.18 mmol)
19 in MeOH (0.8 mL), AcOH (0.2 mL), and EtOAc (1.0 mL) was sparged with argon gas for 10
20 minutes. To this solution was added palladium hydroxide (3 mg, 10 mol%) and the solution was
21 sparged with hydrogen gas for 10 minutes. The reaction as stirred under a hydrogen atmosphere
22 overnight at room temperature. The suspension was sparged with argon gas for 10 minutes then
23 filtered through a pad of celite with MeOH to afford crude (3'*R*,4'*S*)-3'-methyl-1,4'-bipiperidine
24 which was used in the next step without further purification. LRMS-ESI⁺: *m/z* calcd for
25 C₁₁H₂₂N₂ [M+H]⁺ = 183.2; found, 183.
26
27
28
29
30
31
32
33
34
35
36

37 Step 6. The SnAr was performed following general procedure A using (*R*)-6-chloro-1-(1-
38 (2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazine (**67**, 180 mg, 0.51 mmol),
39 (3'*R*,4'*S*)-3'-methyl-1,4'-bipiperidine (95 mg, 0.51 mmol), and *N,N*-diisopropylethylamine (0.17
40 mL, 1.0 mmol) in DMF (2 mL) at 80 °C for 16 h. The residue was purified by reverse phase
41 preparative HPLC (Gemini-NX, 10 μ m, 250 x 30 mm, C18 column, Phenomenex, Torrance,
42 CA), eluent: 0 to 100% acetonitrile in water, both eluents containing 0.1% TFA, gradient elution
43 over 30 minutes to give a mixture of stereoisomers. The mixture of stereoisomers was basified
44 using a basification column (PL-HCO₃ MP SPE, 500 mg per 5 mL tube, Agilent) using
45 dichloromethane and methanol (4:1) as the eluent and concentrated under reduced pressure. The
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 residue was dissolved in ethanol, treated with 2 N HCl in Et₂O (0.2 mL), and concentrated under
4
5 reduced pressure to afford 1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-6-((3'*R*,4'*S*)-3'-methyl-
6
7 [1,4'-bipiperidin]-1'-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine (**16**, 46% yield). ¹H NMR (400 MHz,
8
9 CD₃CN; HCl Salt) δ 8.17 (s, 1H), 7.47 (d, *J* = 2.2 Hz, 1H), 7.41 (d, *J* = 8.6 Hz, 1H), 7.26 (dd, *J* =
10
11 8.5, 2.2 Hz, 1H), 6.25 (q, *J* = 7.1 Hz, 1H), 4.53 (ddt, *J* = 13.3, 4.8, 2.5 Hz, 1H), 4.40 (dt, *J* = 13.5,
12
13 2.7 Hz, 1H), 3.00 (dd, *J* = 13.5, 2.9 Hz, 1H), 2.82 (td, *J* = 13.2, 3.1 Hz, 1H), 2.43 (s, 3H), 2.42 –
14
15 2.35 (m, 4H), 2.31 – 2.21 (m, 1H), 2.20 – 2.15 (m, 1H), 1.88 – 1.82 (m, 1H), 1.84 (d, *J* = 7.1 Hz,
16
17 3H), 1.58 – 1.47 (m, 4H), 1.47 – 1.35 (m, 3H), 0.81 (d, *J* = 7.0 Hz, 3H). LRMS-ESI⁺: *m/z* calcd
18
19 for C₂₅H₃₂Cl₂N₆ [M+H]⁺ = 487.2; found, 487.
20
21
22
23
24

25 **1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-6-((3'*S*,4'*R*)-3'-methyl-[1,4'-**
26 **bipiperidin]-1'-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine hydrochloride (**17**). The inactive isomer was
27 prepared via the same route, however during the chiral resolution (-)-*O*,*O'*-dibenzoyl-*L*-tartaric
28 acid was used in place of (+)-*O*,*O'*-dibenzoyl-*D*-tartaric acid to afford (3*S*,4*S*)-3-methylpiperidin-
29 4-yl pivalate (a single crystal x-ray of this tartrate was also obtained, Cambridge
30
31 Crystallographic Data Centre Deposition Number 1915202, authors will release the atomic
32
33 coordinates upon article publication). This side chain was found to yield the less active
34
35 isomer **17**, and thus the stereochemistry of this series was based on these results. ¹H NMR (400
36
37 MHz, CD₃CN; HCl Salt) δ 8.16 (s, 1H), 7.46 (d, *J* = 2.2 Hz, 1H), 7.39 (dd, *J* = 8.5, 2.2 Hz, 1H),
38
39 7.25 (dd, *J* = 8.5, 2.2 Hz, 1H), 6.25 (q, *J* = 7.1 Hz, 1H), 4.53 (ddt, *J* = 13.1, 4.5, 2.4 Hz, 1H), 4.45
40
41 (dt, *J* = 13.5, 2.7 Hz, 1H), 3.00 (dd, *J* = 13.4, 2.8 Hz, 1H), 2.82 (td, *J* = 13.2, 3.1 Hz, 1H), 2.43 (s,
42
43 3H), 2.46 – 2.37 (m, 4H), 2.29 – 2.22 (m, 1H), 2.21 – 2.15 (m, 1H), 1.90 – 1.84 (m, 1H), 1.83 (d,
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60**

1
2
3 $J = 7.1$ Hz, 3H), 1.57 – 1.47 (m, 4H), 1.47 – 1.36 (m, 3H), 0.75 (d, $J = 6.9$ Hz, 3H). LRMS-ESI⁺:
4
5 m/z calcd for C₂₅H₃₂Cl₂N₆ [M+H]⁺ = 487.2; found, 487.
6
7

8
9 **1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-6-((3*R*,4*S*)-3-methyl-4-(pyrrolidin-1-**
10 **yl)piperidin-1-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine hydrochloride (18).** Step 1. The reductive
11 amination was performed following general procedure D using 1-*tert*-butoxycarbonyl-3-methyl-
12 4-piperidone (213 mg, 1.0 mmol), pyrrolidine (0.12 mL, 1.5 mmol), sodium
13 triacetoxyborohydride (316 mg, 1.5 mmol) in 1,2-dichloroethane (2.5 mL) at room temperature
14 for 16 h. The crude residue was dissolved in methanol (0.5 mL) and HCl (4 N in 1,4-dioxane, 2
15 mL, 8 mmol) was added. The mixture was stirred at room temperature for 1 h and then
16 concentrated under reduced pressure to afford 3-methyl-4-(pyrrolidin-1-yl)piperidine
17 hydrochloride which was used without further purification. LRMS-ESI⁺: m/z calcd for C₁₀H₂₀N₂
18 [M+H]⁺ = 168.2; found, 168.
19
20
21
22
23
24
25
26
27
28
29
30
31

32 Step 2. The SnAr was performed following general procedure A using (*R*)-6-chloro-1-(1-
33 (2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazine (**67**, 75 mg, 0.22 mmol), 3-
34 methyl-4-(pyrrolidin-1-yl)piperidine hydrochloride (41 mg, 0.2 mmol), and *N,N*-
35 diisopropylethylamine (0.15 mL, 0.86 mmol) in DMF (1.0 mL) at 80 °C for 1 h. The
36 residue was purified by reverse phase preparative HPLC (Gemini-NX, 10 μm, 250 x 30
37 mm, C18 column, Phenomenex, Torrance, CA), eluent: 0 to 100% acetonitrile in water,
38 both eluents containing 0.1% TFA, gradient elution over 30 minutes to give a mixture of
39 stereoisomers. The stereoisomers were basified using a basification column (PL-HCO₃
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 MP SPE, 500 mg per 5 mL tube, Agilent) using dichloromethane and methanol (4:1) as
4
5
6
7 the eluent and concentrated under reduced pressure. The residue was azeotroped with
8
9
10 ethanol and the residue was further purified using chiral HPLC (OZ-H, Chiralpak®,
11
12
13 Daicel Corporation, West Chester, PA), eluent: 20% ethanol in heptanes, with heptanes
14
15
16 containing 0.1% diethylamine, 30 min. The first eluting isomer was isolated and the free
17
18
19 base was dissolved in ethanol, treated with 2 N HCl in Et₂O (0.2 mL), and concentrated
20
21
22 under reduced pressure to afford 1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-6-
23
24
25 ((3*R*,4*S*)-3-methyl-4-(pyrrolidin-1-yl)piperidin-1-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine
26
27
28 hydrochloride (**18**, 7% yield). ¹H NMR (400 MHz, CD₃OD; HCl salt) δ 8.31 (s, 1H), 7.46
29
30
31 (d, *J* = 2.1 Hz, 1H), 7.43 (d, *J* = 8.5 Hz, 1H), 7.28 (dd, *J* = 8.5, 2.1 Hz, 1H), 6.32 (q, *J* =
32
33
34 6.9 Hz, 1H), 4.75 (dm, *J* = 14.1 Hz, 1H), 4.60 (dm, *J* = 13.8 Hz, 1H), 3.76 – 3.67 (m,
35
36
37 2H), 3.52 – 3.45 (m, 1H), 3.26 – 3.11 (m, 3H), 3.01 (td, *J* = 13.6, 3.0 Hz, 1H), 2.57 –
38
39
40 2.50 (m, 1H), 2.52 (s, 3H), 2.24 – 2.16 (m, 2H), 2.14 – 1.98 (m, 3H), 1.96 – 1.83 (m, 1H),
41
42
43 1.89 (d, *J* = 7.1 Hz, 3H), 1.05 (d, *J* = 6.9 Hz, 3H). LRMS-ESI⁺: *m/z* calcd for C₂₄H₃₀Cl₂N₆
44
45
46
47
48
49
50
51
52 [M+H]⁺ = 473.2; found, 473.
53
54
55
56
57
58
59
60

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

6-((3*R*,4*S*)-4-(azetidin-1-yl)-3-methylpiperidin-1-yl)-1-((*R*)-1-(2,4-

dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazine hydrochloride (19). Step 1.

The reductive amination was performed following general procedure D using 1-*tert*-butoxycarbonyl-3-methyl-4-piperidone (213 mg, 1.0 mmol), azetidine (0.10 mL, 1.5 mmol), and sodium triacetoxyborohydride (316 mg, 1.5 mmol) in 1,2-dichloroethane (2.5 mL) at room temperature for 16 h. The crude residue was dissolved in methanol (0.5 mL) and HCl (4 N in 1,4-dioxane, 2 mL, 8 mmol) was added. The mixture was stirred at room temperature for 1 h and then concentrated under reduced pressure to afford 4-(azetidin-1-yl)-3-methylpiperidine hydrochloride which was used without further purification. LRMS-ESI⁺: *m/z* calcd for C₉H₁₈N₂ [M+H]⁺ = 155.2; found, 155.

Step 2. The SnAr was performed following general procedure A using (*R*)-6-chloro-1-(1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazine (67, 75 mg, 0.22 mmol), 4-(azetidin-1-yl)-3-methylpiperidine hydrochloride (38 mg, 0.2 mmol), and *N,N*-diisopropylethylamine (0.15 mL, 0.86 mmol) in DMF (1.0 mL) at 80 °C for 1 h. The residue was purified by reverse phase preparative HPLC (Gemini-NX, 10 μm, 250 x 30

1
2
3 mm, C18 column, Phenomenex, Torrance, CA), eluent: 0 to 100% acetonitrile in water,
4
5
6
7 both eluents containing 0.1% TFA, gradient elution over 30 minutes to afford a mixture
8
9
10 of stereoisomers. The stereoisomers were basified using a basification column (PL-
11
12
13 HCO3 MP SPE, 500 mg per 5 mL tube, Agilent) using dichloromethane and methanol
14
15
16
17 (4:1) as the eluent and concentrated under reduced pressure. The residue was
18
19
20 azeotroped with ethanol and the residue was further purified using chiral HPLC (OZ-H,
21
22
23 Chiralpak®, Daicel Corporation, West Chester, PA), eluent: 20% ethanol in heptanes,
24
25
26
27 with heptanes containing 0.1% diethylamine, 30 min. The first eluting isomer was
28
29
30
31 isolated and the free base was dissolved in ethanol, treated with 2 N HCl in Et₂O (0.2
32
33
34 mL), and concentrated under reduced pressure to afford 6-((3*R*,4*S*)-4-(azetidin-1-yl)-3-
35
36
37 methylpiperidin-1-yl)-1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-
38
39
40
41 *b*]pyrazine hydrochloride (**19**, 5% yield). ¹H NMR (400 MHz, CD₃OD; HCl salt) δ 8.32 (s,
42
43
44 1H), 7.46 (s, *J* = 2.1 Hz, 1H), 7.44 (d, *J* = 8.5 Hz, 1H), 7.29 (dd, *J* = 8.5, 2.1 Hz, 1H),
45
46
47 6.31 (q, *J* = 7.1 Hz, 1H), 4.70 (dm, *J* = 13.5 Hz, 1H), 4.57 (dt, *J* = 13.8, 2.4 Hz, 1H), 4.37
48
49
50
51 – 4.09 (m, 4H), 3.64 (dt, *J* = 12.2, 4.3 Hz, 1H), 3.18 (dd, *J* = 14.0, 2.6 Hz, 1H), 3.06 –
52
53
54
55 2.97 (m, 1H), 2.74 – 2.58 (m, 1H), 2.52 (s, 3H), 2.47 – 2.32 (m, 2H), 1.96 – 1.88 (m,
56
57
58
59
60

1
2
3
4 1H),. 1.89 (d, $J = 7.1$ Hz, 3H), 1.71 – 1.56 (m, 1H), 0.92 (d, $J = 7.0$ Hz, 3H). LRMS-

5
6
7 ESI⁺: m/z calcd for C₂₃H₂₈Cl₂N₆ [M+H]⁺ = 459.2; found, 459.
8
9

10
11 **6-((3*R*,4*S*)-4-(azepan-1-yl)-3-methylpiperidin-1-yl)-1-((*R*)-1-(2,4-**
12
13
14 **dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazine hydrochloride (20).** Step 1.
15
16

17
18 The reductive amination was performed following general procedure D using 1-*tert*-
19
20
21
22 butoxycarbonyl-3-methyl-4-piperidone (213 mg, 1.0 mmol), azepane (0.17 mL, 1.5
23
24
25 mmol), and sodium triacetoxyborohydride (316 mg, 1.5 mmol) in 1,2-dichloroethane (2.5
26
27
28 mL) at room temperature for 16 h. The crude residue was dissolved in methanol (0.5
29
30
31 mL) and HCl (4 N in 1,4-dioxane, 2 mL, 8 mmol) was added. The mixture was stirred at
32
33
34
35 room temperature for 1 h and then concentrated under reduced pressure to afford 1-(3-
36
37
38 methylpiperidin-4-yl)azepane hydrochloride which was used without further purification.
39
40
41

42
43 LRMS-ESI⁺: m/z calcd for C₁₂H₂₄N₂ [M+H]⁺ = 197.2; found, 197.
44
45

46
47 Step 2. The SnAr was performed following general procedure A (*R*)-6-chloro-1-
48
49
50 (1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazine (**67**, 75 mg, 0.22
51
52
53 mmol), 1-(3-methylpiperidin-4-yl)azepane hydrochloride (47 mg, 0.2 mmol), and *N,N*-
54
55
56
57
58
59
60

1
2
3 diisopropylethylamine (0.15 mL, 0.86 mmol) in DMF (1.0 mL) at 80 °C for 1 h. The
4
5
6
7 residue was purified by reverse phase preparative HPLC (Gemini-NX, 10 μm, 250 x 30
8
9
10 mm, C18 column, Phenomenex, Torrance, CA), eluent: 0 to 100% acetonitrile in water,
11
12
13 both eluents containing 0.1% TFA, gradient elution over 30 minutes to give a mixture of
14
15
16
17 stereoisomers. The stereoisomers were basified using a basification column (PL-HCO₃
18
19
20 MP SPE, 500 mg per 5 mL tube, Agilent) using dichloromethane and methanol (4:1) as
21
22
23
24 the eluent and concentrated under reduced pressure. The residue was azeotroped with
25
26
27
28 ethanol and the residue was further purified using chiral HPLC (OZ-H, Chiralpak®,
29
30
31 Daicel Corporation, West Chester, PA), eluent: 20% ethanol in heptanes, with heptanes
32
33
34 containing 0.1% diethylamine, 30 min. The first eluting isomer was isolated and the free
35
36
37
38 base was dissolved in ethanol, treated with 2 N HCl in Et₂O (0.2 mL), and concentrated
39
40
41
42 under reduced pressure to afford 6-((3*R*,4*S*)-4-(azepan-1-yl)-3-methylpiperidin-1-yl)-1-
43
44
45 ((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazine hydrochloride
46
47
48 (**20**, 10% yield). ¹H NMR (400 MHz, CD₃OD; HCl salt) δ 8.11 (s, 1H), 7.21 (d, *J* = 2.1
49
50
51 Hz, 1H), 7.14 (d, *J* = 8.5 Hz, 1H), 7.04 (dd, *J* = 8.5, 2.2 Hz, 1H), 6.09 (q, *J* = 7.2 Hz, 1H),
52
53
54
55 4.56 – 4.38 (m, 2H), 3.42 (dt, *J* = 7.0, 3.7 Hz, 1H), 3.35 – 3.07 (m, 4H), 2.95 (dd, *J* =

1
2
3 14.0, 2.2 Hz, 1H), 2.87 – 2.78 (m, 1H), 2.37 (s, 1H), 2.33 – 2.27 (m, 1H), 2.29 (s, 3H),
4
5
6
7 1.94 (br d, $J = 11.0$ Hz, 1H), 1.74 (d, $J = 25.2$ Hz, 4H), 1.65 (d, $J = 7.1$ Hz, 3H), 1.61 –
8
9
10 1.45 (m, 4H), 0.73 (d, $J = 6.9$ Hz, 3H). LRMS-ESI⁺: m/z calcd for C₂₆H₃₄Cl₂N₆ [M+H]⁺ =
11
12
13
14 501.2; found, 501.
15
16
17

18 **1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-6-((3*R*,4*S*)-3-methyl-4-((*R*)-2-**
19
20
21 **methylpyrrolidin-1-yl)piperidin-1-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine hydrochloride (21). Step**
22
23
24

25 1. The reductive amination was performed following general procedure D using 1-*tert*-
26
27
28
29 butoxycarbonyl-3-methyl-4-piperidone (213 mg, 1.0 mmol), (*R*)-2-methylpyrrolidine (128
30
31
32 mg, 1.5 mmol), and sodium triacetoxyborohydride (316 mg, 1.5 mmol) in 1,2-
33
34
35
36 dichloroethane (2.5 mL) at room temperature for 16 h. The crude residue was dissolved
37
38
39 in methanol (0.5 mL) and HCl (4 N in 1,4-dioxane, 2 mL, 8 mmol) was added. The
40
41
42
43 mixture was stirred at room temperature for 1 h and then concentrated under reduced
44
45
46 pressure to give 3-methyl-4-((*R*)-2-methylpyrrolidin-1-yl)piperidine hydrochloride which
47
48
49 was used without further purification. LRMS-ESI⁺: m/z calcd for C₁₁H₂₂N₂ [M+H]⁺ =
50
51
52
53 183.2; found, 183.
54
55
56
57
58
59
60

1
2
3
4 Step 2. The SnAr was performed following general procedure A using (*R*)-6-
5
6
7 chloro-1-(1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazine (**67**, 75 mg,
8
9
10 0.22 mmol), 3-methyl-4-((*R*)-2-methylpyrrolidin-1-yl)piperidine hydrochloride (44 mg, 0.2
11
12
13 mmol), and *N,N*-diisopropylethylamine (0.15 mL, 0.86 mmol) in DMF (1.0 mL) at 80 °C
14
15
16
17 for 1 h. The residue was purified by reverse phase preparative HPLC (Gemini-NX, 10
18
19
20 μm, 250 x 30 mm, C18 column, Phenomenex, Torrance, CA), eluent: 0 to 100%
21
22
23 acetonitrile in water, both eluents containing 0.1% TFA, gradient elution over 30 minutes
24
25
26
27 to give a mixture of stereoisomers. The stereoisomers were basified using a basification
28
29
30 column (PL-HCO3 MP SPE, 500 mg per 5 mL tube, Agilent) using dichloromethane and
31
32
33 methanol (4:1) as the eluent and concentrated under reduced pressure. The residue
34
35
36
37 was azeotroped with ethanol and the residue was further purified using chiral HPLC (ID-
38
39
40 3, Chiralpak®, Daicel Corporation, West Chester, PA), eluent: 20% ethanol in heptanes,
41
42
43
44 with heptanes containing 0.1% diethylamine, 30 min. The first eluting isomer was
45
46
47
48 isolated and the free base was dissolved in ethanol, treated with 2 N HCl in Et₂O (0.2
49
50
51 mL), and concentrated under reduced pressure to afford 1-((*R*)-1-(2,4-
52
53
54
55 dichlorophenyl)ethyl)-3-methyl-6-((3*R*,4*S*)-3-methyl-4-((*R*)-2-methylpyrrolidin-1-

1
2
3
4 yl)piperidin-1-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine hydrochloride (**21**, 5% yield). ¹H NMR (400
5
6 MHz, CDCl₃, HCl salt): δ 8.09 (s, 1H), 7.40 (d, *J* = 8.5 Hz, 1H), 7.35 (d, *J* = 2.1 Hz, 1H),
7
8
9
10 7.14 (dd, *J* = 8.5, 2.2 Hz, 1H), 6.31 (q, *J* = 7.1 Hz, 1H), 4.55 – 4.47 (m, 1H), 4.37 – 4.31
11
12
13 (m, 1H), 3.15 – 3.07 (m, 1H), 3.05 – 2.98 (m, 1H), 2.86 (td, *J* = 12.8, 3.6 Hz, 1H), 2.79 –
14
15
16
17 2.72 (m, 1H), 2.68 – 2.60 (m, 1H), 2.58 – 2.53 (m, 4H), 2.18 – 2.11 (m, 1H), 1.90 (d, *J* =
18
19
20
21 7.1 Hz, 3H), 1.83 – 1.63 (m, 5H), 1.50 – 1.42 (m, 1H), 0.99 – 0.93 (m, 6H). LRMS-ESI⁺:
22
23
24 *m/z* calcd for C₂₅H₃₂Cl₂N₆ [M+H]⁺ = 487.2; found, 487.
25
26
27
28

29 **1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-6-((3*R*,4*S*)-3-methyl-4-((*S*)-2-**
30
31
32 **methylpyrrolidin-1-yl)piperidin-1-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine hydrochloride (**22**). Step**
33
34
35
36 1. The reductive amination was performed following general procedure D using 1-*tert*-
37
38
39 butoxycarbonyl-3-methyl-4-piperidone (213 mg, 1.0 mmol), (*S*)-2-methylpyrrolidine (128
40
41
42 mg, 1.5 mmol), and sodium triacetoxyborohydride (316 mg, 1.5 mmol) in 1,2-
43
44
45
46 dichloroethane (2.5 mL) at room temperature for 16 h. The crude residue was dissolved
47
48
49
50 in methanol (0.5 mL) and HCl (4 N in 1,4-dioxane, 2 mL, 8 mmol) was added. The
51
52
53
54 mixture was stirred at room temperature for 1 h and then concentrated under reduced
55
56
57
58
59
60

1
2
3 pressure to give 3-methyl-4-((*S*)-2-methylpyrrolidin-1-yl)piperidine hydrochloride which
4
5
6
7 was used without further purification. LRMS-ESI⁺: *m/z* calcd for C₁₁H₂₂N₂ [M+H]⁺ =
8
9
10 183.2; found, 183.
11
12
13

14
15 Step 2. The SnAr was performed following general procedure A using (*R*)-6-
16
17 chloro-1-(1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazine (**67**, 75 mg,
18
19 0.22 mmol), 3-methyl-4-((*S*)-2-methylpyrrolidin-1-yl)piperidine hydrochloride (44 mg, 0.2
20
21 mmol), and *N,N*-diisopropylethylamine (0.15 mL, 0.86 mmol) in DMF (1.0 mL) at 80 °C
22
23
24
25
26
27
28 for 1 h. The residue was purified by reverse phase preparative HPLC (Gemini-NX, 10
29
30
31 μm, 250 x 30 mm, C18 column, Phenomenex, Torrance, CA), eluent: 0 to 100%
32
33
34
35 acetonitrile in water, both eluents containing 0.1% TFA, gradient elution over 30 minutes
36
37
38
39 to give a mixture of stereoisomers. The stereoisomers were basified using a basification
40
41
42 column (PL-HCO₃ MP SPE, 500 mg per 5 mL tube, Agilent) using dichloromethane and
43
44
45 methanol (4:1) as the eluent and concentrated under reduced pressure. The residue
46
47
48
49 was azeotroped with ethanol and the residue was further purified using chiral HPLC (ID-
50
51
52
53 3, Chiralpak®, Daicel Corporation, West Chester, PA), eluent: 20% ethanol in heptanes,
54
55
56
57
58
59
60

1
2
3 with heptanes containing 0.1% diethylamine, 30 min. The first eluting isomer was
4
5
6
7 isolated and the free base was dissolved in ethanol, treated with 2 N HCl in Et₂O (0.2
8
9
10 mL), and concentrated under reduced pressure to afford 1-((*R*)-1-(2,4-
11
12
13 dichlorophenyl)ethyl)-3-methyl-6-((3*R*,4*S*)-3-methyl-4-((*S*)-2-methylpyrrolidin-1-
14
15
16 yl)piperidin-1-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine hydrochloride (**22**, 5% yield). ¹H NMR (400
17
18 MHz, CDCl₃; HCl salt): δ 8.08 (s, 1H), 7.37 (d, *J* = 8.5 Hz, 1H), 7.34 (d, *J* = 2.1 Hz, 1H),
19
20
21 7.15 – 7.11 (m, 1H), 6.35 – 6.29 (m, 1H), 4.58 – 4.51 (m, 1H), 4.38 – 4.33 (m, 1H), 3.18
22
23
24 – 2.98 (m, 2H), 2.92 – 2.61 (m, 3H), 2.56 (s, 3H), 2.19 – 2.10 (m, 1H), 1.90 (d, *J* = 7.1
25
26
27 Hz, 4H), 1.83 – 1.60 (m, 2H), 1.49 – 1.42 (m, 1H), 1.32 – 1.23 (m, 3H), 0.95 (d, *J* = 6.3
28
29
30 Hz, 3H), 0.87 (d, *J* = 6.7 Hz, 3H). LRMS-ESI⁺: *m/z* calcd for C₂₅H₃₂Cl₂N₆ [M+H]⁺ =
31
32
33
34
35
36
37
38 487.2; found, 487.
39
40
41

42
43 **(*S*)-1-((3*R*,4*S*)-1-(1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-
44
45
46 *b*]pyrazin-6-yl)-3-methylpiperidin-4-yl)pyrrolidine-2-carboxamide hydrochloride (**23**).**
47
48

49
50 Step 1. The reductive amination was performed following general procedure D using 1-
51
52
53 *tert*-butoxycarbonyl-3-methyl-4-piperidone (1.5 g, 7.03 mmol), *L*-prolinamide (1.2 g, 10.5
54
55
56
57
58
59
60

1
2
3 mmol), AcOH (0.6 mL, 10.5 mmol), and sodium triacetoxyborohydride (2.22 g, 10.7
4
5
6 mmol) in 1,2-dichloroethane (20 mL) at room temperature 16 h. The residue was
7
8
9
10 purified by silica gel chromatography (0 to 5% methanol in dichloromethane) to afford a
11
12
13 mixture of diastereomers. The residue was dissolved in dichloromethane (20 mL) and
14
15
16 HCl (4 N in 1,4-dioxane, 5 mL) was added. The mixture was stirred at room temperature
17
18
19
20 for 1 h and then concentrated under reduced pressure to afford (2*S*)-1-(3-
21
22
23 methylpiperidin-4-yl)pyrrolidine-2-carboxamide hydrochloride which was used in the
24
25
26
27 next step without further purification. LRMS-ESI⁺: *m/z* calcd for C₁₁H₂₀N₂O₂ [M+H]⁺ =
28
29
30
31 212.2; found, 212.
32
33
34

35
36 Step 2. The SnAr was performed following general procedure A using (*R*)-6-
37
38 chloro-1-(1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazine (**67**, 204 mg,
39
40 0.60 mmol), (2*S*)-1-(3-methylpiperidin-4-yl)pyrrolidine-2-carboxamide hydrochloride
41
42 (150 mg, 0.61 mmol), and *N,N*-diisopropylethylamine (0.31 mL, 1.8 mmol) in DMSO (1.2
43
44
45 mL) at 80 °C for 16 h. The residue was purified by silica gel chromatography (0% to
46
47
48
49 10% methanol in dichloromethane) to afford a mixture of diastereomers. The mixture
50
51
52
53
54
55
56
57
58
59
60

1
2
3 was further purified by preparative SFC (AD-H (2 x 25 cm), 35% isopropanol with 0.1%
4
5
6
7 DEA and CO₂ at 100 bar, 55 mL/min). The first eluting isomer was isolated from this
8
9
10 purification and converted to the HCl salt by diluting in dichloromethane (2 mL), adding
11
12
13 HCl (2 N in diethyl ether, 0.5 mL), and concentrating to afford 1-((*R*)-1-(2,4-
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

15% yield). ¹H NMR (400 MHz, CDCl₃; TFA Salt) δ 8.07 (s, 1H), 7.39 (d, *J* = 8.3 Hz, 1H), 7.36 (d, *J* = 2.1 Hz, 1H), 7.13 (dd, *J* = 8.5, 2.1 Hz, 1H), 7.08 (d, *J* = 5.8 Hz, 1H), 6.30 (q, *J* = 7.1 Hz, 1H), 5.37 (d, *J* = 5.7 Hz, 1H), 4.49 (d, *J* = 13.4 Hz, 1H), 4.37 (d, *J* = 13.6 Hz, 1H), 4.07 – 3.95 (m, 1H), 3.48 (s, 1H), 3.32 – 3.15 (m, 2H), 3.04 (dd, *J* = 13.5, 2.9 Hz, 1H), 2.82 (td, *J* = 12.9, 3.2 Hz, 1H), 2.45 – 2.30 (m, 1H), 2.28 – 2.11 (m, 2H), 2.02 – 1.94 (m, 1H), 1.89 (d, *J* = 7.2 Hz, 3H), 1.88 – 1.56 (m, 4H), 1.35 (d, *J* = 4.4 Hz, 1H), 1.23 – 1.16 (m, 1H), 0.99 (d, *J* = 6.9 Hz, 3H). LRMS-ESI⁺: *m/z* calcd for C₂₅H₃₁Cl₂N₇O₂ [M+H]⁺ = 516.2; found, 516.

51
52
53
54
55
56
57
58
59
60

**3-((*S*)-1-((3*R*,4*S*)-1-(1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-
b]pyrazin-6-yl)-3-methylpiperidin-4-yl)pyrrolidin-2-yl)propenamide hydrochloride (24).**

1
2
3 Step 1. To a cooled suspension of sodium *tert*-butoxide (2.41 g, 25.1 mmol) in THF (100 mL) at
4
5 0 °C was added trimethylphosphonoacetate (4.5 mL, 27.6 mmol). After 1 h, the solution was
6
7 transferred via cannula into a solution of *N*-Boc-*L*-prolinal (5.0 g, 25.1 mmol) in THF (50 mL).
8
9 After 30 min, the mixture was poured into saturated aqueous sodium bicarbonate solution and
10
11 the aqueous layer was extracted with ethyl acetate. The combined organic layers were dried over
12
13 sodium sulfate and concentrated under reduced pressure to afford the intermediate *tert*-butyl
14
15 (*S,E*)-2-(3-methoxy-3-oxoprop-1-en-1-yl)pyrrolidine-1-carboxylate which was used without
16
17 further purification (99% yield). LRMS-ESI⁺: *m/z* calcd for C₁₃H₂₁NO₄ [M+H]⁺ = 256.2; found,
18
19 256.
20
21
22
23
24

25 Step 2. A solution of *tert*-butyl (*S,E*)-2-(3-methoxy-3-oxoprop-1-en-1-yl)pyrrolidine-1-
26
27 carboxylate (6.38 g, 25.0 mmol) and 10% palladium on carbon (1.3 g, 1.25 mmol) in methanol
28
29 (100 mL) was stirred under a hydrogen atmosphere for 16 h. Nitrogen gas was bubbled through
30
31 the solution for 15 minutes before it was carefully filtered through Celite using MeOH and
32
33 concentrated under reduced pressure to afford *tert*-butyl (*S*)-2-(3-methoxy-3-
34
35 oxopropyl)pyrrolidine-1-carboxylate which was used without further purification. To a cooled
36
37 solution of *tert*-butyl (*S*)-2-(3-methoxy-3-oxopropyl)pyrrolidine-1-carboxylate (25.0 mmol) in
38
39 dichloromethane (100 mL) at 0 °C was added trifluoroacetic acid (19 mL, 250 mmol). The
40
41 mixture was stirred at room temperature for 16 h and concentrated via rotary evaporation. The
42
43 residue was re-dissolved in dichloromethane, and potassium carbonate (17.3 g, 125 mmol) was
44
45 added. After 30 min of stirring, the solution was filtered and the solvent was removed under
46
47 reduced pressure to afford methyl (*S*)-3-(pyrrolidin-2-yl)propanoate which was used without
48
49 further purification (99% yield). LRMS-ESI⁺: *m/z* calcd for C₈H₁₅NO₂ [M+H]⁺ = 158.1; found,
50
51 158.
52
53
54
55
56
57
58
59
60

1
2
3 Step 3. The reductive amination was performed following general procedure D using *tert*-
4 butyl 3-methyl-4-oxopiperidine-1-carboxylate (2.24 g, 10.6 mmol), methyl (*S*)-3-(pyrrolidin-2-
5 yl)propanoate (2 g, 12.73 mmol), sodium triacetoxyborohydride (3.4 g, 15.8 mmol), and acetic
6 acid (0.9 mL, 15.8 mmol) in dichloromethane (25 mL) at room temperature for 16 h. The residue
7 was loaded onto silica gel and purified by normal-phase column chromatography (0 to 50% ethyl
8 acetate in hexanes) to afford *tert*-butyl 4-((*S*)-2-(3-methoxy-3-oxopropyl)pyrrolidin-1-yl)-3-
9 methylpiperidine-1-carboxylate (75% yield). LRMS-ESI⁺: *m/z* calcd for C₁₉H₃₄N₂O₄ [M+H]⁺ =
10 355.3; found, 355.
11
12
13
14
15
16
17
18
19
20
21

22 Step 4. To a solution of *tert*-butyl 4-((*S*)-2-(3-methoxy-3-oxopropyl)pyrrolidin-1-yl)-3-
23 methylpiperidine-1-carboxylate (2.03 g, 5.97 mmol) in THF (20 mL), and water (10 mL) was
24 added lithium hydroxide (215 mg, 8.96 mmol). The reaction was heated to 50 °C for 16 h, cooled
25 to room temperature and the solvent was removed under reduced pressure to afford 3-((2*S*)-1-(1-
26 (*tert*-butoxycarbonyl)-3-methylpiperidin-4-yl)pyrrolidin-2-yl)propanoic acid which was used
27 without further purification. The residue was dissolved in dichloromethane (30 mL) and
28 trifluoroacetic acid (4.6 mL, 59.7 mmol) was added. The reaction was stirred at room
29 temperature for 16 h and concentrated via rotary evaporation to afford 3-((2*S*)-1-(3-
30 methylpiperidin-4-yl)pyrrolidin-2-yl)propanoic acid 2,2,2-trifluoroacetate which was used
31 without further purification (99% yield). LRMS-ESI⁺: *m/z* calcd for C₁₉H₃₄N₂O₄ [M+H]⁺ =
32 355.3; found, 355.
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47

48 Step 5. The SnAr was performed following general procedure A using (*R*)-6-chloro-1-(1-
49 (2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazine (**67**, 512 mg, 1.50 mmol), 3-
50 ((2*S*)-1-(3-methylpiperidin-4-yl)pyrrolidin-2-yl)propanoic acid 2,2,2-trifluoroacetate (721 mg,
51 3.00 mmol), and *N,N*-diisopropylethylamine (0.78 mL, 4.50 mmol) in DMSO (3.0 mL) at 80 °C
52
53
54
55
56
57
58
59
60

1
2
3 for 16 h. The residue was purified by reverse phase preparative HPLC (Gemini-NX, 10 μ m, 250
4 x 30 mm, C18 column, Phenomenex, Torrance, CA), eluent: 35 to 40 % acetonitrile in water,
5
6 both eluents containing 0.1% ammonium formate, gradient over 20 minutes, to afford the
7
8 formate salt of the desired product as the second eluting isomer. The formate salt was suspended
9
10 in saturated aqueous sodium bicarbonate solution and extracted three times with
11
12 dichloromethane. The combined organic layers were dried over sodium sulfate and concentrated
13
14 under reduced pressure. The residue was re-dissolved in dichloromethane (3.0 mL), treated with
15
16 2M HCl in Et₂O (0.3 mL), and concentrated under reduced pressure to afford 3-((*S*)-1-((3*R*,4*S*)-
17
18 1-(1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazin-6-yl)-3-
19
20 methylpiperidin-4-yl)pyrrolidin-2-yl)propanoic acid hydrochloride (17% yield). ¹H NMR (400
21
22 MHz, CD₃CN, HCl salt) δ 8.31 (s, 1H), 8.17 (s, 1H), 7.46 (d, *J* = 2.2 Hz, 1H), 7.43 (d, *J* = 8.5
23
24 Hz, 1H), 7.27 – 7.22 (m, 1H), 6.26 (q, *J* = 7.1 Hz, 1H), 4.63 – 4.53 (m, 1H), 4.45 (dt, *J* = 13.6,
25
26 2.6 Hz, 1H), 3.66 – 3.57 (m, *J* = 5.9, 3.2 Hz, 1H), 3.31 – 3.20 (m, 1H), 3.06 – 2.80 (m, 4H), 2.49
27
28 (ddd, *J* = 17.6, 9.9, 3.0 Hz, 1H), 2.43 (s, *J* = 1.4 Hz, 3H), 2.41 – 2.33 (m, 2H), 2.15 – 1.96 (m,
29
30 3H), 1.93 – 1.85 (m, 2H), 1.83 (d, *J* = 7.1 Hz, 3H), 1.81 – 1.68 (m, 3H), 0.98 (d, *J* = 6.9 Hz, 3H).
31
32 LRMS-ESI⁺: *m/z* calcd for C₂₇H₃₅Cl₂N₆O₂ [M+H]⁺ = 545.2; found, 545.
33
34
35
36
37
38
39
40

41 Step 6. The HATU coupling was performed following general procedure C using
42
43 3-((*S*)-1-((3*R*,4*S*)-1-(1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-
44
45 *b*]pyrazin-6-yl)-3-methylpiperidin-4-yl)pyrrolidin-2-yl)propanoic acid hydrochloride (30
46
47 mg, 0.055 mmol), ammonia gas (excess), *N,N*-diisopropylethylamine (19 μ L, 0.110
48
49 mmol), and HATU (23 mg, 0.061 mmol) in DMF (1.0 mL) at room temperature for 10
50
51
52
53
54
55
56
57
58
59
60

1
2
3 min. The residue was purified by reverse phase preparative HPLC (Gemini-NX, 10 μ m,
4
5
6
7 250 x 30 mm, C18 column, Phenomenex, Torrance, CA), eluent: 0 to 100% acetonitrile
8
9
10 in water, both eluents containing 0.1% TFA, gradient elution over 30 minutes to afford 3-
11
12
13
14 ((*S*)-1-((3*R*,4*S*)-1-(1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-
15
16
17 *b*]pyrazin-6-yl)-3-methylpiperidin-4-yl)pyrrolidin-2-yl)propenamide (**24**, 99% yield).). LRMS-
18
19
20
21 ESI⁺: *m/z* calcd for C₂₇H₃₅Cl₂N₇O [M+H]⁺ = 544.2; found, 544.
22
23

24 **1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-6-((3*R*,4*S*)-3-methyl-4-((*S*)-2-(2-
25
26 (methylsulfonyl)ethyl)pyrrolidin-1-yl)piperidin-1-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine
27
28 **hydrochloride (25)**. Step 1. Dimethyl ((methylsulfonyl)methyl)phosphonate (1.22 g, 6.02 mmol)
29
30 was dissolved in THF (20 mL) and the solution was cooled to 0 °C. Sodium *tert*-butoxide (0.579
31
32 g, 6.02 mmol) was added and then the mixture was stirred for 30 min at 0 °C. *Tert*-Butyl (*S*)-2-
33
34 formylpyrrolidine-1-carboxylate was then added (dissolved in 5 mL of THF). The reaction was
35
36 stirred vigorously for 2 h at room temperature and then quenched with saturated aqueous sodium
37
38 bicarbonate. The mixture was extracted with CH₂Cl₂ and the combined organic fractions were
39
40 dried over Na₂SO₄, filtered and concentrated under reduced pressure to provide *tert*-butyl (*S,E*)-
41
42 2-(2-(methylsulfonyl)vinyl)pyrrolidine-1-carboxylate (98% yield) as a colorless oil which was
43
44 used without further purification. LRMS-ESI⁺: *m/z* calcd for C₁₂H₂₁NO₄S [M+H]⁺ = 276.1;
45
46
47
48 found, 276.
49
50**

51
52 Step 2. *Tert*-Butyl (*S,E*)-2-(2-(methylsulfonyl)vinyl)pyrrolidine-1-carboxylate (1.2 g,
53
54 4.36 mmol) was dissolved in MeOH (20 mL) and then PtO₂ (9.9 mg, 0.044 mmol) was added.
55
56
57
58
59
60

1
2
3 The mixture was purged twice with nitrogen and then twice with hydrogen. The reaction was
4 then placed under an atmosphere of hydrogen and stirred for 1 h at room temperature. The
5 mixture was flushed with nitrogen and then filtered carefully through a silica gel plug and
6 concentrated under reduced pressure to provide *tert*-butyl (*S*)-2-(2-
7 (methylsulfonyl)ethyl)pyrrolidine-1-carboxylate (99% yield) as a colorless oil which was used in
8 the next step without further purification. LRMS-ESI⁺: *m/z* calcd for C₁₂H₂₃NO₄S [M+H]⁺ =
9 278.1; found, 278.
10
11
12
13
14
15
16
17
18
19

20 Step 3. *Tert*-Butyl (*S*)-2-(2-(methylsulfonyl)ethyl)pyrrolidine-1-carboxylate (1.3 g, 4.69
21 mmol) was dissolved in CH₂Cl₂ (10 mL) and HCl (4 N in 1,4-dioxane, 3.5 mL, 14.1 mmol) was
22 added. The reaction was stirred for 4 h at room temperature. The mixture was diluted with
23 dichloromethane and washed with saturated aqueous sodium carbonate. The organic fraction was
24 dried over Na₂SO₄, filtered and concentrated under reduced pressure to provide (*S*)-2-(2-
25 (methylsulfonyl)ethyl)pyrrolidine hydrochloride (99% yield) which was used without further
26 purification. LRMS-ESI⁺: *m/z* calcd for C₇H₁₅NO₂S [M+H]⁺ = 178.1; found, 178.
27
28
29
30
31
32
33
34
35
36

37 Step 4. The reductive amination was performed following general procedure D using 1-
38 *tert*-butoxycarbonyl-3-methyl-4-piperidone (1.0 g, 4.69 mmol), (*S*)-2-(2-
39 (methylsulfonyl)ethyl)pyrrolidine hydrochloride (1.0 g, 4.69 mmol), and sodium
40 triacetoxyborohydride (2.98 g, 14.07 mmol) in 1,2-dichloroethane (10 mL) at room temperature
41 for 6 h. The crude residue was used without further purification. The residue was dissolved in
42 dichloromethane (20 mL) and HCl (4 N in 1,4-dioxane, 3.5 mL) was added. The mixture was
43 stirred at room temperature for 4 h and then concentrated under reduced pressure to afford (2*S*)-
44 1-(3-methylpiperidin-4-yl)pyrrolidine-2-carboxamide hydrochloride (96% yield) which was used
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 in the next step without further purification. LRMS-ESI⁺: *m/z* calcd for C₁₃H₂₆N₂O₂S [M+H]⁺ =
4
5 275.2; found, 275.
6
7

8
9 Step 5. The SnAr was performed following general procedure A using (*R*)-6-chloro-1-(1-
10 (2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazine (**67**, 500 mg, 1.46 mmol), (*2S*)-
11 1-(3-methylpiperidin-4-yl)pyrrolidine-2-carboxamide hydrochloride (500 mg, 1.61 mmol), and
12
13 *N,N*-diisopropylethylamine (0.76 mL, 4.39 mmol) in DMF (15 mL) at 80 °C for 8 h. The residue
14
15 was purified by reverse phase preparative HPLC (Gemini-NX, 10 μm, 250 x 30 mm, C18
16
17 column, Phenomenex, Torrance, CA), eluent: 0 to 100% acetonitrile in water, both eluents
18
19 containing 0.1% TFA, gradient elution over 30 minutes to give a mixture of stereoisomers. The
20
21 stereoisomers were basified using a basification column (PL-HCO₃ MP SPE, 500 mg per 5 mL
22
23 tube, Agilent) using dichloromethane and methanol (4:1) as the eluent and concentrated under
24
25 reduced pressure. The residue was azeotroped with ethanol and the residue was further purified
26
27 using chiral HPLC (IF-3, Chiralpak®, Daicel Corporation, West Chester, PA), eluent: 45%
28
29 ethanol in heptanes, with heptanes containing 0.1% diethylamine, 30 min. The first eluting
30
31 isomer was isolated and the free base was dissolved in ethanol, treated with 2 N HCl in Et₂O (0.2
32
33 mL), and concentrated under reduced pressure to afford 1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-
34
35 methyl-6-((3*R*,4*S*)-3-methyl-4-((*R*)-2-methylpyrrolidin-1-yl)piperidin-1-yl)-1*H*-pyrazolo[3,4-
36
37 *b*]pyrazine hydrochloride (**25**, 6% yield). ¹H NMR (400 MHz, CDCl₃; HCl Salt) δ 8.09 (s, 1H),
38
39 7.41 (d, *J* = 8.5 Hz, 1H), 7.37 (d, *J* = 2.0 Hz, 1H), 7.15 (dd, *J* = 8.5, 2.0 Hz, 1H), 6.31 (q, *J* = 7.1
40
41 Hz, 1H), 4.52 (d, *J* = 13.3 Hz, 1H), 4.35 (d, *J* = 13.3 Hz, 1H), 3.22 – 3.07 (m, 2H), 3.06 – 2.92
42
43 (m, 3H), 2.90 (s, 3H), 2.89 – 2.80 (m, 1H), 2.57 (s, 3H), 2.49 – 2.43 (m, 1H), 2.21 – 2.09 (m,
44
45 1H), 2.00 – 1.92 (m, 3H), 1.91 (d, *J* = 7.1 Hz, 3H), 1.87 – 1.62 (m, 5H), 1.59 – 1.50 (m, 1H),
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

0.95 (d, J = 6.8 Hz, 3H). LRMS-ESI⁺: *m/z* calcd for C₂₇H₃₆Cl₂N₆O₂S [M+H]⁺ = 579.2; found, 579.

2-((*S*)-1-((3*R*,4*S*)-1-(1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazin-6-yl)-3-methylpiperidin-4-yl)pyrrolidin-2-yl)ethane-1-sulfonamide (26). Step 1. Triethylamine (1.0 mL, 7.4 mmol) was added to *tert*-butyl (*S*)-2-(2-hydroxyethyl)pyrrolidine-1-carboxylate (800 mg, 3.7 mmol) in dichloromethane (10 mL). The mixture was cooled in to 0 °C and methanesulfonyl chloride (0.35 mL, 4.44 mmol) was added. After 1.5 h, the reaction mixture was quenched with water (10 mL) and the layers were separated. The organic layer was dried over sodium sulfate, filtered, and concentrated via rotary evaporation to afford an oil which was used directly in the following step without further purification. The intermediate mesylate (~3.7 mmol) was dissolved in DMF (10 mL) and potassium thioacetate (845 mg, 7.4 mmol) was added. The mixture was heated to 50 °C, upon which copious precipitate appeared. An additional 10 mL of DMF was added to the mixture and the reaction was stirred for an additional 1 h. The reaction mixture was then partitioned between 50% ethyl acetate in hexanes (100 mL) and water (100 mL). The organic layer was washed with water (2 x 50 mL), dried over sodium sulfate, filtered, and concentrate via rotary evaporation. The residue was purified by silica gel chromatography (0 to 50% ethyl acetate in hexanes) to afford *tert*-butyl (*S*)-2-(2-(acetylthio)ethyl)pyrrolidine-1-carboxylate as a white solid (68% over 2 steps). LRMS-ESI⁺: *m/z* calcd for C₁₃H₂₃NO₃S [M+H]⁺ = 274.2; found, 274.

Step 2. *N*-chlorosuccinimide (1.33 g, 10 mmol) was added to a mixture of 2 N aqueous HCl (1.24 mL, 2.5 mmol) in acetonitrile (16.5 mL) at room temperature. The mixture was stirred for 10 min before being cooled to 0 °C. *Tert*-butyl (*S*)-2-(2-(acetylthio)ethyl)pyrrolidine-1-carboxylate (678 mg, 2.5 mmol) was then added to the reaction mixture dropwise as a solution in

1
2
3 acetonitrile (4 mL). The reaction was warmed to room temperature and stirred for 10 min before
4
5 diluting with ethyl acetate (100 mL) and water (100 mL). The organic layer was washed with
6
7 brine (50 mL), dried over sodium sulfate, filtered, and concentrated via rotary evaporation to
8
9 afford the sulfonyl chloride intermediate as a white solid which was used in the next step without
10
11 further purification. The sulfonyl chloride intermediate (~2.5 mmol) was dissolved in acetonitrile
12
13 (16.5 mL) and concentrated aqueous ammonia (33 %, 2.48 mL) was added. The mixture was
14
15 stirred for 15 min and then diluted with ethyl acetate (50 mL) and water (50 mL). The organic
16
17 layer was dried over sodium sulfate, filtered, and concentrated via rotary evaporation. The
18
19 residue was treated with HCl (4 N in 1,4-dioxane, 10 mL) and stirred overnight. After
20
21 concentrating, the solid was taken up in methanol (10 mL) and filtered through a basification
22
23 column (PL-HCO₃ MP SPE, Agilent) to afford (*S*)-2-(pyrrolidin-2-yl)ethane-1-sulfonamide
24
25 which was used directly in the next step without further purification (93% yield). LRMS-ESI⁺:
26
27 *m/z* calcd for C₆H₁₄N₂O₂S [M+H]⁺ = 179.1; found, 179.
28
29
30
31
32
33

34 Step 3. The reductive amination was performed following general procedure D using 1-
35
36 *tert*-butoxycarbonyl-3-methyl-4-piperidone (213 mg, 1.0 mmol), (*S*)-2-(pyrrolidin-2-yl)ethane-1-
37
38 sulfonamide (267 mg, 1.5 mmol), and sodium triacetoxyborohydride (316 mg, 1.5 mmol) in 1,2
39
40 dichloroethane at room temperature for 16 h. The crude residue was dissolved in
41
42 dichloromethane (6 mL) and trifluoroacetic acid (2 mL) was added. The reaction was stirred at
43
44 room temperature for 1 h and concentrated by rotary evaporation. The residue was purified by
45
46 silica gel chromatography (0 to 30% methanol in dichloromethane) to afford 2-((*S*)-1-((3*R*,4*S*)-3-
47
48 methylpiperidin-4-yl)pyrrolidin-2-yl)ethane-1-sulfonamide as the first eluting diastereomer (22%
49
50 yield). LRMS-ESI⁺: *m/z* calcd for C₆H₁₄N₂O₂S [M+H]⁺ = 275.2; found, 275.
51
52
53
54
55
56
57
58
59
60

Step 4. The SnAr was performed following general procedure A using (*R*)-6-chloro-1-(1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazine (**67**, 75 mg, 0.22 mmol), 2-((*S*)-1-((3*R*,4*S*)-3-methylpiperidin-4-yl)pyrrolidin-2-yl)ethane-1-sulfonamide (55 mg, 0.2 mmol), and *N,N*-diisopropylethylamine (0.15 mL, 0.861 mmol) in DMF (0.5 mL) at 80 °C for 1 h. The residue was purified by reverse phase preparative HPLC (Gemini-NX, 10 μm, 250 x 30 mm, C18 column, Phenomenex, Torrance, CA), eluent: 0 to 100% acetonitrile in water, both eluents containing 0.1% TFA, gradient elution over 30 minutes to afford the trifluoroacetate salt. The salt was dissolved in methanol, filtered through a basification column (PL-HCO₃ MP SPE, Agilent), the filtrate was treated with HCl (2 N in diethyl ether, 0.5 mL), and concentrated under reduced pressure to afford 2-((*S*)-1-((3*R*,4*S*)-1-(1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazin-6-yl)-3-methylpiperidin-4-yl)pyrrolidin-2-yl)ethane-1-sulfonamide (**26**, 15% yield). ¹H NMR (400 MHz, CD₃OD, HCl salt) δ 8.25 (s, 1H), 7.40 (d, *J* = 2.1 Hz, 1H), 7.37 (d, *J* = 8.5 Hz, 1H), 7.22 (dd, *J* = 8.5, 2.1 Hz, 1H), 6.28 (q, *J* = 7.1 Hz, 1H), 4.75 (dm, *J* = 13.7 Hz, 1H), 4.59 (dm, *J* = 13.8 Hz, 1H), 4.19 – 4.04 (m, 1H), 3.78 – 3.57 (m, 2H), 3.43 – 3.32 (m, 1H), 3.29 – 3.25 (m, 2H), 3.15 (br d, *J* = 13.6 Hz, 1H), 3.02 (br t, *J* = 11.9 Hz, 1H), 2.55 (br s, 1H), 2.49 (s, 3H), 2.39 – 1.91 (m, 10H), 1.85 (d, *J* = 7.1 Hz, 3H), 1.07 (d, *J* = 6.9 Hz, 3H). LRMS-ESI⁺: *m/z* calcd for C₂₆H₃₅Cl₂N₇O₂S [M+H]⁺ = 580.2; found, 580.

3-((*S*)-1-((3*R*,4*S*)-1-(1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazin-6-yl)-3-methylpiperidin-4-yl)pyrrolidin-2-yl)propanenitrile 2,2,2-trifluoroacetate (27**). To a solution of 3-((*S*)-1-((3*R*,4*S*)-1-(1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazin-6-yl)-3-methylpiperidin-4-**

1
2
3
4 yl)pyrrolidin-2-yl)propanamide (**24**, 30 mg, 0.055 mmol) was added Burgess reagent (26
5
6
7 mg, 0.110 mmol). The reaction was stirred at room temperature for 16 h before being
8
9
10 concentrated under reduced pressure. The residue was purified by reverse phase
11
12
13 preparative HPLC (Gemini-NX, 10 μ m, 250 x 30 mm, C18 column, Phenomenex,
14
15
16 Torrance, CA), eluent: 0 to 100% acetonitrile in water, both eluents containing 0.1%
17
18
19 TFA, gradient elution over 30 minutes to afford 3-((*S*)-1-((3*R*,4*S*)-1-(1-((*R*)-1-(2,4-
20
21
22 dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazin-6-yl)-3-methylpiperidin-4-
23
24
25
26
27
28 yl)pyrrolidin-2-yl)propanenitrile 2,2,2-trifluoroacetate (**27**, 30% yield). ¹H NMR (400 MHz,
29
30
31 CDCl₃, TFA salt) δ 8.11 (s, 1H), 7.39 – 7.34 (m, 2H), 7.15 (dd, J = 8.5, 2.1 Hz, 1H), 6.31
32
33
34 (q, J = 7.1 Hz, 1H), 4.65 (d, J = 13.7 Hz, 1H), 4.52 (d, J = 13.8 Hz, 1H), 3.89 (s, 1H),
35
36
37
38 3.66 (s, 1H), 3.47 (d, J = 11.9 Hz, 1H), 3.15 (s, 1H), 3.06 (d, J = 13.6 Hz, 1H), 2.97 (t, J
39
40
41 = 12.9 Hz, 1H), 2.79 – 2.66 (m, 1H), 2.58 (s, 3H), 2.50 – 1.92 (m, 10H), 1.90 (d, J = 7.1
42
43
44 Hz, 3H), 1.20 (d, J = 6.7 Hz, 3H). LRMS-ESI⁺: *m/z* calcd for C₂₇H₃₄Cl₂N₇ [M+H]⁺ =
45
46
47
48 526.2; found, 526.
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3
4 **((3*R*,4*S*)-1-(1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-**

5
6
7 ***b*]pyrazin-6-yl)-3-methylpiperidin-4-yl)-*L*-proline 2,2,2-trifluoroacetate (28).** Step 1. The
8
9
10 reductive amination was performed following general procedure D using 1-*tert*-
11
12
13
14 butoxycarbonyl-3-methyl-4-piperidone (2.83 g, 13.3 mmol), *L*-proline (1.84 g, 16 mmol),
15
16
17 AcOH (1.14 mL, 20 mmol), and sodium triacetoxyborohydride (4.24 g, 20 mmol) in 1,2-
18
19
20 dichloroethane (30 mL) at room temperature for 16 h. The residue was purified by silica
21
22
23
24 gel chromatography (0 to 50% ethyl acetate in hexanes) to afford a mixture of
25
26
27
28 diastereomers. The residue was dissolved in dichloromethane (50 mL) and
29
30
31 trifluoroacetic acid (16.5 mL) was added. The mixture was stirred at room temperature
32
33
34
35 for 16 h and then concentrated under reduced pressure to afford (3-methylpiperidin-4-
36
37
38 yl)-*L*-proline trifluoroacetate which was used in the next step without further purification.
39
40

41
42 LRMS-ESI⁺: *m/z* calcd for C₁₁H₂₀CN₂O₂ [M+H]⁺ = 213.2; found, 213.
43
44
45

46
47 Step 2. The SnAr was performed following general procedure A using (*R*)-6-
48
49
50 chloro-1-(1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazine (67, 75 mg,
51
52
53 0.22 mmol), (3-methylpiperidin-4-yl)-*L*-proline trifluoroacetate (65 mg, 0.2 mmol), and
54
55
56
57
58
59
60

1
2
3
4 *N,N*-diisopropylethylamine (0.15 mL, 0.86 mmol) in DMSO (1.0 mL) at 80 °C for 1 h.
5
6

7 The residue was purified by reverse phase preparative HPLC (Gemini-NX, 10 μm, 250 x
8
9

10 30 mm, C18 column, Phenomenex, Torrance, CA), eluent: 0 to 100% acetonitrile in
11
12

13 water, both eluents containing 0.1% TFA, gradient elution over 30 minutes to afford
14
15

16
17 ((3*R*,4*S*)-1-(1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazin-6-
18
19

20 yl)-3-methylpiperidin-4-yl)-*L*-proline trifluoroacetate as the second eluting isomer (**28**, 7%
21
22

23 yield). ¹H NMR (400 MHz, CD₃CN; TFA salt): δ 8.20 (s, 1H), 7.48 – 7.43 (m, 2H), 7.27 (dd, *J* =
24
25

26 8.5, 2.1 Hz, 1H), 6.29 (q, 1H), 4.66 – 4.51 (m, 2H), 4.47 (dd, *J* = 10.2, 4.0 Hz, 1H), 3.90 – 3.80
27
28

29 (m, 1H), 3.63 – 3.50 (m, 1H), 3.32 – 3.17 (m, 1H), 3.07 (d, *J* = 13.7 Hz, 1H), 2.97 – 2.85 (m,
30
31

32 1H), 2.56 – 2.46 (m, 2H), 2.46 (s, 3H), 2.35 – 2.24 (m, 1H), 2.20 – 2.09 (m, 1H), 2.06 – 1.95 (m,
33
34

35 1H), 1.93 – 1.87 (m, 2H), 1.85 (d, *J* = 7.1 Hz, 3H), 1.09 (d, *J* = 6.9 Hz, 3H). LRMS-ESI⁺: *m/z*
36
37

38 calcd for C₂₅H₃₀Cl₂N₆O₂ [M+H]⁺ = 517.2; found, 517.
39
40

41 **((S)-1-((3*R*,4*S*)-1-(1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-
42
43 *b*]pyrazin-6-yl)-3-methylpiperidin-4-yl)pyrrolidin-2-yl)methanol (29)**. Step 1. The reductive
44 amination was performed following general procedure D using 1-*tert*-butoxycarbonyl-3-methyl-
45 4-piperidone (213 mg, 1.0 mmol), (*S*)-pyrrolidin-2-ylmethanol (152 mg, 1.5 mmol), and sodium
46 triacetoxyborohydride (316 mg, 1.5 mmol) in 1,2-dichloroethane (2.5 mL) at room temperature
47
48 for 16 h. The residue was purified by silica gel chromatography (0 to 20% methanol in
49
50 dichloromethane) to afford the *cis*-diastereomer of *tert*-butyl 4-((*S*)-2-
51
52

53 (hydroxymethyl)pyrrolidin-1-yl)-3-methylpiperidine-1-carboxylate (40% yield). The residue was
54
55
56
57
58
59
60

1
2
3 dissolved in dichloromethane (2.0 mL) and trifluoroacetic acid (0.5 mL) was added. The mixture
4
5 was stirred at room temperature for 1 h and then concentrated under reduced pressure to afford
6
7 the *cis*-diastereomer of ((2*S*)-1-(3-methylpiperidin-4-yl)pyrrolidin-2-yl)methanol 2,2,2-
8
9 trifluoroacetate which was used without further purification. LRMS-ESI⁺: *m/z* calcd for
10
11 C₁₁H₂₂N₂O [M+H]⁺ = 199.2; found, 199.
12
13
14

15
16 Step 2. The SnAr was performed following general procedure A using (*R*)-6-chloro-1-(1-
17
18 (2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazine (**67**, 75 mg, 0.22 mmol), the
19
20 *cis*-diastereomer of ((2*S*)-1-(3-methylpiperidin-4-yl)pyrrolidin-2-yl)methanol 2,2,2-
21
22 trifluoroacetate (62 mg, 0.2 mmol), and *N,N*-diisopropylethylamine (0.15 mL, 0.86 mmol) in
23
24 DMF (1.0 mL) at 80 °C for 1 h. The residue was purified by reverse phase preparative HPLC
25
26 (Gemini-NX, 10 μm, 250 x 30 mm, C18 column, Phenomenex, Torrance, CA), eluent: 0 to
27
28 100% acetonitrile in water, both eluents containing 0.1% TFA, gradient elution over 30 minutes
29
30 to afford the 2,2,2-trifluoroacetate salt of the title compound. The residue was dissolved in
31
32 dichloromethane, washed with saturated aqueous sodium bicarbonate, and concentrated under
33
34 reduced pressure. The organic layer was treated with HCl (2 N in diethyl ether, 1.0 mL) and the
35
36 volatiles were removed to afford ((*S*)-1-((3*R*,4*S*)-1-(1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-
37
38 methyl-1*H*-pyrazolo[3,4-*b*]pyrazin-6-yl)-3-methylpiperidin-4-yl)pyrrolidin-2-yl)methanol (**29**,
39
40 15% yield). ¹H NMR (400 MHz, CD₃CN; HCl salt): δ 8.20 (s, 1H), 7.47 – 7.42 (m, 2H), 7.26
41
42 (dd, *J* = 8.5, 2.2 Hz, 1H), 6.27 (q, *J* = 7.1 Hz, 1H), 4.66 – 4.57 (m, 1H), 4.52 (dt, *J* = 13.8, 2.5,
43
44 1H), 4.02 – 3.92 (m, 1H), 3.71 (dd, *J* = 12.3, 4.2 Hz, 1H), 3.66 – 3.52 (m, 2H), 3.46 – 3.35 (m,
45
46 1H), 3.34 – 3.22 (m, 1H), 3.04 (dd, *J* = 13.7, 2.4 Hz, 1H), 2.53 – 2.46 (m, 1H), 2.44 (s, 3H), 2.19
47
48 – 2.08 (m, 2H), 2.06 – 1.85 (m, 4H), 1.84 (d, *J* = 7.1 Hz, 3H), 1.85 – 1.77 (m, 1H), 1.01 (d, *J* =
49
50 6.9 Hz, 3H). LRMS-ESI⁺: *m/z* calcd for C₂₅H₃₂Cl₂N₆O [M+H]⁺ = 503.2; found, 503.
51
52
53
54
55
56
57
58
59
60

1
2
3
4 **1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-6-((3*R*,4*S*)-4-((*S*)-2-(hydroxymethyl)pyrrolidin-**
5
6
7 **1-yl)-3-methylpiperidin-1-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine-3-carbonitrile (30).** The S_NAr
8
9
10 was performed following general procedure A using (*R*)-6-chloro-1-(1-(2,4-
11
12 dichlorophenyl)ethyl)-1*H*-pyrazolo[3,4-*b*]pyrazine-3-carbonitrile (**46**, 75 mg, 0.22 mmol),
13
14 the cis-diastereomer of ((2*S*)-1-(3-methylpiperidin-4-yl)pyrrolidin-2-yl)methanol 2,2,2-
15
16 trifluoroacetate (**42**, 62 mg, 0.2 mmol), and *N,N*-diisopropylethylamine (0.15 mL, 0.86
17
18 mmol) in DMF (1.0 mL). The mixture was heated at 80 °C for 1 h and concentrated. The
19
20 residue was purified by reverse phase preparative HPLC (Gemini-NX, 10 μm, 250 x 30
21
22 mm, C18 column, Phenomenex, Torrance, CA), eluent: 0 to 100% acetonitrile in water,
23
24 both eluents containing 0.1% TFA, gradient elution over 30 minutes. The residue was
25
26 dissolved in dichloromethane, washed with saturated aqueous sodium bicarbonate, and
27
28 concentrated under reduced pressure. The organic layer was treated with HCl (2 N in
29
30 diethyl ether, 1.0 mL) and the volatiles were removed to afford 1-((*R*)-1-(2,4-
31
32 dichlorophenyl)ethyl)-6-((3*R*,4*S*)-4-((*S*)-2-(hydroxymethyl)pyrrolidin-1-yl)-3-
33
34 methylpiperidin-1-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine-3-carbonitrile hydrochloride (**30**, 10%
35
36 yield). ¹H NMR (400 MHz, CD₃CN; HCl salt): δ 8.43 (s, 1H), 7.52 (d, J = 2.2 Hz, 1H),
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 7.46 (d, J = 8.5 Hz, 1H), 7.33 (dd, J = 8.5, 2.2 Hz, 1H), 6.45 (q, J = 7.0 Hz, 1H), 4.72 –
4
5
6 4.54 (m, 2H), 4.02 – 3.90 (m, 1H), 3.74 – 3.68 (m, 2H), 3.65 – 3.55 (m, 1H), 3.46 – 3.36
7
8
9
10 (m, 1H), 3.35 – 3.25 (m, 1H), 3.09 (dd, J = 13.7, 1.9 Hz, 1H), 2.99 (dt, J = 13.4, 3.0 Hz,
11
12
13 1H), 2.59 – 2.50 (m, 1H), 2.28 – 2.20 (m, 1H), 2.19 – 1.93 (m, 4H), 1.91 (d, J = 7.1 Hz,
14
15
16 3H), 1.88 – 1.78 (m, 1H), 1.06 (d, J = 6.9 Hz, 3H). LRMS-ESI⁺: m/z calcd for
17
18
19
20
21 C₂₅H₂₉Cl₂N₇O [M+H]⁺ = 514.2; found, 514.
22
23

24 **1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-6-((4*S*,5*R*)-4-((*S*)-2-(hydroxymethyl)pyrrolidin-**
25
26
27
28 **1-yl)-5-methylcyclohex-1-en-1-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine-3-carbonitrile**
29
30
31 **hydrochloride (31).** 1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-6-(5-methyl-4-oxocyclohex-1-en-
32
33
34 1-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine-3-carbonitrile (**55**, 350 mg, 0.82 mmol), L-prolinol (**47**,
35
36
37 330 mg, 3.2 mmol), NaBH₃CN (130 mg, 1.98 mmol) and AcOH (0.146 mL) were
38
39
40
41 dissolved in 1,2-dichloroethane (3 mL) and stirred at room temperature for 16 h. After
42
43
44
45 concentrating, the residue was purified by reverse phase preparative HPLC (Gemini-
46
47
48 NX, 10 μm, 250 x 30 mm, C18 column, Phenomenex, Torrance, CA), eluent: 0 to 100%
49
50
51 acetonitrile in water, both eluents containing 0.1% TFA, gradient elution over 30 minutes
52
53
54
55 to afford a 4:1:1 mixture of diastereomers. The mixture was further purified by
56
57
58
59
60

1
2
3 preparative SFC (AD-H (2 x 25 cm), 40% isopropanol with 0.1% DEA and CO₂ at 100
4
5
6
7 bar, 50 mL/min). The third eluting isomer was isolated from this purification and
8
9
10 converted to the HCl salt by diluting in dichloromethane (2 mL), adding HCl (2 N in
11
12
13 diethyl ether, 0.5 mL), and concentrating to afford 1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-6-
14
15 ((4*S*,5*R*)-4-((*S*)-2-(hydroxymethyl)pyrrolidin-1-yl)-5-methylcyclohex-1-en-1-yl)-1*H*-
16
17 pyrazolo[3,4-*b*]pyrazine-3-carbonitrile hydrochloride (**31**, 10% yield). ¹H NMR (400 MHz,
18
19 CD₃OD; HCl salt) δ 9.08 (s, 1H), 7.46 (dd, J = 6.7, 2.1 Hz, 1H), 7.43 (d, J = 8.5 Hz, 1H),
20
21 7.31 (dd, J = 8.5, 2.1 Hz, 1H), 7.02 – 6.92 (m, 1H), 6.72 (q, J = 7.0 Hz, 1H), 4.01 (tt, J =
22
23 8.7, 4.2 Hz, 1H), 3.81 (dd, J = 12.0, 4.8 Hz, 1H), 3.71 (dd, J = 12.0, 7.8 Hz, 2H), 3.65 –
24
25 3.57 (m, 1H), 3.44 (td, J = 10.6, 6.4 Hz, 1H), 3.02 (dt, J = 10.7, 3.8 Hz, 1H), 2.97 – 2.48
26
27 (m, 4H), 2.31 – 2.02 (m, 2H), 1.99 (d, J = 7.0 Hz, 3H), 1.97 – 1.87 (m, 2H), 1.14 (d, J =
28
29 6.7 Hz, 3H); m/z 511.1 (M+H⁺). ¹³C NMR (101 MHz; CDCl₃; HCl salt) δ 152.2, 142.0,
30
31 141.5, 136.2, 135.0, 133.4, 133.0, 132.5, 129.6, 129.1, 128.8, 128.0, 118.6, 111.9, 72.1,
32
33 66.7, 62.0, 53.6, 52.9, 33.4, 28.0, 26.8, 26.6, 24.7, 20.3, 13.6. LRMS-ESI⁺: m/z calcd for
34
35 C₂₆H₂₈Cl₂N₆O [M+H]⁺ = 511.2; found, 511.
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3
4 **2nd Generation Synthesis of 1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-6-((4*S*,5*R*)-4-((*S*-**
5
6
7 **2-(hydroxymethyl)pyrrolidin-1-yl)-5-methylcyclohex-1-en-1-yl)-1*H*-pyrazolo[3,4-**
8
9
10 ***b*]pyrazine-3-carbonitrile hydrochloride (31).** To a solution of 6-((4*S*,5*R*)-4-((*S*-2-(((tert-
11
12
13
14 butyldimethylsilyl)oxy)methyl)pyrrolidin-1-yl)-5-methylcyclohex-1-en-1-yl)-1-((*R*)-1-(2,4-
15
16
17 dichlorophenyl)ethyl)-1*H*-pyrazolo[3,4-*b*]pyrazine-3-carbonitrile (**66**, 432 mg, 0.69 mmol)
18
19
20
21 in dichloromethane (6.9 mL) was added 4 N HCl in 1,4-dioxane (0.69 mL, 2.76 mmol).
22
23
24
25 After 4 h at room temperature, the solution was concentrated under reduced pressure,
26
27
28 and the residue was purified by silica gel chromatography (0 to 20% methanol in
29
30
31 dichloromethane) to afford 1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-6-((4*S*,5*R*)-4-((*S*-2-
32
33
34 (hydroxymethyl)pyrrolidin-1-yl)-5-methylcyclohex-1-en-1-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine-
35
36
37
38 **3-carbonitrile (31, 82% yield). ¹H NMR and LCMS data matched previous prepared lots.**
39
40
41

42 **((2*S*)-1-(4-(1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazin-**
43
44 **6-yl)-6-methylcyclohex-3-en-1-yl)pyrrolidin-2-yl)methanol 2,2,2-trifluoroacetate (32).** Step
45
46
47 1. A 300 mL sealed tube was charged with a mixture of 4,4,5,5-tetramethyl-2-(10-methyl-1,4-
48
49 dioxaspiro[4.5]dec-7-en-8-yl)-1,3,2-dioxaborolane and 4,4,5,5-tetramethyl-2-(6-methyl-1,4-
50
51 dioxaspiro[4.5]dec-7-en-8-yl)-1,3,2-dioxaborolane (**53**, 1.40 g, 5.00 mmol), (*R*)-6-chloro-1-(1-
52
53 (2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazine (**67**, 1.14 g, 3.33 mmol),
54
55
56
57
58
59
60

1
2
3 Pd(dppf)Cl₂ (183 mg, 0.250 mmol), 4:1 THF:H₂O (11.2 mL), and Na₂CO₃ (882 mg, 8.33 mmol).
4
5 Nitrogen gas was bubbled through the reaction mixture for 35 min before placing the reaction
6
7 vessel in a pre-heated oil bath at 90 °C for 4 h. The reaction mixture was quenched with
8
9 saturated aqueous NaHCO₃ and extracted with EtOAc (2 x). The combined organic layers were
10
11 washed with brine, dried over sodium sulfate, concentrated, and purified by silica gel
12
13 chromatography (10% to 25% EtOAc in hexanes) to afford 1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-
14
15 3-methyl-6-(10-methyl-1,4-dioxaspiro[4.5]dec-7-en-8-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine as the
16
17 second eluting peak (21% yield). ¹H NMR (400 MHz, CDCl₃) δ 8.69 (d, *J* = 1.1 Hz, 1H), 7.41
18
19 (dd, *J* = 8.4, 5.9 Hz, 1H), 7.35 (d, *J* = 2.1 Hz, 1H), 7.16 – 7.12 (m, 1H), 6.71 – 6.67 (m, 1H), 6.54
20
21 (dd, *J* = 8.4, 5.9 Hz, 1H), 7.35 (d, *J* = 2.1 Hz, 1H), 7.16 – 7.12 (m, 1H), 6.71 – 6.67 (m, 1H), 6.54
22
23 (q, *J* = 7.1 Hz, 1H), 4.06 – 3.96 (m, 4H), 3.03 – 2.87 (m, 1H), 2.65 (s, 2H), 2.63 – 2.42 (m, 3H),
24
25 2.23 – 2.12 (m, 1H), 1.94 (d, *J* = 7.1 Hz, 3H), 1.06 (d, *J* = 6.8, 3.4, 3H). LRMS-ESI⁺: *m/z* calcd
26
27 for C₂₃H₂₄Cl₂N₄O₂ [M+H]⁺ = 459.1; found, 459.
28
29
30

31
32 Step 2. 1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-6-(10-methyl-1,4-
33
34 dioxaspiro[4.5]dec-7-en-8-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine (320 mg, 0.670 mmol) was diluted
35
36 with dichloromethane (6 mL) and TFA (1.5 mL). The resulting clear, red solution was stirred at
37
38 room temperature for 20 h. The reaction was then carefully quenched with saturated aqueous
39
40 NaHCO₃ and extracted with dichloromethane (3 x). The combined organic layers were dried
41
42 over Na₂SO₄ and concentrated to afford 4-(1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-
43
44 pyrazolo[3,4-*b*]pyrazin-6-yl)-6-methylcyclohex-3-en-1-one (92% yield). ¹H NMR (400 MHz,
45
46 CDCl₃) δ 8.74 (d, *J* = 1.4 Hz, 1H), 7.45 (dd, *J* = 8.5, 1.4 Hz, 1H), 7.37 (dd, *J* = 2.9, 2.1 Hz, 1H),
47
48 7.21 – 7.14 (m, 1H), 6.79 (ddt, *J* = 5.9, 4.0, 2.0 Hz, 1H), 6.57 (qd, *J* = 7.0, 1.2 Hz, 1H), 3.45 –
49
50 3.32 (m, 1H), 3.31 – 3.07 (m, 2H), 2.80 (dq, *J* = 12.3, 6.3 Hz, 1H), 2.68 (d, *J* = 0.5 Hz, 3H), 2.65
51
52
53
54
55
56
57
58
59
60

1
2
3 – 2.53 (m, 1H), 1.96 (d, $J = 7.1$ Hz, 3H), 1.24 (d, $J = 6.6$ Hz, 3H). LRMS-ESI⁺: m/z calcd for
4
5 C₂₁H₂₀Cl₂N₄O₁ [M+H]⁺ = 415.1; found, 415.
6
7

8
9 Step 3. The reductive amination was performed following general procedure D using 4-
10 (1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazin-6-yl)-6-
11 methylcyclohex-3-en-1-one (287 mg, 0.690 mmol), *L*-prolinol (349 mg, 3.46 mmol), NaBH₃CN
12 (87 mg, 1.4 mmol), and AcOH (0.19 mL) in 1,2-dichloroethane (2 mL) at room temperature
13 overnight. The residue was purified by reverse phase preparative HPLC (Gemini-NX, 10 μm,
14 250 x 30 mm, C18 column, Phenomenex, Torrance, CA), eluent: 45% to 75% acetonitrile in
15 water, both eluents containing 0.1% TFA, gradient elution over 30 min to afford ((2*S*)-1-(4-(1-
16 ((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazin-6-yl)-6-methylcyclohex-
17 3-en-1-yl)pyrrolidin-2-yl)methanol 2,2,2-trifluoroacetate (**32**, 5% yield). ¹H NMR (400 MHz,
18 CD₃OD; TFA salt) δ 8.81 (s, 0.5H), 8.80 (s, 0.5H), 7.54 – 7.40 (m, 2H), 7.27 (ddd, $J = 8.5, 3.7,$
19 2.0 Hz, 1H), 6.88 – 6.75 (m, 1H), 6.54 (qd, $J = 7.1, 2.5$ Hz, 1H), 4.15 – 3.35 (m, 6H), 3.13 – 2.73
20 (m, 3.5H), 2.73 – 2.50 (m, 4H), 2.38 – 1.99 (m, 3.5H), 1.99 – 1.84 (m, 4H), 1.22 – 1.01
21 (m, 3H). LRMS-ESI⁺: m/z calcd for C₂₆H₃₁Cl₂N₅O [M+H]⁺ = 500.2; found, 500.
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41

42 **1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-6-(4-((*S*)-2-(2-hydroxypropan-2-yl)pyrrolidin-1-
43 yl)-5-methylcyclohex-1-en-1-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine-3-carbonitrile 2,2,2-
44 trifluoroacetate (**33**). The reductive amination was performed following general procedure D
45 using 1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-6-(5-methyl-4-oxocyclohex-1-en-1-yl)-1*H*-
46 pyrazolo[3,4-*b*]pyrazine-3-carbonitrile (**55**, 256 mg, 0.60 mmol), (*S*)-2-(pyrrolidin-2-yl)propan-
47 2-ol (78 mg, 0.60 mmol), and sodium triacetoxyborohydride (381 mg, 1.8 mmol) in 1,2-
48 dichloroethane (2.0 mL) at room temperature for 16 h. The residue was purified by reverse phase
49
50
51
52
53
54
55
56
57
58
59
60**

1
2
3 preparative HPLC (Gemini-NX, 10 μ m, 250 x 30 mm, C18 column, Phenomenex, Torrance,
4 CA), eluent: 0 to 100% acetonitrile in water, both eluents containing 0.1% TFA, gradient elution
5 over 30 minutes to afford 1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-6-(4-((*S*)-2-(2-hydroxypropan-2-
6 yl)pyrrolidin-1-yl)-5-methylcyclohex-1-en-1-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine-3-carbonitrile
7 trifluoroacetate as a mixture of diastereomers (**33**, 3% yield). ¹H NMR (400 MHz, CDCl₃; TFA
8 Salt) δ 9.10 (s, 1H), 7.52 – 7.49 (m, 1H), 7.47 – 7.41 (m, 1H), 7.36 – 7.31 (m, 1H), 7.04 – 6.95
9 (m, 1H), 6.74 (q, *J* = 7.0 Hz, 1H), 4.04 – 3.88 (m, 1H), 3.72 – 3.60 (m, 2H), 3.56 – 3.40 (m, 1H),
10 3.23 – 2.92 (m, 2H), 2.91 – 2.62 (m, 2H), 2.33 – 2.20 (m, 2H), 2.19 – 2.03 (m, 4H), 2.00 (d, *J* =
11 7.2 Hz, 3H), 1.41 – 1.27 (m, 9H). LRMS-ESI⁺: *m/z* calcd for C₂₈H₃₂Cl₂F₃N₆O [M+H]⁺ = 538.2;
12 found, 538.
13
14
15
16
17
18
19
20
21
22
23
24
25

26
27 **6-((4*S*,5*R*)-4-((*S*)-2-(aminomethyl)pyrrolidin-1-yl)-5-methylcyclohex-1-en-1-yl)-1-**
28 **((*R*)-1-(2,4-dichlorophenyl)ethyl)-1*H*-pyrazolo[3,4-*b*]pyrazine-3-carbonitrile hydrochloride**
29 **(34)**. The reductive amination was performed following general procedure D using 1-((*R*)-1-(2,4-
30 dichlorophenyl)ethyl)-6-(5-methyl-4-oxocyclohex-1-en-1-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine-3-
31 carbonitrile (**55**, 120 mg, 0.28 mmol), *tert*-butyl (*S*)-(pyrrolidin-2-ylmethyl)carbamate (62 mg,
32 0.31 mmol), and sodium triacetoxyborohydride (120 mg, 0.56 mmol) in 1,2-dichloroethane (1.1
33 mL) at room temperature for 16 h. The residue was purified by reverse phase preparative HPLC
34 (Gemini-NX, 10 μ m, 250 x 30 mm, C18 column, Phenomenex, Torrance, CA), eluent: 0 to
35 100% acetonitrile in water, both eluents containing 0.1% TFA, gradient elution over 30 minutes
36 to afford the desired isomer as the second eluting peak. The residue was diluted with
37 dichloromethane, washed with saturated aqueous sodium bicarbonate, dried over sodium sulfate,
38 and concentrated by rotary evaporation. The free base was treated with HCl (4 N in 1,4-dioxane,
39 1 mL), stirred for 1 h, and concentrated under reduced pressure to afford 6-((4*S*,5*R*)-4-((*S*)-2-
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

(aminomethyl)pyrrolidin-1-yl)-5-methylcyclohex-1-en-1-yl)-1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-1*H*-pyrazolo[3,4-*b*]pyrazine-3-carbonitrile hydrochloride (**34**, 5% yield). ¹H NMR (400 MHz; CD₃OD; HCl salt) δ 9.10 (s, 1H), 7.50 (d, *J* = 2.1 Hz, 1H), 7.45 (d, *J* = 8.5 Hz, 1H), 7.34 (dd, *J* = 8.5, 2.1 Hz, 1H), 6.99 – 6.93 (m, 1H), 6.74 (q, *J* = 7.0 Hz, 1H), 4.27 – 4.18 (m, 1H), 3.86 – 3.77 (m, 2H), 3.77 – 3.70 (m, 2H), 3.69 – 3.63 (m, 4H), 3.60 – 3.54 (m, 2H), 3.49 – 3.38 (m, 2H), 2.96 – 2.82 (m, 2H), 2.74 – 2.62 (m, 1H), 2.44 – 2.30 (m, 1H), 2.28 – 2.08 (m, 2H), 2.01 (d, *J* = 7.1 Hz, 3H), 1.18 (d, *J* = 6.0 Hz, 3H). LRMS-ESI⁺: *m/z* calcd for C₂₆H₂₉Cl₂N₇ [M+H]⁺ = 510.2; found, 510.

(*S*)-1-((1*S*,6*R*)-4-(3-cyano-1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-1*H*-pyrazolo[3,4-*b*]pyrazin-6-yl)-6-methylcyclohex-3-en-1-yl)pyrrolidine-2-carboxamide hydrochloride (35**).**

The reductive amination was performed following general procedure D using 1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-6-(5-methyl-4-oxocyclohex-1-en-1-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine-3-carbonitrile (**55**, 120 mg, 0.28 mmol), *L*-prolinamide (46 mg, 0.40 mmol), and sodium triacetoxyborohydride (119 mg, 0.56 mmol) in 1,2-dichloroethane (1.1 mL) at room temperature for 16 h. The residue was purified by reverse phase preparative HPLC (Gemini-NX, 10 μm, 250 x 30 mm, C18 column, Phenomenex, Torrance, CA), eluent: 0 to 100% acetonitrile in water, both eluents containing 0.1% TFA, gradient elution over 30 minutes to afford the desired isomer. The residue was diluted with dichloromethane, washed with saturated aqueous sodium bicarbonate, dried over sodium sulfate, and concentrated by rotary evaporation. The free base was treated with HCl (4 N in 1,4-dioxane, 1 mL), stirred for 1 h, and concentrated under reduced pressure to afford (*S*)-1-((1*S*,6*R*)-4-(3-cyano-1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-1*H*-pyrazolo[3,4-*b*]pyrazin-6-yl)-6-methylcyclohex-3-en-1-yl)pyrrolidine-2-carboxamide hydrochloride (**35**, 8% yield). ¹H NMR (400 MHz, CD₃CN; HCl salt) δ 9.02 (s, 1H), 7.55 – 7.48

1
2
3 (m, 2H), 7.36 (dd, $J = 8.5, 2.2$ Hz, 1H), 7.08 (s, 1H), 6.92 – 6.78 (m, 1H), 6.77 – 6.63 (m, 2H),
4
5 4.43 (dd, $J = 10.4, 3.6$ Hz, 1H), 4.02 – 3.89 (m, 1H), 3.69 – 3.61 (m, 1H), 2.89 – 2.35 (m, 8H),
6
7 2.25 – 2.13 (m, 2H), 1.99 (d, $J = 7.0$ Hz, 3H), 1.14 (d, $J = 6.9$ Hz, 3H). LRMS-ESI⁺: m/z calcd
8
9 for C₂₆H₂₇Cl₂N₇O [M+H]⁺ = 524.2; found, 524.

10
11
12
13 **1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-6-((4*S*,5*R*)-5-methyl-4-((*R*)-2-methylpyrrolidin-**
14
15
16
17 **1-yl)cyclohex-1-en-1-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine-3-carbonitrile hydrochloride (36).**

18
19
20 The reductive amination was performed following general procedure D using 1-((*R*)-1-
21
22 (2,4-dichlorophenyl)ethyl)-6-(5-methyl-4-oxocyclohex-1-en-1-yl)-1*H*-pyrazolo[3,4-
23
24 *b*]pyrazine-3-carbonitrile (**55**, 120 mg, 0.28 mmol), (*R*)-2-methylpyrrolidine (34 mg, 0.4
25
26 mmol), and sodium triacetoxyborohydride (119 mg, 0.56 mmol) in 1,2-dichloroethane
27
28 (1.1 mL) at room temperature for 16 h. The residue was purified by reverse phase
29
30 preparative HPLC (Gemini-NX, 10 μm, 250 x 30 mm, C18 column, Phenomenex,
31
32 Torrance, CA), eluent: 0 to 100% acetonitrile in water, both eluents containing 0.1%
33
34 TFA, gradient elution over 30 minutes to afford a mixture of diastereomers. The mixture
35
36 was further purified using chiral preparative SFC (OD-H (2 x 25 cm), 30% methanol with
37
38 0.1% diethyl amine and CO₂ at 100 bar, 60 mL/min). The third eluting isomer was
39
40 isolated from this purification and converted to the HCl salt by diluting in
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

dichloromethane (2 mL), adding HCl (2 N in diethyl ether, 0.5 mL), and concentrating to afford 1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-6-((4*S*,5*R*)-5-methyl-4-((*R*)-2-methylpyrrolidin-1-yl)cyclohex-1-en-1-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine-3-carbonitrile hydrochloride (**36**, 7% yield). ¹H NMR (400 MHz, CDCl₃, HCl salt) δ 8.94 (s, 1H), 7.43 (d, *J* = 8.5 Hz, 1H), 7.39 (d, *J* = 2.2 Hz, 1H), 7.22 (dd, *J* = 8.5, 2.1 Hz, 1H), 6.90 – 6.84 (m, 1H), 6.74 (q, *J* = 7.1 Hz, 1H), 3.34 – 3.07 (m, 1H), 2.86 – 2.24 (m, 8H), 1.99 (d, *J* = 7.1 Hz, 4H), 1.90 – 1.71 (m, 2H), 1.55 – 1.42 (m, 1H), 0.99 (t, *J* = 7.4 Hz, 6H). ¹³C NMR (101 MHz; CDCl₃; HCl salt) δ 152.4, 142.0, 141.5, 136.1, 134.9, 133.4, 132.8, 132.3, 129.6, 129.2, 129.1, 128.0, 118.4, 111.9, 67.1, 60.1, 57.6, 53.6, 49.6, 32.6, 30.1, 28.1, 25.7, 20.3, 19.9, 13.7, 13.5. LRMS-ESI⁺: *m/z* calcd for C₂₆H₂₈Cl₂N₆ [M+H]⁺ = 495.2; found, 495.

1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-6-(5-methyl-4-((*S*)-2-(trifluoromethyl)pyrrolidin-1-yl)cyclohex-1-en-1-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine-3-carbonitrile hydrochloride (37**). The reductive amination was performed following general procedure D using 1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-6-(5-methyl-4-oxocyclohex-1-en-1-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine-3-carbonitrile (**55**, 120 mg, 0.28 mmol), (*S*)-2-(trifluoromethyl)pyrrolidine (43 mg, 0.31 mmol), and sodium triacetoxyborohydride (120 mg, 0.56 mmol) in 1,2-dichloroethane (1.1 mL) at room temperature for 16 h. The residue was purified by reverse phase preparative HPLC (Gemini-NX, 10 μm, 250 x 30 mm, C18 column, Phenomenex, Torrance, CA), eluent: 0 to 100% acetonitrile in water, both eluents containing 0.1% TFA, gradient elution over 30 minutes to afford the desired product as a mixture of isomers. The residue was diluted with dichloromethane, washed**

1
2
3 with saturated aqueous sodium bicarbonate, dried over sodium sulfate, and concentrated by
4
5 rotary evaporation. The free base was treated with HCl (4 N in 1,4-dioxane, 1 mL), stirred for 1
6
7 h, and concentrated under reduced pressure to afford 1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-6-(5-
8
9 methyl-4-((*S*)-2-(trifluoromethyl)pyrrolidin-1-yl)cyclohex-1-en-1-yl)-1*H*-pyrazolo[3,4-
10
11 *b*]pyrazine-3-carbonitrile hydrochloride (**37**, 6% yield). ¹H NMR (400 MHz, CD₃OD; HCl Salt)
12
13 δ 9.04 (s, 1H), 7.47 (d, *J* = 2.1 Hz, 1H), 7.45 (d, *J* = 8.5 Hz, 1H), 7.31 (dd, *J* = 8.5, 2.1 Hz, 1H),
14
15 7.01 (dd, *J* = 5.9, 2.1 Hz, 1H), 6.72 (q, *J* = 7.0 Hz, 1H), 4.39 – 4.23 (m, 1H), 3.54 – 3.43 (m, 1H),
16
17 3.28 – 3.21 (m, 1H), 3.20 – 3.09 (m, 1H), 2.98 – 2.88 (m, 2H), 2.70 – 2.56 (m, 1H), 2.30 – 2.11
18
19 (m, 3H), 2.10 – 2.00 (m, 2H), 1.99 (d, *J* = 7.1 Hz, 3H), 1.99 – 1.85 (m, 1H). LRMS-ESI⁺: *m/z*
20
21 calcd for C₂₆H₂₅Cl₂F₃N₆ [M+H]⁺ = 549.2; found, 549.

22
23
24
25
26
27 **(*R*)-6-chloro-*N*-(1-(2,4-dichlorophenyl)ethyl)pyrazin-2-amine (38)**. The S_NAr was
28
29 performed according to general procedure A using 2,6-dichloropyrazine (3.0 g, 20.1 mmol) and
30
31 (*R*)-1-(2,4-dichlorophenyl)ethan-1-amine (1.0 equiv) in DMSO (40 mL) along with the
32
33 addition of CsF (3.0 equiv) at 75 °C for 45 minutes. The residue was purified by silica
34
35 gel chromatography (50% ethyl acetate in hexanes) to afford (*R*)-6-chloro-*N*-(1-(2,4-
36
37 dichlorophenyl)ethyl)pyrazin-2-amine (**38**, 79% yield). LRMS-ESI⁺: *m/z* calcd for
38
39 C₁₂H₁₀Cl₃N₃ [M+H]⁺ = 302.0; found, 302.

40
41
42
43
44
45
46
47
48
49
50
51 **6-chloro-1-(1-(2,4-dichlorophenyl)ethyl)-1*H*-pyrazolo[3,4-*b*]pyrazine (39)**. Step 1.

52
53
54
55 To a solution 1-(2,4-dichlorophenyl)ethan-1-one (10.3 g, 54.4 mmol) in EtOH (50 mL) at
56
57
58
59
60

1
2
3 room temperature was added tert-butyl carbazate (21.6 g, 163.5 mmol). The reaction
4
5
6
7 was stirred at room temperature for 2 d, then at 50 °C for 1 h. The mixture was cooled
8
9
10 to 0 °C and filtered. The filtrate was concentrated, and the residue was recrystallized
11
12
13 from EtOH. The solids were combined and purified using silica gel chromatography (0 to
14
15
16 25% ethyl acetate in hexanes). The residue was dissolved in EtOH (125 mL) and one
17
18
19 crystal of bromocresol green was added. NaCNBH₃ (11.8 g, 188 mmol) was added and
20
21
22
23
24 AcOH was added dropwise to maintain a yellow color. The reaction was stirred at 60 °C
25
26
27
28 for 2 d and AcOH was added dropwise to maintain a yellow color. The mixture was
29
30
31 cooled to room temperature, concentrated, and purified by silica gel chromatography
32
33
34 (10 to 20% MTBE in hexanes). The residue was dissolved in dioxane (0.17 M) and HCl
35
36
37
38 (4 N in 1,4-dioxane, 10 equiv) was added. The mixture was stirred at 50 °C for 16 h, and
39
40
41
42 a white precipitate formed. The mixture was cooled to room temperature and
43
44
45 concentrated under reduced pressure to afford (1-(2,4-dichlorophenyl)ethyl)hydrazine
46
47
48 hydrochloride (75% yield). LRMS-ESI⁺: m/z calcd for C₈H₁₀Cl₂N₂ [M+H]⁺ = 205.0; found,
49
50
51
52 205.
53
54
55
56
57
58
59
60

1
2
3
4 Step 2. To a solution of 2,2,6,6-tetramethylpiperidine (22.9 mL, 134.3 mmol) in
5
6
7 THF (200 mL) at -40 °C was added n-BuLi (2.5 M in hexanes, 56.4 mL, 141.0 mmol).
8
9
10 The mixture was stirred at -40 °C for 30 min. In a separate flask, ethyl formate (10.9
11
12 mL, 134.3 mmol) and 2,6-dichloropyrazine (10.0 g, 67.1 mmol) were dissolved in THF
13
14 (200 mL) and cooled to -90 °C. The lithium 2,2,6,6-tetramethylpiperidine solution was
15
16
17 added to the 2,6-dichloropyrazine solution via cannula over 30 min at -90 °C. The
18
19
20 mixture was stirred at -90 °C for 1 h and then acetic acid (7.68 mL, 134.3 mmol) was
21
22
23 added, followed by (1-(2,4-dichlorophenyl)ethyl)hydrazine hydrochloride (8.11 g, 33.56
24
25
26 mmol). The reaction was warmed to room temperature and stirred at room temperature
27
28
29
30
31 for 6 h. The mixture was filtered through a silica gel-celite plug, concentrated under
32
33
34 reduced pressure and then the residue was purified by normal-phase column
35
36
37
38 chromatography on silica gel (20 to 100% ethyl acetate in hexanes) to afford a mixture
39
40
41
42 of (*Z*)-3,5-dichloro-2-((2-(1-(2,4-dichlorophenyl)ethyl)hydrazono)methyl)pyrazine and
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
66
67
68
69
70
71
72
73
74
75
76
77
78
79
80
81
82
83
84
85
86
87
88
89
90
91
92
93
94
95
96
97
98
99
100
101
102
103
104
105
106
107
108
109
110
111
112
113
114
115
116
117
118
119
120
121
122
123
124
125
126
127
128
129
130
131
132
133
134
135
136
137
138
139
140
141
142
143
144
145
146
147
148
149
150
151
152
153
154
155
156
157
158
159
160
161
162
163
164
165
166
167
168
169
170
171
172
173
174
175
176
177
178
179
180
181
182
183
184
185
186
187
188
189
190
191
192
193
194
195
196
197
198
199
200
201
202
203
204
205
206
207
208
209
210
211
212
213
214
215
216
217
218
219
220
221
222
223
224
225
226
227
228
229
230
231
232
233
234
235
236
237
238
239
240
241
242
243
244
245
246
247
248
249
250
251
252
253
254
255
256
257
258
259
260
261
262
263
264
265
266
267
268
269
270
271
272
273
274
275
276
277
278
279
280
281
282
283
284
285
286
287
288
289
290
291
292
293
294
295
296
297
298
299
300
301
302
303
304
305
306
307
308
309
310
311
312
313
314
315
316
317
318
319
320
321
322
323
324
325
326
327
328
329
330
331
332
333
334
335
336
337
338
339
340
341
342
343
344
345
346
347
348
349
350
351
352
353
354
355
356
357
358
359
360
361
362
363
364
365
366
367
368
369
370
371
372
373
374
375
376
377
378
379
380
381
382
383
384
385
386
387
388
389
390
391
392
393
394
395
396
397
398
399
400
401
402
403
404
405
406
407
408
409
410
411
412
413
414
415
416
417
418
419
420
421
422
423
424
425
426
427
428
429
430
431
432
433
434
435
436
437
438
439
440
441
442
443
444
445
446
447
448
449
450
451
452
453
454
455
456
457
458
459
460
461
462
463
464
465
466
467
468
469
470
471
472
473
474
475
476
477
478
479
480
481
482
483
484
485
486
487
488
489
490
491
492
493
494
495
496
497
498
499
500
501
502
503
504
505
506
507
508
509
510
511
512
513
514
515
516
517
518
519
520
521
522
523
524
525
526
527
528
529
530
531
532
533
534
535
536
537
538
539
540
541
542
543
544
545
546
547
548
549
550
551
552
553
554
555
556
557
558
559
560
561
562
563
564
565
566
567
568
569
570
571
572
573
574
575
576
577
578
579
580
581
582
583
584
585
586
587
588
589
590
591
592
593
594
595
596
597
598
599
600
601
602
603
604
605
606
607
608
609
610
611
612
613
614
615
616
617
618
619
620
621
622
623
624
625
626
627
628
629
630
631
632
633
634
635
636
637
638
639
640
641
642
643
644
645
646
647
648
649
650
651
652
653
654
655
656
657
658
659
660
661
662
663
664
665
666
667
668
669
670
671
672
673
674
675
676
677
678
679
680
681
682
683
684
685
686
687
688
689
690
691
692
693
694
695
696
697
698
699
700
701
702
703
704
705
706
707
708
709
710
711
712
713
714
715
716
717
718
719
720
721
722
723
724
725
726
727
728
729
730
731
732
733
734
735
736
737
738
739
740
741
742
743
744
745
746
747
748
749
750
751
752
753
754
755
756
757
758
759
760
761
762
763
764
765
766
767
768
769
770
771
772
773
774
775
776
777
778
779
780
781
782
783
784
785
786
787
788
789
790
791
792
793
794
795
796
797
798
799
800
801
802
803
804
805
806
807
808
809
810
811
812
813
814
815
816
817
818
819
820
821
822
823
824
825
826
827
828
829
830
831
832
833
834
835
836
837
838
839
840
841
842
843
844
845
846
847
848
849
850
851
852
853
854
855
856
857
858
859
860
861
862
863
864
865
866
867
868
869
870
871
872
873
874
875
876
877
878
879
880
881
882
883
884
885
886
887
888
889
890
891
892
893
894
895
896
897
898
899
900
901
902
903
904
905
906
907
908
909
910
911
912
913
914
915
916
917
918
919
920
921
922
923
924
925
926
927
928
929
930
931
932
933
934
935
936
937
938
939
940
941
942
943
944
945
946
947
948
949
950
951
952
953
954
955
956
957
958
959
960
961
962
963
964
965
966
967
968
969
970
971
972
973
974
975
976
977
978
979
980
981
982
983
984
985
986
987
988
989
990
991
992
993
994
995
996
997
998
999
1000

1
2
3
4 Step 3. (*Z*)-3,5-dichloro-2-((2-(1-(2,4-
5
6
7 dichlorophenyl)ethyl)hydrazono)methyl)pyrazine and (*E*)-3,5-dichloro-2-((2-(1-(2,4-
8
9
10 dichlorophenyl)ethyl)hydrazono)methyl)pyrazine (1.0 g, 2.75 mmol) were dissolved in
11
12
13 DMF (50 mL) and the solution was degassed. 1,8-Diazabicyclo[5.4.0]undec-7-ene (0.82
14
15
16 mL, 5.49 mmol) was added and the mixture was heated to 140 °C for 2 h. After cooling,
17
18
19 the mixture was diluted with water and ethyl acetate and the layers were separated. The
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
100% ethyl acetate in hexanes) to afford 6-chloro-1-(1-(2,4-dichlorophenyl)ethyl)-1*H*-
pyrazolo[3,4-*b*]pyrazine (**39**, 10% yield). LRMS-ESI⁺: *m/z* calcd for C₁₃H₉Cl₃N₄ [M+H]⁺ =
327.0; found, 327.

41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
(*R*)-(1-(2,4-dichlorophenyl)ethyl)hydrazine hydrochloride (40). Step 1. To a 6 L flask
equipped with overhead stirrer was added (*R*)-1-(2,4-dichlorophenyl)ethan-1-amine (211 g, 1.11
mol), water (3.4 L), and concentrated HCl (92.5 mL, 1.11 mol). To the slurry was added solid
KOCN (90 g, 1.11 mol) in one portion at room temperature. All solids went into solution and
a white precipitate began to form after 1 h. The white precipitate was isolated by
filtration. The filtrate was left standing at room temperature and more precipitate formed.

1
2
3
4 The precipitate was again isolated by filtration. This was repeated several times until no
5
6
7 more precipitate formed in the filtrate upon standing at room temperature for 1 d. All the
8
9
10 solids were combined and dried under high vacuum yielding (*R*)-1-(1-(2,4-
11
12
13 dichlorophenyl)ethyl)urea (78% yield). LRMS-ESI⁺: *m/z* calcd for C₉H₁₀N₂O [M+H]⁺ =
14
15
16
17 232.0; found, 232.
18
19
20
21

22 Step 2. (*R*)-1-(1-(2,4-dichlorophenyl)ethyl)urea (50 g, 214.6 mmol) was milled
23
24
25 into a fine powder and placed into an oven dried 2 L flask. The 2 L flask was purged
26
27
28 with nitrogen gas and a degassed mixture of 1 L of toluene and 375 mL of *t*-BuOH was
29
30
31 added via cannula under nitrogen. Solid *t*-BuOK (240.3 g, 2146 mmol) was milled into
32
33
34 fine powder and added to a separate 5 L, 3 neck flask. The 5 L flask was purged with
35
36
37 nitrogen, and a degassed mixture of 1 L of toluene and 650 mL of *t*-BuOH was added
38
39
40 via cannula under nitrogen gas. The 2 L and the 5 L mixtures were slurries and were
41
42
43 cooled to -20 °C. The lights inside the hood were turned off before *t*-BuOCl (23.18 g, 24
44
45
46 mL, 214.6 mmol) was added to the 2 L flask at -20 °C. The -20 °C bath was removed
47
48
49
50 and the mixture was placed in a 0 °C bath. As soon as the slurry went all into solution,
51
52
53
54
55
56
57
58
59
60

1
2
3 the mixture was transferred to the 5 L flask via cannula under nitrogen at -20 °C. The
4
5
6
7 lights in the hood were turned on, the -20 °C bath was removed, and the mixture was
8
9
10 placed into 0 °C bath. The mixture was stirred at 0 °C for 10 min and then warmed to
11
12
13 room temperature, at which time the mixture was poured onto ice. The mixture was
14
15
16 extracted with EtOAc (x2) and the combined organic layers were washed with 1 L of
17
18
19 water, 500 mL of saturated sodium thiosulfate, and 1 L of brine solution. The solvents
20
21
22 were concentrated under reduced pressure to afford *tert*-butyl (*R*)-2-(1-(2,4-
23
24
25 dichlorophenyl)ethyl)hydrazine-1-carboxylate (99% yield). Ee: 99% Chiralpak® IF-3;
26
27
28 250mmx4.6mm, 5% iPrOH in heptanes; flow rate 1 mL/min; detection at 254 nm; $t_1 =$
29
30
31 4.5 min (minor) $t_2 = 5.3$ min (major). LRMS-ESI⁺: m/z calcd for C₁₃H₁₈Cl₂N₂O₂ [M+H]⁺ =
32
33
34 306.2; found, 306. Note: vigorous stirring of the solution is critical for the success of this
35
36
37
38
39
40
41
42 reaction.

43
44
45
46 The residue was dissolved in 250 mL of dioxane and HCl (4 N in 1,4-dioxane, 161 mL,
47
48
49 643.8 mmol) was added at room temperature. The mixture was stirred at room
50
51
52
53 temperature overnight and then concentrated under reduced pressure. The residue was
54
55
56
57
58
59
60

1
2
3 triturerated from 25% ethyl acetate in hexanes (1 mL of solvent per 1 g of residue) to
4
5
6 afford (*R*)-(1-(2,4-dichlorophenyl)ethyl)hydrazine hydrochloride (**40**, 78% yield). ¹H NMR
7
8 (300 MHz, Methanol-*d*) δ 7.63 (d, *J* = 8.4 Hz, 1H), 7.52 (d, *J* = 2.1 Hz, 1H), 7.42 (dd, *J* =
9
10 8.4, 2.1 Hz, 1H), 4.67 (q, *J* = 6.9 Hz, 1H), 1.40 (d, *J* = 6.9 Hz, 3H). Ee: 98% Chiralpak®
11
12 IF-3; 250mmx4.6mm, 20% iPrOH in heptanes; flow rate 1 mL/min; detection at 254 nm;
13
14
15
16
17
18
19
20
21 t₁ = 4.7 min (major) t₂ = 6.9 min (minor). LRMS-ESI⁺: *m/z* calcd for C₈H₁₀Cl₂N₂ [M+H]⁺ =
22
23
24 206.2; found, 206.
25
26
27

28
29 **(*R*)-6-chloro-1-(1-(2,4-dichlorophenyl)ethyl)-1*H*-pyrazolo[3,4-*b*]pyrazine-3-**
30
31
32 **carboxylate (44)**. Step 1. Ethyl 2-(3,5-dichloropyrazin-2-yl)-2-oxoacetate (**41**, 14.5 g, 58.4
33
34 mmol) and (*R*)-(1-(2,4-dichlorophenyl)ethyl)hydrazine hydrochloride (**40**, 11.7 g, 48.7 mmol)
35
36 were dissolved in THF (97 mL), heated to 80 °C for 2 h, and stirred at room temperature for
37
38
39
40
41 16 h. The mixture was diluted with brine, extracted with ethyl acetate, and the combined
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60 organic layers were dried over magnesium sulfate and concentrated to afford a mixture
of ethyl (*R,E*)-2-(2-(1-(2,4-dichlorophenyl)ethyl)hydrazineylidene)-2-(3,5-dichloropyrazin-
2-yl)acetate (**42**) and (*R,Z*)-2-(2-(1-(2,4-dichlorophenyl)ethyl)hydrazineylidene)-2-(3,5-
dichloropyrazin-2-yl)acetate (**43**) as a red oil, which was used directly in the next

1
2
3
4 reaction without further purification. LRMS-ESI⁺: m/z calcd for C₁₆H₁₄Cl₄N₄O₂ [M+H]⁺ =
5
6
7 435.0; found, 435.
8
9

10 Step 2. The mixture of ethyl (*R,E*)-2-(2-(1-(2,4-dichlorophenyl)ethyl)hydrazono)-
11
12
13
14 2-(3,5-dichloropyrazin-2-yl)acetate (**42**) and (*R,Z*)-2-(2-(1-(2,4-
15
16
17 dichlorophenyl)ethyl)hydrazineylidene)-2-(3,5-dichloropyrazin-2-yl)acetate (**43**) was
18
19
20 diluted with THF (240 mL), cooled to 0 °C, and NaH (60% dispersion in mineral oil, 3.89
21
22
23 g, 97.4 mmol) was added carefully. The reaction was stirred overnight, monitoring by
24
25
26
27 TLC and LC-MS. The reaction was slowly poured into a mixture of crushed ice (~500
28
29
30 mL) and saturated aqueous ammonium chloride (300 mL) under vigorous stirring. The
31
32
33
34 aqueous layer was extracted with EtOAc (2 x 400mL). The combined organic layers
35
36
37
38 were dried over sodium sulfate and concentrated by rotary evaporation. The residue
39
40
41
42 was purified by silica gel chromatography (10% to 20% ethyl acetate in hexanes) to
43
44
45 afford ethyl (*R*)-6-chloro-1-(1-(2,4-dichlorophenyl)ethyl)-1*H*-pyrazolo[3,4-*b*]pyrazine-3-
46
47
48 carboxylate (**44**) as a sticky orange oil (50% yield). LRMS-ESI⁺: m/z calcd for
49
50
51
52 C₁₆H₁₃Cl₃N₄O₂ [M+H]⁺ = 399.0; found, 399.
53
54
55
56
57
58
59
60

1
2
3
4 **(*R*)-6-chloro-1-(1-(2,4-dichlorophenyl)ethyl)-1*H*-pyrazolo[3,4-*b*]pyrazine-3-**
5
6
7 **carboxamide (45).** Ethyl (*R*)-6-chloro-1-(1-(2,4-dichlorophenyl)ethyl)-1*H*-pyrazolo[3,4-
8
9
10 *b*]pyrazine-3-carboxylate (**44**, 4.0 g, 10 mmol) was dissolved in 1,4-dioxane (40 mL) in a
11
12 sealed tube. The reaction solution was diluted with 29% ammonium hydroxide in water
13
14 (40 mL), and the tube was quickly sealed. The reaction was stirred at room temperature
15
16
17 for 3 days, monitoring by LC-MS. The reaction was driven to completion by bubbling
18
19 ammonia gas gently through the solution. The crude mixture was poured into 200 mL of
20
21 water and a white precipitate formed. The white precipitate was collected and rinsed
22
23 with 200 mL of water and 50 mL of hexanes. The resulting solid was dried overnight on
24
25 high vacuum to afford (*R*)-6-chloro-1-(1-(2,4-dichlorophenyl)ethyl)-1*H*-pyrazolo[3,4-
26
27 *b*]pyrazine-3-carboxamide (**45**) as an off-white powder (95% yield). LRMS-ESI⁺: *m/z*
28
29 calcd for C₁₄H₁₀Cl₃N₅O [M+H]⁺ = 370.0; found, 370.
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44

45 **(*R*)-6-chloro-1-(1-(2,4-dichlorophenyl)ethyl)-1*H*-pyrazolo[3,4-*b*]pyrazine-3-**
46
47
48 **carbonitrile (46).** (*R*)-6-chloro-1-(1-(2,4-dichlorophenyl)ethyl)-1*H*-pyrazolo[3,4-
49
50 *b*]pyrazine-3-carboxamide (**45**, 4.47 g, 12.1 mmol) was dissolved in dry
51
52 dichloromethane (30 mL) under argon gas before adding Burgess' reagent (4.3 g, 18.1
53
54
55
56
57
58
59
60

1
2
3 mmol). The reaction was stirred at room temperature and monitored by TLC. Upon the
4
5
6
7 disappearance of starting material, the reaction was diluted with saturated aqueous
8
9
10 sodium bicarbonate and dichloromethane. The organic layer was separated, and the
11
12
13 aqueous layer was extracted with dichloromethane. The combined organic layers were
14
15
16
17 dried over magnesium sulfate and concentrated. The crude product was purified by
18
19
20 silica gel chromatography (5% to 20% ethyl acetate in hexanes) to afford (*R*)-6-chloro-1-
21
22
23 (1-(2,4-dichlorophenyl)ethyl)-1*H*-pyrazolo[3,4-*b*]pyrazine-3-carbonitrile (**46**) as a clear,
24
25
26
27 colorless, sticky solid (88% yield). ¹H NMR (400 MHz, Chloroform-*d*) δ 8.69 (s, 1H), 7.43 (d, *J*
28
29 = 8.5 Hz, 1H), 7.41 (d, *J* = 2.2 Hz, 1H), 7.27 (dd, *J* = 8.5, 2.2 Hz, 1H), 6.68 (q, *J* = 7.0 Hz, 1H),
30
31 1.98 (d, *J* = 7.0 Hz, 3H). LRMS-ESI⁺: *m/z* calcd for C₁₄H₈Cl₃N₅ [M+H]⁺ = 352.0; found, 352.

32
33
34
35
36 **((2*S*)-1-(3-methylpiperidin-4-yl)pyrrolidin-2-yl)methanol 2,2,2-trifluoroacetate (49).**

37
38
39 To a solution of L-prolinol (**47**, 152 mg, 1.5 mmol) and 1-*tert*-butoxycarbonyl-3-methyl-4-
40
41
42 piperidone (**48**, 213 mg, 1.0 mmol) in 1,2-dichloroethane (0.3 M) was added acetic acid
43
44
45 (1.5 equiv) and sodium triacetoxyborohydride (316 mg, 1.5 mmol). The solution was
46
47
48
49 stirred at room temperature for 16 h and the residue was purified by silica gel
50
51
52
53 chromatography (0 to 20% methanol in dichloromethane) to afford the *cis*-diastereomer
54
55
56
57
58
59
60

1
2
3 of *tert*-butyl 4-((*S*)-2-(hydroxymethyl)pyrrolidin-1-yl)-3-methylpiperidine-1-carboxylate
4
5
6
7 (40% yield). The residue was dissolved in dichloromethane (2.0 mL) and trifluoroacetic
8
9
10 acid (0.5 mL) was added. The mixture was stirred at room temperature for 1 h and then
11
12
13 concentrated under reduced pressure to afford the *cis*-diastereomer of ((2*S*)-1-(3-
14
15 methylpiperidin-4-yl)pyrrolidin-2-yl)methanol 2,2,2-trifluoroacetate (**49**) which was used
16
17
18 without further purification. LRMS-ESI⁺: *m/z* calcd for C₁₁H₂₂N₂O [M+H]⁺ = 199.2; found,
19
20
21 199.
22
23
24
25
26
27

28 **6-methyl-1,4-dioxaspiro[4.5]decan-8-one (51)**. To a 2 L flask equipped with a stir
29
30 bar was added CuCN (58.1 g, 0.649 mol) and Et₂O (1.6 L). The solution was cooled to -
31
32 40 °C before slowly adding MeLi (209 mL, 3.1 M in DME, 0.649 mol). As time passed
33
34
35 the reaction produced a bright yellow precipitate. In a separate 5 L flask, 1,4-
36
37
38 dioxaspiro[4.5]dec-6-en-8-one (**50**, 50 g, 0.324 mol) was dissolved in a mixture of Et₂O
39
40
41 (640 mL) and THF (640 mL). The solution was cooled to -40 °C before slowly adding
42
43
44
45
46
47
48 TMSCl (80 mL, 0.63 mol). After the cuprate solution had stirred for 30 min, it was
49
50
51
52 cannulated into the enone solution at -40 °C (some yellow solid remained in cuprate
53
54
55
56 flask). The reaction was stirred at -40 °C for 1 h, then quenched with 800 mL of 3:1
57
58
59
60

1
2
3 saturated aqueous $\text{NH}_4\text{Cl}/\text{NH}_4\text{OH}$ solution and slowly warmed to room temperature.
4
5

6
7 The organic layer was separated, and the aqueous layer was extracted with EtOAc. The
8

9
10 combined organic layers were washed with brine, dried over Na_2SO_4 , and concentrated
11

12
13 via rotary evaporation. The residue was purified by silica gel chromatography (50%
14

15
16 EtOAc in hexanes) to afford a mixture of desired product and the corresponding silyl
17

18
19 enol ether. The mixture was resuspended dichloromethane and washed twice with 1 N
20

21
22 aqueous HCl solution, dried over Na_2SO_4 , and concentrated to afford 6-methyl-1,4-
23

24
25 dioxaspiro[4.5]decan-8-one (**51**) as an orange oil, which was carried forward without
26

27
28 further purification (98% yield). ^1H NMR (400 MHz, CDCl_3) δ 4.03 (s, 4H), 2.57 – 2.31
29

30
31 (m, 4H), 2.29 – 2.15 (m, 1H), 2.10 – 1.98 (m, 1H), 1.88 – 1.78 (m, 1H), 0.96 (d, J = 6.7
32

33
34 Hz, 3H). LRMS-ESI⁺: m/z calcd for $\text{C}_9\text{H}_{15}\text{O}_3$ [M+H]⁺ = 171.1; found, 171.
35
36
37

38
39
40
41
42 **1-((R)-1-(2,4-dichlorophenyl)ethyl)-6-(10-methyl-1,4-dioxaspiro[4.5]dec-7-en-8-**

43
44
45 **yl)-1H-pyrazolo[3,4-b]pyrazine-3-carbonitrile (54)**. Step 1. To a solution of $i\text{-Pr}_2\text{NH}$ (53.5
46

47
48 mL, 0.381 mol) in THF (200 mL) cooled to $-78\text{ }^\circ\text{C}$ was added $n\text{-BuLi}$ (152 mL, 2.5 M in
49

50
51 hexanes, 0.380 mol). The mixture was warmed to $0\text{ }^\circ\text{C}$ and stirred for 30 min. In a
52

53
54
55 separate flask 6-methyl-1,4-dioxaspiro[4.5]decan-8-one (**51**, 54 g, 0.317 mol) was
56
57
58
59
60

1
2
3 dissolved in THF (1.15 L). The solution was cooled to -78 °C before slowly adding the
4
5
6
7 LDA solution. After stirring for 30 min, a solution of PhNTf₂ (136 g, 0.381 mol) in THF
8
9
10 (400 mL) was added and the resulting mixture was warmed to room temperature and
11
12
13 stirred overnight. The reaction was partitioned between EtOAc and saturated aqueous
14
15
16 NaHCO₃. The organic layer was separated, and the aqueous layer was extracted with
17
18
19
20
21 EtOAc. The combined organic layers were dried over Na₂SO₄ and concentrated by
22
23
24 rotary evaporation. The residue was purified by silica gel chromatography (5 to 10%
25
26
27 EtOAc in hexanes) to provide a mixture of desired product and PhNHTf. The mixture
28
29
30
31 was resuspended in EtOAc, washed three times with 1 N aqueous NaOH, washed with
32
33
34 brine, dried over Na₂SO₄, and concentrated to afford a 2:1 mixture of olefin isomers 10-
35
36
37 methyl-1,4-dioxaspiro[4.5]dec-7-en-8-yl trifluoromethanesulfonate and 6-methyl-1,4-
38
39
40
41 dioxaspiro[4.5]dec-7-en-8-yl trifluoromethanesulfonate (**52**) as a clear, colorless oil
42
43
44
45 (36% yield). ¹H NMR (400 MHz, CDCl₃) δ 5.61 (tt, J = 3.7, 1.4 Hz, 0.66H; diagnostic
46
47
48 peak for major isomer), 5.56 (dt, J = 3.6, 1.4 Hz, 0.34H; diagnostic peak for minor
49
50
51 isomer), 4.04 – 3.92 (m, 4H), 2.64 – 2.56 (m, 1H), 2.54 – 2.35 (m, 1H), 2.34 – 2.25 (m,
52
53
54
55 1H), 2.18 – 1.74 (m, 2H), 1.06 (d, J = 7.1 Hz, 1H; diagnostic peak for minor isomer),
56
57
58
59
60

1
2
3
4 1.00 (d, J = 6.8 Hz, 2H; diagnostic peak for major isomer). LRMS-ESI⁺: m/z calcd for
5
6
7 C₁₀H₁₄F₃O₅S [M+H]⁺ = 303.1; found, 303.
8
9

10 Step 2. To a solution of 10-methyl-1,4-dioxaspiro[4.5]dec-7-en-8-yl
11
12 trifluoromethanesulfonate (**52**, 6.5 g, 21.5 mmol) in 1,4-dioxane (108 mL) was added
13
14 bis(pinacolato)diboron (13.7 g, 53.8 mmol), KOAc (6.33 g, 64.5 mmol), and KBr (2.81 g,
15
16
17 23.7 mmol). The mixture was degassed by bubbling nitrogen gas through the solution
18
19
20
21 for 10 min, then Pd(dppf)Cl₂ (787 mg, 1.08 mmol) was added and the resulting solution
22
23
24 was degassed for another 15 min. The reaction was heated to 95 °C for 16 h. After the
25
26
27 mixture was cooled to room temperature, it was partitioned between EtOAc and water.
28
29
30
31
32 The organic layer was separated, and the aqueous layer was extracted with EtOAc. The
33
34
35 combined organic layers were washed with brine, dried over Na₂SO₄, and concentrated
36
37
38 by rotary evaporation. The residue was purified by silica gel chromatography (0 to 10%
39
40
41 EtOAc in dichloromethane) to afford a 2:1 mixture of olefin isomers 4,4,5,5-tetramethyl-
42
43
44 2-(10-methyl-1,4-dioxaspiro[4.5]dec-7-en-8-yl)-1,3,2-dioxaborolane and 4,4,5,5-
45
46
47 tetramethyl-2-(6-methyl-1,4-dioxaspiro[4.5]dec-7-en-8-yl)-1,3,2-dioxaborolane (**53**) as a
48
49
50
51
52 yellow oil (81% yield). ¹H NMR (400 MHz, CDCl₃) δ 6.41 (tt, J = 3.9, 1.9 Hz, 0.66H;
53
54
55
56
57
58
59
60

1
2
3
4 diagnostic peak for major isomer), 6.32 (dt, $J = 3.2, 2.0$ Hz, 0.33H; diagnostic peak for
5
6
7 minor isomer), 4.01 – 3.90 (m, 4H), 2.53 – 2.21 (m, 3H), 2.17 – 2.07 (m, 1H), 2.00 – 1.55
8
9
10 (m, 1H), 1.24 (s, 12H), 1.02 (d, $J = 7.3$ Hz, 1H; diagnostic peak for minor isomer), 0.92
11
12
13
14 (d, $J = 6.7$ Hz, 2H; diagnostic peak for major isomer). LRMS-ESI⁺: m/z calcd for
15
16
17 $C_{15}H_{26}BO_4$ [M+H]⁺ = 281.2; found, 281.
18
19
20

21 Step 3. To a solution of (*R*)-6-chloro-1-(1-(2,4-dichlorophenyl)ethyl)-1*H*-
22
23
24 pyrazolo[3,4-*b*]pyrazine-3-carbonitrile (**46**, 1.67 g, 4.74 mmol), 4,4,5,5-tetramethyl-2-
25
26
27 (10-methyl-1,4-dioxaspiro[4.5]dec-7-en-8-yl)-1,3,2-dioxaborolane (**53**, 1.99 g, 7.10
28
29
30 mmol), and 1 N aqueous Na_2CO_3 (14.2 mL, 14.2 mmol) in 1,4-dioxane (15.8 mL) was
31
32
33
34 added $Pd(dppf)_2Cl_2$ (0.20 g, 0.240 mmol). The reaction mixture was degassed by
35
36
37
38 bubbling nitrogen through the solution for 15 min before heating the reaction to 95 °C for
39
40
41
42 75 min. The reaction was concentrated to dryness and the residue was partitioned
43
44
45
46 between EtOAc and saturated aqueous $NaHCO_3$. The organic layer was separated, and
47
48
49 the aqueous was extracted with EtOAc. The combined organic layers were washed with
50
51
52
53 brine, dried over Na_2SO_4 , and concentrated by rotary evaporation. The residue was
54
55
56 purification by silica gel chromatography (20 to 30% EtOAc in pentanes) to afford 1-
57
58
59
60

1
2
3
4 ((*R*)-1-(2,4-dichlorophenyl)ethyl)-6-(10-methyl-1,4-dioxaspiro[4.5]dec-7-en-8-yl)-1*H*
5
6
7 pyrazolo[3,4-*b*]pyrazine-3-carbonitrile (**54**) as the second eluting isomer as a pale
8
9
10 orange foam (47% yield). ¹H NMR (400 MHz, CDCl₃) δ 8.95 (d, J = 1.8 Hz, 1H), 7.42
11
12
13 (dd, J = 8.5, 4.5 Hz, 1H), 7.39 (dd, J = 2.2, 0.4 Hz, 1H), 7.23 (ddt, J = 8.5, 2.2, 0.5 Hz,
14
15
16 1H), 6.82 (tt, J = 4.2, 2.2 Hz, 1H), 6.73 (q, J = 7.0 Hz, 1H), 4.10 – 3.95 (m, 4H), 3.03 –
17
18
19 2.86 (m, 1H), 2.66 – 2.46 (m, 3H), 2.19 (h, J = 7.2 Hz, 1H), 1.99 (d, J = 7.1 Hz, 3H),
20
21
22 1.07 (dd, J = 6.8, 3.8 Hz, 3H). LRMS-ESI⁺: m/z calcd for C₂₃H₂₁Cl₂N₅O₂ [M+H]⁺ = 470.1;
23
24
25
26
27
28 found, 470.
29
30

31
32 1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-6-(5-methyl-4-oxocyclohex-1-en-1-yl)-1*H*
33
34
35 pyrazolo[3,4-*b*]pyrazine-3-carbonitrile (**55**). To a solution of 1-((*R*)-1-(2,4-
36
37
38 dichlorophenyl)ethyl)-6-(10-methyl-1,4-dioxaspiro[4.5]dec-7-en-8-yl)-1*H*-pyrazolo[3,4-
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
66
67
68
69
70
71
72
73
74
75
76
77
78
79
80
81
82
83
84
85
86
87
88
89
90
91
92
93
94
95
96
97
98
99
100
101
102
103
104
105
106
107
108
109
110
111
112
113
114
115
116
117
118
119
120
121
122
123
124
125
126
127
128
129
130
131
132
133
134
135
136
137
138
139
140
141
142
143
144
145
146
147
148
149
150
151
152
153
154
155
156
157
158
159
160
161
162
163
164
165
166
167
168
169
170
171
172
173
174
175
176
177
178
179
180
181
182
183
184
185
186
187
188
189
190
191
192
193
194
195
196
197
198
199
200
201
202
203
204
205
206
207
208
209
210
211
212
213
214
215
216
217
218
219
220
221
222
223
224
225
226
227
228
229
230
231
232
233
234
235
236
237
238
239
240
241
242
243
244
245
246
247
248
249
250
251
252
253
254
255
256
257
258
259
260
261
262
263
264
265
266
267
268
269
270
271
272
273
274
275
276
277
278
279
280
281
282
283
284
285
286
287
288
289
290
291
292
293
294
295
296
297
298
299
300
301
302
303
304
305
306
307
308
309
310
311
312
313
314
315
316
317
318
319
320
321
322
323
324
325
326
327
328
329
330
331
332
333
334
335
336
337
338
339
340
341
342
343
344
345
346
347
348
349
350
351
352
353
354
355
356
357
358
359
360
361
362
363
364
365
366
367
368
369
370
371
372
373
374
375
376
377
378
379
380
381
382
383
384
385
386
387
388
389
390
391
392
393
394
395
396
397
398
399
400
401
402
403
404
405
406
407
408
409
410
411
412
413
414
415
416
417
418
419
420
421
422
423
424
425
426
427
428
429
430
431
432
433
434
435
436
437
438
439
440
441
442
443
444
445
446
447
448
449
450
451
452
453
454
455
456
457
458
459
460
461
462
463
464
465
466
467
468
469
470
471
472
473
474
475
476
477
478
479
480
481
482
483
484
485
486
487
488
489
490
491
492
493
494
495
496
497
498
499
500
501
502
503
504
505
506
507
508
509
510
511
512
513
514
515
516
517
518
519
520
521
522
523
524
525
526
527
528
529
530
531
532
533
534
535
536
537
538
539
540
541
542
543
544
545
546
547
548
549
550
551
552
553
554
555
556
557
558
559
560
561
562
563
564
565
566
567
568
569
570
571
572
573
574
575
576
577
578
579
580
581
582
583
584
585
586
587
588
589
590
591
592
593
594
595
596
597
598
599
600
601
602
603
604
605
606
607
608
609
610
611
612
613
614
615
616
617
618
619
620
621
622
623
624
625
626
627
628
629
630
631
632
633
634
635
636
637
638
639
640
641
642
643
644
645
646
647
648
649
650
651
652
653
654
655
656
657
658
659
660
661
662
663
664
665
666
667
668
669
670
671
672
673
674
675
676
677
678
679
680
681
682
683
684
685
686
687
688
689
690
691
692
693
694
695
696
697
698
699
700
701
702
703
704
705
706
707
708
709
710
711
712
713
714
715
716
717
718
719
720
721
722
723
724
725
726
727
728
729
730
731
732
733
734
735
736
737
738
739
740
741
742
743
744
745
746
747
748
749
750
751
752
753
754
755
756
757
758
759
760
761
762
763
764
765
766
767
768
769
770
771
772
773
774
775
776
777
778
779
780
781
782
783
784
785
786
787
788
789
790
791
792
793
794
795
796
797
798
799
800
801
802
803
804
805
806
807
808
809
810
811
812
813
814
815
816
817
818
819
820
821
822
823
824
825
826
827
828
829
830
831
832
833
834
835
836
837
838
839
840
841
842
843
844
845
846
847
848
849
850
851
852
853
854
855
856
857
858
859
860
861
862
863
864
865
866
867
868
869
870
871
872
873
874
875
876
877
878
879
880
881
882
883
884
885
886
887
888
889
890
891
892
893
894
895
896
897
898
899
900
901
902
903
904
905
906
907
908
909
910
911
912
913
914
915
916
917
918
919
920
921
922
923
924
925
926
927
928
929
930
931
932
933
934
935
936
937
938
939
940
941
942
943
944
945
946
947
948
949
950
951
952
953
954
955
956
957
958
959
960
961
962
963
964
965
966
967
968
969
970
971
972
973
974
975
976
977
978
979
980
981
982
983
984
985
986
987
988
989
990
991
992
993
994
995
996
997
998
999
1000

1
2
3 organic layers were washed with brine, dried over Na₂SO₄, and concentrated by rotary
4
5
6
7 evaporation. The residue was purification by silica gel chromatography (25 to 30%
8
9
10 EtOAc in hexanes) to afford 1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-6-(5-methyl-4-
11
12
13 oxocyclohex-1-en-1-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine-3-carbonitrile (**55**) as an orange
14
15
16
17 solid (87% yield). ¹H NMR (400 MHz, CDCl₃) δ 9.00 (d, J = 2.7 Hz, 1H), 7.45 – 7.43 (m,
18
19
20 1H), 7.41 (dd, J = 3.6, 2.1 Hz, 1H), 7.26 – 7.23 (m, 1H), 6.92 (ddt, J = 6.4, 4.1, 2.1 Hz,
21
22
23 1H), 6.76 (q, J = 7.1 Hz, 1H), 3.45 – 3.10 (m, 3H), 2.86 – 2.75 (m, 1H), 2.69 – 2.54 (m,
24
25
26
27 1H), 2.00 (dd, J = 7.1, 0.9 Hz, 3H), 1.26 (d, J = 6.6 Hz, 3H). LRMS-ESI⁺: m/z calcd for
28
29
30
31 C₂₁H₁₈Cl₂N₅O₂ [M+H]⁺ = 426.1; found, 426.
32
33

34
35 **(7*R*,8*S*)-7-methyl-*N*((*S*)-1-phenylethyl)-1,4-dioxaspiro[4.5]decan-8-amine (**58**).**
36

37
38 To a solution of 7-methyl-1,4-dioxaspiro[4.5]decan-8-one (**56**, 20.0 g, 118 mmol) in
39
40
41 benzene (14.7 mL) was added (1*S*)-1-phenylethanamine (**57**, 15.2 mL, 118 mmol). The
42
43
44
45 solution was heated to reflux overnight using a Dean-Stark apparatus for removal of
46
47
48
49 water. After 24 h, the solution was concentrated under reduced pressure to afford (*R,E*)-
50
51
52 7-methyl-*N*((*S*)-1-phenylethyl)-1,4-dioxaspiro[4.5]decan-8-imine which was used
53
54
55
56 without further purification. Sodium triacetoxyborohydride (316 mg, 1.5 mmol) was
57
58
59
60

1
2
3 added portion-wise to a solution of (*R,E*)-7-methyl-*N*-((*S*)-1-phenylethyl)-1,4-
4
5
6
7 dioxaspiro[4.5]decan-8-imine (31.7 g, 117 mmol) in dichloromethane (283 mL) and
8
9
10 acetic acid (10 mL). The resulting solution was stirred for 16 h at room temperature
11
12
13
14 before being diluted with dichloromethane (300 mL) and quenched with saturated
15
16
17 aqueous NaHCO₃. The organic layer was washed with brine, dried over Na₂SO₄, and
18
19
20
21 concentrated by rotary evaporation. The residue was purified by silica gel
22
23
24 chromatography (20 to 30% EtOAc in hexanes) to afford (*7R,8S*)-7-methyl-*N*-((*S*)-1-
25
26
27 phenylethyl)-1,4-dioxaspiro[4.5]decan-8-amine (**58**, 65% yield; 10:1 dr) as a pale yellow
28
29
30
31 oil. ¹H NMR (400 MHz, Methanol-d₄) δ 7.35 – 7.25 (m, 4H), 7.22 – 7.16 (m, 1H), 3.91 –
32
33
34 3.79 (m, 4H), 3.76 (q, *J* = 7.1 Hz, 1H), 2.57 – 2.52 (m, 1H), 1.70 – 1.49 (m, 3H), 1.47 –
35
36
37 1.32 (m, 3H), 1.31 (d, *J* = 6.6 Hz, 3H), 1.25 – 1.20 (m, 1H), 1.01 (d, *J* = 7.1 Hz, 3H;
38
39
40
41 diagnostic peak for major isomer), 0.90 (d, *J* = 7.1 Hz, 0.3H; diagnostic peak for minor
42
43
44
45 isomer). LRMS-ESI⁺: *m/z* calcd for C₁₇H₂₆NO₂ [M+H]⁺ = 276.2; found, 276.
46
47
48

49 **Tert-butyl ((*7R,8S*)-7-methyl-1,4-dioxaspiro[4.5]decan-8-yl)carbamate (**59**). A**

50
51
52
53 suspension of (*7R,8S*)-7-methyl-*N*-((*S*)-1-phenylethyl)-1,4-dioxaspiro[4.5]decan-8-amine
54
55
56
57
58
59
60

1
2
3 (58, 12 g, 43.6 mmol) and 20% Pd(OH)₂ on carbon (1.2 g, 1.71 mmol, 0.04 equiv) in
4
5
6
7 methanol (80 mL) was placed under a hydrogen atmosphere using a Parr apparatus (40
8
9
10
11 psi) for 72 h. After sparging with nitrogen gas for 30 minutes, the mixture was filtered
12
13
14 through Celite with methanol and the filtrate was concentrated under reduced pressure
15
16
17 to afford (7*R*,8*S*)-7-methyl-1,4-dioxaspiro[4.5]decan-8-amine (99% yield) as a colorless
18
19
20 oil. LRMS-ESI⁺: m/z calcd for C₉H₁₈NO₂ [M+H]⁺ = 172.1; found, 172. To a solution of
21
22
23 (7*R*,8*S*)-7-methyl-1,4-dioxaspiro[4.5]decan-8-amine (7.88 g, 46.0 mmol) and
24
25
26 triethylamine (6.4 mL, 46.0 mmol) in THF (92 mL) was added di-tert-butyl dicarbonate
27
28
29 (10.0 g, 46.0 mmol) at room temperature. The resulting solution was stirred for 16 h at
30
31
32 room temperature before being diluted with water (200 mL). The aqueous layer was
33
34
35 extracted 3 times with dichloromethane, the combined organic layers were dried over
36
37
38 Na₂SO₄ and concentrated by rotary evaporation to afford tert-butyl ((7*R*,8*S*)-7-methyl-
39
40
41 1,4-dioxaspiro[4.5]decan-8-yl)carbamate (59, 98% yield) as a white solid that was used
42
43
44 without further purification. LRMS-ESI⁺: m/z calcd for C₁₄H₂₆NO₄ [M+H]⁺ = 272.2; found,
45
46
47
48
49
50
51
52 272.
53
54
55
56
57
58
59
60

1
2
3
4 **Tert-butyl *N*[(1*S*,2*R*)-2-methyl-4-oxo-cyclohexyl]carbamate (60).** To a solution of
5
6
7 tert-butyl ((7*R*,8*S*)-7-methyl-1,4-dioxaspiro[4.5]decan-8-yl)carbamate (**59**, 6.54 g, 24.0
8
9
10 mmol) in THF (42 mL) and water (42 mL) was added *p*-toluenesulfonic acid
11
12
13
14 monohydrate (9.67 g, 50.8 mmol) at room temperature. The resulting solution was
15
16
17 stirred for 16 h at room temperature before being quenched with saturated aqueous
18
19
20 NaHCO₃. The aqueous layer was extracted twice with ethyl acetate, then the combined
21
22
23 organic layers were dried over Na₂SO₄ and concentrated by rotary evaporation. The
24
25
26 residue was purified by silica gel chromatography (0 to 100% EtOAc in hexanes) to
27
28
29 afford tert-butyl *N*[(1*S*,2*R*)-2-methyl-4-oxo-cyclohexyl]carbamate (**60** 73% yield) as a
30
31
32 white amorphous solid. ¹H NMR (400 MHz, CDCl₃) δ 5.28 (br s, 1H), 3.82 – 3.76 (m,
33
34
35 1H), 2.25 – 1.95 (m, 5H), 1.85 – 1.74 (m, 1H), 1.73 – 1.63 (m, 1H), 1.20 (s, 9H), 0.72 (d,
36
37
38 J = 6.5 Hz, 3H). LRMS-ESI⁺: *m/z* calcd for C₁₂H₂₂NO₃ [M+H]⁺ = 228.2; found, 228.
39
40
41
42
43
44
45

46 **(4*S*,5*R*)-4-((tert-butoxycarbonyl)amino)-5-methylcyclohex-1-en-1-yl**
47
48 **trifluoromethanesulfonate (61).** To a cooled (-78 °C) solution of tert-butyl *N*[(1*S*,2*R*)-2-
49
50 methyl-4-oxo-cyclohexyl]carbamate (**60**, 1.68 g, 7.39 mmol) in THF (25 mL) was added
51
52
53 LiHMDS (1 M in THF, 18.5 mL, 18.5 mmol) dropwise. After 45 min at this temperature, a
54
55
56 solution of PhNTf₂ (3.17 g, 8.87 mmol) in THF (10 mL) was added. The reaction was stirred for
57
58
59
60

1
2
3 15 mins at $-78\text{ }^{\circ}\text{C}$ and it was warmed to room temperature. After stirring for 1 h at room
4
5 temperature, the reaction was quenched with water. The aqueous layer was extracted twice with
6
7 ethyl acetate, then the combined organic layers were washed with 1 M aq. NaOH (5 times) and
8
9 brine (once), dried over Na_2SO_4 and concentrated by rotary evaporation to afford (4*S*,5*R*)-4-
10
11 ((tert-butoxycarbonyl)amino)-5-methylcyclohex-1-en-1-yl trifluoromethanesulfonate (**61**, 97%
12
13 yield, >20:1 dr) as a tan colored solid that was used without further purification. ^1H NMR (400
14
15 MHz, CDCl_3) δ 5.66 (br s, 1H), 4.51 (d, $J = 9.2\text{ Hz}$, 1H), 3.95 – 3.83 (m, 1H), 2.51 – 2.42 (m,
16
17 2H), 2.21 – 2.04 (m, 3H), 1.44 (s, 9H), 0.98 (d, $J = 6.3\text{ Hz}$, 3H). LRMS-ESI $^+$: m/z calcd for
18
19 $\text{C}_{13}\text{H}_{21}\text{F}_3\text{NO}_5\text{S}$ $[\text{M}+\text{H}]^+ = 360.1$; found, 360.
20
21
22
23

24
25 **(*R*)-1-(1-(2,4-dichlorophenyl)ethyl)-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-**
26
27 **yl)-1*H*-pyrazolo[3,4-*b*]pyrazine-3-carbonitrile (**62**). A sealed tube was charged with a
28
29 solution of 6-chloro-1-[(1*R*)-1-(2,4-dichlorophenyl)ethyl]pyrazolo[3,4-*b*]pyrazine-3-
30
31 carbonitrile (**46**, 17.6 g, 49.8 mmol) in 1,4-dioxane (245 mL), followed by potassium
32
33 acetate (16.7 g, 170 mmol), bis(pinacolato)diboron (12.7 g, 49.8 mmol), and 1,1'-
34
35 Bis(diphenylphosphino)ferrocene-palladium(II) dichloride (1.82 g, 2.49 mmol). Nitrogen
36
37 gas was bubbled through the reaction solution for 15 min. The sealed tube was then
38
39 placed in an oil bath and heated to $90\text{ }^{\circ}\text{C}$ for 2 h. After cooling to room temperature, the
40
41 reaction mixture was filtered through a pad of Celite and silica gel (1:1), and the pad
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60**

1
2
3 was rinsed with ethyl acetate (1 L). The residue was purified by silica gel
4
5
6 chromatography (0 to 100% EtOAc in hexanes) to afford (*R*)-1-(1-(2,4-
7
8 dichlorophenyl)ethyl)-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazolo[3,4-
9
10
11 *b*]pyrazine-3-carbonitrile (**62**, 72% yield). ¹H NMR (400 MHz, CDCl₃) δ 9.12 (s, 1H), 7.48
12
13
14 (d, *J* = 8.5 Hz, 1H), 7.39 (d, *J* = 2.1 Hz, 1H), 7.26 (dd, *J* = 8.5, 2.1 Hz, 1H), 6.95 (q, *J* =
15
16
17 7.0 Hz, 1H), 1.97 (d, *J* = 7.0 Hz, 3H), 1.43 (s, 12H). LRMS-ESI⁺: *m/z* calcd for
18
19
20 C₂₀H₂₁BCl₂N₅O₂ [M+H]⁺ = 444.1; found, 444.
21
22
23
24
25

26
27 **Tert-butyl ((1*S*,6*R*)-4-(3-cyano-1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-1*H*-**
28
29 **pyrazolo[3,4-*b*]pyrazin-6-yl)-6-methylcyclohex-3-en-1-yl)carbamate (**63**). A sealed tube**
30
31 was charged with a solution of 1-[(1*R*)-1-(2,4-dichlorophenyl)ethyl]-6-(4,4,5,5-
32
33
34 tetramethyl-1,3,2-dioxaborolan-2-yl)pyrazolo[3,4-*b*]pyrazine-3-carbonitrile (**62**, 8.2 g,
35
36
37 18.5 mmol) and [(4*S*,5*R*)-4-(tert-butoxycarbonylamino)-5-methyl-cyclohexen-1-yl]
38
39
40 trifluoromethanesulfonate (**61**, 6.64g, 18.5 mmol) in water (6 mL) and toluene (62 mL).
41
42
43
44 Sodium carbonate (5.87 g, 55.4 mmol) and 1,1'-bis(diphenylphosphino)ferrocene-
45
46
47
48 palladium(II) dichloride (1.35 g, 1.85 mmol) were added and nitrogen gas was bubbled
49
50
51
52 through the reaction solution for 15 min. The sealed tube was then placed in an oil bath
53
54
55
56
57
58
59
60

1
2
3 and heated to 90 °C for 16 h. After cooling to room temperature, the reaction mixture
4
5
6
7 was filtered through a pad of Celite and silica gel (1:1), and the pad was rinsed with
8
9
10 ethyl acetate (500 mL). The residue was purified by silica gel chromatography (30 to
11
12
13 100% EtOAc in hexanes) to afford tert-butyl ((1*S*,6*R*)-4-(3-cyano-1-((*R*)-1-(2,4-
14
15 dichlorophenyl)ethyl)-1*H*-pyrazolo[3,4-*b*]pyrazin-6-yl)-6-methylcyclohex-3-en-1-
16
17
18 yl)carbamate (**63**, 83% yield). ¹H NMR (400 MHz, CDCl₃) δ 8.94 (s, 1H), 7.43 (d, J = 8.5
19
20 Hz, 1H), 7.40 (d, J = 2.1 Hz, 1H), 7.23 (dd, J = 8.5, 2.1 Hz, 1H), 6.88 (s, 1H), 6.74 (q, J
21
22 = 7.1 Hz, 1H), 4.58 (d, J = 9.3 Hz, 1H), 4.04 – 3.96 (m, 1H), 2.80 – 2.70 (m, 1H), 2.69 –
23
24 2.59 (m, 1H), 2.46 – 2.28 (m, 2H), 1.99 (d, J = 7.1 Hz, 3H), 1.45 (s, 9H), 1.07 (d, J = 6.8
25
26 Hz, 3H). LRMS-ESI⁺: m/z calcd for C₂₆H₂₉Cl₂N₆O₂ [M+H]⁺ = 527.2; found, 527.
27
28
29
30
31
32
33
34
35
36
37
38

39 **6-((4*S*,5*R*)-4-amino-5-methylcyclohex-1-en-1-yl)-1-((*R*)-1-(2,4-**
40
41
42 **dichlorophenyl)ethyl)-1*H*-pyrazolo[3,4-*b*]pyrazine-3-carbonitrile hydrochloride (**64**). To a
43
44
45 solution of tert-butyl ((1*S*,6*R*)-4-(3-cyano-1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-1*H*-
46
47
48 pyrazolo[3,4-*b*]pyrazin-6-yl)-6-methylcyclohex-3-en-1-yl)carbamate (**63**, 0.6 g, 1.14
49
50
51 mmol) in dichloromethane (8 mL) was added 4 N HCl in 1,4-dioxane (1.3 mL, 5.05
52
53
54
55
56
57
58
59
60**

1
2
3 mmol). After 2 h at room temperature, the solution was concentrated under reduced
4
5
6
7 pressure to afford 6-((4*S*,5*R*)-4-amino-5-methylcyclohex-1-en-1-yl)-1-((*R*)-1-(2,4-
8
9
10 dichlorophenyl)ethyl)-1*H*-pyrazolo[3,4-*b*]pyrazine-3-carbonitrile hydrochloride (**64**, 100%
11
12
13 yield) which was used without further purification. LRMS-ESI⁺: *m/z* calcd for C₂₁H₂₁Cl₂N₆
14
15
16
17 [M+H]⁺ = 427.1; found, 427.
18
19
20

21
22 **[(4*R*)-5-[tert-butyl(dimethyl)silyl]oxy-4-methylsulfonyloxy-pentyl]**

23
24 **methanesulfonate (65)**. To a cooled (0 °C) solution of (4*R*)-5-[tert-
25
26 butyl(dimethyl)silyl]oxypentane-1,4-diol⁴³ (2.14 g, 9.13 mmol) and triethylamine (3.8 mL, 27.4
27
28 mmol) in dichloromethane (33 mL) was added methanesulfonyl chloride (1.3 mL, 16.6 mmol)
29
30 dropwise. The reaction mixture was warmed to room temperature and stirred for 16 h before
31
32 quenching with saturated aqueous NaHCO₃. The aqueous layer was extracted with ethyl acetate,
33
34 the combined organic layers were dried over Na₂SO₄, and concentrated by rotary evaporation.
35
36 The residue was purified by silica gel chromatography (0 to 100% EtOAc in hexanes) to afford
37
38 [(4*R*)-5-[tert-butyl(dimethyl)silyl]oxy-4-methylsulfonyloxy-pentyl] methanesulfonate (**65**, 79%
39
40 yield) as a white amorphous solid. ¹H NMR (400 MHz, CDCl₃) δ 4.70 – 4.63 (m, 1H), 4.30 –
41
42 4.18 (m, 2H), 4.11 – 4.04 (m, 1H), 3.76 – 3.66 (m, 2H), 3.04 (s, 3H), 2.98 (s, 3H), 1.95 – 1.67
43
44 (m, 4H), 0.86 (s, 9H), 0.05 (s, 3H), 0.04 (s, 3H). LRMS-ESI⁺: *m/z* calcd for C₁₃H₃₁O₇S₂Si
45
46
47 [M+H]⁺ = 391.1; found, 391.
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

6-((4*S*,5*R*)-4-((*S*)-2-(((tert-butyl)dimethylsilyl)oxy)methyl)pyrrolidin-1-yl)-5-methylcyclohex-1-en-1-yl)-1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-1*H*-pyrazolo[3,4-*b*]pyrazine-3-carbonitrile (66). To a solution of 6-[(4*S*,5*R*)-4-amino-5-methyl-cyclohexen-1-yl]-1-[(1*R*)-1-(2,4-dichlorophenyl)ethyl]pyrazolo[3,4-*b*]pyrazine-3-carbonitrile (**64**, 482 mg, 1.13 mmol) and [(4*R*)-5-[tert-butyl(dimethyl)silyl]oxy-4-methylsulfonyloxy-pentyl]methanesulfonate (**65**, 881 mg, 2.26 mmol) in acetonitrile (5 mL) was added *N,N*-diisopropylethylamine (1.0 mL, 5.64 mmol). The solution was heated to reflux for 24 h before cooling to room temperature and concentrating under reduced pressure. The residue was purified by silica gel chromatography (0 to 20% methanol in dichloromethane) to afford 6-((4*S*,5*R*)-4-((*S*)-2-(((tert-butyl)dimethylsilyl)oxy)methyl)pyrrolidin-1-yl)-5-methylcyclohex-1-en-1-yl)-1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-1*H*-pyrazolo[3,4-*b*]pyrazine-3-carbonitrile (**66**, 75% yield). LRMS-ESI⁺: *m/z* calcd for C₃₂H₄₃Cl₂N₆OSi [M+H]⁺ = 625.3; found, 625.

(*R*)-6-chloro-1-(1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazine (67). Step 1. (*R*)-1-(2,4-dichlorophenyl)hydrazine hydrochloride (**40**, 47.3 g, 195.8

1
2
3
4 mmol) was dissolved in ethanol (356 mL) at room temperature and 1-(3,5-
5
6
7 dichloropyrazin-2-yl)ethan-1-one (34.0 g, 178.0 mmol) was added. The mixture was
8
9
10 stirred at room temperature for 8 h and then concentrated under reduced pressure. The
11
12
13 residue was suspended in 20% ethyl acetate in hexanes (200 mL) and filtered through a
14
15
16 silica gel plug eluting with 20% ethyl acetate in hexanes. The filtrate was concentrated
17
18
19 under reduced pressure to give (*R,Z*)-3,5-dichloro-2-(1-(2-(1-(2,4-
20
21
22 dichlorophenyl)ethyl)hydrazono)ethyl)pyrazine and (*R,E*)-3,5-dichloro-2-(1-(2-(1-(2,4-
23
24
25 dichlorophenyl)ethyl)hydrazono)ethyl)pyrazine (9:1) as a viscous orange oil. LRMS-
26
27
28 dichlorophenyl)ethyl)hydrazono)ethyl)pyrazine (9:1) as a viscous orange oil. LRMS-
29
30
31 ESI⁺: *m/z* calcd for C₁₄H₁₂Cl₄N₄ [M+H]⁺ = 377.0; found, 377.
32
33
34

35
36 Step 2. A mixture of (*R,Z*)-3,5-dichloro-2-(1-(2-(1-(2,4-
37
38
39 dichlorophenyl)ethyl)hydrazono)ethyl)pyrazine and (*R,E*)-3,5-dichloro-2-(1-(2-(1-(2,4-
40
41
42 dichlorophenyl)ethyl)hydrazono)ethyl)pyrazine (9:1) (33 g, 87.3 mmol) was dissolved in
43
44
45 *N*-methyl-2-pyrrolidone (218 mL) at room temperature and 2,6-lutidine (30.3 mL, 261.9
46
47
48 mmol) was added. The mixture was degassed with nitrogen and heated to 100 °C under
49
50
51 nitrogen for 8 h. The reaction mixture was cooled to room temperature and poured into
52
53
54
55
56
57
58
59
60

1
2
3 a separatory funnel containing 500 mL of 1 N HCl in water and 500 mL of ethyl acetate.
4
5

6
7 The layers were separated, and the organic layer was washed with 500 mL of 1 N HCl
8
9

10 in water, dried over sodium sulfate, and concentrated under reduced pressure. The
11
12

13
14 residue was purified by silica gel chromatography (0 to 20% (1:1
15
16

17 MTBE:dichloromethane) in hexanes) to provide (*R*)-6-chloro-1-(1-(2,4-
18
19

20 dichlorophenyl)ethyl)-3-methyl-1H-pyrazolo[3,4-*b*]pyrazine (**67**) as off-white solid (67%
21
22

23
24 yield). Ee: 98% Chiralpak® OZ-H; 250mmx4.6mm, 5% iPrOH in heptanes; flow rate 1
25
26

27 mL/min; detection at 254 nm; R_t = 6.9 min. ^1H NMR (300 MHz, Chloroform-*d*) δ 8.45 (s,
28
29

30
31 1H), 7.43 (d, J = 8.4 Hz, 1H), 7.38 (d, J = 2.1 Hz, 1H), 7.19 (dd, J = 8.4, 2.1 Hz, 1H),
32
33

34
35 6.48 (q, J = 6.9 Hz, 1H), 1.93 (d, J = 6.9 Hz, 3H). LRMS-ESI⁺: m/z calcd for C₁₄H₁₁Cl₃N₄
36
37

38 [M+H]⁺ = 341.0; found, 341.
39
40
41
42
43
44
45
46

47 ASSOCIATED CONTENT

48
49
50

51 **Supporting Information.** The Supporting Information is available free of charge via the
52
53

54
55 Internet at the [ACS Publications website](#).
56
57
58
59
60

1
2
3 Assay conditions; hepatocyte stability assay; in vitro safety panel; X-ray
4 structures of synthetic intermediates of **16** and **17**; Molecular Formula Strings
5
6
7
8
9
10

11
12 **AUTHOR INFORMATION**
13

14
15
16 **Corresponding Authors**
17

18
19
20 *dwustrow@rapt.com
21

22
23
24 *mzibinsky@rapt.com
25

26
27
28 **Present Addresses**
29

30
31
32 §J. M. K.: Mirati Therapeutics, 9393 Towne Centre Drive, Suite 200, San Diego, CA
33

34
35
36 92121.
37

38
39
40 ‡B. B.: Pharmacyclics, an AbbVie Company, 995 East Arques Avenue, Sunnyvale, CA 94085.
41

42
43 †H. P. B.: IDEAYA Biosciences, 7000 Shoreline Court, Suite 350, South San Francisco, CA
44
45 94080.
46

47
48 #M. H. T. B.: Exelixis Inc., 1851 Harbor Bay Parkway, Alameda, CA 94502.
49

50
51 †J. M. & H. P. S.: Nurix Therapeutics Inc., 1700 Owens Street, Suite 205, San Francisco, CA
52
53 94158.
54
55
56
57
58
59
60

1
2
3 ∇C. M.: Arcus Biosciences, 26118 Research Road, Hayward, CA 94545.
4
5

6 ∟A. O: Takeda Oncology, 40 Landsdowne Street, Cambridge, MA 02139.
7
8

9 ∓M. K. R.: Pliant Therapeutics Inc., 260 Littlefield Avenue, South San Francisco, CA
10
11
12
13 94080.
14
15

16
17 ∆J. R. W.: Sigma Aldrich Fine Chemicals, 645 Science Drive, Madison, WI 53711.
18
19

20 **Author Contributions**

21
22
23 †These authors contributed equally to the preparation of this manuscript. The manuscript was
24
25 written through contributions from all authors. All authors have given approval to the final
26
27 version of the manuscript.
28
29

30 **Notes**

31
32
33
34 X-ray structures of intermediates for compounds **16** and **17** were deposited in the
35
36
37
38 Cambridge Crystallographic Data Centre under deposition numbers 1915201 (**16**) and
39
40
41 1915202 (**17**). The authors will release the atomic coordinates upon article publication.
42
43
44

45 RAPT Therapeutics was formerly known as FLX Bio Inc. The authors declare the
46
47
48 following competing financial interests: All authors of this manuscript are or were
49
50
51 employees of RAPT Therapeutics.
52
53
54
55
56
57
58
59
60

1
2
3
4 **ACKNOWLEDGMENT**
5
6

7 We would like to thank all current and former employees for all their hard work and
8
9
10 dedication to our CCR4 program. We are grateful to Dr. Antonio DiPasquale for
11
12
13
14 assistance with X-ray crystallography.
15
16
17

18 **ABBREVIATIONS**
19
20

21 CCR4, CC chemokine receptor 4; CCL17, CC chemokine ligand 17; CCL22, CC
22
23
24 chemokine ligand 22; T_{reg}, regulatory T cells; IO, immuno-oncology; T_{eff}, effector T cells;
25
26
27
28 TME, tumor microenvironment; Th2, T helper type 2 cell; GPCR, G protein-coupled
29
30
31 receptor; CTCL, Cutaneous T-Cell Lymphoma; ADCC, antibody-dependent cell
32
33
34
35 mediated cytotoxicity; CTX, chemotaxis; CD4, cluster of differentiation 4; CD8, cluster of
36
37
38 differentiation 8; CD25, cluster of differentiation 25; FOXP3, forkhead box P3; HS,
39
40
41 human serum; hHep, human hepatocytes; rHep, rat hepatocytes; V_{ss}, volume of
42
43
44
45 distribution; CL, clearance; miTreg, mouse induced regulatory T cells; GFP, green
46
47
48 fluorescent protein; HATU, 1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-
49
50
51
52 b]pyridinium 3-oxid hexafluorophosphate; HCl, hydrochloric acid; Pd, palladium; DIPEA,
53
54
55
56
57
58
59
60

1
2
3 *N,N*-diisopropylethylamine; NaH, sodium hydride; NH₄OH, ammonium hydroxide; S_NAr,
4
5
6
7 nucleophilic aromatic substitution.
8
9

10
11 REFERENCES
12

- 13 (1) Ishida, T.; Ueda, R. CCR4 as a novel molecular target for immunotherapy of cancer.
14
15 *Cancer Sci.* **2006**, *97*, 1139-1146.
16
17
18 (2) Sugiyama, D.; Nishikawa, H.; Maeda, Y.; Nishioka, M.; Tanemura, A.; Katayama, I.;
19
20 Ezoe, S.; Kanakura, Y.; Sato, E.; Fukumori, Y.; Karbach, J.; Jäger, E.; Sakaguchi, S. Anti-
21
22 CCR4 mAb selectively depletes effector-type FoxP3⁺CD4⁺ regulatory T cells, evoking
23
24 antitumor immune responses in humans. *Proc. Natl. Acad. Sci. USA* **2013**, *110*, 17945-
25
26 17950.
27
28
29 (3) Martinenaite, E.; Munir Ahmad, S.; Hansen, M.; Met, O.; Westergaard, M. W.; Larsen, S.
30
31 K.; Klausen, T. W.; Donia, M.; Svane, I. M.; Andersen, M. H. CCL22-specific T cells:
32
33 Modulating the immunosuppressive tumor microenvironment. *Oncoimmunology* **2016**, *5*,
34
35 e1238541.
36
37
38 (4) Kurowska-Stolarska, M.; Stolarski, B.; Kewin, P.; Murphy, G.; Corrigan, C. J.; Ying, S.;
39
40 Pitman, N.; Mirchandani, A.; Rana, B.; van Rooijen, N.; Shepherd, M.; McSharry, C.;
41
42 McInnes, I. B.; Xu, D.; Liew, F. Y. IL-33 amplifies the polarization of alternatively
43
44 activated macrophages that contribute to airway inflammation. *J. Immunol.* **2009**, *183*,
45
46 6469-6477.
47
48
49 (5) Curiel T. J.; Coukos, G.; Zou, L.; Alvarez, X.; Cheng, P.; Mottram, P.; Evdemon-Hogan,
50
51 M.; Conejo-Garcia, J. R.; Zhang, L.; Burow, M.; Zhu, Y.; Wei, S.; Kryczek, I.; Daniel, B.;
52
53 Gordon, A.; Myers, L.; Lackner, A.; Disis, M. L.; Knutson, K. L.; Chen, L.; Zou, W.
54
55
56
57
58
59
60

- 1
2
3 Specific recruitment of regulatory T cells in ovarian carcinoma fosters immune privilege
4 and predicts reduced survival. *Nat. Med.* **2004**, *10*, 942-949.
5
6
7
8 (6) Hadrup, S.; Donia, M.; Straten, P. Effector CD4 and CD8 T cells and their role in the
9 tumor microenvironment. *Cancer Microenviron.* **2013**, *6*, 123-133.
10
11
12 (7) Yu, P.; Lee, Y.; Liu, W.; Krausz, T.; Chong, A.; Schreiber, H.; Fu, Y. X. Intratumor
13 depletion of CD4+ cells unmasks tumor immunogenicity leading to the rejection of late-
14 stage tumors. *J. Exp. Med.* **2005**, *201*, 779-791.
15
16
17
18 (8) Munir, S.; Andersen, G. H.; Met, O.; Donia, M.; Frosig, T. M.; Larsen, S. K.; Klausen, T.
19 W.; Svane, I. M.; Andersen, M. H. HLA-restricted cytotoxic T cells that are specific for the
20 immune checkpoint ligand PD-L1 occur with high frequency in cancer patients. *Cancer*
21 *Res.* **2013**, *73*, 1764-1776.
22
23
24
25
26
27
28 (9) Gobert, M.; Treilleux, I.; Bendriss-Vermare, N.; Bachelot, T.; Goddard-Leon, S.; Arfi, V.;
29 Biota, C.; Doffin, A. C.; Duran, I.; Olive, D.; Perez, S.; Pasqual, M.; Faure, C.; Ray-
30 Coquard, I.; Puisieux, A.; Caux, C.; Blay, J. Y.; Menetrier-Caux, C. Regulatory T cells
31 recruited through CCL22/CCR4 are selectively activated in lymphoid infiltrates
32 surrounding primary breast tumors and lead to an adverse clinical outcome. *Cancer Res.*
33 **2009**, *69*, 2000-2009.
34
35
36
37
38
39
40
41
42 (10) Kumai, T.; Nagato, T.; Kobayashi, H.; Komabayashi, Y.; Ueda, S.; Kishibe, K.; Ohkuri,
43 T.; Takahara, M.; Celis, E.; Harabuchi, Y. CCL17 and CCL22/CCR4 signaling is a strong
44 candidate for novel target therapy against nasal natural killer/T-cell lymphoma. *Cancer*
45 *Immunol. Immunother.* **2015**, *64*, 697-705.
46
47
48
49
50
51 (11) Zhou, M.; Bracci, P. M.; McCoy, L. S.; Hsuang, G.; Wiemels, J. L.; Rice, T.; Zheng, S.;
52 Kelsey, K. T.; Wensch, M. R.; Wiencke, J. K. Serum macrophage-derived
53
54
55
56
57
58
59
60

- 1
2
3 chemokine/CCL22 levels are associated with glioma risk, CD4 T cell lymphopenia and
4 survival time. *Int. J. Cancer* **2015**, *137*, 826-836.
5
6
7
8 (12) Bayry, J.; Tartour, E.; Tough, D. F. Targeting CCR4 as an emerging strategy for cancer
9 therapy and vaccines. *Trends Pharmacol.* **2014**, *35*, 163-165.
10
11
12 (13) Solari, R.; Pease, J. E. Targeting chemokine receptors in disease – a case study of CCR4.
13
14 *Eur. J. Pharmacol.* **2015**, *763*, 169-177.
15
16
17 (14) Zhang, Y.; Wu, Y.; Qi, H.; Xiao, J.; Gong, H.; Zhang, Y.; Xu, E.; Li, S.; Ma, D.; Wang,
18 Y.; Li, W.; Shen, H. A new antagonist for CCR4 attenuates allergic lung inflammation in a
19 mouse model of asthma. *Sci. Rep.* **2017**, *7*, 15038.
20
21
22
23
24 (15) Hall, D.; Ford, A.; Hodgson, S. Therapeutic Potential of CCR4 Antagonists. In *New*
25 *Drugs and Targets for Asthma and COPD*; Karger Medical and Scientific Publishers,
26 2010; Vol. 39, pp 161-165.
27
28
29
30
31 (16) Pease, J. E.; Horuk, R. Recent progress in the development of antagonists to the
32 chemokine receptors CCR3 and CCR4. *Expert Opin. Drug Discov.* **2014**, *9*, 467-483.
33
34
35
36 (17) Andrews, G.; Jones, C.; Wregett, K. A. An intracellular allosteric site for a specific class
37 of antagonists of the CC chemokine G protein-coupled receptors CCR4 and CCR5. *Mol.*
38 *Pharmacol.* **2008**, *73*, 855-867.
39
40
41
42 (18) Slack, R. J.; Russell, L. J.; Barton, N. P.; Weston, C.; Nalesso, G.; Thompson, S.-A.;
43 Allen, M; Chen, Y. H.; Barnes, A.; Hodgson, S. T.; Hall, D. A. Antagonism of human CC-
44 chemokine receptor 4 can be achieved through three distinct binding sites on the receptor.
45
46
47
48
49 *Pharma. Res. Per.* **2013**, *1*, e00019.
50
51
52 (19) Ajram, L; Begg, M.; Slack, R.; Cryan, J.; Hall, D.; Hodson, S.; Ford, A.; Branes, A.;
53 Swieboda, D.; Mousnier, A.; Solari, R. Internalization of the chemokine receptor CCR4
54
55
56
57
58
59
60

- 1
2
3 can be evoked by orthosteric and allosteric receptor antagonists. *Eur. J. Pharmacol.* **2014**,
4
5 729, 75-85.
6
7
8 (20) Heifetz, A.; Schertler, G. F. X.; Seifert, R.; Tate, C. G.; Sexton, P. M. Gurevich, V. V.;
9
10 Fourmy, D.; Cherezov, V.; Marshall, F. H.; Storer, R. I.; Moraes, I.; Tikhonova, I. G.;
11
12 Tautermann, C. S.; Hunt, P.; Ceska, T.; Hodgson, S.; Bodkin, M. J.; Singh, S.; Law, R. J.;
13
14 Biggin, P.C. GPCR structure, function, drug discovery and crystallography: report from
15
16 Academia-Industry International Conference (UK Royal Society) Chicheley Hall, 1-2
17
18 September 2014. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **2015**, 388, 883-903.
19
20
21 (21) Kontani, T.; Kawano, N.; Masuda, N.; Kato, K.; Nagata, H.; Inami, H.; Terasaka, T.;
22
23 Kazuhir, Y.; Miyazaki, T. Preparation of Amino(acylaminopiperidiny)quinazoline
24
25 Derivatives as CCR4 Inhibitors for Treatment of Inflammation, Allergy, and Autoimmune
26
27 Disease. WO2007111227A, 2007.
28
29
30 (22) Yokoyama, K.; Ishikawa, N.; Igarashi, S.; Kawano, N.; Masuda, N.; Hamaguchi, W.;
31
32 Yamasaki, S.; Koganemaru, Y.; Hattori, K.; Miyazaki, T.; Ogino, S.; Matsumoto, Y.;
33
34 Takeuchi, M.; Ohta, M. Potent and orally bioavailable CCR4 atagonists: Synthesis and
35
36 structure-activity relationship study of 2-aminoquinazolines. *Bioorg. Med. Chem.* **2009**, 17,
37
38 64-73.
39
40
41 (23) Purandare, A. V.; Wan, H.; Somerville, J. E.; Burke, C.; Vaccaro, W.; Yang, X.;
42
43 McIntyre, K. W.; Poss, M. A. Core exploration in optimization of chemokine receptor
44
45 CCR4 antagonists. *Bioorganic Med. Chem. Lett.* **2007**, 17, 679-682.
46
47
48 (24) Nakagami, Y.; Kawase, Y.; Yonekubo, K.; Nosaka, E.; Etori, M.; Takahashi, S.; Takagi,
49
50 N.; Takeshi, F.; Kuribayashi, T.; Nara, F.; Yamashita, M. RS-1748, a novel CC chemokine
51
52
53
54
55
56
57
58
59
60

- receptor 4 antagonist, inhibits ovalbumin-induced airway inflammation in guinea pigs.
Biol. Pharm. Bull. **2010**, *33*, 1067-1069.
- (25) Kindon, N.; Andrews, G.; Baxter, A.; Cheshire, D.; Hemsley, P.; Johnson, T.; Liu, Y. Z.; McGinnity, D.; McHale, M.; Mete, A.; Reuberson, J.; Roberts, B.; Steele, J.; Teobald, B.; Unitt, J.; Vaughan, D. Walters, I.; Stocks, M. Discovery of AZD-2098 and AZD-1678, two potent and bioavailable CCR4 receptor antagonists. *ACS Med. Chem. Lett.* **2017**, *8*, 981-986.
- (26) Habashita, H.; Kokubo, M.; Shibayama, S.; Tada, H.; Sagawa, K. Preparation of Pyrazine and Quinoxaline Derivatives as Chemokine Receptor CCR4 Antagonists and Medicinal Use Thereof. W02004007472, 2004.
- (27) Procopiou, P. A.; Barrett, J. W.; Barton, N. P.; Begg, M.; Clapham, D.; Copley, R. C. B.; Ford, A. J.; Graves, R. H.; Hall, D. A.; Hancock, A. P.; Hill, A. P.; Hobbs, H.; Hodgson, S. T.; Jumeaux, C.; Lacroix, Y. M. L.; Miah, A. H.; Morriss, K. M. L.; Needham, D.; Sheriff, E. B.; Slack, R. J.; Smith, C. E.; Sollis, S. L.; Staton, H. Synthesis and structure–activity relationships of indazole arylsulfonamides as allosteric CC-chemokine receptor 4 (CCR4) antagonists. *J. Med. Chem.* **2013**, *56*, 1946-1960.
- (28) Cahn, A.; Hodgson, S.; Wilson, R.; Robertson, J.; Watson, J.; Beerahee, M.; Hughes, S. C.; Young, G.; Graves, R.; Hall, D.; van Marle S.; Solari, R. Safety, tolerability, pharmacokinetics and pharmacodynamics of GSK2239633, a CC-chemokine receptor 4 antagonist, in healthy male subjects: results from an open-label and from a randomized study. *BMC Pharmacol. Toxicol.* **2013**, *14*, 1-11.
- (29) Toogood, P. Small molecule immuno-oncology therapeutic agents. *Bioorg. Med. Chem. Lett.* **2017**, *28*, 319-329.

- 1
2
3 (30) FDA approves treatment for two rare types of non-Hodgkin lymphoma
4
5 <https://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm616176.htm>
6
7 (accessed November 16th, 2018).
8
9
- 10 (31) Ishida, T.; Ito, A.; Sato, F.; Kusumoto, S.; Iida, S.; Inagaki, H.; Morita, A.; Akinaga, S.;
11
12 Ueda, R. Stevens-Johnson Syndrome associated with mogamulizumab treatment of adult
13
14 T-cell leukemia / lymphoma. *Cancer Sci.* **2013**, *104*, 647-650.
15
16
- 17 (32) Miyagawa, F.; Yamamoto, S.; Miyao, M.; Nishikawa, M.; Ogawa, K.; Asada, H.
18
19 Predisposition to multi-drug hypersensitivity after administration of magamulizumab. *Eur.*
20
21 *J. Dermatol.* **2018**, *28*, 526-528.
22
23
- 24 (33) Saulite, I.; Guenova, E.; Hoetzenecker, W. Adverse Reactions of Antibody-Therapy for
25
26 Primary Cutaneous Lymphomas: Rituximan, Bentuximab Vedotin, Alemtuzumab, and
27
28 Mogamulizumab. In *Current Problems in Dermatology. Adverse Reactions of Biologics*;
29
30 Puig, L.; Gulliver, W. Eds.; Karger: Basel, 2018: Vol. 53, pp 70-81.
31
32
- 33 (34) Zhang, R.; Xie, X. Tools for GPCR drug discovery. *Acta Pharmacol. Sin.* **2012**, *33*, 372-
34
35 384.
36
37
- 38 (35) Proudfoot, A. E. I.; Power, C. A.; Church, D. J.; Soler, D.; Mack, M. Cellular assays of
39
40 chemokine receptor activation. *Curr. Protoc. Pharmacol.* **2001**, *14*, 12.4.1-12.4.26.
41
42
- 43 (36) Beck, H.; Bui, M.; Chian, D.; Diokno, R.; Hu, X. D.; Jacobson, S.; Karbarz, E.; Kassner,
44
45 P. D.; Ketcham, J. M.; Marshall, L.; McKinnell, J.; Meleza, C.; Reilly, M. K.; Robles, O.;
46
47 Shunatona, H. P.; Talay, O.; Walker, J. R.; Wustrow, D. J.; Zibinsky, M. Unpublished
48
49 results.
50
- 51 (37) Mosely, S. I. S.; Prime, J. E.; Sainson, R. C. A.; Koopman, J.-O.; Wang, D. Y. Q.;
52
53 Greenawalt, D. M.; Ahdesmaki, M. J.; Leyland, R.; Stefanie, M.; Pacelli, L.; Marcus, D.;
54
55
56
57
58
59
60

- 1
2
3 Anderton, J.; Watkins, A.; Ulrichsen, J. C.; Brohawn, P.; Higgs, B. W.; McCourt, M.;
4
5 Jones, H.; Harper, J. A.; Morrow, M.; Valge-Archer, V.; Stewart, R.; Dovedi, S. J.;
6
7 Wilkinson, R. W. Rational selection of syngeneic preclinical tumor models for
8
9 immunotherapeutic drug discovery. *Cancer Immunol. Res.* **2017**, *5*, 29-41.
10
11
12 (38) Fantini, M. C.; Dominitzki, S.; Rizzo, A.; Neurath, M. F.; Becker, C. In vitro generation
13
14 of CD4⁺CD25⁺ regulatory cells from murin naive T cells. *Nat. Protoc.* **2007**, *2*, 1789-
15
16 1794.
17
18
19 (39) Schmitt, E. G.; Williams, C. B. Generation and function of induced regulatory T cells.
20
21 *Front Immunol.* **2013**, *4*, 1-13.
22
23
24 (40) More detailed studies on the biology of CCR4 antagonism in the context of various
25
26 immune cells and anti-tumor activity will be published in another manuscript.
27
28
29 (41) Schall, T. J.; Proudfoot, A. E. I. Overcoming hurdles in developing successful drugs
30
31 targeting chemokine receptors. *Nat. Rev. Immunol.* **2011**, *11*, 355-363.
32
33
34 (42) Hersperger, R.; Janser, P.; Pfenninger, E.; Wuethrich, H. J.; Miltz, W. Preparation of 1H-
35
36 indole-2-carboxylic acid N-(piperidin-4-yl)amides and related derivatives as chemokine
37
38 receptor, particularly CCR2 and CCR5 antagonists. WO2005077932, 2005.
39
40
41 (43) Chakraborty, T. K.; Samanta, R.; Kumar, P. K. A radical mediated approach to the
42
43 stereoselective formal total synthesis of (+)-Sch 642305. *Tetrahedron* **2009**, 6925-6931.
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Table of Contents graphic.

